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Integrated Science Assessment for Ozone and Related Photochemical Oxidants

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ACRONYMS AND ABBREVIATIONS

Acronym/Abbreviation	Meaning
^{13}C	carbon-13
^{15}N	nitrogen-15 stable isotope of nitrogen
3D	three-dimensional
3-PG	Physiological Principles Predicting Growth, a process-based stand level model which is a transition model between growth and carbon balance models
5HT	5-Hydroxytryptamine (serotonin)
5HT2aR	5-Hydroxytryptamine (serotonin) receptor 2a
5Ht4R	5-Hydroxytryptamine (serotonin) receptor 4
5HTT	5-Hydroxytryptamine (serotonin) transporter
A1c	Hemoglobin A1C
ABA	abscisic acid
AC	air conditioning
ACCMIP	Atmospheric Chemistry and Climate Model Intercomparison Project
ACE-1	angiotensin converting enzyme
ACM2	Asymmetric Convective Model Version 2
ACS	American Cancer Society
ACTH	Adrenocorticotropic hormone
ADA	Americans with Disabilities Act
ADAM	a disintegrin and metalloproteinase
ADF	acid digestible fiber
ADL	acid digestible lignin
ADMS	Advanced Dispersion Modeling System
ADMS-Urban	Atmospheric Dispersion Modelling System-Urban
ADOS	Autism Diagnostic Observation Schedule
ADREX, ADX	adrenalectomy
AER	air exchange rate

AERMIC	AMS/EPA Regulatory Model Improvement Committee
AERMOD	AERMIC Model
AERO5	fifth-generation modal CMAQ aerosol model with extensions
AF	atrial fibrillation
AHI	apnea-hypopnea Index
AHR	airway hyperresponsiveness, arylhydrocarbon receptor
AHS	Agricultural Health Study
AIC	Akaike's information criterion
AIRDATA	(U.S. EPA) Air quality data collected at outdoor monitors across the U.S
AIRPACT-3	Air Information Report for Public Access and Community Tracking Version 3
AIRS	Aerometric Information Retrieval System
AK	averaging kernel
AKT	Protein kinase B (PKB), also known as Akt, is a serine/threonine-specific protein kinase
ALRI	acute lower respiratory infection
AM	alveolar macrophage(s)
AM3	Atmospheric Model 3 (chemical transport model from Geophysical Fluid Dynamics Laboratory)
AMI	acute myocardial infarction
AMO	Atlantic Multidecadal Oscillation
AMPK	Adenosine monophosphate-activated protein kinase
AMS	American Meteorological Society
AMX	adrenal medullarectomy
ANPP	annual net primary productivity
AO	arctic oscillation
AOD	aerosol optical depth
AOT0	seasonal sum of the difference between an hourly concentration at the threshold value of 0 ppb, minus the threshold value of 0 ppb
AOT40	seasonal sum of the difference between an hourly concentration at the threshold value of 40 ppb, minus the threshold value of 40 ppb
AOT60	seasonal sum of the difference between an hourly concentration at the threshold value of 60 ppb minus the threshold value of 60 ppb

AOTx	sum of the differences between hourly concentrations greater than a specified threshold (x) during a specified daily and seasonal time window
APEX	Air Pollutants Exposure Model
APHEA	Air Pollution and Health: a European Approach
APHEA2	Air Pollution and Health: A European Approach 2
APHENA	Air Pollution and Health: A Combined European and North American Approach
API	Air Pollution Index
APMoSPHERE	Air Pollution Modeling for Support to Policy on Health and Environmental Risk in Europe
ApoA1	apolipoprotein A-I
ApoB	Apolipoprotein B
ApoE-	apolipoprotein E-deficient
APP+	amyloid beta precursor protein
APT	Advanced Plume Treatment
AQCD	Air Quality Criteria Document
AQMEII	Air Quality Model Evaluation International Initiative
AQS	U.S. EPA Air Quality System database
AR	airway responsiveness
AR4	Fourth Assessment Report (AR4) from the IPCC
AR5	Fifth Assessment Report (AR5) from the IPCC
ARDS	adult respiratory distress syndrome
ARG	arginase
ARI	acute respiratory infection
As	Air Resource Specialists
ASD	Autism Spectrum Disorder
ASHRAE	American Society of Heating, Refrigeration, and Air Conditioning Engineers
AT	Apparent Temperature
ATA	American Trucking Association
ATPase	Adenosinetriphosphotase
AUC	area under the curve

AUP	unpaired predicted to-observed peak ozone ratio
AURAMS	Unified Regional Air Quality Modeling System
AV	atrioventricular
AVCD	atrioventricular conduction disorders
avg	average
BAD	bronchial artery diameter
BAL	bronchoalveolar lavage
BALF	bronchoalveolar lavage fluid
BAMSE	Children, Allergy, Milieu, Stockholm, Epidemiology (Swedish Abbreviation)
BASIC	Brain Attack Surveillance in Corpus Christi
BC	black carbon
BCSC	Breast Cancer Surveillance Consortium
BEIS	Biogenic Emission Inventory System
BELD	Biogenic Emissions Land use Database
BME	Bayesian maximum-entropy
BMI	body mass index
BN	Brown Norway rat
BP	blood pressure
bpm	beats per minute
BRAVO	Big Bend Regional Aerosol and Visibility Observational
BrdU	bromodeoxyuridine
BSA	body surface area
bun	blood urea nitrogen
BVAIT	B-Vitamin Atherosclerosis Intervention Trial
BVOC	biogenic volatile organic compound
BW	birth weight; body weight
BWHS	Black Women's Health Study
C	carbon; degrees Celsius; the product of microenvironmental concentration
C3	plants that only use the Calvin cycle for fixing the carbon dioxide from the air

C4	plants that use the Hatch-Slack cycle for fixing the carbon dioxide from the air
C57BL	wild type c57 black mouse
C_a	ambient ozone concentration
CAA	Clean Air Act
CAD	coronary artery disease
CALGRID	California Grid Simulations
CalNEX	California Research at the Nexus of Air Quality and Climate Change
CAM	Community Atmosphere Model; plants that use crassulacean acid metabolism for fixing the carbon dioxide from the air
CAMP	Constant Air Quality Model Performance
CAMS	Continuous Monitoring Station
CAMx	Comprehensive Air Quality Model with extensions
CanCHEC	Canadian Census Health and Environment Cohort
CAP	concentrated ambient particle(s)
CAPPS	Canadian Asthma Primary Prevention Study
CARB	California Air Resources Board
CASAC	Clean Air Scientific Advisory Committee
CASTNet	Clean Air Status and Trends Network
CAT	catalase
CATHGEN	catheterization genetics
CB05	carbon bond mechanism developed in 2005
CBVD	cerebrovascular disease
CCL	chemokine ligand
CCR2	chemokine receptor type 2
CCSP	U.S. Climate Change Science Program, forerunner to the U.S. Global Change Research Program; club cell secretory protein
CD	cluster of differentiation; confidence distribution
CD36	cluster of differentiation 36
CD4	cluster of differentiation 4
cd56	neural cell adhesion molecule

CDC	Centers for Disease Control and Prevention
CDR	Clinical Dementia Rating Sum of Boxes
CEM	continuous emissions modeling
CF	charcoal filter; carbon filtered
CFR	Code of Federal Regulations
CH_2O	carbohydrate
CH_4	methane
CHAD	Consolidated Human Activity Database
CHARGE	Childhood Autism Risks from Genetics and the Environment
CHASER	CHemical Atmospheric general circulation model for Study of atmospheric Environment and Radiative Forcing (chemical transport model)
CHD	chronic heart disease; coronary heart disease
CHE	controlled human exposure
CHF	congestive heart failure
Chil4	chitinase-like-4 protein
CHIMERE	regional chemistry transport model Multi-scale chemistry-transport model for atmospheric composition analysis and forecast
CHRONOS	Canadian Hemispheric and Regional Ozone and NOX System
CHS	Children's Health Study
CI	Confidence interval
Ci	intra-cellular carbon dioxide; substrate concentrations
cIMT	carotid intimal-medial thickness
CL	critical level
Cl_2	chlorine (gas)
Clca1	Chloride channel accessory 1
CLM	chemiluminescence method
$ClNO_2$	nitryl chloride
CLPPs	community-level physiological profiles
cm	centimeter
CM	conditioned medium

CMAQ	Community Multi-scale Air Quality modeling system
CMAQ-HBM	Community Multi-scale Air Quality-Hierarchical Bayesian Model
CMIP6	Coupled Model Intercomparison Project Phase 6
CMP	Central Mean Arterial Pressure
C:N	carbon nitrogen ratio
CNS	central nervous system
CO	carbon monoxide
CO_2	carbon dioxide
COFI	Cohorte Fibrose
CONUS	Continental United States
COPD	Chronic Obstructive Pulmonary Disease
CP	coverage prediction interval
CPC	condensation particle counter
cPOM	course particulate organic matter
CPSII	(ACS) Cancer Prevention Study II
CRH	Corticotropin-releasing hormone
CRP	C-reactive protein
CSAPR	Cross-State Air Pollution Rule
CSI	critical success index
CSS	calculated severity score (ADOS-CSS)
CSTRs	continuous stirred tank reactor
CTM	chemical transport model
CV	cardiovascular; coefficient of variation
CVD	cardiovascular disease
CX3CL1	Fractalkine
CX3CR1	Fractalkine receptor
CXC	cys-xxx-cys - (amino acid motif)
CXCL	chemokine family of cytokines with highly conserved motif: cys-xxx-cys (CXC)
CXCR	receptor for chemokine family of receptors

Cypb5	cytochrome p450 b5
D	dose
DA24	daily 24-hour average concentration
db	decibel
DBP	diastolic blood pressure
DC3	Deep Convective Cloud and Chemistry (field study)
DDM	Decoupled Direct Method
DDS	Department of Developmental Services
DECSO	Daily Emission estimates Constrained by Satellite Observations (algorithm)
DEHM	Danish Eulerian Hemispheric Model
DEMED	Adrenal demedullation
df	degrees of freedom
dg	decigram
DISCOVER-AQ	Deriving Information on Surface conditions from COlumn and VERtically resolved observations relevant to Air Quality
DL	distributed lag
dL	deciliters
DLEM	Dynamic Land Ecosystem Model
DLNM	distributed lag nonlinear model
DM	Dry Moderate; dry matter
DM8H	8-hour daily maximum ozone
DNA	deoxyribonucleic acid
DOC	dissolved organic carbon
DOE	Department of Energy
DOHaD	Developmental Origins of Health and Disease
dP	change in pressure
DP	Dry Polar
DSM-IV-R	Diagnostic and Statistical Manual of Mental Disorders, 4th edition Revised
DT	Dry Tropical

Ea	ambient ozone exposure
EBC	exhaled breath condensate
EC	elemental carbon
ECG	electrocardiographic
ED	emergency department
EDGAR	Emissions Database for Global Atmospheric Research
EDMUS	European Database for Multiple Sclerosis
EGAS	Economic Growth Analysis System
EGF	epidermal growth factor
EGUs	electricity generating units
EH	redox potential
EI	emissions inventory
EIB	emissions influenced background
ELITE	Early Versus Late Intervention Trial with Estradiol
EMEP	European Monitoring and Evaluation Program
EMF	ectomycorrhizal fungal
EMS	emergency medical service
EnKF	Ensemble Kalman Filter
eNO	exhaled nitric oxide
eNOS	endothelial nitric oxide synthase
ENSO	El Niño Southern Oscillation
E-O_3	elevated ozone
EPA	U.S. Environmental Protection Agency
ER	emergency room; estrogen receptor
ERF	effective radiative forcing
ET-1	endothelin-1
EUgrow	forest productivity model
F344	Fisher 344 strain of rats
FA	filtered air; adjusted forcings; fatty acid

FACE	free-air carbon dioxide/ozone enrichment
FACS	florescence activated cell sorting
FAR	false alarm ratio
FB	fractional bias
FBG	fasting blood glucose
FDDA	four-dimensional data assimilation
FE	fractional error
FEF25-75	mean forced expiratory flow over the middle half of the forced vital
FEM	Federal Equivalent Method
FeNO	fractional exhaled nitric oxide
FEPS	Fire Emissions Production Simulator
FEV_1	forced expiratory volume in one second
FFA	free fatty acid
FHH	fawn-hooded hypertensive
FIA	(United States Department of Agriculture Forest Service) Forest Inventory and Analysis Program
FLN	flower number
FMD	flow-mediated dilation
FN	fruit number
FOR	fecundability odds ratio
Fp	percentage of cases where simulation results were close to observations
FPG	fasting plasma glucose
fPOM	fine particulate organic matter
FR	Federal Register; fecundity risk
FRM	Federal Reference Method
FW	fruit weight
g	grams
GAM	generalized additive model
GB	gross bias
GCM	General Circulation Model

GD	gestational day
GDAS	Global Data Assimilation System
GDM	gestational diabetes mellitus
GE	gross error
GEM-MACH	Global Environmental Multi-scale coupled with Model of Air quality and Chemistry
GEMS	Global and regional Earth-system Monitoring using Satellite and in-situ data
GEOS	Goddard Earth Observing System
GEOS-Chem	GEOS-Chemistry
GFAP	glial fibrillatory acidic protein
GFDL	Geophysical Fluid Dynamics Laboratory
GGT	gamma-glutamyltranspeptidase
GHG	greenhouse gas
GHGs	Greenhouse Gases
GINI	German Infant Nutritional Intervention study
GINIplus	German Infant Nutritional Intervention plus environmental and genetic influences
GIS	Geographic Information System
GISS	Goddard Institute for Space Studies
GLM	generalized linear model
GLP	good laboratory practices
GLVs	green leaf volatiles
GMRF	Gaussian Markov random field
GOES	Geostationary Operational Environmental Satellite
GOME2a	Global Ozone Monitoring Experiment 2A (instrument system borne on the European Remote Sensing Satellite)
GPP	gross primary productivity
GPS	Global Positioning System
GPx	glutathione peroxidase
GRP	gastrin-releasing peptide
GRPR	gastrin-releasing peptide receptor

Gs	stomatal conductance
GSH	reduced glutathione
GSHGSSG	ratio of reduced to oxidized glutathione
GSS	gas-sensitive semiconducting oxide; glutathione synthetase
GSSG	oxidized glutathione
GST	glutathione-S-transferase
GSTM1	glutathione S-transferase polymorphism Mu 1
GSTP1	glutathione S-transferase pi gene
GTT	glucose tolerance test
GVRD	Greater Vancouver Regional District
h	hour
H_2O	water
H_2O_2	hydrogen peroxide
HA	hospital admission
HAWC	Health Assessment Workspace Collaborative
HbA1c	Hemoglobin A1C
HBM	Hierarchical Bayesian Model
HCHO	Formaldehyde
HDDM	higher order decoupled direct method; Hierarchical Bayesian Diffusion Drift Model
HDL	high density lipoprotein
HDM	house dust mite; house dust mite allergen
HDMA	house dust mite allergen
HEI	Health Effects Institute
HERO	Health and Environmental Research Online
HF	high frequency component of HRV; heart failure; high fat diet
HFD	high fat diet
HFr	right heart failure
Hg	mercury
HGB	Houston–Galveston–Brazoria

HIRA	Health Insurance Review and Assessment Service
HISA	Highly Influential Scientific Assessment
HMOX	heme oxygenase
HMS	Hazard Mapping System; Hospital Morbidity Survey
HNO_3	nitric acid
HO	heme oxygenase
HO_2	hydroperoxyl radical
HOCl	hypochlorous acid
HOMA	Homeostatic model assessment
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
HOMOVA	homogeneity of molecular variance
HONO	nitrous acid
HO_X	hydrogen & oxygen containing radicals (sum of hydroxyl and hydroperoxyl radicals)
HPA	hypothalamic pituitary adrenal axis
HR	heart rate; hazard ratio
HRV	heart rate variability
HSP70	heat shock protein 70
HTAP	hemispheric transport of air pollutants
HVAC	heating, ventilation, and air conditioning
HYSPLIT	hybrid single particle lagrangian integrated trajectory
ICAM	intercellular adhesion molecule
ICARTT	International Consortium for Atmospheric Research on Transport and Transformation
ICC	interclass correlation coefficient
ICD	International Classification of Diseases; implantable cardioverter defibrillators
ICD10	International Classification of Diseases - version 10
ICD9	International Classification of Diseases - version 9
ICU	intensive care unit
IDW	inverse distance weighting
IFN	interferon

IGAC	International Global Atmospheric Chemistry
IgE	immunoglobulin E
IHD	ischemic heart disease
IL	interleukin
ILC	immune lymphoid cell
IMPROVE	Interagency Monitoring of Protected Visual Environments
iNOS	inducible nitric oxide synthase
IO	iodine monoxide
IOA	index of agreement
IOM	institute of medicine
IP	inhalable particle; intraperitoneal injection
IPCC	Intergovernmental Panel on Climate Change
IQR	interquartile range
IR	infrared; incidence rate
IRB	Institutional Review Board
IRP	Integrated Review Plan
ISA	Integrated Science Assessment
ISAM	Integrated Source Apportionment Method (in CMAQ)
IST	Insulin sensitivity test
IT	intra-tracheal instillation
ITT	Insulin tolerance test
IVDMD	in vitro dry matter digestibility
IVF	in vitro fertilization
IVND	in vitro nitrogen digestibility
JA	jasmonic acid
JCR	A/JCr mice
JNK	c-Jun N-terminal kinase
K_2SO_4	potassium sulfate-measurement of extractable soil carbon
KC	local neutrophil chemoattractant protein

KCLurban	King's College London urban
KEEP	The Kidney Early Evaluation Program
kg	kilogram
KK	KK mouse strain
KKAY	KKAY strain of mouse
km	kilometer
kPa	kilopascal
KROFEX	Kranzberg Ozone Fumigation Experiment
L	location
LAEI	large artery elasticity index
LAI	leaf area index
LANDIS	forest landscape model
lbs	pounds
LC_{50}	median lethal concentration
LDH	lactate dehydrogenase
LE	Lake Elsinore; Long Evans rat
LF	low-frequency component of HRV
LHID2000-NHIRD	Longitudinal Health Insurance Database 2000 - National Health Insurance Research Database
LIF	leukemia inhibitory factor
LIFE	Longitudinal Investigation of Fertility and the Environment
LISA	Lifestyle-Related factors on the Immune System and the Development of Allergies in Childhood
LISAplus	Lifestyle-Related factors on the Immune System and the Development of Allergies in Childhood plus the influence of traffic emissions and genetics
LNO_X	nitrogen oxides generated by lightning
LOOCV	leave-one-out cross-validation
LOTOS-EUROS	Long Term Ozone Simulation European Operational Smog
LPS	lipopolysaccharide
LT_{50}	median lethal time
LTB4	leukotriene B4

LUR	land use regression
LV	left ventricle
LVDP	left ventricular developed pressure
LVOS	Las Vegas ozone study
LWRE	longwave radiative effect
m	meter
M1	Month 1
M2	Month 2
M3	Month 3
M7	seven hour seasonal mean
MA	moving average
MADRID	Model of Aerosol Dynamics, Reaction, Ionization and Dissolution
MAE	mean absolute error
MAGE	mean absolute gross error
MAP	mean arterial pressure
MAQSIP	multiscale air quality simulation platform
MASAES	Moderate and Severe Asthmatics and Their Environment Study
max	maximum
MB	mean bias
MBE	mean bias error
MCh	methacholine
MCM	master chemical mechanism
MCP	monocyte chemoattractant protein; monocyte chemotactic protein
Mcp-1	Monocyte chemotactic protein 1
MDA	malondialdehyde
MDA1	daily maximum 1-hour average
MDA8	daily maximum 8-hour average
MDL	method detection limit
ME	microenvironmental exposure; mean error

MEGAN	Model of Emissions of Gases and Aerosols from Nature
MEIC	Multiresolution Emissions Inventory for China
MERRA	Modern-Era Retrospective analysis for Research and Applications (a NASA reanalysis of satellite ozone data)
MESA	Multi-Ethnic Study of Atherosclerosis
MESA-Air	Multi-Ethnic Study of Atherosclerosis and Air Pollution
MFB	mean fractional bias
MFE	mean fractional error
mg	milligrams
MI	myocardial infarction; myocardial ischemia
min	minute(s); minimum
MINAP	Myocardial Ischaemia National Audit Project
MIP	macrophage inflammatory protein
MIROC	Model for Interdisciplinary Research On Climate
mL	milliliter
ML	Mira Loma
MLN	mediastinal lymph node
mm	millimeters
MM5	Mesoscale Model Version 5
MNB	mean normalized bias
MNE	mean normalized error
MNGE	mean normalized gross error
MO	month
MODIS	MODerate resolution Imaging Spectroradiometer
MOSES	Met Office Surface Exchange Scheme
MOVES	U.S. EPA Motor Vehicle Emission Simulator
MOZART	MOdel for Ozone and Related chemical Tracers
MP	mid polar; myelopeptide; moist polar
MPAN	peroxymethacrylic nitrate

MPO	myeloperoxidase
MRI-CCM2	Meteorological Research Institute Chemistry-Climate Model, version 2
mRNA	messenger ribonucleic acid
MSA	metropolitan statistical area
MSE	mean squared error
MSEL	Mullen Scales of Early Learning
MT	metric ton; Moist Tropical
mtDNA	mitochondrial DNA
MT-nx	total nonoxygenated terpenes
MUC5AC	mucin 5AC glycoprotein
MUC5B	mucin 5B
MUSCAT	MUltiScale Chemistry Aerosol Transport
MW	midwest
MX	mean metric
MYJ	Mellore-Yamadae-Janjic
n	sample size; number
N100	number of hours when the measured ozone concentration is greater than or equal to 0.100 ppm
N_2	Nitrogen (gas)
N_2O	nitrous oxide
NA	not available
NAAQS	National Ambient Air Quality Standards
NAB	North American background (ozone)
NACC	National Alzheimer's Coordinating Center
NACRS	National Ambulatory Care Reporting System
NADP	National Atmospheric Deposition Program
NADPH	reduced form of nicotinamide adenine dinucleotide phosphate
NAEPP	National Asthma Education and Prevention Program
NAG	N-acetyl-β-D-glucosaminidase

NAI	net annual increment
NAM	Northern Annular Mode; North American mesoscale
NAMS	National Air Monitoring Stations
NAPCA	U.S. National Air Pollution Control Administration
NAPDH	reduced form of nicotinamide adenine dinucleotide phosphate
NAPS	National Air Pollution Surveillance
NAQFC	National Air Quality Forecasting Capability
NASA	U.S. National Aeronautics and Space Administration
NASEM	U.S. National Academy of Science, Engineering, and Medicine
NB	normalized bias
NBDPS	National Birth Defects Prevention Study
NCAR	National Center for Atmospheric Research
NCDENR	North Carolina Department of Environment and Natural Resources
NCEA	U.S. EPA National Center for Environmental Assessment
NCLAN	National Crop Loss Assessment Network
NCore	National Core network
ND	non-detectable
nDer f 1	Dermatophagoides farinae allergen
NE	normalized error
NECSS	National Enhanced Cancer Surveillance System
NEI	U.S. EPA National Emissions Inventory
NES2	Neurobehavioral Evaluation System-2
NEu	Northern Europe
NF	non-filtered air
NFkB	nuclear factor kappa light-chain-enhancer of activated B cells
ng	nanogram
NGE	normalized gross error
NGF	nerve growth factor
NH_3	ammonia

NH_4^+	ammonium
NH_4NO_3	ammonium nitrate
NHANES	National Health and Nutrition Examination Survey
NHEERL	U.S. EPA National Health and Environmental Effects Research Laboratory
NHIRD	National Health Insurance Research Database
NHIS	National Health Insurance Service
NHIS-NCI	National Health Insurance Service - National Sample Cohort
NHIS-NSC	National Health Insurance Service - National Sample Cohort
NHLBI	National Heart, Lung, and Blood Institute
NHS	Nurses' Health Study
NK	neurokinin
nL	nanoliter
NLDN	National Lightning Detection Network
Nlrp	Nucleotide-binding oligomerization domain, Leucine rich Repeat and Pyrin domain containing
nm	nanometer(s)
NMB	normalized mean bias
NME	normalized mean error
NMMAPS	U.S. National Morbidity, Mortality, and Air Pollution Study
NNs	the interval between normal beats
NO	nitric oxide
NO_2	Nitrogen dioxide
NO_3^-	nitrate
NOAA	U.S. National Oceanic and Atmospheric Administration
NOS	nitric oxide synthase
Notch3	neurogenic locus notch homolog protein 3
Notch4	neurogenic locus notch homolog protein 4
NO_X	oxides of nitrogen (NO + NO2)
NO_Y	the sum of NOx with its related reservoir forms (gas- phase HNO3, PAN, HONO, NO3, N2O5, organic nitrates [RNO3], and nitrate in particles [pNO3])

NO_Z	Abbreviation for the sum of NOy minus NOx, i.e. NOx reservoir species, only.
NPP	net primary production
NQO1	NADPH-quinone oxidoreductase (genotype)
NR	not reported
NRC	National Research Council
NRCS	USDA National Resources Conservation Service
NRF2	Nuclear factor (erythroid-derived 2)-like 2
NS	not statistically significant; natural spline
NTS	neurotensin
NU	NASA-Unified
NUR	nuclear receptor subfamily
NW	northwest
O	outdoor ozone air concentration
O1D	The oxygen "singlet D" radical (a high energy, electronically excited form of the monatomic oxygen radical)
O_2	oxygen
O_3	ozone
OA	objective analysis
obs	observed
OC	organic carbon
ODSs	Ozone Depleting Substances
OE	elevated ozone treatment
OGG1	8 oxo-guanine repair enzyme
OGTT	oral glucose tolerance test
OH	hydroxide; hydroxyl radical
OHCA	out-of-hospital cardiac arrests
OI	optimal interpolation
OII	ozone injury index
OK	ordinary kriging

OMB	U.S. EPA Office of Management and Budget
OMI	Ozone Monitoring Instrument
ONPHEC	Ontario Population Health and Environment Cohort
OPEC	Outdoor Plant Environment Chamber
OR	odds ratio(s)
ORD	U.S. EPA Office of Research and Development
OSAT	Ozone Source Apportionment Tool (in CAMx)
OTC	open-top chamber
OTUs	operational taxonomic units
OZOVEG	Ozone Vegetation Database
P	population; probability value
PA	photoacoustic analyzer; physical activity; plasminogen activator; pascal(s); policy assessment
PAH	polycyclic aromatic hydrocarbons
PAI	plasminogen activator inhibitor, (e.g. PAI-1)
PAMS	Photochemical Assessment Monitoring Stations
PAN	peroxyacetyl nitrate; peroxyacl nitrate
PAR	photosynthetically active radiation
PAT	pulse amplitude tonometry; paroxysmal
PBL	planetary boundary layer
PCA	principal component analysis
PCI	percutaneous coronary intervention
PCR	polymerase chain reaction
PD	provocative dose
PDLR	partial derivative linear regression
PDO	Pacific Decadal Oscillation
PE	post exposure; post exercise; phenylephrine; pulmonary embolism
PECOS	Population, Exposure, Comparison, Outcome and Study Design
PEF	peak expiratory flow
Per	perylene

PFT	pulmonary function test
Pg	Petagram, equal to 1015 grams or one billion tonnes
Pgam5	phosphoglycerate mutase 7
PGE2	prostaglandin E2
PGF2α	prostoglandin 2 alpha
pH	measure of hydrogen ion concentration
PI	prediction interval
PIAMA	prevention and incidence of asthma and mite allergy
PKS	polyketide synthases
PLANTS	USDA-National Resource Conservation Services Plant List of Accepted Nomenclature, Taxonomy and Symbols
PLFA	phospholipid fatty acid
PLS	partial least squares
PM	particulate matter
PM_{10}	particulate matter with an aerodynamic diameter less than or equal to 10 µm
$PM_{2.5}$	particulate matter with an aerodynamic diameter less than or equal to 2.5 µm
PMAE	Predictive Mean Absolute Error
PMN	polymorphonuclear leukocytes
PMNs	polymorphic neutrophil
PMSE	Predictive Mean Squared Error
PN	particle number
PND	postnatal day
PND10	postnatal day 10
PND15	postnatal day 15
PND21	postnatal day 21
PND28	postnatal day 28
pNN50	proportion of pairs of successive normal simus intervals exceeds 50 milliseconds divided by the total number of successive pairs of normal simus intervals
POD	probability of detection; phytotoxic ozone dose
POD6	phytotoxic ozone dose above a threshold of 6 nmol/m2/s

ppb	parts per billion
ppbv	parts per billion by volume
PPL	potential productivity loss
ppm	parts per million
ppm-h	parts per million per hours: weighted concentration values based on hourly concentrations: usually summed over a certain number of hours, day(s), months, and/or season
PPN	peroxypropionyl nitrate
PPROM	preterm premature rupture of membranes
ppt	parts per trillion
PQAPP	Program-level Quality Assurance Project Plan
PR	time interval between the beginning of the P wave to the peak of the R wave
PR protein	pathogenesis related protein
PRB	Policy-relevant Background, typically used in the phrase, "PRB ozone."
PROM	premature rupture of membranes
Prxd	peroxiredoxin
PTB	preterm birth
pts	points
PTT	partial thomboplastin time
PVD	peripheral vascular disease
PVN	paraventricular nucleus
PWA	population weighted average
Q1	1st quartile or quintile
Q2	2nd quartile or quintile
Q3	3rd quartile or quintile
Q4	4th quartile or quintile
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QBME	quantile-based Bayesian maximum entropy
QC	quality control

QNSE	Quasi Normal Scale Elimination
qPCR	quantitative polymerase chain reaction
QRS	time interval between the beginning of the Q wave and the peak of the S wave
QT interval	time interval between the beginning of the Q wave to end of the T wave
QTc	QT interval corrected for heart rate
r	correlation coefficient
R6MA1	running 6-month average of the 1 hour daily max
Rag	Recombination activating gene
RAMP	Regionalized Air Quality Model Performance
RCGC	Research and Development Center for Global Change
RCTs	randomized clinical trials
REA	risk and exposure assessment
REAS	Regional Emissions inventory in Asia
redox	reduction-oxidation
RF	radiative forcing(s)
RFLP	restriction fragment length polymorphism
RH	relative humidity
RISCAT	Cardiovascular Risk and Air Pollution in Tuscany
rMSSD	root-mean-square of successive differences
RMSE	root mean squared error
RNA	ribonucleic acid
RNS	reactive nitrogen species
ROCK	rho associated kinase
ROS	reactive oxygen species
RP-N	reducing power of protein-binding compounds on nitrogen digestibility
RR	risk ratio, relative risk
rRNA	ribosomal ribonucleic acid
RuBisCO	riibulose-1,5-bisphosphate carboxylase/oxygenase
RV	right ventricular

s	second
S07	Statewide Air Pollution Research Center 2007
S99	Statewide Air Pollution Research Center 1999
SAEI	small artery elasticity index
SAGE	Stratospheric Aerosol and Gas Experiment
SAM	s-adenosyl methionine
SAPRC07	Statewide Air Pollution Research Center 2007
SAPRC07T	SAPRC07 Toxics
SAPRC99	Statewide Air Pollution Research Center 1999
SAT	satellite
SBP	systolic blood pressure
SD	standard deviation; Sprague-Dawley rat
SD-Fire	satellite-derived fire emissions
SDNN	standard deviation normal-to-normal (NN or RR) time interval
SEARCH	Southeastern Aerosol Research and Characterization
SEBAS	Social Environment and Biomarkers of Aging Study
SED	sedimentation rate
SES	socioeconomic status
SETIL	population-based case-control study of childhood cancer in Italy
SEu	Southern Europe
SF	seeds per fruiting structure
sFlt	soluble fms-like tyrosine kinase 1
sfpd	surfactant protein D
sGAW	specific airway conductance
SGDS	Korean Geriatric Depression Scale (short-form)
SH	spontaneously hypertensive
SHAM	Sham surgery (placebo surgery)
SHEDS	Stochastic Human Exposure and Dose Simulation
SHHF	spontaneously hypertensive heart failure

SHIS	Shanghai Health Insurance Study
Si	silicon
SILAM	System for Integrated modeLling of Atmospheric coMposition
SIP	State Implementation Plan
SJV	San Joaquin Valley
SLAMS	State and Local Air Monitoring Stations
SMARTFIRE	Satellite Mapping Automated Reanalysis Tool for Fire Incident Reconciliation
SMBD	Spanish Minimum Basic Data
SMOKE	Spare-Matrix Operator Kernel Emissions system
SO_2	sulfur dioxide
SOC	semi-volatile organic compound
SOD	superoxide dismutase
SOEP	Socioeconomic Panel
SOLVEG	atmosphere-soil-vegetation land surface model
SO_x	sulfur oxides
SoyFACE	Soybean Free Air gas Concentration Enrichment (Facility)
sp	species
SP	surfactant protein (e.g., SPA, SPD)
SP+	substance-P-positive
spp	several species
SPSH	stroke-prone spontaneously hypertensive
sRaw	specific airway resistance
ST	spatiotemporal
STAT3	signal transducer and activator of transcription 3
std	standard
STE	stratosphere-troposphere exchange
STEMI	ST-Elevation Myocardial Infarction
STN	Speciation Trends Network
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology

SUM00	sum of all hourly average concentrations
SUM06	seasonal sum of all average hourly concentrations > 0.06 ppm
SUM60	seasonal sum of all hourly average concentrations ≥ 60 ppb
SVT	supraventricular tachycardia
SW	southwest
SWAN	Study of Women's Health Across Nations
t	time
T1D	type 1 diabetes
T2D	type 2 diabetes
T3	thyroid hormone triiodothyronine
T4	thyroid hormone Thyroxine
TAC1	Tachykinin, precursor 1
TAG	traffic, asthma, and genetics
TB	tracheobronchial
TBARS	thiobarbituric acid reactive substances
TC	total hydrocarbon
TCCON	Total Carbon Column Observing Network
TCEQ	Texas Commission on Environmental Quality
TCR	T cell receptor
TES	Tropospheric Emission Spectrometer; Tropospheric Emissions System
TES L3	TES Level 3
TF	tissue factor
Tg	Teragram (Tg), 1 X 1012 g, a unit of mass
TGF	transforming growth factor
Th	thorium
Th2	T-derived lymphocyte helper 2
TIA	transient ischemic attack
TID	ter in die, three times per day
TIMP	tissue inhibitor of metalloproteinase

TLC	Total lung capacity
TLR	Toll-like receptor
TM5	Tracer Model version 5
TNC	total nonstructural carbohydrates
TNF	tumor necrosis factor
TNF-α	Tumor Necrosis Factor alpha
TNFR	tumor necrosis factor receptor
TNHIP	Taiwan National Health Insurance Program
TNHIP-NHIRD	Taiwan National Health Insurance Program - National Health Insurance Research Database
TOA	top of the atmosphere
TOAR	Tropospheric Ozone Assessment Report
TOPP	Tropospheric Ozone Pollution Project
tPA	tissue plasminogen activator
TPWA	"true" population weighted average
TROY	Testing Responses on Youth
TRPA1	transient receptor potential cation channel, subfamily A, member 1
TRPV1	transient receptor potential vanilloid-1 receptor
TSH	thyroid stimulating hormone
TSLP	thymic stromal lymphopoietin
U	zonal velocity of wind vector
UA	unweighted average
UAM	Urban Airshed Model
UB	Uinta Basin
UCD-CIT	University of California at Davis California Institute of Technology
UFP	ultrafine particle
UGRB	Upper Green River Basin
UK	universal kriging; United Kingdom
UNEP	United Nations Environment Programme
UNFCCC	United Nations Framework Convention on Climate Change

UPA	unpaired normalized bias
URI	upper respiratory infection
URTI	upper respiratory tract infection
USB	United States Background
USBAB	U.S. Background apportionment-based
USDA	United States Department of Agriculture
UV	ultraviolet radiation
UVAFME	University of Virginia Forest Model Enhanced
V	meridional velocity of wind vector
VABS	Vineland Adaptive Behavior Scales
VBS	volatility basis set
VCAM	vascular cell adhesion protein
VEGF	vascular endothelial growth factor
VIIc	Factor VII coagulant activity
VIS	visible (spectrum)
VOC	volatile organic compound
VPD	vapor pressure deficit
VPSC(s)	volatile plant signaling compound(s)
VSD	Very Simple Dynamic Model-soil biogeochemical process model
VT	tidal volume
VTI	velocity time interval
VW	Volkswagen
vWF	von Willebrand factor
W126	cumulative integrated exposure index with a sigmoidal weighting function
WBC	white blood cell(s)
WDCGG	World Data Centre for Greenhouse Gases
WED	U.S. EPA National Health and Environmental Effects Research Laboratory Western Ecology Division
WHI-OS	Women's Health Initiative Observational Study

WHO	World Health Organization
WISH	Women's Isoflavone Soy Health
WKY	Wistar-Kyoto rat strain
W/m_2	watts per meters squared
WMO	World Meteorological Organization
WP	seed weight per plant
WP-3D	Lockheed WP-3D Orion Aircraft operated by NOAA
WRF	Weather Research and Forecasting
WRF-ARW	WRF-Advanced Research WRF
WRF-Chem	WRF with Chemistry
WRF-NMM	WRF-Nonhydrostatic Mesoscale Model
WS	wood smoke
WT	wild type
WUE	Water Use Efficiency
XO	radical containing a halogen atom and an oxygen atom, X = I or Br
YIBs	Yale Interactive Terrestrial Biosphere Model
Ym2	chitinase-like-4 protein
yr	year(s)
YSU	Yonsei University
ZCTAs	zip-code tabulation areas

PREFACE

The Preface to the Integrated Science Assessment for Ozone and Related Photochemical Oxidants (Ozone ISA) outlines the legislative requirements of a National Ambient Air Quality Standard (NAAQS) review and the history of the Ozone NAAQS. This information details the general purpose and function of the ISA. The Preface presents the basis for the decisions that supported the previous Ozone NAAQS review. In addition, it details specific issues pertinent to the evaluation of the scientific evidence that takes place within this ISA, including the scope of the ISA and discipline-specific decisions that governed parts of the review.

Legislative Requirements for the Review of the National Ambient Air Quality Standards

Two sections of the Clean Air Act (CAA) govern the establishment, review, and revision of the National Ambient Air Quality Standards (NAAQS). Section 108 (42 U.S. Code [U.S.C.] 7408) directs the Administrator to identify and list certain air pollutants and then to issue air quality criteria for those pollutants. The Administrator is to list those air pollutants that in their "judgment, cause or contribute to air pollution which may reasonably be anticipated to endanger public health or welfare," "the presence of which in the ambient air results from numerous or diverse mobile or stationary sources," and "for which …[the Administrator] plans to issue air quality criteria …" [42 U.S.C. 7408(a)(1); CAA (1990)]. Air quality criteria are intended to "accurately reflect the latest scientific knowledge useful in indicating the kind and extent of all identifiable effects on public health or welfare, which may be expected from the presence of [a] pollutant in the ambient air …" (42 U.S.C. 7408[b]). Section 109 [42 U.S.C. 7409; CAA (1990)] directs the Administrator to propose and promulgate "primary" and "secondary" NAAQS for pollutants for which air quality criteria are issued. Section 109(b)(1) defines a primary standard as one "the attainment and maintenance of which in the judgment of the Administrator, based on such criteria and allowing an adequate margin of safety, are requisite to protect the public health."[1] A secondary standard, as defined in Section 109(b)(2), must "specify a level of air quality the attainment and maintenance of which, in the judgment of the Administrator, based on such criteria, is requisite to protect the public welfare from any known or anticipated adverse effects associated with the presence of [the] air pollutant in the ambient air."[2]

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

[2] Section 302(h) of the Act [42 U.S.C. 7602(h)] provides that all language referring to effects on welfare includes, but is not limited to, "effects on soils, water, crops, vegetation, man-made materials, animals, wildlife, weather, visibility and climate, damage to and deterioration of property, and hazards to transportation, as well as effects on economic values and on personal comfort and well-being…" (CAA, 2005).

The requirement that primary standards provide an adequate margin of safety was intended to address uncertainties associated with inconclusive scientific and technical information available at the time of standard setting. It was also intended to provide a reasonable degree of protection against hazards that researchers have not yet identified.[1] Both kinds of uncertainty are components of the risk associated with pollution at levels below those at which human health effects can be said to occur with reasonable scientific certainty. Thus, in selecting primary standards that provide an adequate margin of safety, the Administrator is seeking not only to prevent pollutant levels that have been demonstrated to be harmful but also to prevent lower pollutant levels that may pose an unacceptable risk of harm, even if the risk is not precisely identified as to nature or degree. The CAA does not require the Administrator to establish a primary NAAQS at a zero-risk level or at background concentration levels, but rather at a level that reduces risk sufficiently so as to protect public health with an adequate margin of safety.[2] In so doing, protection is provided for both the population as a whole and those groups and lifestages potentially at increased risk for health effects from exposure to the air pollutant for which each NAAQS is set.

In addressing the requirement for an adequate margin of safety, the U.S. Environmental Protection Agency (U.S. EPA) considers such factors as the nature and severity of the health effects involved, the size of the sensitive group(s), and the kind and degree of the uncertainties. The selection of any particular approach to providing an adequate margin of safety is a policy choice left specifically to the Administrator's judgment.[3]

In setting standards that are "requisite" to protect public health and welfare as provided in Section 109(b), the U.S. EPA's task is to establish standards that are neither more nor less stringent than necessary for these purposes. In so doing, the U.S. EPA may not consider the costs of implementing the standards.[4] Likewise, "[a]ttainability and technological feasibility are not relevant considerations in the promulgation of national ambient air quality standards."[5]

Section 109(d)(1) requires that "not later than December 31, 1980, and at 5-year intervals thereafter, the Administrator shall complete a thorough review of the criteria published under Section 108 and the national ambient air quality standards…and shall make such revisions in such criteria and standards and promulgate such new standards as may be appropriate…." Section 109(d)(2) requires that an independent scientific review committee "shall complete a review of the criteria…and the national

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[1] See *Lead Industries Association v. EPA*, 647 F.2d 1130, 1154 [District of Columbia Circuit (D.C. Cir.) 1980]; *American Petroleum Institute v. Costle*, 665 F.2d 1176, 1186 (D.C. Cir. 1981); *American Farm Bureau Federation v. EPA*, 559 F. 3d 512, 533 (D.C. Cir. 2009); *Association of Battery Recyclers v. EPA*, 604 F. 3d 613, 617−18 (D.C. Cir. 2010).

[2] See *Lead Industries v. EPA*, 647 F.2d at 1156 n.51*; Mississippi v. EPA*, 744 F. 3d 1334, 1339, 1351, 1353 (D.C. Cir. 2013).

[3] See *Lead Industries Association v. EPA*, 647 F.2d at 1161−62; *Mississippi v. EPA*, 744 F. 3d at 1353.

[4] See generally, *Whitman v. American Trucking Associations*, 531 U.S. 457, 465−472, 475−476 (2001).

[5] See *American Petroleum Institute v. Costle*, 665 F. 2d at 1185.

primary and secondary ambient air quality standards…and shall recommend to the Administrator any new…standards and revisions of existing criteria and standards as may be appropriate….” Since the early 1980s, this independent review function has been performed by the Clean Air Scientific Advisory Committee (CASAC).[1]

History of the Reviews of the Primary and Secondary National Ambient Air Quality Standard for Ozone

NAAQS are defined by four basic elements: indicator, averaging time, level, and form. The indicator defines the pollutant to be measured in the ambient air for the purpose of determining compliance with the standard. The averaging time defines the time period over which air quality measurements are to be obtained and averaged or cumulated. The level of a standard defines the air quality concentration used (i.e., a specific concentration of the indicator pollutant in ambient air) in determining whether the standard is achieved. The form of the standard defines the air quality statistic that is compared to the level of the standard in determining whether an area attains the standard. For example, the form of the current primary and secondary annual Ozone NAAQS is 0.070 ppm as a 3-year avg of the annual fourth-highest daily max 8-hour concentration. The Administrator considers these four elements collectively in evaluating the protection to public health provided by the primary and secondary NAAQS.

Tropospheric ozone is produced near the earth’s surface due to chemical interactions involving solar radiation and specific ozone precursors, such as nitrogen oxides (NO_X), volatile organic compounds (VOC), and carbon monoxide (CO), which can be emitted from both natural and anthropogenic sources.[2] The chemistry that leads to ozone formation is complex and can vary depending upon the relative proportions of different types of precursor pollutants, as well as external conditions such as temperature and sunlight. Over most areas of the U.S., summer daytime ozone production typically increases as NO_X concentrations increase (2013 ISA, Section 3.2.4). Formation of ozone in this regime is described as “NO_X limited.” At other times and locations, where NO_X concentrations are higher or when meteorological conditions do not favor photochemical production, ozone formation may be only weakly dependent on NO_X emissions, or even inversely correlated (i.e., NO_X emissions actually deplete ozone locally[3]). Ozone formation in these regimes increases as VOC concentrations increase and is described as “VOC-limited.” Once formed, ozone near the Earth’s surface can be transported by the prevailing winds

[1] The List of CASAC members is available at: https://yosemite.epa.gov/sab/sabpeople.nsf/WebExternalCommitteeRosters?OpenView&committee=CASAC&secondname=Clean%20Air%20Scientific%20Advisory%20Committee%20.

[2] Methane (CH_4) emissions can also contribute to ozone formation, but its effects are more frequently observed at the global scale over longer time periods (e.g., decadal scale).

[3] In these cases, NO_X generally results in eventual net ozone production downwind of the emissions sources over longer timescales.

before eventually being removed from the atmosphere over the course of hours to weeks via chemical reactions or deposition to surfaces.

The U.S. EPA initially set primary and secondary NAAQS for photochemical oxidants in 1971, with a 1-hour averaging time and a level of 0.08 ppm not to be exceeded more than 1 hour per year (36 FR 8186, April 30, 1971). These standards were based on scientific information contained in the 1970 air quality criteria document (AQCD). The U.S. EPA initiated the first periodic review of the NAAQS for photochemical oxidants in 1977. Based on the 1978 AQCD[1] (U.S. EPA, 1978), the U.S. EPA published proposed revisions to the original NAAQS in 1978 (43 FR 26962, June 22, 1978) and final revisions in 1979 (44 FR 8202, February 8, 1979). At that time, the U.S. EPA changed the indicator from photochemical oxidants to ozone, revised the level of the primary and secondary standards from 0.08 to 0.12 ppm and revised the form of both standards from a deterministic (i.e., not to be exceeded more than 1 hour per year) to a statistical form. With these changes, attainment of the standards was defined to occur when the average number of days per calendar year (across a 3-year period) with maximum hourly average ozone concentration greater than 0.12 ppm equaled one or less (44 FR 8202, February 8, 1979; 43 FR 26962, June 22, 1978). Since then, the Agency has completed multiple reviews of the air quality criteria standards, as summarized in Table I.

The next periodic reviews of the criteria and standards for ozone and other photochemical oxidants began in 1982 and 1983, respectively (47 FR 11561, March 17, 1982; 48 FR 38009, August 22, 1983). The U.S. EPA subsequently published the 1986 AQCD (U.S. EPA, 1986a) and the 1989 Staff Paper[2] (U.S. EPA, 1989). Following publication of the 1986 AQCD, a number of scientific abstracts and articles were published that appeared to be of sufficient importance concerning the potential health and welfare effects of ozone to warrant preparation of a supplement to the 1986 AQCD. In August of 1992, the U.S. EPA proposed to retain the existing primary and secondary standards based on the health and welfare effects information contained in the 1986 AQCD and its 1992 Supplement (57 FR 35542, August 10, 1992). In March 1993, the U.S. EPA announced its decision to conclude this review by affirming its proposed decision to retain the standards, without revision (58 FR 13008, March 9, 1993).

[1] The AQCD served the same purpose as the ISA in the current review.

[2] The Staff Paper served the same purpose as the Policy Assessment (PA) in the current review.

Table I History of the National Ambient Air Quality Standards for Ozone, 1971−2015.

Final Rule/Decision	Indicator	Averaging Time (h)	Level (ppm)	Form
36 FR 8186 April 30, 1971	Total photochemical oxidants	1	0.08	Not to be exceeded more than 1 h per year
44 FR 8202 February 8, 1979	Ozone	1	0.12	Attainment is defined when the expected number of days per calendar year, with maximum hourly average concentration greater than 0.12 ppm, is equal to or less than 1
58 FR 13008 March 9, 1993	U.S. EPA decided revisions to the standard were not warranted at the time.			
62 FR 38856 July 18, 1997	Ozone	8	0.08	Annual fourth-highest daily max 8-h concentration averaged over 3 yr
73 FR 16483 March 27, 2008	Ozone	8	0.075	Annual fourth-highest daily max 8-h concentration averaged over 3 yr
80 FR 65292 October 26, 2015	Ozone	8	0.070	Annual fourth-highest daily max 8-h concentration averaged over 3 yr

ppm = parts per million.

Note: Primary and secondary standards are identical.

In the 1992 notice of its proposed decision in that review, the U.S. EPA announced its intention to proceed as rapidly as possible with the next review of the air quality criteria and standards for ozone and other photochemical oxidants in light of emerging evidence of health effects related to 6- to 8-hour ozone exposures (57 FR 35542, August 10, 1992). The U.S. EPA subsequently published the AQCD and Staff Paper for that next review (U.S. EPA, 1996a, b). In December 1996, the U.S. EPA proposed revisions to both the primary and secondary standards (61 FR 65716, December 13, 1996). With regard to the primary standard, the U.S. EPA proposed replacing the then-existing 1-hour primary standard with an 8-hour standard to be set at a level of 0.08 ppm (equivalent to 0.084 ppm based on the proposed data handling convention) as a 3-year avg of the annual third-highest daily max 8-hour concentration. The U.S. EPA proposed to revise the secondary standard either by setting it identical to the proposed new primary standard or by setting it as a distinct standard with a cumulative seasonal form. The U.S. EPA completed this review in 1997 by setting both the primary and secondary standards at a level of 0.08 ppm, based on the annual fourth-highest daily max 8-hour avg concentration, averaged over 3 years (62 FR 38856, July 18, 1997).

On May 14, 1999, in response to challenges by industry and others to the U.S. EPA's 1997 decision, the D.C. Circuit remanded the Ozone NAAQS to the U.S. EPA, finding that Section 109 of the CAA, as interpreted by the U.S. EPA, effected an unconstitutional delegation of legislative authority (*American Trucking Assoc. v. EPA*, 175 F.3d 1027, 1,034−1,040 [D.C. Cir. 1999]). In addition, the court directed that, in responding to the remand, the U.S. EPA should consider the potential beneficial health effects of ozone pollution in shielding the public from the effects of solar ultraviolet (UV) radiation, as well as adverse health effects (*id.* at 1,051−53). In 1999, the U.S. EPA petitioned for a rehearing *en banc* on several issues related to that decision. The court granted the request for rehearing in part and denied it in part, but declined to review its ruling with regard to the potential beneficial effects of ozone pollution (*American Trucking Assoc. v. EPA*,195 F.3d 4, 10 [D.C. Cir., 1999]). On January 27, 2000, the U.S. EPA petitioned the U.S. Supreme Court for *certiorari* on the constitutional issue (and two other issues), but did not request review of the ruling regarding the potential beneficial health effects of ozone. On February 27, 2001, the U.S. Supreme Court unanimously reversed the judgment of the D.C. Circuit on the constitutional issue. *Whitman v. American Trucking Assoc*., 531 U. S. 457, 472−74 (2001) (holding that Section 109 of the CAA does not delegate legislative power to the U.S. EPA in contravention of the Constitution). The Court remanded the case to the D.C. Circuit to consider challenges to the Ozone NAAQS that had not been addressed by that court's earlier decisions. On March 26, 2002, the D.C. Circuit issued its final decision on the remand, finding the 1997 Ozone NAAQS to be "neither arbitrary nor capricious," and so denying the remaining petitions for review. See *American Trucking Associations, Inc. v. EPA*, 283 F.3d 355, 379 (D.C. Cir. 2002, hereafter referred to as "*ATA III*").

Coincident with the continued litigation of the other issues, the U.S. EPA responded to the court's 1999 remand to consider the potential beneficial health effects of ozone pollution in shielding the public from effects of UV radiation (66 FR 57268, November 14, 2001; 68 FR 614, January 6, 2003). The U.S. EPA provisionally determined that the information linking changes in patterns of ground-level ozone concentrations to changes in relevant patterns of exposures to UV radiation of concern to public health was too uncertain, at that time, to warrant any relaxation of the 1997 Ozone NAAQS. The U.S. EPA also expressed the view that any plausible changes in UV-B radiation exposures from changes in patterns of ground-level ozone concentrations would likely be very small from a public health perspective. In view of these findings, the U.S. EPA proposed to leave the 1997 primary standard unchanged (66 FR 57268, November 14, 2001). After considering public comment on the proposed decision, the U.S. EPA published its final response to this remand in 2003, reaffirming the 8-hour primary standard set in 1997 (68 FR 614, January 6, 2003).

The U.S. EPA initiated the fourth periodic review of the air quality criteria and standards for ozone and other photochemical oxidants with a call for information in September 2000 (65 FR 57810, September 26, 2000). In 2007, the U.S. EPA proposed to revise the level of the primary standard within a range of 0.070 to 0.075 ppm (72 FR 37818, July 11, 2007). The U.S. EPA proposed to revise the secondary standard either by setting it identical to the proposed new primary standard or by setting it as a new seasonal standard using a cumulative form. The U.S. EPA completed the review in March 2008 by

revising the levels of both the primary and secondary standards from 0.08 to 0.075 ppm, while retaining the other elements of the prior standards (73 FR 16436, March 27, 2008). On September 16, 2009, the U.S. EPA announced its intention to reconsider the 2008 Ozone NAAQS,[1] and initiated a rulemaking to do so.

In January 2010, the U.S. EPA issued a notice of proposed rulemaking to reconsider the 2008 final decision (75 FR 2938, January 19, 2010). In that notice, the U.S. EPA proposed that further revisions to the primary and secondary standards were necessary to provide a requisite level of protection to public health and welfare. The U.S. EPA proposed to revise the level of the primary standard from 0.075 ppm to a level within the range of 0.060 to 0.070 ppm, and to revise the secondary standard to one with a cumulative, seasonal form. In view of delays in reaching a final decision, and the fact that the Agency's next periodic review of the Ozone NAAQS required under CAA Section 109 had already begun (as announced on September 29, 2008), the U.S. EPA decided to consolidate the reconsideration with its statutorily required periodic review.[2]

On July 23, 2013, the court upheld the U.S. EPA's 2008 primary ozone standard but remanded the 2008 secondary standard to the U.S. EPA (*Mississippi v. EPA*, 744 F. 3d 1,334 [D.C. Cir. 2013]). With respect to the secondary standard, the court held that the U.S. EPA's explanation for setting the secondary standard identical to the revised 8-hour primary standard was inadequate under the CAA because the U.S. EPA had not adequately explained how that standard provided the required public welfare protection.

At the time of the court's decision, the U.S. EPA had already completed significant portions of its next statutorily required periodic review of the Ozone NAAQS. This review had been formally initiated in 2008 with a call for information in the *Federal Register* (73 FR 56581, September 29, 2008). In late 2014, based on the Integrated Science Assessment (ISA), Risk and Exposure Assessments (REAs) for health and welfare, and PA[3] developed for this review, the U.S. EPA proposed to revise the 2008 primary and secondary standards by reducing the level of both standards to within the range of 0.065 to 0.070 ppm (79 FR 75234, December 17, 2014).

The U.S. EPA's final decision in this review was published in October 2015, establishing the now-current standards (80 FR 65292, October 26, 2015). In this decision, based on consideration of the health effects evidence on respiratory effects of ozone in at-risk populations, the U.S. EPA revised the primary standard from a level of 0.075 ppm to a level of 0.070 ppm, while retaining all the other elements of the standard (80 FR 65292, October 26, 2015). The level of the secondary standard was also revised

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[1] The press release of this announcement is available at: https://archive.epa.gov/epapages/newsroom_archive/newsreleases/85f90b7711acb0c88525763300617d0d.html.

[2] This rulemaking, completed in 2015, concluded the reconsideration process.

[3] The final versions of these documents, released in August 2014, were developed with consideration of the comments and recommendations from the CASAC, as well as comments from the public on the draft documents (Frey, 2014a 2014, 5408574 2014, 5408574, c; U.S. EPA, 2014a, b, c).

from 0.075 to 0.070 ppm based on the scientific evidence of ozone effects on welfare, particularly the evidence of ozone effects on vegetation, and quantitative analyses available in the review.[1] The other elements of the standard were retained. This decision on the secondary standard also incorporated the U.S. EPA's response to the D.C. Circuit's remand of the 2008 secondary standard in *Mississippi v. EPA*, 744 F.3d 1,344 (D.C. Cir. 2013).

After publication of the final rule, a number of industry groups, environmental and public health organizations, and certain states filed petitions for judicial review in the D.C. Circuit. The industry and state petitioners filed briefs arguing that the revised standards are too stringent, while the environmental and health petitioners' brief argued that the revised standards are not stringent enough to protect public health and welfare as the Act requires. On August 23, 2019, the court issued an opinion that denied all the petitions for review with respect to the 2015 primary standard while also concluding that the EPA had not provided a sufficient rationale for aspects of its decision on the 2015 secondary standard and remanding that standard to the U.S. EPA (*Murray Energy v. EPA*, No. 15-1,385, Order, Doc. No. 1803352 [D.C. Cir. August 23, 2019]).

Purpose and Overview of the Integrated Science Assessment

The Integrated Science Assessment (ISA) is a comprehensive evaluation and synthesis of the policy-relevant science "useful in indicating the kind and extent of identifiable effects on public health or welfare which may be expected from the presence of [a] pollutant in ambient air," as described in Section 108 of the Clean Air Act (CAA, 1990). This ISA communicates critical science judgments of the health and welfare criteria for ozone, and serves as the scientific foundation for the review of the current primary (health-based) and secondary (welfare-based) National Ambient Air Quality Standards (NAAQS) for ozone.

As stated in the Ozone IRP (Section 4.1), the purpose of this ISA is to draw upon the existing body of evidence to synthesize and provide a critical evaluation of the current state of scientific knowledge on the most relevant issues pertinent to the review of the NAAQS for ozone and other photochemical oxidants, to identify changes in the scientific evidence bases since the previous review, and to describe remaining or newly identified uncertainties. The ISA identifies, critically evaluates, and synthesizes the most policy-relevant current scientific literature (e.g., epidemiology, controlled human exposure, animal toxicology, atmospheric science, exposure science, ecology, and climate-related science), including key science judgments that are important to inform the development of risk and exposure analyses (as warranted) and the policy assessment, as well as other aspects of the NAAQS review process.

This ISA evaluates relevant scientific literature published since the 2013 Ozone ISA (U.S. EPA, 2013), integrating key information and judgments contained in the 2013 Ozone ISA and previous

[1] The current NAAQS for ozone are specified at 40 CFR 50.19.

assessments of ozone, specifically, the 2006 AQCD for Ozone and Related Photochemical Oxidants (U.S. EPA, 2004), the 2007 Staff Paper (U.S. EPA, 2007), the 1996 AQCD and Staff Paper for Ozone and Other Photochemical Oxidants (U.S. EPA, 1996a, b), the 1986 AQCD for ozone (U.S. EPA, 1982) and its Supplement (U.S. EPA, 1986b), and the 1978 AQCD for Ozone and Other Photochemical Oxidants (NAPCA, 1969). Thus, this ISA updates the state of the science from that available for the 2013 Ozone ISA, which informed decisions on the primary and secondary Ozone NAAQS in the review completed in 2015.

This new review of the primary and secondary Ozone NAAQS is guided by several policy-relevant questions identified in the Integrated Review Plan for the National Ambient Air Quality Standards for Ozone (https://www.epa.gov/naaqs/ozone-o3-standards-planning-documents-current-review). To address these questions and update the scientific judgments in the 2013 Ozone ISA (U.S. EPA, 2013), this ISA aims to:

- Assess whether new information (since the last Ozone NAAQS review) further informs the relationship between exposure to ozone and specific health and welfare effects.
- Provide new information as to whether the NAAQS (comprised of indicator, averaging time, form, and level) are appropriate.

In addressing policy-relevant questions, this ISA aims to characterize the health and welfare effects of ozone independent from co-occurring air pollutants. In the characterization of whether there is evidence of an independent health and welfare effect due to ozone, the ISA considers possible influences of other atmospheric pollutants, including both gaseous (i.e., NO_2, SO_2, and CO) and various particulate matter (PM) size fractions. The information summarized in this ISA will serve as the scientific foundation for the review of the current primary and secondary Ozone NAAQS.

Process for Developing Integrated Science Assessments

The U.S. EPA uses a structured and transparent process for evaluating scientific information and determining the causal nature of relationships between air pollution exposures and health effects [details provided in the Preamble to the Integrated Science Assessments (U.S. EPA, 2015a)]. The ISA development process describes approaches for literature searches, criteria for selecting and evaluating relevant studies, and a framework for evaluating the weight of evidence and forming causality determinations. Table II provides a description of each of the five causality determinations and the types of scientific evidence that is considered for each category for both health and welfare effects.

Table II Weight of evidence for causality determinations.

	Health Effects	Ecological and Other Welfare Effects
Causal relationship	Evidence is sufficient to conclude that there is a causal relationship with relevant pollutant exposures (e.g., doses or exposures generally within one to two orders of magnitude of recent concentrations). That is, the pollutant has been shown to result in health effects in studies in which chance, confounding, and other biases could be ruled out with reasonable confidence. For example: (1) controlled human exposure studies that demonstrate consistent effects, or (2) observational studies that cannot be explained by plausible alternatives or that are supported by other lines of evidence (e.g., animal studies or mode-of-action information). Generally, the determination is based on multiple high-quality studies conducted by multiple research groups.	Evidence is sufficient to conclude that there is a causal relationship with relevant pollutant exposures. That is, the pollutant has been shown to result in effects in studies in which chance, confounding, and other biases could be ruled out with reasonable confidence. Controlled exposure studies (laboratory or small- to medium-scale field studies) provide the strongest evidence for causality, but the scope of inference may be limited. Generally, the determination is based on multiple studies conducted by multiple research groups, and evidence that is considered sufficient to infer a causal relationship is usually obtained from the joint consideration of many lines of evidence that reinforce each other.
Likely to be a causal relationship	Evidence is sufficient to conclude that a causal relationship is likely to exist with relevant pollutant exposures. That is, the pollutant has been shown to result in health effects in studies where results are not explained by chance, confounding, and other biases, but uncertainties remain in the evidence overall. For example: (1) observational studies show an association, but copollutant exposures are difficult to address and/or other lines of evidence (controlled human exposure, animal, or mode-of-action information) are limited or inconsistent, or (2) animal toxicological evidence from multiple studies from different laboratories demonstrate effects, but limited or no human data are available. Generally, the determination is based on multiple high-quality studies.	Evidence is sufficient to conclude that there is a likely causal association with relevant pollutant exposures. That is, an association has been observed between the pollutant and the outcome in studies in which chance, confounding, and other biases are minimized but uncertainties remain. For example, field studies show a relationship, but suspected interacting factors cannot be controlled, and other lines of evidence are limited or inconsistent. Generally, the determination is based on multiple studies by multiple research groups.
Suggestive of, but not sufficient to infer, a causal relationship	Evidence is suggestive of a causal relationship with relevant pollutant exposures but is limited, and chance, confounding, and other biases cannot be ruled out. For example: (1) when the body of evidence is relatively small, at least one high-quality epidemiologic study shows an association with a given health outcome and/or at least one high-quality toxicological study shows effects relevant to humans in animal species, or (2) when the body of evidence is relatively large, evidence from studies of varying quality is generally supportive but not entirely consistent, and there may be coherence across lines of evidence (e.g., animal studies or mode-of-action information) to support the determination.	Evidence is suggestive of a causal relationship with relevant pollutant exposures, but chance, confounding, and other biases cannot be ruled out. For example, at least one high-quality study shows an effect, but the results of other studies are inconsistent.

Table II (Continued): Weight of evidence for causality determinations.

	Health Effects	Ecological and Other Welfare Effects
Inadequate to infer a causal relationship	Evidence is inadequate to determine that a causal relationship exists with relevant pollutant exposures. The available studies are of insufficient quantity, quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an effect.	Evidence is inadequate to determine that a causal relationship exists with relevant pollutant exposures. The available studies are of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an effect.
Not likely to be a causal relationship	Evidence indicates there is no causal relationship with relevant pollutant exposures. Several adequate studies, covering the full range of levels of exposure that human beings are known to encounter and considering at-risk populations and lifestages, are mutually consistent in not showing an effect at any level of exposure.	Evidence indicates there is no causal relationship with relevant pollutant exposures. Several adequate studies examining relationships with relevant exposures are consistent in failing to show an effect at any level of exposure.

Source: U.S. EPA (2015a).

As part of this process, the ISA is reviewed by the CASAC, which is a formal independent panel of scientific experts, and by the public. Because this ISA informs the review of the primary and secondary Ozone NAAQS, it integrates and synthesizes information characterizing exposure to ozone and potential relationships with health and welfare effects. Relevant studies include those examining atmospheric chemistry, spatial and temporal trends, and exposure assessment, as well as U.S. EPA analyses of air quality and emissions data. Relevant health research includes epidemiologic, controlled human exposure, and toxicological studies on health effects, as well as the biological plausibility that ozone could cause such health effects. Additionally, relevant welfare research includes studies examining effects on environmental biota and ecosystems, as well as climate.

Scope of the ISA

This ISA updates the state of the science from that available for the 2013 Ozone ISA, which informed decisions on the primary and secondary ozone NAAQS in the review completed in 2015. The previous ISA for ozone was published in 2013 (U.S. EPA, 2013) and included peer-reviewed literature published through July 2011. Search techniques for the current ISA identified and evaluated studies and reports that have undergone scientific peer review and were published or accepted for publication between January 1, 2011 (providing some overlap with the cutoff date from the last review) and March 30, 2018. Studies published after the literature cutoff date for this review were also considered if they were submitted in response to the Call for Information (83 FR 29785, June 26, 2018) or identified in subsequent phases of ISA development (e.g., peer-input consultation, CASAC review of draft Integrated Review Plan), particularly to the extent that they provide new information that affects key scientific conclusions. Section 10.2, "Literature Search and Initial Screen," details the study selection process in further detail.

For human health effects, the U.S. EPA concluded in the 2013 Ozone ISA that the findings of epidemiologic, controlled human exposure, and animal toxicological studies collectively provided evidence of a "causal relationship" for short-term ozone exposures and respiratory effects. In evaluating a broader range of health effects for ozone, the 2013 Ozone ISA concluded there was evidence of a "likely to be causal relationship" for long-term ozone exposures and respiratory effects and for short-term ozone exposures and cardiovascular effects and mortality. Additionally, there was evidence "suggestive of a causal relationship" for ozone exposures and other health effects, including developmental and reproductive effects (e.g., low birth weight, infant mortality) and central nervous system effects (e.g., cognitive development).

For welfare effects, the evidence in the 2013 Ozone ISA indicated a "causal relationship" between ozone exposure and visible foliar injury effects on vegetation, reduced vegetation growth, reduced productivity in terrestrial ecosystems, reduced yield and quality of agricultural crops, and alteration of below-ground biogeochemical cycles. The evidence indicated a "likely to be causal relationship" for reduced carbon sequestration in terrestrial ecosystems, alteration of terrestrial ecosystem water cycling, and alteration of terrestrial community composition. For climate, there was a causal relationship between changes in tropospheric ozone concentration and radiative forcing and likely to be a causal relationship between changes in tropospheric ozone concentration and effects on climate. For this current review, specific science questions related to the causality determinations that were addressed include:

- Does the evidence base from recent studies contain new information to support or call into question the causality determinations made for relationships between ozone exposure and various health and welfare effects in the 2013 Ozone ISA?
- Is there new information to extend causality determinations to other ecological endpoints?
- Does new evidence confirm or extend biological plausibility of ozone-related health effects?
- What is the strength of inference from epidemiologic studies based on the extent to which they have:
    - Examined exposure metrics that capture the spatial and/or temporal pattern of ozone in the study area?
    - Assessed potential confounding by other pollutants and factors?
- What does the available information indicate regarding changes in population health status that may be associated with a decrease in ambient air ozone concentrations that might inform causality determinations?

Evaluation of the Evidence

The Preamble to the ISAs (U.S. EPA, 2015a) describes the general framework for evaluating scientific information, including criteria for assessing study quality and developing scientific conclusions. Aspects specific to evaluating studies of ozone are described in Appendix 10 of the ISA, which were

applied to studies that fit the overall scope of this Ozone ISA. Appendix 10 complements the Preamble by providing additional details regarding methods used in the literature search, study quality evaluations, and quality assurance. Categories of health and welfare effects were considered for evaluation in this ISA if they were examined in previous U.S. EPA assessments for ozone or in multiple recent studies. Therefore, in this ISA, the broad health effects categories evaluated include those considered in the 2013 Ozone ISA (i.e., respiratory effects, cardiovascular effects, central nervous system effects, cancer, and mortality), along with the addition of metabolic effects. Further, new research indicates it is appropriate to refine the category of reproductive and developmental effects to focus overall conclusions specifically on birth outcomes and on fertility and pregnancy effects separately.

In the 2013 Ozone ISA, the welfare effects evidence for ozone focused on the effects of ozone on vegetation and ecosystems and the role of tropospheric ozone on climate change. In this ISA, the U.S. EPA builds on the 2013 Ozone ISA by evaluating the newly available literature related to ozone exposures and welfare effects, specifically ecological effects and effects on climate. With regards to ecological effects, this ISA evaluates the literature related to ozone exposures at levels of biological organization from the organism to the ecosystem level, including effects on biodiversity. Evidence from experimental (e.g., laboratory, greenhouse, open-top chamber [OTC], free-air carbon dioxide enrichment [FACE]), field, gradient, and modeling studies that address effects of ozone on ecological endpoints are considered to identify concentrations at which effects are observed.

Peer review is an important component of any scientific assessment. U.S. EPA has formal guidance about peer review in the *Peer Review Handbook* (U.S. EPA, 2015b), and this ISA follows all the policies and procedures identified therein. Additionally, this ISA follows all of the U.S. EPA's Information Quality Guidelines (U.S. EPA, 2002).

In forming the key science judgments for each of the health and welfare effects categories evaluated, the Ozone ISA draws conclusions about relationships between ozone exposure and health effects by integrating information across scientific disciplines and related health outcomes and synthesizing evidence from previous and recent studies. To impart consistency in the evaluation of health effects evidence for epidemiologic studies, additional parameters to those outlined in the scope were developed. To help compare results across epidemiologic studies, risk estimates were standardized to a defined increment for both short- and long-term exposure to ozone, unless otherwise noted in the text. All epidemiologic results are standardized to a 15-ppb increase in 24-hour avg, a 20-ppb increase in 8-hour daily max, a 25-ppb increase in 1-hour daily max ozone concentrations, or a 10-ppb increase in seasonal/annual ozone concentrations. These increments are loosely based on the 50th−95th percentile of concentrations observed for each averaging time and exposure duration. Additionally, while assessing copollutants or other variables in epidemiologic studies, high, moderate, or low correlations are defined as the following: low correlation, $r < 0.40$; moderate correlation, $r \geq 0.40$ and $r < 0.70$; and high correlation, $r \geq 0.70$. Consistency in interpreting the epidemiologic evidence through approaches such as the standardization of risk estimates and the evaluation of correlations, in combination with the integration of

evidence across scientific disciplines, supports a thorough evaluation of the current state of the science for ozone.

In evaluating the evidence, determinations are made about causation, not just association, and are based on judgments of aspects such as the consistency of evidence within a discipline, coherence of effects across disciplines, and biological plausibility of observed effects. Determinations account for related uncertainties. The ISA uses a formal causal framework [Table II of the Preamble to the ISAs (U.S. EPA, 2015a)] to classify the weight of evidence according to the five-level hierarchy.

References for Preface

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

CAA (Clean Air Act). (2005). Clean Air Act, section 302: Definitions, 42 USC 7602. http://www.gpo.gov/fdsys/pkg/USCODE-2005-title42/pdf/USCODE-2005-title42-chap85-subchapIII-sec7602.pdf.

Frey, HC. (2014a). Letter from Dr. H. Christopher Frey, Chair, Clean Air Scientific Advisory Committee, to Administrator Gina McCarthy. CASAC Review of the EPAs Second Draft Policy Assessment for the Review of the Ozone National Ambient Air Quality Standards. EPA-CASAC-14-004. Available online at https://yosemite.epa.gov/sab/sabproduct.nsf/264cb1227d55e02c85257402007446a4/5EFA320CCAD326E885257D030071531C/$File/EPA-CASAC-14-004+unsigned.pdf (accessed

Frey, HC. (2014b). Letter from Dr. H. Christopher Frey, Chair, Clean Air Scientific Advisory Committee, to Administrator Gina McCarthy. CASAC Review of the EPAs Welfare Risk and Exposure Assessment for Ozone (Second External Review Draft). EPACASAC-14-003. Available online at https://yosemite.epa.gov/sab/sabproduct.nsf/264cb1227d55e02c85257402007446a4/3D561C9413E49E8E85257CFB0069E0DF/$File/EPA-CASAC-14-003+unsigned.pdf (accessed

NAPCA (National Air Pollution Control Administration). (1969). Air quality criteria for particulate matter. Washington, DC.

U.S. EPA (U.S. Environmental Protection Agency). (1978). Air quality criteria for ozone and other photochemical oxidants [EPA Report]. (EPA/600/8-78/004). Washington, DC. http://nepis.epa.gov/exe/ZyPURL.cgi?Dockey=200089CW.txt.

U.S. EPA (U.S. Environmental Protection Agency). (1982). Air quality criteria for particulate matter and sulfur oxides (final, 1982) [EPA Report]. (EPA 600/8-82/029a). Washington, DC: Environmental Criteria and Assessment Office. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=46205.

U.S. EPA (U.S. Environmental Protection Agency). (1986a). Air quality criteria for ozone and other photochemical oxidants [EPA Report]. (EPA-600/8-84-020aF - EPA-600/8-84-020eF). Research Triangle Park, NC. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=PB87142949.

U.S. EPA (U.S. Environmental Protection Agency). (1986b). Second addendum to air quality criteria for particulate matter and sulfur oxides (1982): Assessment of newly available health effects information [EPA Report]. (EPA/600/8-86/020F). Research Triangle Park, NC: Environmental Criteria and Assessment Office.

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

U.S. EPA (U.S. Environmental Protection Agency). (1996a). Air quality criteria for ozone and related photochemical oxidants, Vol. III of III [EPA Report]. (EPA/600/P-93/004cF). Research Triangle Park, NC. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=PB96185608.

U.S. EPA (U.S. Environmental Protection Agency). (1996b). Review of national ambient air quality standards for ozone: Assessment of scientific and technical information. OAQPS staff paper [EPA Report]. (EPA/452/R-96/007). Research Triangle Park, NC. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=PB96203435.

U.S. EPA (U.S. Environmental Protection Agency). (2002). Guidelines for ensuring and maximizing the quality, objectivity, utility, and integrity of information disseminated by the Environmental Protection Agency. (EPA/260/R-02/008). Washington, DC: U.S. Environmental Protection Agency, Office of Environmental Information. https://www.epa.gov/sites/production/files/2017-03/documents/epa-info-quality-guidelines.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2004). Air quality criteria for particulate matter [EPA Report]. (EPA/600/P-99/002aF-bF). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environemntal Assessment- RTP Office. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=87903.

U.S. EPA (U.S. Environmental Protection Agency). (2007). Review of the national ambient air quality standards for ozone: Policy assessment of scientific and technical information: OAQPS staff paper [EPA Report]. (EPA/452/R-07/007). Research Triangle Park, NC. https://www3.epa.gov/ttn/naaqs/standards/ozone/data/2007_07_ozone_staff_paper.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2013). Integrated science assessment for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-10/076F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment-RTP Division. http://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=247492.

U.S. EPA (U.S. Environmental Protection Agency). (2014a). Health Risk and Exposure Assessment for Ozone. Final Report. (EPA-452/P-14-004a). Research Triangle Park, NC: Office of Air Quality Planning and Standards. http://nepis.epa.gov/exe/ZyPURL.cgi?Dockey=P100KBUF.txt.

U.S. EPA (U.S. Environmental Protection Agency). (2014b). Policy assessment for the review of the ozone national ambient air quality standards (pp. EPA-452/R-414-006). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Air Quality Planning and Standards. https://www3.epa.gov/ttn/naaqs/standards/ozone/data/20140829pa.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2014c). Welfare Risk and Exposure Assessment for Ozone, Final. (EPA-452/P-14-005a). Research Triangle Park, NC: Office of Air Quality Planning and Standards. https://www3.epa.gov/ttn/naaqs/standards/ozone/data/20141021welfarerea.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2015a). Preamble to the integrated science assessments [EPA Report]. (EPA/600/R-15/067). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, RTP Division. https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=310244.

U.S. EPA (U.S. Environmental Protection Agency). (2015b). Science policy council peer review handbook [EPA Report] (4th ed.). (EPA/100/B-15/001). Washington, DC: U.S. Environmental Protection Agency, Science Policy Council. https://www.epa.gov/osa/peer-review-handbook-4th-edition-2015.

EXECUTIVE SUMMARY

ES.1 Purpose and Scope of the Integrated Science Assessment

This Integrated Science Assessment (ISA)[1] is a comprehensive evaluation and synthesis of the policy-relevant science aimed at characterizing the health and welfare[2] effects caused by ozone. It communicates critical science judgments of the health-based and welfare-based criteria for ozone and related photochemical oxidants in ambient air. In 2015, the U.S. EPA lowered the health- and welfare-based National Ambient Air Quality Standards (NAAQS) for ozone to 0.070 ppm (annual fourth-highest daily max 8-hour concentration averaged over 3 years[3]). The health-based ozone NAAQS is meant to protect public health, including at-risk populations such as children and people with asthma, with an adequate margin of safety. The welfare-based ozone standard is intended to protect the public welfare from known or anticipated adverse effects associated with the presence of ozone in the ambient air.

The ISA identifies and critically evaluates the most policy-relevant scientific literature across scientific disciplines, including epidemiology, controlled human exposure studies, animal toxicology, atmospheric science, exposure science, vegetation studies, agricultural science, ecology, and climate-related science. Key scientific conclusions (i.e., causality determinations; Section ES.4) are presented and explained. They provide the scientific basis for developing risk and exposure analyses, policy evaluations, and policy decisions for the review. This ISA draws conclusions about the causal nature of the relationships between ozone exposure and health and welfare effects by integrating information across scientific disciplines and building off the evidence base evaluated in previous reviews. The ISA thus provides the policy-relevant scientific information that supports the review of the NAAQS.

This executive summary provides an overview of the important conclusions drawn in the ISA across the scientific disciplines, beginning with information on sources, concentrations, estimated background and exposure, followed by health and welfare effects. A more detailed summary of the evidence is presented in the Integrated Synthesis, and individual appendices for each topic area include study-level information and an in-depth characterization of the weight-of-evidence conclusions.

[1] The general process for developing an ISA, including the framework for evaluating weight of evidence and drawing scientific conclusions and causal judgments, is described in a companion document, Preamble to the Integrated Science Assessments (U.S. EPA, 2015), www.epa.gov/isa.

[2] Under Clean Air Act section 302(h), effects on welfare include, but are not limited to, "effects on soils, water, crops, vegetation, manmade materials, animals, wildlife, weather, visibility, and climate, damage to and deterioration of property, and hazards to transportation, as well as effects on economic values and on personal comfort and well-being."

[3] Final rule signed October 1, 2015, and effective December 28, 2015 (80 FR 65291).

ES.2 Ozone in Ambient Air

The general photochemistry of tropospheric ozone is well-established. Ozone is produced in urban areas and downwind of sources mainly by the reaction of volatile organic compounds (VOCs) with oxides of nitrogen (NO_X) in the presence of sunlight, and throughout the troposphere also by reactions of CO and CH_4 with oxides of nitrogen (Section 1.4). Recent developments in understanding ozone chemistry include observations of high ozone concentrations during the winter in some western U.S. mountain basins (Section 1.4.1) and new research on the role of marine halogen chemistry in suppressing coastal ozone concentrations (Section 1.4.2). Air monitoring data for the period 2015−2017 show that U.S. daily max 8-hour avg concentrations of ozone (MDA8) are higher in spring and summer (median = 46 ppb) than in autumn (median = 38 ppb) and winter (median = 34 ppb). Figure ES-1 shows the highest values of the 3-year avg of annual fourth-highest MDA8 ozone concentrations (design values above 70 ppb) occur in central and southern California, Arizona, Colorado, Utah, Texas, along the shore of Lake Michigan, and in the Northeast Corridor, typically during the ozone season between May and September (Section 1.2.1.1).

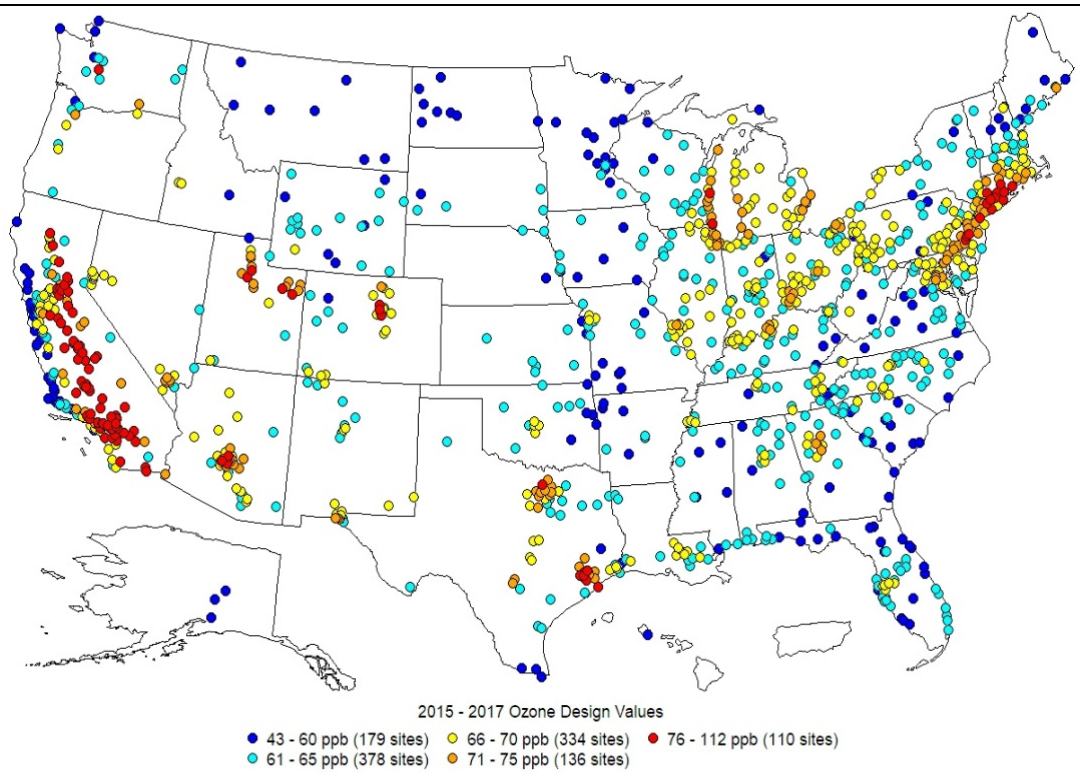


Figure ES-1 Individual monitor ozone concentrations in terms of design values (i.e., 3-year avg of annual fourth-highest max daily 8-hour avg ozone concentration) for 2015−2017.

A better understanding of the origins of ground-level U.S. background (USB) ozone and its concentration trends has emerged since the 2013 Ozone ISA. USB ozone concentration is defined as the ozone concentration that would occur if all U.S. anthropogenic ozone precursor emissions were removed (Section IS.2.2). Major contributors to USB ozone concentrations are stratospheric exchange, international transport, wildfires, lightning, global methane emissions, and natural biogenic and geogenic precursor emissions. USB is not measured directly but is estimated based on models. The estimates of USB ozone concentrations include uncertainties of about 10 ppb for seasonal average concentrations, with higher uncertainty for MDA8 concentrations. Models consistently estimate higher USB ozone concentrations at higher elevations of the western U.S. than in the eastern U.S. or along the Pacific coast. The estimated seasonal pattern in USB ozone concentrations tends to indicate lower USB in the summer than during the rest of the year. Several modeling studies using different approaches indicate that for MDA8 concentrations above 50−60 ppb, USB concentration estimates generally do not increase with increasing total ozone concentration (i.e., USB ozone concentrations are no higher on high ozone days than on low or moderate ozone days). The temporal trend in estimated USB ozone concentrations indicates increasing concentrations at high elevation western U.S. sites through approximately 2010. Recently, however, this trend has shown signs of slowing or even reversing, possibly due to decreasing East Asian precursor emissions.

ES.3 Exposure to Ozone

Ambient air ozone concentrations, either measured at fixed-site monitors or estimated by models, is often used as a surrogate for personal exposure in epidemiologic studies. Exposure measurement error can lead to reduced precision and an underestimation of the association between short-term ambient ozone exposure and a health effect (Section 2.6.1). For studies of long-term exposure, the true effect of exposure to ambient ozone may be underestimated or overestimated when the exposure model respectively overestimates or underestimates ozone exposure. It is much more common for the effect to be underestimated, and bias in the effect estimate is typically small in magnitude (Section 2.6.2). The availability and sophistication of models to predict ambient ozone concentrations to estimate exposure have increased substantially in recent years (Section 2.3.2). For effects elicited by ozone, the use of exposure estimates that do not account for population behavior and mobility (e.g., via use of time-activity data) may result in underestimation of the true effect and reduced precision (Section 2.4.1).

Tropospheric ozone can cause plant damage, which can then have negative impacts on terrestrial ecosystems. Robust exposure indices that quantify exposure as it relates to measured plant response (e.g., growth) have been in use for decades and only require ambient air quality data. Exposure duration influences the degree of plant response, and ozone effects on plants are cumulative. Cumulative indices summarize ozone concentrations over time and provide a consistent metric for reviewing and comparing exposure-response effects obtained from various studies. Cumulative indices of exposure that

differentially weight hourly concentrations are, therefore, best suited to characterize vegetation exposure to ozone (Section 8.1.2.1).

ES.4 Health and Welfare Effects of Ozone Exposure

Broad health and welfare effect categories are evaluated independently in the appendices of this ISA. Determinations are made about causation by evaluating evidence across scientific disciplines and are based on judgments of consistency, coherence, and biological plausibility of observed effects, as well as related uncertainties. The ISA uses a formal causal framework to classify the weight of evidence using a five-level hierarchy described in Table II of the Preamble (U.S. EPA, 2015). The subsequent sections characterize the evidence that forms the basis of causality determinations for health and welfare effect categories of a "causal relationship" or a "likely to be causal relationship", or describe instances where a causality determination has changed (i.e., "likely to be causal" changed to "suggestive of, but not sufficient to infer, a causal relationship"). Other relationships between ozone and health effects are *"suggestive of, but not sufficient to infer*" and "*inadequate*". These causality determinations appear in Table ES-1, and are more fully discussed in the respective health effects appendices.

ES.4.1 Health Effects of Ozone Exposure

Ozone-induced effects can occur through a variety of complex pathways within the body. After inhalation, ozone reacts with lipids, proteins, and antioxidants in the epithelial lining fluid of the respiratory tract, creating secondary oxidation products (Section 5.2.3). Initial ozone exposure leads to physiological reactions that may induce a host of autonomic, endocrine, immune, and inflammatory responses throughout the body at the cellular, tissue, and organ level. Recent evidence continues to support ozone-induced effects on the respiratory system. In addition, recent evidence indicates ozone-induced metabolic effects, as shown in Figure ES-2. There is also some evidence that ozone exposure can affect the cardiovascular and nervous systems, reproduction and development, and mortality, although there are more uncertainties associated with interpretation of the evidence for these effects.

Table ES-1 Summary of causality determinations by exposure duration and health outcome.

Health Outcome[a]	Conclusions from 2013 Ozone ISA	Conclusions in the Current ISA
Short-term exposure to ozone		
Respiratory effects	Causal relationship	Causal relationship
Metabolic effects	No determination made	Likely to be a causal relationship[b]
Cardiovascular effects	Likely to be a causal relationship	Suggestive of, but not sufficient to infer, a causal relationship[c]
Total mortality	Likely to be a causal relationship	Suggestive of, but not sufficient to infer, a causal relationship
Central nervous system effects	Suggestive of a causal relationship	Suggestive of, but not sufficient to infer, a causal relationship
Long-term exposure to ozone		
Respiratory effects	Likely to be a causal relationship	Likely to be a causal relationship
Metabolic effects	No determination made	Likely to be a causal relationship[b]
Cardiovascular effects	Suggestive of a causal relationship	Suggestive of, but not sufficient to infer, a causal relationship
Total mortality	Suggestive of a causal relationship	Suggestive of, but not sufficient to infer, a causal relationship
Reproductive effects	Suggestive of a causal relationship	Effects on fertility and reproduction: suggestive of a causal relationship[b]
		Effects on pregnancy and birth outcomes: suggestive of a causal relationship[b]
Central nervous system effects	Suggestive of a causal relationship	Suggestive of, but not sufficient to infer, a causal relationship
Cancer	Inadequate to infer a causal relationship	Inadequate to infer a causal relationship

[a]Health effects (e.g., respiratory effects, cardiovascular effects) include the spectrum of outcomes, from measurable subclinical effects (e.g., decrements in lung function, blood pressure) to observable effects (e.g., medication use, hospital admissions) and cause-specific mortality. Total mortality includes all-cause (nonaccidental) mortality, as well as cause-specific mortality.

[b]Denotes new causality determination.

[c]Denotes change in causality determination from 2013 Ozone ISA.

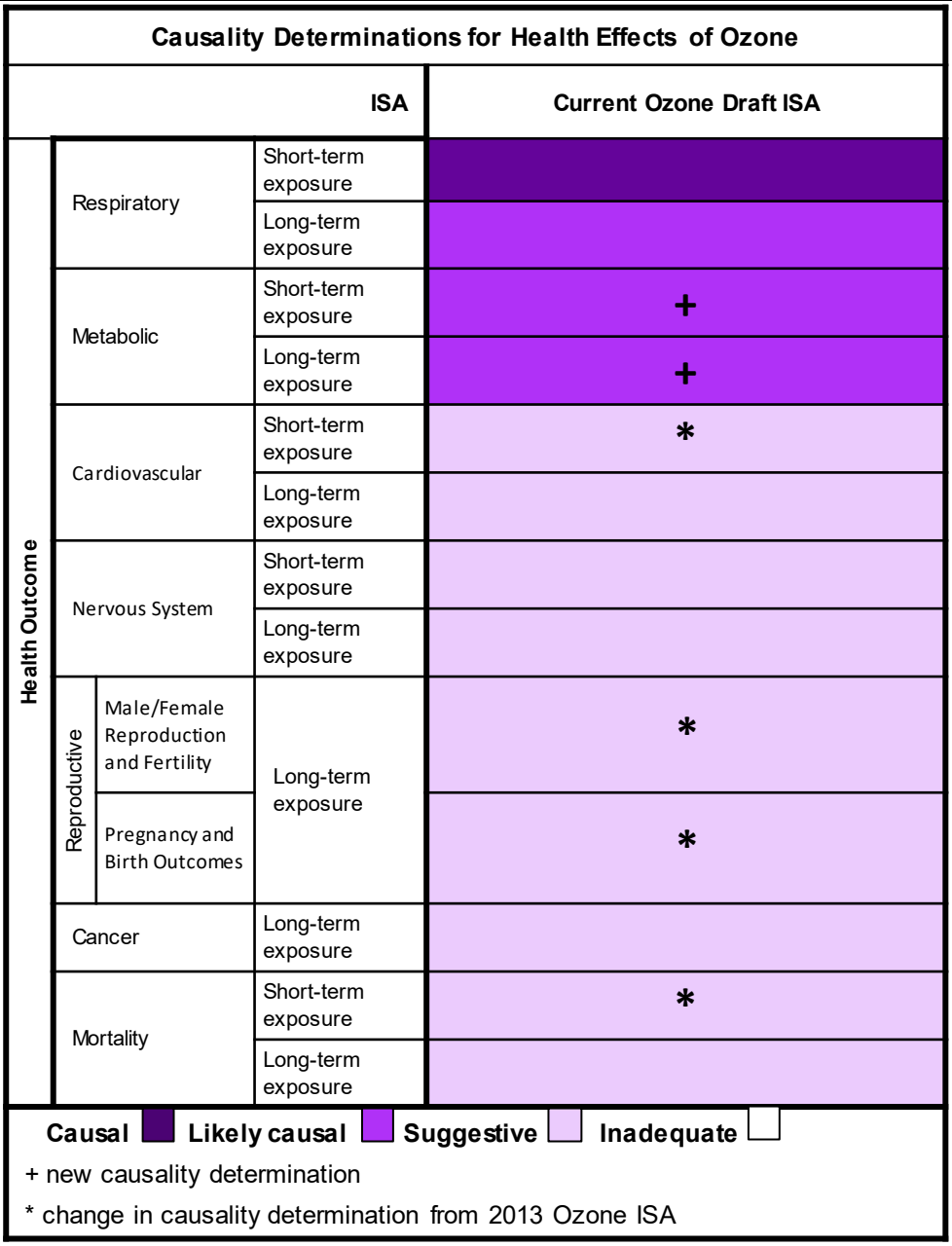


Figure ES-2 Causality determinations for health effects of short- and long-term exposure to ozone.

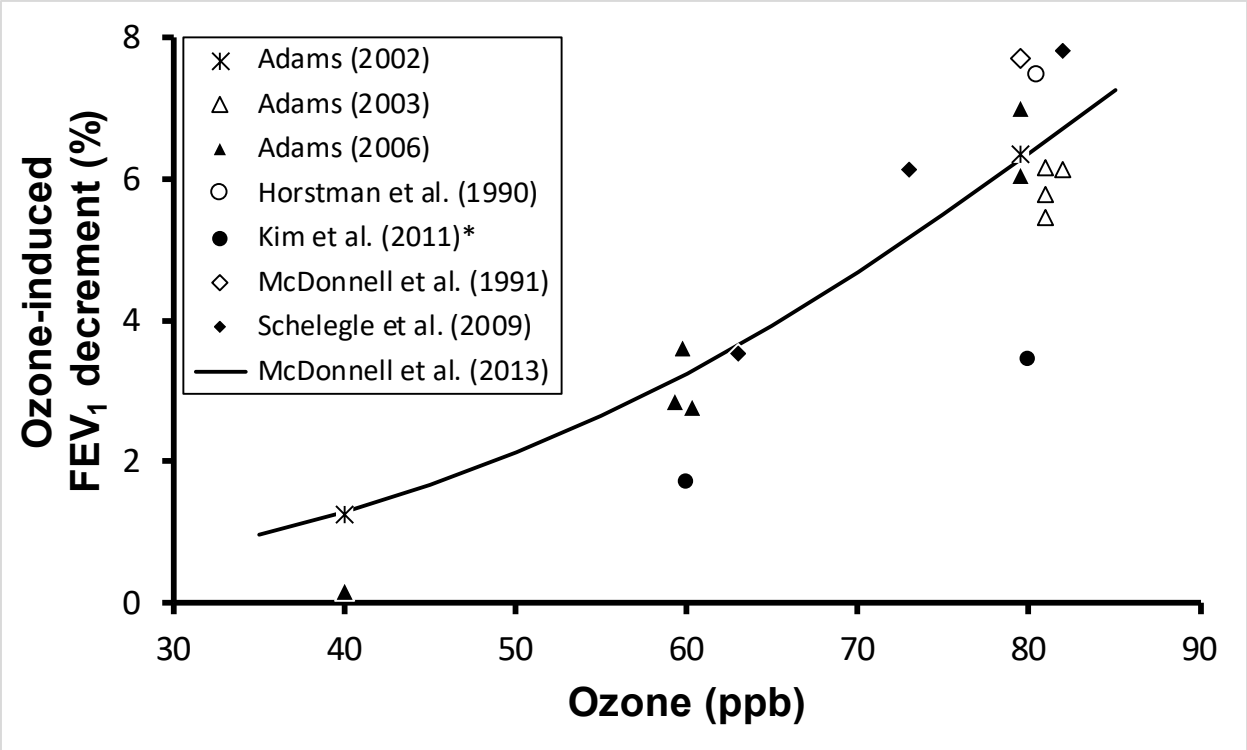
The strongest evidence for health effects due to ozone exposure continues to come from studies of short- and long-term ozone exposure and respiratory health, and this evidence is detailed in Appendix 3. Consistent with conclusions from the 2013 Ozone ISA (Table ES-1), **there is a "causal relationship" between short-term ozone exposure and respiratory effects** (Section 3.1.11), and **there**

is a "likely to be causal relationship" between long-term ozone exposure and respiratory effects (Section 3.2.6).

For short-term ozone exposure, controlled human exposure studies conducted over many decades provide experimental evidence for ozone-induced lung function decrements (Figure ES-3), respiratory symptoms, and respiratory tract inflammation. Epidemiologic studies continue to provide evidence that ozone concentrations are associated with a range of respiratory effects, including asthma exacerbation, chronic obstructive pulmonary disease (COPD) exacerbation, respiratory infection, and hospital admissions and emergency department (ED) visits for combined respiratory diseases.

A large body of animal toxicological studies demonstrate ozone-induced alterations in lung function, inflammation, increased airway responsiveness, and impaired lung host defense. These animal toxicological studies also aid in our understanding of potential mechanisms underlying respiratory effects at the population level and the biological plausibility of epidemiologic associations between short-term ozone exposure and respiratory-related ED visits and hospital admissions.

With respect to long-term ozone exposure, there is strong coherence between animal toxicological studies of changes in lung morphology and epidemiologic studies reporting positive associations between long-term ozone exposure and new-onset asthma, respiratory symptoms in children with asthma, and respiratory mortality. Furthermore, the experimental evidence provides biologically plausible pathways through which long-term ozone exposure could lead to respiratory effects reported in epidemiologic studies.



All responses at and above 70 ppb (targeted concentration) were statistically significant ($p < 0.05$). Adams (2006) found statistically significant responses to square-wave chamber exposures at 60 ppb based on the analysis of Brown et al. (2008) and Kim et al. (2011). During each hour of the exposures, subjects were engaged in moderate quasi-continuous exercise (20 L/min per m^2 BSA) for 50 minutes and rest for 10 minutes. Following the 3rd hour, subjects had an additional 35-minute rest period for lunch. The data at 60 and 80 ppb have been offset for illustrative purposes. The McDonnell et al. (2013) illustrates the predicted FEV_1 decrements using Model 3 coefficients at 6.6 hours as a function of ozone concentration for a 23.8-year-old with a BMI of 23.1 kg/m^2.

*80 ppb data for 30 health subjects were collected as part of the Kim et al. (2011) study, but only published in Figure 5 of McDonnell et al. (2012).

Adapted from Figure 6-1 of 2013 Ozone ISA (U.S. EPA, 2013). Studies appearing in the figure legend are: Adams (2006), Adams (2003), Adams (2002), Folinsbee et al. (1988), Horstman et al. (1990), Kim et al. (2011), McDonnell et al. (2013), McDonnell et al. (1991), and Schelegle et al. (2009).

Figure ES-3 Cross-study comparisons of mean decrements in ozone-induced forced expiratory volume in 1 second (FEV_1) in young, healthy adults following 6.6 hours of exposure to ozone.

Metabolic effects related to ozone exposure are evaluated as a separate health endpoint category for the first time in this ISA (Appendix 5). Recent evidence from animal toxicological, controlled human exposure, and epidemiologic studies indicate that **there is a "likely to be causal relationship" between short-term ozone exposure and metabolic effects** (Section 5.1.8). The strongest evidence for this determination is provided by animal toxicological studies that demonstrate impaired glucose tolerance, increased triglycerides, fasting hyperglycemia, and increased hepatic gluconeogenesis in various strains of animals across multiple laboratories. Biological plausibility is provided by results from controlled human exposure and animal toxicological studies that demonstrate activation of sensory nerve pathways following ozone exposure triggers the central neuroendocrine stress response, which includes increased

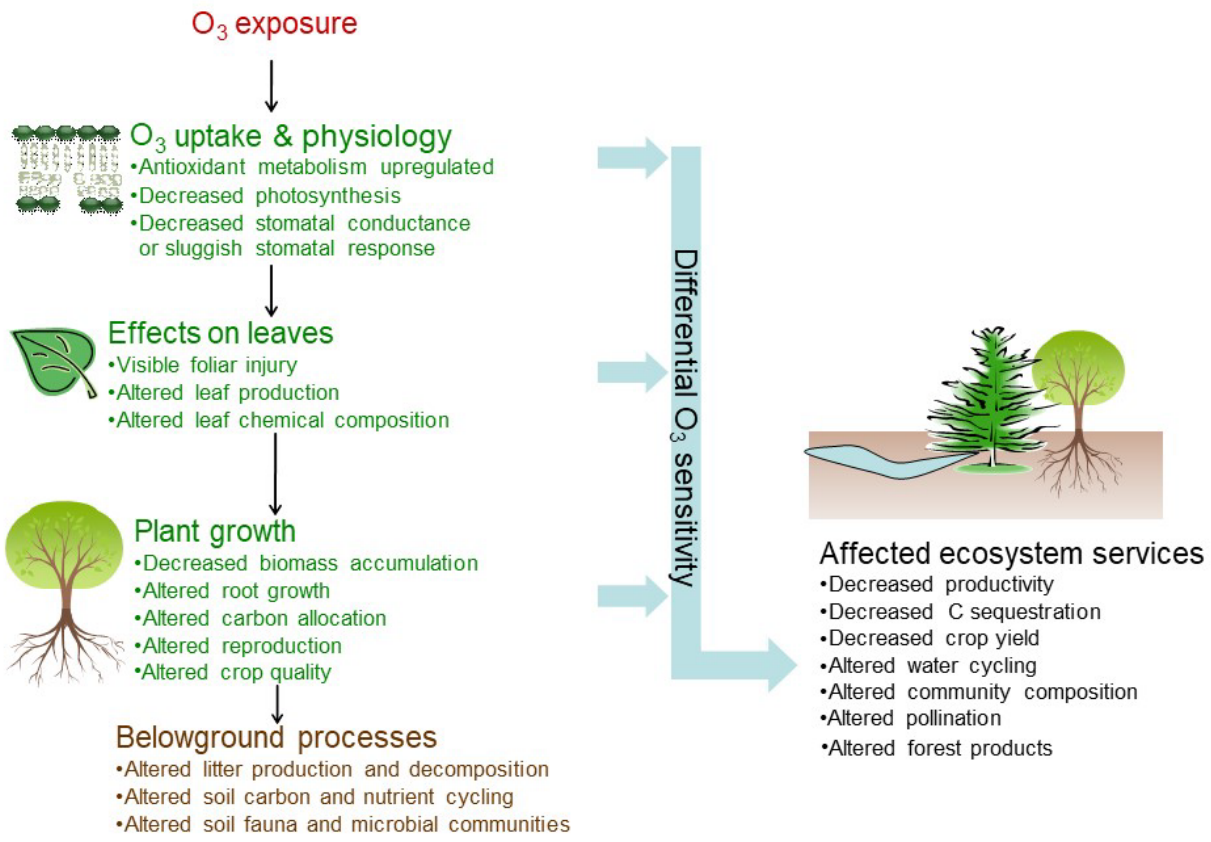
corticosterone, cortisol, and epinephrine production. These findings are coherent with epidemiologic studies that report associations between ozone exposure and perturbations in glucose and insulin homeostasis. In addition, these pathophysiological changes are often accompanied by increased inflammatory markers in peripheral tissues and by activation of the neuroendocrine system.

Similarly, **there is a "likely to be causal relationship" between long-term ozone exposure and metabolic effects** (Section 5.2.11). Animal toxicological studies of long term-ozone exposure also provide evidence for impaired insulin signaling, glucose intolerance, hyperglycemia, and insulin resistance. In prospective cohort studies conducted in the U.S. and Europe, increased incidence of type 2 diabetes was observed with long-term ozone exposure. In a large, population-based study in China, the risk of metabolic syndrome was also increased. These results are also consistent with two long-term ozone exposure studies in China that observed increased risk of obesity (a risk factor for type 2 diabetes) in both adults and children. In epidemiologic studies, positive associations between long-term exposure to ozone and diabetes-related mortality were reported in well-established cohorts in the U.S. and Canada. The results of the morbidity and mortality studies are supported by epidemiologic and experimental studies reporting effects on glucose homeostasis and serum lipids, as well as other indicators of metabolic function (e.g., peripheral inflammation and neuroendocrine activation).

Notably, there are changes in the causality determinations for short-term ozone exposure and cardiovascular effects (Appendix 4) as well as total mortality (Appendix 6). In both instances, the 2013 Ozone ISA concluded that the evidence was sufficient to conclude a "likely to be causal relationship", but after integrating the previous evidence with recent data, **the collective evidence is "suggestive of, but not sufficient to infer, a causal relationship" between short-term ozone exposure and cardiovascular effects (Section 4.1.17) or total mortality (Section 6.1.8) in** this ISA. The evidence that supports this change in the causality determinations includes: (1) a growing body of controlled human exposure studies providing less consistent evidence for an effect of short-term ozone exposure on cardiovascular health endpoints; (2) a paucity of positive evidence from epidemiologic studies for more severe cardiovascular morbidity endpoints (i.e., heart failure, ischemic heart disease and myocardial infarction, arrhythmia and cardiac arrest, and stroke); and (3) uncertainties due to a lack of control for potential confounding by copollutants in epidemiologic studies. Although there is generally consistent evidence for a limited number of ozone-induced cardiovascular endpoints in animal toxicological studies and for cardiovascular mortality in epidemiologic studies, these results are not coherent with results from controlled human exposure and epidemiologic studies examining cardiovascular morbidity endpoints. There remains evidence for ozone-induced cardiovascular mortality from epidemiologic studies. However, inconsistent results from a larger number of recent controlled human exposure studies that do not provide evidence of cardiovascular effects in response to short-term ozone exposure introduce additional uncertainties.

ES.4.2 Ozone Exposure and Welfare Effects

The scientific evidence for welfare effects of ozone consists mainly of effects on vegetation and ecosystems (Appendix 8) and effects on climate (Appendix 9). For ecological effects, damage to terrestrial ecosystems caused by ozone is largely a function of uptake of ozone into the leaf via stomata (gas exchange openings on leaves). Subsequent reactions with plant tissues alter whole-plant responses that cascade up to effects at higher levels of biological organization (i.e., from the cellular and subcellular level to the individual organism up to ecosystem level processes and services; Figure ES-4). At the leaf level, ozone uptake produces reactive oxygen species that affect cellular function (Section 8.1.3 and Figure 8-2). Reduced photosynthesis, altered carbon allocation, and impaired stomatal function lead to observable responses in plants. Observed vegetation responses to ozone include visible foliar injury (Section IS.5.1.1), and whole-plant level responses (Section IS.5.1.2), which encompass reduction in aboveground and belowground growth, reproduction and yield. Plant-fauna linkages affected by ozone include herbivores that feed on ozone-damaged vegetation and interactions of ozone with compounds emitted by plants that can alter attraction of pollinators to plants (Section IS.5.1.3). Ozone can result in broad changes in ecosystems such as decreased productivity and carbon sequestration (Section IS.5.1.4), altered belowground processes (Section IS.5.1.5), terrestrial community composition (Section IS.5.1.6), and water cycling (Section IS.5.1.7).



Source: Adapted from U.S. EPA (2013).

Figure ES-4 Illustrative diagram of ozone effects cascading from the cellular level to plants and ecosystems.

There are 12 causality determinations for ecological effects of ozone generally organized from the individual-organism scale to the ecosystem scale in Figure ES-5. Like the findings of the 2013 Ozone ISA (Table ES-2), five are causal relationships (i.e., visible foliar injury, reduced vegetation growth, reduced crop yield, reduced productivity, and altered belowground biogeochemical cycles) and two are likely to be causal relationships (i.e., reduced carbon sequestration, altered ecosystem water cycling). One of the endpoints, alteration of terrestrial community composition, is now concluded to be a causal relationship whereas in the 2013 Ozone ISA this endpoint was classified as a likely to be causal relationship. Three new endpoint categories (i.e., increased tree mortality, alteration of herbivore growth and reproduction, alteration of plant-insect signaling) not evaluated in the 2013 Ozone ISA, are all determined to have a likely to be causal relationship with ozone. Plant reproduction, previously considered as part of the evidence for growth effects, is now a stand-alone causal relationship.

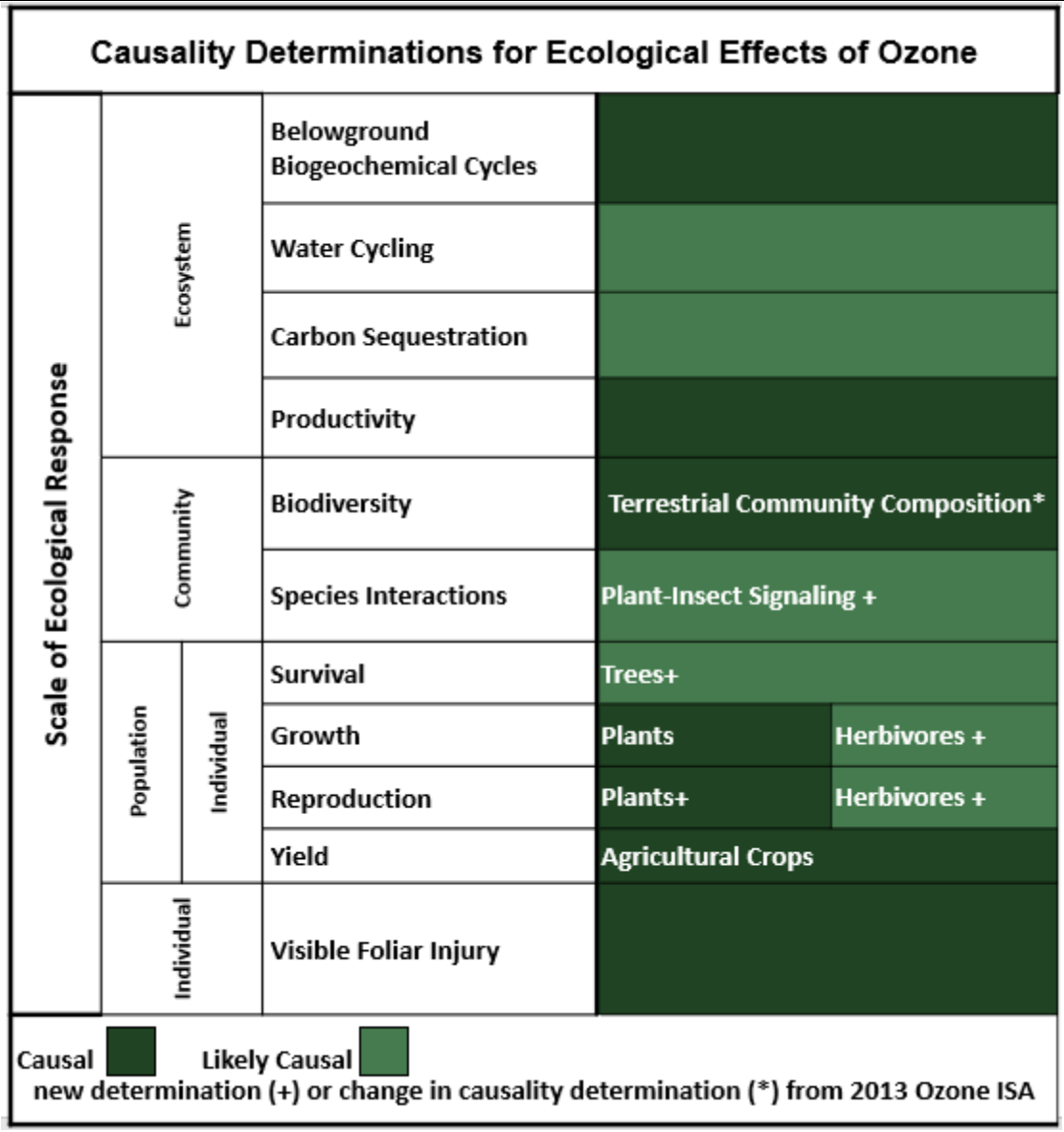


Figure ES-5 Causality determinations for ozone across biological scales of organization and taxonomic groups.

Table ES-2 Summary of causality determinations for ecological effects.

Endpoint	Conclusions from 2013 Ozone ISA	Conclusions in the current ISA
Visible foliar injury	Causal relationship	Causal relationship
Reduced vegetation growth	Causal relationship	Causal relationship
Reduced plant reproduction	No separate causality determination; included with plant growth	Causal relationship[a]
Increased tree mortality	Causality not assessed	Likely to be a causal relationship[a]
Reduced yield and quality of agricultural crops	Causal relationship	Causal relationship
Alteration of herbivore growth and reproduction	Causality not assessed	Likely to be a causal relationship[a]
Alteration of plant-insect signaling	Causality not assessed	Likely to be a causal relationship[a]
Reduced productivity in terrestrial ecosystems	Causal relationship	Causal relationship
Reduced carbon sequestration in terrestrial ecosystems	Likely to be a causal relationship	Likely to be a causal relationship
Alteration of belowground biogeochemical cycles	Causal relationship	Causal relationship
Alteration of terrestrial community composition	Likely to be a causal relationship	Causal relationship[b]
Alteration of ecosystem water cycling	Likely to be a causal relationship	Likely to be a causal relationship

[a]Denotes new causality determination.
[b]Denotes change in causality determination from 2013 Ozone ISA.

Visible foliar injury resulting from exposure to ozone has been well characterized and documented in over six decades of research involving many tree, shrub, herbaceous, and crop species and using both long-term field studies and laboratory approaches. Recent experimental evidence (Section 8.2) continues to show a consistent association between visible injury and ozone exposure supporting the conclusion of the 2013 Ozone ISA that, **there is a "causal relations hip" between ozone and visible foliar injury.** Changes in photosynthesis and carbon allocation in ozone-exposed plants scale up to reduced growth documented in natural and managed (e.g., agriculture, forestry, landscaping) species (Section 8.3), as well as impaired reproduction in individual plants (Section 8.4.1). Consistent with the

conclusions in the 2013 Ozone ISA, there is **a "causal relationship" between ozone and reduced plant growth** and **a "causal relationship" between ozone and reduced crop yield and quality**. In the 2013 Ozone ISA, reproduction was considered in the same category with plant growth. Increased information on metrics of plant reproduction (e.g., flower number, fruit number, fruit weight, seed number, rate of seed germination) and evidence for direct negative effects on reproductive tissues as well as for indirect negative effects (resulting from decreased photosynthesis and other whole-plant physiological changes) warrants a separate causality determination of **a "causal relationship" between ozone exposure and reduced plant reproduction**. Since the 2013 Ozone ISA, a large-scale multivariate analysis of factors contributing to tree mortality (1971−2005) concluded that county-level ozone concentrations averaged over the study period significantly increased tree mortality in 7 out of 10 plant functional types in the eastern and central U.S. (Section 8.4.3). This evidence, combined with observations of long-term declines of conifer forests in several high ozone regions and new experimental evidence that sensitive genotypes of aspen have increased mortality with ozone exposure support **a "likely to be causal relationship" between ozone exposure and tree mortality.**

In addition to effects on plants, ozone can alter ecological interactions between plants and other species including herbivores consuming ozone-exposed vegetation. Studies of insect herbivores in previous ozone assessments and newer studies covering a range of species at varying levels of ozone exposure frequently show statistically significant effects; however, they do not provide any consistent pattern of response across endpoints of growth or reproduction (Section 8.6). **The collective evidence supports "a likely to be causal relationship" between ozone exposure and altered herbivore growth and reproduction.** Many plant-insect interactions are mediated through volatile plant signaling compounds which plants use to signal other community members. In the 2013 Ozone ISA, a few experimental and modeling studies reported altered insect-plant interactions that are mediated through chemical signaling. New evidence from multiple studies show altered/degraded emissions of chemical signals from plants and reduced detection of volatile plant signaling compounds by insects, including pollinators, in the presence of ozone (Section 8.7). **The collective evidence supports "a likely to be causal relationship" between ozone exposure and alteration of plant-insect signaling.**

At the ecosystem scale, ozone-caused decreases in plant photosynthesis can lead to reduced ecosystem carbon content. Changes in patterns of aboveground and belowground carbon allocation associated with ozone effects on plants can alter ecosystem properties of storage (e.g., productivity, carbon sequestration) and cycling (e.g., biogeochemistry). Consistent with the conclusions of the 2013 Ozone ISA, there is **a "causal relationship" between ozone exposure and reduced productivity and a "likely to be causal relationship" between ozone and reduced carbon sequestration** (Section 8.8). As described in the 2013 Ozone ISA and new studies, processes such as carbon and nitrogen cycling and decomposition in soils are indirectly affected via ozone effects on the quality and quantity of carbon supply from plants and leaf litter (Section 8.9). **Recent evidence continues to support a "causal relationship" between ozone exposure and the alteration of belowground biogeochemical cycles**. Ozone can affect water use in plants through several mechanisms including damage to stomatal

functioning, loss of leaf area, and changes in wood anatomy (e.g., vessel size and density) that can affect plant and stand evapotranspiration and may lead, in turn, to possible effects on hydrological cycling (Section 8.11). Evidence continues to support the conclusion of the 2013 Ozone ISA that, **there is a "likely to be causal relationship" between ozone and alteration of ecosystem water cycling.** In terrestrial ecosystems, ozone may alter community composition by uneven effects on co-occurring species, decreasing the abundance of sensitive species, and giving tolerant species a competitive advantage. Alteration of community composition of some ecosystems including conifer forests, broadleaf forests, and grasslands and altered fungal and bacterial communities in soils reported in the 2013 Ozone ISA is augmented by additional evidence for effects in forest and grassland communities (Section 8.10); collective evidence indicates a change in the causality determination to **a "causal relationship" between ozone exposure and altered terrestrial community composition of some ecosystems.**

For effects on climate, changes in the abundance of tropospheric ozone perturb the radiative balance of the atmosphere by interacting with incoming solar radiation and outgoing longwave radiation. This effect is quantified by radiative forcing.[1] Through this effect on the Earth's radiation balance, tropospheric ozone plays a major role in the climate system and increases in tropospheric ozone abundance contribute to climate change. **Recent evidence continues to support a "causal relationship" between tropospheric ozone and radiative forcing and a "likely to be causal relationship," via radiative forcing, between tropospheric ozone and temperature, precipitation, and related climate variables** (referred to as "climate change" in the 2013 Ozone ISA; the revised title for this causality determination provides a more accurate reflection of the available evidence; Table ES-3). The new evidence comes from the Intergovernmental Panel on Climate Change (IPCC) Fifth Assessment Report (AR5) and its supporting references, as well as a limited number of more recent studies, and builds on evidence presented in the 2013 Ozone ISA. The new studies further support the causality determinations included in the 2013 Ozone ISA.

Table ES-3 Summary of causality determinations for tropospheric ozone effects on climate.

	Conclusions in 2013 Ozone ISA	Conclusions in the current ISA
Radiative forcing	Causal relationship	Causal relationship
Temperature, precipitation, and related climate variables	Likely to be a causal relationship	Likely to be a causal relationship

[1] Radiative forcing is the perturbation in net radiative flux at the tropopause (or top of the atmosphere) caused by a change in radiatively active forcing agent(s) after stratospheric temperatures have readjusted to radiative equilibrium [stratospherically adjusted radiative forcing, (Myhre et al., 2013)].

ES.5 Key Aspects of Health and Welfare Effects Evidence

There is extensive scientific evidence that demonstrates health and welfare effects from exposure to ozone. As documented by the evaluation of evidence throughout the subsequent appendices to this ISA, the U.S. EPA carefully considers uncertainties in the evidence, and the extent to which recent studies have addressed or reduced uncertainties from previous assessments, as well as the strengths of the evidence. Uncertainties do not necessarily change the fundamental conclusions of the literature base. In fact, some conclusions are robust to such uncertainties. Where there is clear evidence linking ozone with health and welfare effects—with or despite remaining uncertainties—the U.S. EPA makes a determination of a causal or likely to be causal relationship. The identification of the strengths and limitations in the evidence will help in the prioritization of research efforts to support future ozone NAAQS reviews.

ES.5.1 Health Effects Evidence: Key Findings

A large body of scientific evidence spanning many decades clearly demonstrates there are health effects related to both short- and long-term ozone exposure. The strongest evidence supports a relationship between ozone exposure and respiratory health effects. The collective body of evidence for each health outcome category evaluated in this ISA is systematically considered and assessed, including the inherent strengths, limitations, and uncertainties in the overall body of evidence, resulting in the causality determinations detailed in Table ES-1.

An inherent strength of the evidence integration in this ISA is the extensive amount (in both breadth and depth) of available evidence resulting from decades of scientific research that describes the relationship between both short- and long-term ozone exposure and health effects. The breadth of the enormous database is illustrated by the different scientific disciplines that provide evidence (e.g., controlled human exposure, epidemiologic, animal toxicological studies), the range of health outcomes examined (e.g., respiratory, cardiovascular, metabolic, reproductive, and nervous system effects, as well as cancer and mortality), and the large number of studies within several of these outcome categories. The depth of the literature base is exemplified by the examination of effects that range from biomarkers of exposure, to subclinical effects, to overt clinical effects, and even mortality.

There is strong and consistent experimental evidence linking short- and long-term ozone exposure with respiratory and metabolic health effects. However, several uncertainties should be considered when evaluating and synthesizing evidence from these studies. Experimental animal studies are often conducted at ozone concentrations higher than those observed in ambient air (i.e., 250 to >1,000 ppb) to evoke a response within a reasonable study period. These studies are informative and the conduct of studies at these concentrations is commonly used for identifying potential human hazards. There are also substantial differences in exposure concentrations and exposure durations between animal toxicological and

controlled human exposure studies. Additionally, a number of animal toxicological studies are performed in rodent disease models, while controlled human exposure studies generally are conducted in healthy individuals. Controlled human exposure studies do not typically include unhealthy or diseased individuals for ethical reasons; therefore, this exclusion represents an important uncertainty to consider in interpreting the results of these studies (i.e., that other individuals may be more sensitive and at risk to ozone than those in the study groups). Additionally, exposure concentration and disease status differences in physiology (e.g., rodents are obligate nose breathers), differences in the duration and timing of exposure (e.g., rodents are exposed during the day, during their resting cycle, while humans are exposed during the day when they are normally active), and differences in the temperature at which the exposure was conducted, may contribute to any lack of coherence between results of experimental animal and human studies.

Epidemiologic studies contribute important evidence supporting the relationship between short- and long-term ozone exposure with respiratory and metabolic health effects. Although susceptible to chance, bias, and other potential confounding due to their observational nature, epidemiologic studies have the benefit of evaluating real-world exposure scenarios and can include sensitive populations that cannot typically be included in controlled human exposure studies. Innovations in epidemiologic study designs and methods have substantially reduced the role of chance, bias, and other potential confounders in well-designed, well-conducted epidemiologic studies. The most common source of uncertainty in epidemiologic studies of ozone is exposure measurement error. The exposure assignment methods used in short- and long-term ozone exposure epidemiologic studies have inherent strengths and limitations, and exposure measurement errors associated with those methods contribute bias and uncertainty to health effect estimates. For short-term exposure studies, exposure measurement error generally leads to underestimation and reduced precision of the association, whereas in long-term exposure studies exposure measurement error has the potential to bias effect estimates in either direction, although it is more common that they are underestimated. When combined with coherent evidence from animal toxicological and controlled human exposure studies, the epidemiologic evidence can support and strengthen determinations of the causal nature of the relationship between health effects and exposure to ozone at relevant ambient air concentrations.

ES.5.2 Welfare Effects Evidence: Key Findings

The collective body of evidence for each welfare endpoint evaluated in this ISA was carefully considered and assessed, including the inherent strengths, limitations, and uncertainties in the overall body of evidence, resulting in the causality determinations for ecological effects detailed in Table ES-2 and effects on climate in Table ES-3. A large body of scientific evidence spanning more than 60 years clearly shows effects on vegetation due to ozone exposure. Decades of research on many plant species confirm effects on visible foliar injury, plant growth, reproduction and yield. The use of visible foliar injury to identify phytotoxic levels of ozone is an established and widely used methodology. There are

robust exposure-response functions (i.e., from carefully controlled experimental conditions, involving multiple concentrations and based on multiple studies) for about a dozen important tree species and a dozen major commodity crop species. Newer evidence supports a role for ozone in tree mortality and shifts in community composition of forest tree and grassland species. While the effect of ozone on vegetation is well established in general, there are some knowledge gaps regarding precisely which species are sensitive, what exposures elicit adverse responses for many species and how plant response changes with age and size.

There is high certainty in ozone effects on impairment to leaf physiology as mechanisms for effects at higher levels of biological organization (i.e., from the cellular level through individual organisms to the level of communities and ecosystems) and how those can ultimately affect aboveground and belowground processes such as productivity, carbon sequestration, biogeochemical cycling, and hydrology. However, ecosystems are inherently complex, and it is difficult to partition observed responses within a suite of multiple stressors. Scaling ozone effects to the ecosystem level remains a challenge, but there is a large body of knowledge of how ecosystems work through ecological observations and models. Interactive effects in natural ecosystems with multiple stressors (e.g., drought, disease) are difficult to study, but some have been investigated using different statistical methods. Although models and methods for characterizing ecosystem-level responses to ozone are accompanied by inherent uncertainties, more research will strengthen understanding of scaling across different levels of biological organization.

There are multiple pathways in which ozone can affect plant-insect interactions. Studies that characterize volatile plant signaling compounds in ozone-enriched environments and assess insect response to altered chemical signals suggest that ozone alters scent-mediated interactions in ecological communities. A relatively small number of insect species and plant-insect associations have been assessed, and there are knowledge gaps in the mechanisms and consequences of modulation of signaling by ozone. There are multiple studies demonstrating ozone effects on fecundity and growth in insects that feed on ozone-exposed vegetation. However, no consistent directionality of response is observed across studies and uncertainties remain in regard to different plant consumption methods across species and the exposure conditions associated with particular severities of effects.

Changes in the abundance of tropospheric ozone affect radiative forcing, and thus tropospheric ozone is considered an important greenhouse gas. The recent IPCC AR5 estimates tropospheric ozone radiative forcing to be 0.40 (0.20 to 0.60) W/m^2 and recent studies reinforce the AR5 estimates. Consistent with previous estimates, the effect of tropospheric ozone on global surface temperature, through its impact on radiative forcing, continues to be estimated at roughly 0.1 to 0.3°C since preindustrial times with larger effects regionally. Some new research has explored certain additional aspects of the climate response to ozone radiative forcing beyond global and regional temperature change. Specifically, ozone changes are understood to have impacts on other climate metrics such as precipitation and atmospheric circulation patterns, and new evidence has continued to support and further quantify this

understanding. While the warming effect of tropospheric ozone in the climate system is well established in general, precisely quantifying changes in surface temperature due to tropospheric ozone changes, along with related climate effects, requires complex climate simulations, including important feedbacks and interactions. Current limitations in climate modeling tools, variation across models, and the need for more comprehensive observational data on these effects represent sources of uncertainty in quantifying the precise magnitude of climate responses to ozone changes, particularly at regional scales.

ES.6 References for Executive Summary

Adams, WC. (2002). Comparison of chamber and face-mask 6.6-hour exposures to ozone on pulmonary function and symptoms responses. Inhal Toxicol 14: 745-764. http://dx.doi.org/10.1080/08958370290084610.

Adams, WC. (2003). Comparison of chamber and face mask 6.6-hour exposure to 0.08 ppm ozone via square-wave and triangular profiles on pulmonary responses. Inhal Toxicol 15: 265-281. http://dx.doi.org/10.1080/08958370390168283.

Adams, WC. (2006). Comparison of chamber 6.6-h exposures to 0.04-0.08 ppm ozone via square-wave and triangular profiles on pulmonary responses. Inhal Toxicol 18: 127-136. http://dx.doi.org/10.1080/08958370500306107.

Brown, JS; Bateson, TF; Mcdonnell, WF. (2008). Effects of exposure to 0.06 ppm ozone on FEV1 in humans: A secondary analysis of existing data. Environ Health Perspect 116: 1023-1026. http://dx.doi.org/10.1289/ehp.11396.

Folinsbee, LJ; Mcdonnell, WF; Horstman, DH. (1988). Pulmonary function and symptom responses after 6.6-hour exposure to 0.12 ppm ozone with moderate exercise. J Air Waste Manag Assoc 38: 28-35. http://dx.doi.org/10.1080/08940630.1988.10466349.

Horstman, DH; Folinsbee, LJ; Ives, PJ; Abdul-Salaam, S; Mcdonnell, WF. (1990). Ozone concentration and pulmonary response relationships for 6.6-hour exposures with five hours of moderate exercise to 0.08, 0.10, and 0.12 ppm. Am J Respir Crit Care Med 142: 1158-1163. http://dx.doi.org/10.1164/ajrccm/142.5.1158.

Kim, CS; Alexis, NE; Rappold, AG; Kehrl, H; Hazucha, MJ; Lay, JC; Schmitt, MT; Case, M; Devlin, RB; Peden, DB; Diaz-Sanchez, D. (2011). Lung function and inflammatory responses in healthy young adults exposed to 0.06 ppm ozone for 6.6 hours. Am J Respir Crit Care Med 183: 1215-1221. http://dx.doi.org/10.1164/rccm.201011-1813OC.

McDonnell, WF; Kehrl, HR; Abdul-Salaam, S; Ives, PJ; Folinsbee, LJ; Devlin, RB; O'Neil, JJ; Horstman, DH. (1991). Respiratory response of humans exposed to low levels of ozone for 6.6 hours. Arch Environ Occup Health 46: 145-150. http://dx.doi.org/10.1080/00039896.1991.9937441.

McDonnell, WF; Stewart, PW; Smith, MV. (2013). Ozone exposure-response model for lung function changes: An alternate variability structure. Inhal Toxicol 25: 348-353. http://dx.doi.org/10.3109/08958378.2013.790523.

McDonnell, WF; Stewart, PW; Smith, MV; Kim, CS; Schelegle, ES. (2012). Prediction of lung function response for populations exposed to a wide range of ozone conditions. Inhal Toxicol 24: 619-633. http://dx.doi.org/10.3109/08958378.2012.705919.

Myhre, G; Samset, BH; Schulz, M; Balkanski, Y; Bauer, S; Berntsen, TK; Bian, H; Bellouin, N; Chin, M; Diehl, T; Easter, RC; Feichter, J; Ghan, SJ; Hauglustaine, D; Iversen, T; Kinne, S; Kirkevag, A; Lamarque, JF; Lin, G; Liu, X; Lund, MT; Luo, G; Ma, X; van Noije, T; Penner, JE; Rasch, PJ; Ruiz, A; Seland, O; Skeie, RB; Stier, P; Takemura, T; Tsigaridis, K; Wang, P; Wang, Z; Xu, L; Yu, H; Yu, F; Yoon, JH; Zhang, K; Zhang, H; Zhou, C. (2013). Radiative forcing of the direct aerosol effect from AeroCom Phase II simulations. Atmos Chem Phys 13: 1853-1877. http://dx.doi.org/10.5194/acp-13-1853-2013.

Schelegle, ES; Morales, CA; Walby, WF; Marion, S; Allen, RP. (2009). 6.6-hour inhalation of ozone concentrations from 60 to 87 parts per billion in healthy humans. Am J Respir Crit Care Med 180: 265-272. http://dx.doi.org/10.1164/rccm.200809-1484OC.

U.S. EPA (U.S. Environmental Protection Agency). (2013). Integrated science assessment for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-10/076F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment-RTP Division. http://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=247492.

U.S. EPA (U.S. Environmental Protection Agency). (2015). Preamble to the integrated science assessments [EPA Report]. (EPA/600/R-15/067). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, RTP Division. https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=310244.

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INTEGRATED SYNTHESIS

Overall Conclusions of the Ozone Integrated Science Assessment (ISA)

Human Health Effects

- Recent studies support and expand upon the strong body of evidence, which has been accumulating over the last few decades, that short-term ozone exposure causes respiratory effects. The strongest evidence comes from controlled human exposure studies demonstrating ozone-induced decreases in lung function and inflammation in healthy, exercising adults at concentrations as low as 60 ppb after 6.6 hours of exposure. In addition, epidemiologic studies continue to provide strong evidence that ozone is associated with respiratory effects, including asthma and COPD exacerbations, as well as hospital admissions and emergency department visits for respiratory diseases. The results from toxicological studies further characterize potential mechanistic pathways and provide continued support for the biological plausibility of ozone-induced respiratory effects.
- Emerging evidence indicates that short- and long-term ozone exposure contributes to metabolic disease, including diabetes. Specifically, animal toxicological studies demonstrate impaired glucose tolerance, increased triglycerides, fasting hyperglycemia, and increased hepatic gluconeogenesis in laboratory animals. A limited number of epidemiologic studies observed associations between ozone and increased incidence of type 2 diabetes and mortality from diabetes.
- Because recent evidence from controlled human exposure studies provides inconsistent evidence of ozone-induced cardiovascular effects, the overall body of evidence for an association of short-term ozone exposure with cardiovascular effects and total (nonaccidental) mortality is less certain than reported in the 2013 Ozone ISA, resulting in a change in the causality determinations.

Welfare Effects

Ecological Effects

- A large body of scientific evidence spanning more than 60 years clearly demonstrates that ozone affects vegetation and ecosystems. The strongest evidence comes from vegetation-related endpoints; foliar injury, reduced growth, and decreased yield are well characterized in a variety of crop and noncrop species. Ecological effects of ozone are observed across several scales of biological organization (i.e., from the cellular level through individual organisms to the level of communities and ecosystems), ultimately affecting aboveground and belowground processes including productivity, carbon sequestration, biogeochemical cycling and hydrology. In most cases, new research strengthens the previously reached conclusions in the 2013 Ozone ISA. New endpoints included in this review result from emerging areas of study such as chemical ecology (e.g., plant-insect signaling) or new evidence enabling further refinement of previously understood ozone effects (e.g., plant reproduction, tree mortality, herbivore growth and reproduction, terrestrial community composition).

Effects on Climate

- New research builds on the evidence in the 2013 Ozone ISA and continues to support the previous findings of tropospheric ozone impacts on radiative forcing and climate variables, including temperature and precipitation (referred to as “climate change” in the 2013 Ozone ISA).

IS.1 Introduction

IS.1.1 Purpose and Overview

The Integrated Science Assessment (ISA) serves as the scientific foundation of the National Ambient Air Quality Standard (NAAQS) review process. [1] The ISA is a comprehensive evaluation and synthesis of the policy-relevant science "useful in indicating the kind and extent of identifiable effects on public health or welfare[2] which may be expected from the presence of [a] pollutant in ambient air," as described in Section 108 of the Clean Air Act (CAA, 1990).[3] This ISA reviews and the air quality criteria for the health and welfare effects of ozone and related photochemical oxidants in ambient air. It draws on the existing body of evidence to evaluate and synthesize the current state of scientific knowledge on the most relevant issues pertinent to the current review of the ozone NAAQS,[4] to identify changes in the scientific evidence since the previous review, and to describe remaining or newly identified uncertainties and limitations in the evidence. In 2015, the U.S. EPA lowered the level of the primary and secondary ozone standards to 0.070 ppm which is for the annual fourth-highest daily maximum 8-hour concentration averaged over 3 years[5]. The ozone primary NAAQS is established to protect public health with an adequate margin of safety, including at-risk populations such as children and people with asthma. The ozone secondary standard is intended to protect the public welfare from known or anticipated adverse effects associated with the presence of ozone in the ambient air.

This ISA identifies and critically evaluates the most policy-relevant current scientific literature published since the 2013 Ozone ISA across scientific disciplines, including epidemiology, controlled human exposure studies, animal toxicology, atmospheric science, exposure science, vegetation studies, agricultural science, ecology, and climate-related science. Key scientific conclusions (i.e., causality determinations; Section IS.1.2.4) are presented that provide the basis for developing risk and exposure analyses, evaluating policy, and making environmental health decisions. In characterizing the evidence

[1] Section 109(d)(1) of the Clean Air Act requires periodic review and, if appropriate, revision of existing air quality criteria to reflect advances in scientific knowledge on the effects of the pollutant on public health and welfare. Under the same provision, EPA is also to periodically review and, if appropriate, revise the NAAQS, based on the revised air quality criteria.

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

[3] The general process for developing an ISA, including the framework for evaluating weight of evidence and drawing scientific conclusions and causal judgments, is described in a companion document, Preamble to the Integrated Science Assessments (U.S. EPA, 2015).

[4] The "indicator" of a standard defines the chemical species or mixture that is to be measured in determining whether an area attains the standard. The indicator of the current NAAQS for photochemical oxidants is ozone.

[5] Final rule signed October 1, 2015, and effective December 28, 2015 (80 FR 65291).

for each of the health and welfare effects categories evaluated, this ISA draws conclusions about the causal nature of the relationships between ozone exposure and effects by integrating information across scientific disciplines and related health and welfare outcomes and synthesizing evidence from previous and recent studies. As in previous reviews, the ISA for this review will focus mainly on the assessment of health and welfare effects resulting from exposure to surface-level concentrations of tropospheric ozone. Less emphasis will be accorded to other photochemical oxidants for which there is distinctly much less information. Ozone is currently the NAAQS indicator for photochemical oxidants, and the primary literature evaluating the health and ecological effects of photochemical oxidants includes ozone almost exclusively as an indicator of photochemical oxidants.[1] The ISA thus provides the policy-relevant scientific information that supports the review of the current ozone NAAQS.

IS.1.2 Process and Development

Through iterative NAAQS reviews, ISAs build on evidence and conclusions from previous assessments. The previous ozone ISA was published in 2013 (U.S. EPA, 2013b) and generally included peer-reviewed literature published through July 2011. Prior assessments include the 2006 Air Quality Criteria Document (AQCD) for Ozone and Related Photochemical Oxidants (U.S. EPA, 2006a), the 1996 AQCD for Ozone (U.S. EPA, 1996a), the 1986 AQCD for Ozone (U.S. EPA, 1986), the 1978 Air Quality Criteria for Ozone and Other Photochemical Oxidants (U.S. EPA, 1978), and the 1970 Criteria Document (NAPCA, 1970). This ISA identifies and evaluates studies published since 2011, synthesizing and integrating the new evidence into the information and conclusions from previous assessments.

In the process of developing an ISA, systematic review methodologies are used to identify and evaluate relevant scientific information, which is synthesized into text and figures to communicate the state of the science. The process begins with a "Call for Information" published in the Federal Register that announces the start of the NAAQS review and invites the public to assist in this process by identifying relevant research studies in the subject areas of concern. For this Ozone NAAQS review, this notice was published on June 26, 2018 (83 FR 29785). The subsequent ISA development steps are described in greater detail in the Preamble to the Integrated Science Assessments (U.S. EPA, 2015), which provides a general overview of the ISA development process. The Preamble describes the general framework for evaluating scientific information, including criteria for assessing study quality and developing scientific conclusions. The U.S. EPA uses a structured and transparent process to evaluate scientific information and to determine the causal nature of relationships between air pollution and health and welfare effects [see Preamble (U.S. EPA, 2015)]. Development of the ISA includes approaches for

[1] Ozone is the only photochemical oxidant other than nitrogen dioxide (NO_2) that is routinely monitored in ambient air (i.e., EPA's AQS database; https://www.epa.gov/aqs). Data for other photochemical oxidants (e.g., PAN, H_2O_2, etc.) typically have been obtained only as part of special field studies. Consequently, no data on nationwide patterns of ambient air concentrations are available for these other photochemical oxidants; nor are extensive data available on the relationships of concentrations and patterns of these photochemical oxidants to those of ozone.

literature searches, criteria for selecting and evaluating relevant studies, and a framework for evaluating the weight of evidence and forming causality determinations. As part of this process, the ISA is reviewed by the public and by the Clean Air Scientific Advisory Committee (CASAC), which is a formal, independent scientific committee. The Preamble describes a science and policy workshop that often occurs at the beginning of the NAAQS review process; such a workshop was not convened for the current Ozone NAAQS review. Instead, the "Call for Information" published in the Federal Register requested public input on science and polity issues pertinent to the Ozone NAAQS review.

IS.1.2.1 Scope of the ISA and the Population, Exposure, Comparison, Outcome, and Study Design (PECOS) Tools

The Ozone ISA includes research relevant to characterizing ozone in ambient air (hereafter referred to as ambient ozone) and assessing the health and welfare effects of exposure to ambient ozone. Health effects evidence evaluated in the ISA includes experimental controlled human exposure and animal toxicological studies, and observational epidemiologic studies. Welfare-based evidence included in the Ozone ISA focuses specifically on ecological effects and effects on climate. The evidence connecting tropospheric ozone and UV-B shielding was evaluated in the 2013 Ozone ISA and determined to be inadequate to draw a causal conclusion; this continues to be the case in the current ISA (Section 9.1.3.4), and this topic is not discussed further in this synthesis.

The scope of the health and welfare effects evidence evaluated in this ISA is further refined by scoping that generally defines the relevant Population, Exposure, Comparison, Outcome, and Study Design (PECOS) for each of the scientific disciplines that form the basis of the evaluation of evidence for the broad health and welfare effects categories for which this ISA forms causality determinations. The PECOS tools provide structured frameworks for defining the scope of the ISA. There are discipline-specific PECOS tools for experimental and epidemiologic studies (Section 3.1.2, Section 3.2.2, Section 4.1.2, Section 4.2.1.1, Section 5.1.1, Section 5.2.1, Section 6.1.1.1, Section 6.2.1.1, Section 7.1.1.1, Section 7.2.1.1, Section 7.2.2.1, and Section 7.3.1.1), ecological studies (Table 8-2), and studies of the effects of tropospheric ozone on climate (Table 9-1). These PECOS criteria were developed around the evidence base at the time of the last review (the causality determinations from the 2013 Ozone ISA) and the uncertainties and limitations associated with that evidence. The use of PECOS tools is a widely accepted and rapidly growing approach to systematic review in risk assessment, and their use is consistent with recommendations by the National Academy of Sciences for improving the design of risk assessment through planning, scoping, and problem formulation to better meet the needs of decision makers (NRC, 2009). The PECOS tools serve as guides for the inclusion or exclusion of studies in the ISA. Additional details on the development and use of these PECOS tools can be found in Appendix 10 (Section 10.3.1).

IS.1.2.2 Organization of the ISA

The ISA consists of the Preface (legislative requirements and history of the primary and secondary ozone NAAQS; and purpose and overview of the ISA along with the overall scope, and process for evaluating evidence), Executive Summary, Integrated Synthesis, and 10 appendices. This Integrated Synthesis provides the key information for each topic area, encompassing a description of ozone concentrations in the U.S. (including background sources), conclusions regarding the health and welfare effects associated with ozone exposure (including causality determinations for relationships between exposure to ozone and specific types of health and welfare effects), identification of the human lifestages and populations at increased risk of the effects of ozone, and a discussion of the key strengths, limitations, and uncertainties inherent in this evidence base. The purpose of this Integrated Synthesis is not to summarize each of the appendices; rather it is to synthesize the key findings on each topic considered in characterizing ozone exposure and relationships with health and welfare effects. This Integrated Synthesis also discusses additional policy-relevant issues. These include exposure durations, metrics, and concentrations eliciting health and welfare effects and the concentration-response (C-R) relationships for specific effects, including their overall shapes and the evidence with regard to discernibility of threshold exposures below which effects are unlikely to occur. Subsequent Appendix 1−Appendix 10 are organized by subject area, with the detailed assessment of atmospheric science (Appendix 1), exposure (Appendix 2), health (Appendix 3−Appendix 7), and welfare evidence (Appendix 8−Appendix 9). Each of the appendices contain an evaluation of results from recent studies integrated with evidence from previous reviews. Appendices for each broad health effect category (e.g., respiratory effects) discuss potential biological pathways and conclude with a causality determination describing the strength of the evidence between exposure to ozone and the outcome(s) under consideration. Likewise, the appendices devoted to ecological (Appendix 8) and climate evidence (Appendix 9) for welfare effects will include causality determinations for multiple effects on ecosystems and climate, respectively. Appendix 10 describes the process of developing the ozone ISA, including aspects related to study design, study quality, and quality assurance (QA) and quality control (QC) documentation.

IS.1.2.3 Quality Assurance Summary

The use of QA and peer review helps ensure that the U.S. EPA conducts high quality science that can be used to help policymakers, industry, and the public make informed decisions. Quality assurance activities performed by the U.S. EPA ensure that environmental data is of sufficient quantity and quality to support the Agency's intended use. The U.S. EPA has developed a detailed Program-level QA Project Plan (PQAPP) for the ISA Program to describe the technical approach and associated QA/QC procedures associated with the ISA Program. All QA objectives and measurement criteria detailed in the PQAPP have been employed in developing this draft ISA. Furthermore, the Ozone ISA is classified as providing Highly Influential Scientific Assessment (HISA), which is defined by the Office of Management and

Budget (OMB) as a scientific assessment that is novel, controversial, or precedent-setting, or has significant interagency interest (OMB, 2004). OMB requires a HISA to be peer reviewed before dissemination. To meet this requirement, the U.S. EPA engages the Clean Air Scientific Advisory Committee (CASAC) as an independent federal advisory committee to conduct peer reviews. Both peer-review comments provided by the CASAC panel and public comments submitted to the panel during its deliberations about the external review draft will be considered in the development of a final ISA. For a more detailed discussion of peer review and quality assurance, see Section 10.4 and Section 10.5, respectively.

IS.1.2.4 Evaluation of the Evidence

This ISA draws conclusions about the causal nature of relationships between exposure to ozone and health and welfare effects for categories of related effects (e.g., respiratory effects) by integrating recent evidence across scientific disciplines and building on the evidence from previous assessments. Determinations are made about causation, not just association, and are based on judgments of consistency, coherence, and biological plausibility of observed effects, as well as related uncertainties. The ISA uses a formal causal framework to classify the weight of evidence using a five-level hierarchy [i.e., "causal relationship"; "likely to be a causal relationship"; "suggestive of, but not sufficient to infer, a causal relationship"; "inadequate to infer a causal relationship"; "not likely to be a causal relationship" as described in Table II of the Preamble (U.S. EPA, 2015)] that is based largely on the aspects for causality proposed by Sir Austin Bradford Hill, as well as other frameworks to assess causality developed by other organizations.

IS.1.3 New Evidence Evaluation and Causality Determinations

In the 2013 Ozone ISA, the causality determinations communicated the extent of the then current knowledge of health and welfare effects. Updates to the causality determinations for ozone based on new evidence in this review are summarized below and described in greater detail in Section IS.4 (Health) and Section IS.5 (Welfare).

IS.1.3.1 Human Health

The results from the health studies, supported by the evidence from atmospheric chemistry and exposure assessment studies, contribute to the causality determinations made for the health outcomes. The conclusions from the 2013 Ozone ISA and the causality determinations from this review are summarized in Table IS-1.

Table IS-1 Summary of causality determinations by exposure duration and health outcome.

Health Outcome[a]	Conclusions from 2013 Ozone ISA	Conclusions in the Current ISA
Short-term exposure to ozone		
Respiratory effects	Causal relationship	Causal relationship
Metabolic effects	No determination made	Likely to be a causal relationship[b]
Cardiovascular effects	Likely to be a causal relationship	Suggestive of a causal relationship[c]
Total mortality	Likely to be a causal relationship	Suggestive of a causal relationship[c]
Central nervous system effects	Suggestive of a causal relationship	Suggestive of a causal relationship
Long-term exposure to ozone		
Respiratory effects	Likely to be a causal relationship	Likely to be a causal relationship
Metabolic effects	No determination made	Likely to be a causal relationship[b]
Cardiovascular effects	Suggestive of a causal relationship	Suggestive of a causal relationship
Total mortality	Suggestive of a causal relationship	Suggestive of a causal relationship
Reproductive effects	Suggestive of a causal relationship	Effects on fertility and reproduction: suggestive of a causal relationship[b]
		Effects on pregnancy and birth outcomes: suggestive of a causal relationship[b]
Central nervous system effects	Suggestive of a causal relationship	Suggestive of a causal relationship
Cancer	Inadequate to infer a causal relationship	Inadequate to infer a causal relationship

[a]Health effects (e.g., respiratory effects, cardiovascular effects) include the spectrum of outcomes, from measurable subclinical effects (e.g., FEV_1, blood pressure), to observable effects (e.g., medication use, hospital admissions), and cause-specific mortality. Total mortality includes all-cause (nonaccidental) mortality, as well as cause specific mortality.

[b]Denotes new causality determination.

[c]Denotes change in causality determination from 2013 Ozone ISA.

The strongest evidence for health effects due to ozone exposure continues to come from studies of short- and long-term ozone exposure and respiratory health. Consistent with conclusions from the 2013 Ozone ISA, there is a "causal relationship" between short-term ozone exposure and respiratory effects, and there is a "likely to be causal relationship" between long-term ozone exposure and respiratory effects. For short-term ozone exposure, controlled human exposure studies provide experimental evidence for ozone-induced lung function decrements, respiratory symptoms, and respiratory tract inflammation.

Epidemiologic studies continue to provide evidence that increased ozone concentrations are associated with a range of respiratory effects, including asthma exacerbation, chronic obstructive pulmonary disease (COPD) exacerbation, respiratory infection, and hospital admissions and ED visits for combined respiratory diseases. A large body of animal toxicological studies demonstrates ozone-induced changes in measures of lung function, inflammation, increased airway responsiveness, and impaired lung host defense. These animal studies also inform the potential mechanisms underlying downstream respiratory effects (e.g., respiratory tract inflammation) and thereby provide strong support for the biological plausibility of epidemiologic associations between short-term ozone exposure and respiratory-related ED visits and hospital admissions. With respect to long-term ozone exposure, there is strong coherence between animal toxicological studies of changes in lung morphology and epidemiologic studies reporting positive associations between long-term ozone exposure and new-onset asthma, respiratory symptoms in children with asthma, and respiratory mortality. Furthermore, the experimental evidence provides biologically plausible pathways through which long-term ozone exposure could lead to the types of respiratory effects reported in epidemiologic studies.

Metabolic effects related to ozone exposure are evaluated as a separate health endpoint category for the first time in this ISA. Recent evidence from animal toxicological, controlled human exposure, and epidemiologic studies indicate that there is a "likely to be causal relationship" between short-term ozone exposure and metabolic effects. The strongest evidence is provided by animal toxicological studies that demonstrate impaired glucose tolerance, increased triglycerides, fasting hyperglycemia, and increased hepatic gluconeogenesis in various strains of animals across multiple laboratories. Biological plausibility is provided by controlled human exposure and animal studies that demonstrate activation of sensory pathways following ozone exposure, which triggers the central neuroendocrine stress response, and results in increased corticosterone, cortisol or epinephrine, as noted in the description of the controlled human exposure study. These findings are coherent with epidemiologic studies that report associations between ozone exposure and perturbations to glucose and insulin homeostasis. Animal toxicological studies also provide evidence for impaired insulin signaling, glucose intolerance, hyperglycemia, and insulin resistance after long-term exposure. In addition, these pathophysiological changes are often accompanied by increased inflammatory markers in peripheral tissues and activation of the neuroendocrine system. In prospective cohort studies in the U.S. and Europe, increased incidence of type 2 diabetes was observed with long-term ozone exposure. In China, the odds of metabolic syndrome increased as well. These findings are consistent with two long-term ozone exposure studies in China, one in adults and one in children, that presented increased odds of obesity (a risk factor for type 2 diabetes) in both adults and children. In epidemiologic studies, positive associations between long-term exposure to ozone and diabetes-related mortality were observed in well-established cohorts in the U.S. and Canada. The results of mortality studies are supported by epidemiologic and experimental studies reporting effects on glucose homeostasis and serum lipids, as well as other indicators of metabolic function (e.g., peripheral inflammation and neuroendocrine activation). There is a "likely to be causal relationship" between long-term ozone exposure and metabolic effects.

Notably, there are changes in the causality determinations for short-term ozone exposure and cardiovascular effects and total mortality. In both instances, the 2013 Ozone ISA concluded that the evidence was sufficient to conclude a "likely to be causal relationship", but after integrating the previous evidence with recent evidence, the collective evidence is "suggestive of, but not sufficient to infer, a causal relationship" in this draft ISA. The evidence that supports this change in the causality determination includes (1) a growing body of controlled human exposure studies providing less consistent evidence for an effect of short-term ozone exposure on cardiovascular health endpoints; (2) a paucity of positive evidence from epidemiologic studies for more severe cardiovascular morbidity endpoints (i.e., heart failure [HF], ischemic heart disease [IHD] and myocardial infarction [MI], arrhythmia and cardiac arrest, and stroke); and (3) uncertainties from a lack of control for potential confounding by copollutants in epidemiologic studies. Although there is consistent or generally consistent evidence for several ozone-induced cardiovascular endpoints in animal toxicological studies and for cardiovascular mortality in epidemiologic studies, these results are not coherent with those in controlled human exposure and epidemiologic studies examining cardiovascular morbidity endpoints.

IS.1.3.2 Welfare: Ecological Effects

The 2013 Ozone ISA (U.S. EPA, 2013b) concluded that the responses to ozone exposure occur across multiple biological scales and a broad array of ecological endpoints, with the strongest evidence for effects on vegetation. The focus of the current ISA and literature evaluated herein are those effects observed at the individual-organism level of biological organization and higher (e.g., population, community, ecosystem). New research largely strengthens the previous conclusions on the ecological effects of ozone. The types of ecological effects studies conducted since the 2013 Ozone ISA mostly fall into three categories: (1) empirical research that has refined/reinforced earlier studies, in some cases using new approaches, new species, or larger-scale systems; (2) meta-analyses that have provided a more statistically based understanding of patterns compiled from existing literature; and (3) modeling approaches that have increased in complexity and enabled examination of ozone effects at greater spatial scales (e.g., regional, national). There are 12 causality determinations for ecological effects of ozone (Table IS-2), generally organized from the individual-organism scale to the ecosystem scale. Similar to the findings of the 2013 Ozone ISA, five are causal relationships (i.e., visible foliar injury, reduced vegetation growth, reduced crop yield, reduced productivity, and altered belowground biogeochemical cycles) and two are likely to be causal relationships (i.e., reduced carbon sequestration, altered ecosystem water cycling). One endpoint, alteration of terrestrial community composition, is now concluded to be a causal relationship, whereas this endpoint was classified as likely to be causal in the 2013 Ozone ISA. Three new endpoint categories (i.e., increased tree mortality, alteration of herbivore growth and reproduction, and alteration of plant-insect signaling) not evaluated for causality in the 2013 Ozone ISA all have a likely to be causal relationship. Plant reproduction, previously considered as part of the evidence for growth effects, is now a stand-alone causal relationship.

Table IS-2 Summary of causality determinations for ecological effects.

Endpoint	Conclusions from 2013 Ozone ISA	Conclusions in the Current ISA
Visible foliar injury	Causal relationship	Causal relationship
Reduced vegetation growth	Causal relationship	Causal relationship
Reduced plant reproduction	No separate causality determination; included with plant growth	Causal relationship[a]
Increased tree mortality	Causality not assessed	Likely to be a causal relationship[a]
Reduced yield and quality of agricultural crops	Causal relationship	Causal relationship
Alteration of herbivore growth and reproduction	Causality not assessed	Likely to be a causal relationship[a]
Alteration of plant-insect signaling	Causality not assessed	Likely to be a causal relationship[a]
Reduced productivity in terrestrial ecosystems	Causal relationship	Causal relationship
Reduced carbon sequestration in terrestrial ecosystems	Likely to be a causal relationship	Likely to be a causal relationship
Alteration of belowground biogeochemical cycles	Causal relationship	Causal relationship
Alteration of terrestrial community composition	Likely to be a causal relationship	Causal relationship[b]
Alteration of ecosystem water cycling	Likely to be a causal relationship	Likely to be a causal relationship

[a]Denotes new causality determination.

[b]Denotes change in causality determination from 2013 Ozone ISA.

IS.1.3.3 Welfare: Effects on Climate

Recent evidence continues to support a causal relationship between tropospheric ozone and radiative forcing and a likely to be causal relationship, via radiative forcing, between tropospheric ozone and temperature, precipitation, and related climate variables (referred to as "climate change" in the 2013 Ozone ISA; the revised title for this causality determination provides a more accurate reflection of the available evidence [Table IS-3]). The new evidence comes from the Intergovernmental Panel on Climate

Change (IPCC) Fifth Assessment Report (AR5) (Myhre et al., 2013) and its supporting references—in addition to a few more recent studies—and builds on evidence presented in the 2013 Ozone ISA. The new studies further support the causality determinations included in the 2013 Ozone ISA.

Table IS-3 Summary of causality determinations for tropospheric ozone effects on climate.

	Conclusions in 2013 Ozone ISA	Conclusions in the Current ISA
Radiative forcing	Causal relationship	Causal relationship
Temperature, precipitation, and related climate variables	Likely to be a causal relationship	Likely to be a causal relationship

IS.2 Atmospheric Chemistry, Ambient Air Ozone Concentrations, and Background Ozone

Scientific advances in atmospheric ozone research relevant to the Ozone NAAQS are reviewed in this section, with a primary focus on understanding the relative contribution of precursor emissions due to natural processes and anthropogenic activities to ambient ozone concentrations. The section summarizes recent developments in measurement and modeling methods, atmospheric chemistry, and ambient air concentration trends (Section IS.2.1.). The U.S. background (USB) ozone concentration is defined as the ozone concentration that would occur if all U.S. anthropogenic ozone precursor emissions were removed, as described in Section IS.2.2. This definition facilitates separate consideration of ozone that results from anthropogenic precursor emissions within the U.S. and ozone originating from natural and foreign precursor sources. This discussion is followed by a summary of recent observations and research related to USB ozone, with an emphasis on major sources (Section IS.2.2.1), estimation methods (Section IS.2.2.2), and geographic, seasonal, and long-term ozone concentration trends (Section IS.2.2.3).

IS.2.1 Ambient Air Ozone Anthropogenic Sources, Measurement, and Concentrations

The general photochemistry of tropospheric ozone is described in detail in previous assessments (U.S. EPA, 2013b, 2006a). Anthropogenic ozone in urban settings is primarily produced by the reaction of volatile organic compounds (VOCs) with oxides of nitrogen (NO_X) in the presence of sunlight. Carbon monoxide (CO) and methane (CH_4) also react with NO_X to form ozone in the absence of more reactive

organic compounds, which may be found in the upper troposphere (Section 1.4). The most abundant national and global sources of VOCs are biogenic (U.S. EPA, 2013b), and oxides of nitrogen are predominately emitted from a range of anthropogenic sources, including automobile exhaust, off-road vehicles and engines, electric power generation, industrial activities, and stationary fuel combustion (U.S. EPA, 2016). Recent developments in understanding ozone chemistry include observations of high ozone concentrations during the winter in western U.S. mountain basins (Section 1.4.1), and new research on the role of marine halogen chemistry in suppressing coastal ozone concentrations (Section 1.4.2). For example, wintertime ozone concentrations in the Uintah Basin of Utah and Upper Green River Basin of Wyoming have been measured as high as 150 ppb (1-hour avg), with episodes driven by local concentrations of ozone precursor emissions from oil and gas extraction coinciding with strong mountain valley temperature inversions on cold winter days with snow cover. In addition, incorporating marine halogen chemistry into atmospheric modeling methods for predicting ozone concentrations has improved agreement between model results and observed ozone near marine environments.

Extensive air monitoring data are obtained from the state and local air monitoring site (SLAMS) network for ozone, consisting of more than 1,300 monitors throughout the U.S. (Section 1.7). In the SLAMS network, ozone is measured by ultraviolet spectroscopy using a Federal Equivalency Method (FEM) at most sites (Section 1.6.1.1). A new Federal Reference Method (FRM) for ozone measurement was adopted in 2015 (Section 1.6.1.1) based on chemiluminescence resulting from the reaction of ozone with nitric oxide. In addition to network monitoring, satellite-based remote sensing methods are increasingly used to measure the total ozone column in the atmosphere, and satellite data are used to constrain model estimates of ground-level tropospheric ozone concentrations (Section 1.6.1.2). Because tropospheric concentration estimates based on satellite measurements can have much greater uncertainty than total column ozone measurements, these technologies are most suitable for investigating trends in total column ozone or in the upper troposphere. The 2013 Ozone ISA provided an overview of chemical transport models (CTMs), including the relevant processes, numerical approaches, relevant spatial scales, and methods for evaluation (U.S. EPA, 2013b). Since the 2013 Ozone ISA, numerous improvements to these models have been made. These include the addition of a halogen chemistry mechanism; improvements in the representation of land cover and near surface meteorology; the inclusion of dry deposition and stomatal uptake, stratosphere-troposphere exchange, and biogenic emissions; and the integration of meteorological models and CTMs (Section 1.6.2).

SLAMS network data for the period 2015−2017 show higher nationwide median "maximum daily 8-hour average" (MDA8) ozone concentrations across all monitoring sites in spring (median = 46 ppb) and summer (median = 46 ppb) than in autumn (median = 38 ppb) and winter (median = 34 ppb). The highest values of annual 4th-highest MDA8 ozone concentration (>75 ppb) occur in central and southern California, Arizona, Colorado, Utah, Texas, along the shore of Lake Michigan, and in the Northeast Corridor, typically during the warm season between May and September (Section 1.2.1.1). These results are similar to those reported in the 2013 Ozone ISA (U.S. EPA, 2013b).

The highest values of W126, an example of a cumulative index of plant exposure (Section IS.3.2 and Section 1.2.1.2), occurred in California and the southwestern U.S. Several recent studies have documented a long-term decreasing trend in nationwide average ambient air MDA8 ozone concentration over several decades and a faster decline in the magnitude and frequency of high MDA8 ozone episodes (Section 1.7). Comparison of the difference between 5th and 95th percentile concentrations indicates a compression of the MDA8 ozone concentration distribution occurring widely across the U.S. This compression results from a decrease in 95th percentile concentrations together with a general increase in 5th percentile concentrations. This is consistent with observed reductions in NO_X emissions (Section 1.3.1), because there is less NO available to react with ozone at low ozone concentrations, as well as less NO_2 available to form ozone at high ozone concentrations.

IS.2.2 Background Ozone

Use of the term "background ozone" varies within the air pollution research community. It has generally been used to describe ozone levels that would exist in the absence of anthropogenic emissions within a particular area and has been broadly applied to every geospatial scale: local, regional, national, continental, or global. For instance, on a local scale, ozone that originates from precursor emissions outside of a locality's municipal boundaries could be considered background ozone to that locality. Similarly, on a national scale, background ozone could be defined as ozone that is not formed from anthropogenic emissions within national boundaries.

The USB concentration is defined as the ozone concentration that would occur if all U.S. anthropogenic ozone precursor emissions were removed. It is a hypothetical construct that cannot be measured. The 2006 Ozone AQCD (U.S. EPA, 2006a) and 2013 Ozone ISA (U.S. EPA, 2013b) concluded that background ozone concentrations could not be determined solely from ozone measurements, even at the most remote monitoring sites, because of long-range transport of ozone originating from U.S. anthropogenic precursors. Since then, chemical transport models have been used as the primary tool for estimating USB ozone concentrations.

IS.2.2.1 Sources of U.S. Background Ozone

Major contributors to ground-level USB ozone concentrations are stratospheric exchange, international transport, wildfires, lightning, global methane emissions, and natural biogenic and geogenic precursor emissions. As the USB literature has evolved, much of the discussion has focused on the relative importance of stratospheric ozone and intercontinental transport as major sources.

Tropospheric ozone derived from stratosphere-troposphere dynamics was described in detail in the 2013 Ozone ISA (U.S. EPA, 2013b). Stratospheric air naturally rich in ozone can be transported into the troposphere under certain meteorological circumstances, with maximum contributions observed at

midlatitudes during the late winter and early spring. This process, known as "tropopause folding," is characterized by episodic events typically lasting a few days from late winter through spring when deep stratospheric intrusions rich in ozone can quickly and directly well into the troposphere and, more rarely, reach ground level (U.S. EPA, 2013b). The 2013 Ozone ISA (U.S. EPA, 2013b) also discussed the potential importance of deep convection, another form of stratosphere-troposphere exchange that occurs mainly in summer, as a mechanism for transporting stratospheric ozone into the upper troposphere. Stratospheric ozone contributions from deep intrusion between 17 and 40 ppb have been estimated at ground level for springtime model simulations in the western U.S. (Section 1.3.2.1). Stratospheric intrusion events related to frontal passage and tropopause folding that reach the surface have less influence on surface ozone during the summer months when total ground-level ozone concentrations tend to be highest.

Intercontinental transport from Asia has also been identified as a major source of precursors that contribute about 5 to 7 ppb to USB ozone concentrations over the western U.S. (U.S. EPA, 2013b, 2006a, b). Ozone precursor emissions from China and other Asian countries have been estimated to have more than doubled in the period 1990−2010 (Section 1.3.1.2), and an estimated increase of 0.3 to 0.5 ppb/year of midtropospheric ozone USB in spring over the western U.S. in the two decades after 1990 was largely attributed to a tripling of Asian NO_X emissions (Section 1.3.1). However, after this period, trends in NO_X emissions from China, the largest ozone precursor source in Asia, have declined as confirmed by rapidly decreasing satellite-derived tropospheric NO_2 column measurements over China since 2012. Stringent air quality standards implemented in 2013 within China have markedly reduced national emissions (Section 1.3.1.2).

Other contributors to USB are either smaller or more uncertain than stratospheric and intercontinental contributions. Wildfires have been estimated to contribute a few ppb to seasonal mean ozone concentrations in the U.S., but episodic contributions may be as high as 30 ppb (Section 1.3.1.2). However, estimates of the magnitude of ozone formation form wildfires is highly uncertain with some work showing large overpredictions of modeled wildfire contributions (Section 1.3.1.3). Lightning was estimated to contribute 2 to 3 ppb to ground level ozone concentrations in the southeastern U.S. in the summer (U.S. EPA, 2013b). Eighty percent of the NO_X present in the upper troposphere is generated by lightning where it can have a longer atmospheric residence time than NO_X derived from ground sources (Section 1.3.1.3). There is an approximately linear relationship between anthropogenic methane emissions and tropospheric ozone, which is consistent with the contribution of anthropogenic methane emissions to global annual mean ozone concentration of ~4−5 ppb reported in the 2013 Ozone ISA (U.S. EPA, 2013b). Biogenic emissions of NO_X are estimated to contribute 0.3 Tg N/year, or about 7.5% of total NO_X emissions (Section 1.3.1.3).

IS.2.2.2 Methods for Estimating U.S. Background Ozone

Large uncertainties are associated with estimating USB ozone concentrations. Approaches for estimating USB ozone are described in Section 1.8.1. USB ozone is estimated using either zero-out simulations or source apportionment simulations. The most widely used approach to measuring USB or other measures of background ozone is the zero-out method, in which anthropogenic U.S. or other areas emissions are set to zero in a model simulation to estimate these ozone measures (Section 1.8.1.1). As an alternative to model sensitivity approaches, source apportionment techniques track source contributions to ozone formation without perturbing emissions (Section 1.8.1.2). Tracking techniques use reactive tracer species to tag specific emissions source categories or source regions and then track the ozone produced by emissions from those source groups. Both approaches are essential and complementary for understanding and estimating USB ozone. The zero out approach is suited for determining what ozone levels would have existed in recent modeled years in the absence of all U.S. emissions, while the source apportionment approach is suited for determining the fraction of current ozone originating from background sources in recent modeled years. The difference between estimates from these approaches is small in remote areas that are most strongly affected by USB sources. However, the differences in the estimates given by these methods can be substantial in urban areas strongly affected by anthropogenic sources that influence both production and destruction of ozone.

USB ozone concentrations vary daily and by location and are a function of season, meteorology, and elevation. Quantification of USB ozone on days when MDA8 ozone concentrations exceed 70 ppb is more relevant to understanding USB ozone contributions on those days than are seasonal mean USB ozone estimates, but also more uncertain (Jaffe et al., 2018). Jaffe et al. (2018) reviewed recent modeling results and reported that USB ozone estimates contain uncertainties of about 10 ppb for seasonal average concentrations and 15 ppb for MDA8 avg concentrations. Because of uncertainty in model predictions of USB, model results are often adjusted using simple bias correction approaches. Because such approaches might not be reliable if the model has large errors in USB ozone and locally produced ozone, however, days with poor model performance have sometimes been excluded when using model results to estimate USB or other measures of background ozone. There have been continued efforts to improve model performance and better understand biases and uncertainties involved in the application of CTMs to estimating USB or other measures of background ozone (Section 1.8.1.5).

IS.2.2.3 U.S. Background Concentrations and Trends

A greater variety of approaches for estimating USB concentrations and other measures of background ozone used in recent years have led to a wider range of USB estimates than reported in the 2013 Ozone ISA (U.S. EPA, 2013b), although some of the basic patterns remain consistent. For example, higher USB concentrations (and related measures of background ozone) were estimated in the western U.S. than in the eastern U.S. in the 2013 Ozone ISA (U.S. EPA, 2013b), especially in the intermountain

West and Southwest. Higher USB concentrations were also estimated at elevations greater than 1,500 m than at lower elevations (U.S. EPA, 2013b). New studies since the 2013 Ozone ISA confirm these findings (Section 1.8.2.1).

USB concentrations are relatively constant with increasing total ozone concentration, indicating that days with higher ozone concentrations generally occur because of higher U.S. anthropogenic contributions (Section 1.8.2.3). In the eastern U.S. and in urban and low-elevation areas of the western U.S., there is consistent evidence across several studies that daily USB ozone concentrations are similar to or smaller than seasonal mean USB ozone concentrations on most high ozone concentration days (i.e., days with MDA8 ozone greater than 60 ppb). In contrast, high elevation locations in the western U.S., USB concentration estimates have been consistently predicted to increase with total ozone concentration, consistent with a larger background contribution. Lower USB contributions on days of high ozone concentration can result from meteorological conditions that favor large ozone production from U.S. anthropogenic sources relative to USB sources (Section 1.5.2). The highest ozone concentrations observed in the U.S. have historically occurred during stagnant conditions when an air mass remains stationary over a region abundant in anthropogenic ozone precursor sources (U.S. EPA, 2013b, 2006a, 1996a), while the largest USB contributions often occur under the opposite conditions, when the atmosphere is well mixed and transport of USB ozone generated in the stratosphere or during long-range transport of Asian or natural precursors in the upper troposphere more readily occurs (Section 1.5.2).

Characterizing long-term trends in USB presents numerous challenges (Section 1.8.2.4). Research has mainly focused on high elevation sites in the western U.S. or measurements made aloft, where, until recently, increasing midtropospheric ozone was reported. The most recent analyses suggest that this trend has now slowed or reversed, and there is little evidence to suggest that USB is still increasing, even in the western U.S. (Section 1.8.2.4).

IS.3 Exposure to Ambient Ozone

IS.3.1 Human Exposure Assessment in Epidemiologic Studies

With regard to exposure assessment relevant to human health effects, the 2013 Ozone ISA (U.S. EPA, 2013b) primarily discussed personal exposure to ozone and its relationship to ambient air concentrations.

Its primary conclusions were that personal exposure to ozone is moderately correlated with ambient air concentration (Pearson R = 0.3−0.8), indoor ozone concentrations were roughly 10−30% of ambient air concentrations, and ozone exposure minimization efforts through public messaging (e.g., ozone action days) were effective in reducing exposures for people younger than 20 years old but

did not make an appreciable difference in exposure among those ages 20−64 years old. The 2013 Ozone ISA noted that urban scale ozone concentrations often have low spatial variability except in the vicinity of roadways, where nitrogen oxides emitted from motor vehicles tend to scavenge ozone.

The 2013 Ozone ISA found that exposure measurement error can bias epidemiologic associations between ambient ozone concentrations and health outcomes and widen confidence intervals around effect estimates. Recently published studies agree with these previous findings. Although ozone concentrations measured at fixed-site ambient air monitors are still widely used as surrogates for ozone exposure in epidemiologic studies (Section 2.3.1.1), the availability and sophistication of models to predict ambient ozone concentrations for this purpose have increased substantially in recent years (Section 2.3.2). The greatest expansion in modeling capability has occurred in chemical transport modeling (CTM; Section 2.3.2.3), especially when incorporated into a hybrid spatiotemporal framework that integrates modeling output with monitoring and satellite data over time and space (Section 2.3.2.4). Hybrid methods have produced lower error predictions of ozone concentration compared with spatiotemporal models using land use and other geospatial data alone (Section 2.3.2.2) but may be subject to overfitting given the many different sources of data incorporated into the hybrid framework.

Use of an exposure surrogate in epidemiologic studies generally leads to underestimation of any association between short-term exposure to ozone and a health effect, with reduced precision. Although the magnitude of an association between ambient ozone and a health effect is uncertain, the evidence indicates that the true effect is typically larger than the effect estimate in these cases. Epidemiologic studies evaluating short-term ozone exposure examine how short-term (e.g., hourly, daily, weekly) changes in health effects are associated with short-term changes in exposure (Section 2.6.1). Accurate characterization of temporal variability is more important than accurate characterization of spatial variability for these studies. Use of an exposure surrogate may produce bias when temporal variability in the concentration at the location of the measurement or model prediction differs from temporal variability of the true exposure concentration. As a result, the correlation between the exposure surrogate and the incidence of the effect would decrease due to the additional scatter in that relationship, and the reduced correlation would also likely flatten the slope of the relationship between the effect and exposure surrogate.

For effects elicited by ozone, the use of exposure estimates that do not account for population behavior and mobility (e.g., via use of time-activity data) may underestimate the true effect and have reduced precision. Although the magnitude of association between ozone and such health effects are uncertain, the evidence suggests that the true effect of ambient ozone exposure is larger than the effect estimate when time-activity data are not considered in the analysis. Uncharacterized exposure variability due to omission of time-activity data for short-term studies (Section 2.4.1) creates uncertainty in the exposure estimate that could reduce the correlation between the exposure estimate and the health effect.

Depending on the exposure model and scenario being modeled for application in epidemiology studies, the true effect of long-term exposure to ambient ozone may be underestimated or overestimated

when the exposure model respectively overestimates or underestimates ozone exposure. It is much more common for the effect to be underestimated, and the bias is typically small in magnitude. Long-term epidemiologic studies examine the association between the health effect endpoint and long-term average ambient ozone exposure (Section 2.6.2). For cohort studies of long-term ambient ozone exposure, ambient ozone concentration measured at monitors or estimated by a model is often used as a surrogate for ambient ozone exposure. These studies typically examine differences among cohorts in different locations, at the scale of neighborhoods, cities, or states. Uncharacterized spatial variability in ozone exposure across the study area could lead to bias in the effect estimate if modeled or measured ambient concentration is not representative of ambient exposure. Bias can occur in either direction but more often has been reported to be towards the null in exposure measurement error studies. Uncertainties in time activity and residential patterns of exposed individuals and surface losses of ozone can reduce precision in the effect estimates.

IS.3.2 Ecological Exposure

The key conclusions from the 1996 and 2006 Ozone AQCDs, and the 2013 Ozone ISA in regard to ozone exposure to vegetation, highlighted below, are still valid and most effects observed for nonvegetation biota are mediated through ozone effects on vegetation. Absorption of ozone from the atmosphere into leaves is controlled by the leaf boundary layer and stomatal conductance. Stomata provide the principal pathway for ozone to enter and affect plants, with subsequent oxidative injury to leaf tissue triggering a cascade of physical, biogeochemical, and physiological events that may scale up to responses at the whole-plant scale.

As described in previous ozone assessments, ozone-related injury is a function of flux (i.e., the amount of ozone taken up by the plant over time). Ozone flux is affected by modifying factors such as temperature, vapor pressure deficit, light, soil moisture, and plant growth stage (U.S. EPA, 2013b). Flux is very difficult to measure directly, requiring quantification of stomatal or canopy conductance. While some efforts have been made in the U.S. to calculate ozone flux into leaves and canopies, little information has been published relating these fluxes to effects on vegetation. The scarcity of flux data in the U.S. and lack of understanding of plant detoxification processes have made this technique less viable for risk assessments in the U.S. (U.S. EPA, 2013b). An alternative to flux-based exposure estimates are exposure indices. Exposure indices quantify exposure as it relates to measured plant response (e.g., growth) and only require ambient air quality data rather than more complex indirect calculations of dose to the plant. Cumulative indices summarize ozone concentrations over time to provide a consistent metric for reviewing and comparing exposure-response effects obtained from various studies. For ecological studies in this ISA, emphasis is placed on studies that characterize exposures at concentrations occurring in the environment or experimental ozone concentrations within an order of magnitude of recent concentrations observed in the U.S. (Appendix 1).

It is well established that exposure duration influences the degree of plant response and that ozone effects on plants are cumulative. In previous ozone assessments, effects are clearly demonstrated to be related to the cumulative exposure over the growing season for crops and herbaceous plant species. For long-lived plants, such as trees, exposures occur over multiple seasons and years. Cumulative indices of exposure are, therefore, best suited to assess exposure. Since the 1980s, cumulative-type indices such as threshold weighted (e.g., SUM06, AOTx) and continuous weighted (e.g., W126) functions have been applied to evaluate ozone exposure in plants (U.S. EPA, 2013b). The 2013 Ozone ISA primarily discussed SUM06, AOTx, and W126 exposure metrics. Below are the definitions of the three cumulative index forms:

- SUM06: Sum of all hourly ozone concentrations greater than or equal to 60 ppb observed during a specified daily and seasonal time window.
- AOTx: Sum of the differences between hourly ozone concentrations greater than a specified threshold during a specified daily and seasonal time window. For example, AOT40 is the sum of the differences between hourly concentrations above 40 ppb during a specified period.
- W126: Sigmoidally weighted sum of all hourly ozone concentrations observed during a specified daily and seasonal time window (Lefohn et al., 1988; Lefohn and Runeckles, 1987).

IS.4 Evaluation of the Health Effects of Ozone

IS.4.1 Connections among Health Effects

Broad health effect categories are evaluated independently in the appendices of this ISA, though the mechanisms and disease progression leading to these health effects are not restricted to a single organ system. Here, a high-level overview of how different health effects may be connected, and how insults to one organ system are likely to affect others, is provided. This section provides a more holistic perspective of the relationship between ozone and health than what is found in the individual health appendices.

Ozone-induced injuries can take place via complex pathways within the body. After inhalation, ozone reacts with lipids, proteins, and antioxidants in the respiratory tract epithelial lining fluid to create secondary oxidation products [U.S. EPA (2013b); Section 5.2.3]. The first steps (i.e., initial events) in the cascade of physiological events includes activation of sensory nerves in the respiratory tract and respiratory tract inflammation. These early physiological reactions to ozone may create a host of autonomic, endocrine, immune, and inflammatory responses throughout the body at the cellular, tissue, and organ level. Because the circulatory system is connected to all other body systems, there is the opportunity for insults to multiple organ systems to contribute to a single health effect. The 2006 Ozone AQCD [U.S. EPA (2006a); Chapter 4] and the 2013 Ozone ISA [U.S. EPA (2013b); Section 5.3] provide extensive background on dosimetry and potential pathways and potential pathways underlying health effects for these responses.

Modulations of the autonomic nervous system, which consists of the sympathetic and parasympathetic systems, provide inhibitory or excitatory inputs to tissues to generate organ responses. Some examples of responses from alterations of the autonomic nervous system include changes to heart rate, bronchodilation/bronchoconstriction, blood glucose, glycogenolysis/gluconeogenesis, hormone release, and other organ functions (McCorry, 2007). Endocrine, immune, and inflammatory responses can also send signals capable of altering multiple pathways and eliciting cardiovascular, respiratory, and metabolic health effects.

One example of a multisystem disruption resulting from ozone exposure is the decrease in core body temperature observed in rats. This decrease affects metabolic rate, leading to decreased oxygen consumption, reduced minute ventilation, decreased HR, decreased thyroid hormone concentrations, and lowered blood pressure, among other physiological changes (Watkinson et al., 2003; Mautz and Bufalino, 1989). As discussed in Appendix 5 (Section 5.1), high blood pressure is a component of metabolic syndrome, while obesity, metabolic syndrome, and type 2 diabetes are risk factors for cardiovascular disease, creating a two-way relationship for disease progression between the systems.

While all systems of the body are connected intrinsically, most research presented in the field of air quality examines specific health endpoints resulting from exposure to a pollutant. In an effort to bring together the scientific body of evidence in an easily understandable and relatable way, this document has separated the supporting appendices into Respiratory (Appendix 3), Cardiovascular (Appendix 4), Metabolic (Appendix 5), Mortality (Appendix 6), and Other Health Effects (Appendix 7).

IS.4.2 Biological Plausibility

New to this Ozone ISA are biological plausibility sections for the broad health outcome categories that are included in the human health appendices (Appendix 3-Appendix 7). These sections outline potential pathways along the exposure to outcome continuum and provide plausible links between inhalation of ozone and health outcomes at the population level. Biological plausibility can strengthen the basis for causal inference (U.S. EPA, 2015). In this ISA, biological plausibility is part of the weight-of-evidence analysis that considers the totality of the health effects evidence, including consistency and coherence of effects described in experimental and observational studies. Although there is some overlap in the potential pathways between the appendices, each biological plausibility section is tailored to the specific broad health outcome category and exposure duration for which causality determinations are made.

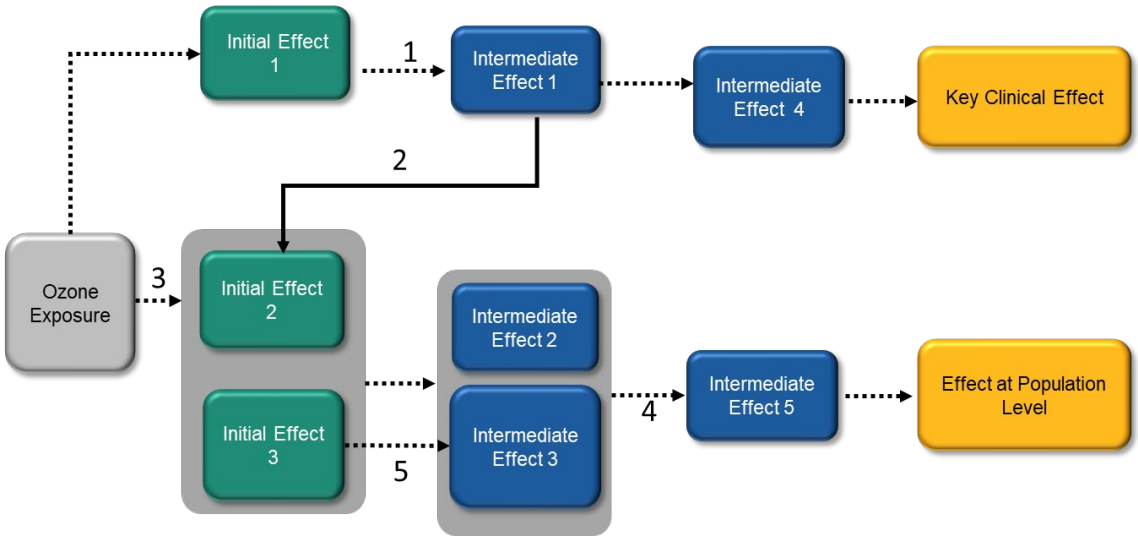
Each of the biological plausibility sections includes a figure depicting potential biological pathways that is accompanied by text. The figures illustrate possible pathways related to ozone exposure that are based on evidence evaluated in previous assessments, both AQCDs and ISAs, as well as evidence from more recent studies. The text characterizes the evidence upon which the figures are based, including results of studies demonstrating specific effects related to ozone exposure and considerations of

physiology and pathophysiology. Together, the figure and text portray the available evidence that supports the biological plausibility of ozone exposure leading to specific health outcomes. Gaps in the evidence base (e.g., health endpoints for which studies have not been conducted) are represented by corresponding gaps in the figures and are identified in the accompanying text.

In the model figure below (Figure IS-1), each box represents evidence that has been demonstrated in a study or group of studies for a particular effect related to ozone exposure. While most of the studies used to develop the figures are experimental studies (i.e., animal toxicological and controlled human exposure studies), some observational epidemiologic studies also contribute to the pathways. These epidemiologic studies are generally (1) panel studies that measure the same or similar effects as the experimental studies (and thus provide supportive evidence) or (2) emergency department and hospital admission studies or studies of mortality, which are effects observed at the population level. The boxes are arranged horizontally, with boxes on the left side representing initial effects that reflect early biological responses and boxes to the right representing intermediate (i.e., subclinical or clinical) effects and effects at the population level. The boxes are color-coded according to their position in the exposure to outcome continuum.

The arrows that connect the boxes indicate a progression of effects resulting from ozone exposure. In most cases, arrows are dotted (arrow 1), denoting a possible relationship between the effects. While most arrows point from left to right, some arrows point from right to left, reflecting progression of effects in the opposite direction or a feedback loop (arrow 2). In a few cases, the arrows are solid (arrow 2), indicating that progression from the upstream to downstream effect occurs as a direct result of ozone exposure. This relationship between the boxes, where the upstream effect is necessary for progression to the downstream effect, is termed *essentiality* (OECD, 2016). Evidence supporting essentiality is generally provided by experimental studies using pharmacologic agents (i.e., inhibitors) or animal models that are genetic knockouts. The use of solid lines, as opposed to dotted lines, reflects the availability of specific experimental evidence that ozone exposure results in an upstream effect which is necessary for progression to a downstream effect.

In the figures, upstream effects are sometimes linked to multiple downstream effects. In order to illustrate this proposed relationship using a minimum number of arrows, downstream boxes are grouped together within a larger shaded box and a single arrow (arrow 3) connects the upstream single box to the outside of the downstream shaded box containing the multiple boxes. Multiple upstream effects may similarly be linked to a single downstream effect using an arrow (arrow 4) that connects the outside of a shaded box, which contains multiple boxes, to an individual box. In addition, arrows sometimes connect one individual box to another individual box that is contained within a larger shaded box (arrow 2) or two individual boxes both contained within larger shaded boxes (arrow 5). Thus, arrows may connect individual boxes, groupings of boxes, and individual boxes within groupings of boxes depending on the proposed relationships between effects represented by the boxes.



Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence related to ozone exposure, and the arrows indicate a proposed relationship between those effects. Solid arrows denote evidence of essentiality as provided, for example, by an inhibitor of the pathway or a genetic knockout model used in an experimental study involving ozone exposure. Shading around multiple boxes is used to denote a grouping of these effects. Arrows may connect individual boxes, groupings of boxes, and individual boxes within groupings of boxes. Progression of effects is generally depicted from left to right and color-coded (gray, exposure; green, initial effect; blue, intermediate effect; orange, effect at the population level or a key clinical effect). Here, population level effects generally reflect results of epidemiologic studies. When there are gaps in the evidence base, there are complementary gaps in the figure and the accompanying text below.

Figure IS-1 Illustrative figure for potential biological pathways for health effects following ozone exposure.

IS.4.3 Summary of Health Effects Evidence

This ISA evaluates the relationships between an array of health effects and short- and long-term exposure to ozone in epidemiologic, controlled human exposure, and animal toxicological studies. Short-term exposures are defined as those with durations of hours up to 1 month, with most studies examining effects related to exposures in the range of several hours to 1 week. Long-term exposures are defined as those with durations of more than 1 month, with many studies spanning a period of years. As detailed in the Preface, the evaluation of the health effects evidence from animal toxicological studies focuses on exposures conducted at concentrations of ozone that are relevant to the range of human exposures associated with ambient air (up to 2 ppm, which is one to two orders of magnitude above recent ambient air concentrations in the U.S.). Drawing from evidence related to the discussion of biological plausibility of ozone-related health effects and the broader health effects evidence spanning scientific disciplines described in detail in Appendix 3–Appendix 7, as well as issues regarding exposure assessment and potential confounding described in Appendix 2, the subsequent sections characterize the

evidence that forms the basis of the causality determinations for health effect categories of a "causal relationship" or a "likely to be causal relationship", or describe instances where a causality determination has been changed (i.e., "likely to be causal" changed to "suggestive of, but not sufficient to infer a causal relationship"). The evidence that supports these causality determinations builds upon the potential biological pathways, which provide evidence of biological plausibility, as well as the broader health effects evidence spanning scientific disciplines for each health effects category, as well as issues related to dosimetry, exposure assessment, and potential confounding. Other relationships between ozone and health effects where a *"suggestive of, but not sufficient to infer"* or "*inadequate*" causality determination has been concluded are noted in Table IS-1, and more fully discussed in the respective health effects appendices.

IS.4.3.1 Short-Term Exposure and Respiratory Health Effects

Section 2.8 of the 2013 Ozone ISA concluded that there is a "causal relationship" between short-term ozone exposure and respiratory health effects (U.S. EPA, 2013b). This conclusion was based largely on controlled human exposure studies demonstrating ozone-related respiratory effects in healthy individuals (Table IS-4). Specifically, statistically significant decreases in group mean pulmonary function in response to 6.6-hour ozone exposures (which included six 50-minutes periods of moderate exertion) to concentrations as low as 60 ppb[1] were observed in young, healthy adults (Figure IS-2). Additionally, controlled human exposure and experimental animal studies demonstrated ozone-induced increases in respiratory symptoms, lung inflammation, airway permeability, and airway responsiveness. The experimental evidence was supported by strong evidence from epidemiologic studies demonstrating associations between ambient ozone concentrations and respiratory hospital admissions and ED visits across the U.S., Europe, and Canada. This evidence was further supported by a large body of individual-level epidemiologic panel studies that demonstrated associations of short-term ozone concentrations with respiratory symptoms in children with asthma. Additional support for a causal relationship was provided by epidemiologic studies that observed ozone-associated increases in indicators of airway inflammation and oxidative stress in children with asthma. Additionally, several multicity studies and a multicontinent study reported associations between short-term increases in ozone concentrations and increases in respiratory mortality.

Evidence from recent controlled human exposure studies augment previously available studies. There are, however, no new 6.6-hour ozone exposure studies since the 2013 Ozone ISA. Evidence in the 2013 Ozone ISA demonstrated increases in FEV_1 decrements, respiratory symptoms, and inflammation following ozone exposures of 6.6 hours, with exercise, as low as 60 to 70 ppb (Section 3.1.4). Evidence from recent epidemiologic studies of short-term ozone exposure and hospital admission or emergency

[1] Concentrations from controlled human exposure studies are target concentrations, unadjusted for study-specific measurement information.

department visits observed associations at concentrations as low as 31 ppb. Controlled human exposure studies also provide consistent evidence of ozone-induced increases in airway responsiveness (Section 3.1.4.3 and Section 3.1.5.5) and inflammation in the respiratory tract (Section 3.1.4.4 and Section 3.1.5.6). Recent animal toxicological studies are consistent with evidence summarized in the 2013 Ozone ISA (U.S. EPA, 2013b); these studies support the evidence observed in healthy humans.

Table IS-4 Summary of evidence from epidemiologic, controlled human exposure, and animal toxicological studies on the respiratory effects of short-term exposure to ozone.

	Conclusions from 2013 Ozone ISA	Results and Conclusions from 2019 ISA[a]
Respiratory effects	Evidence integrated across controlled human exposure, epidemiologic, and animal toxicological studies and across the spectrum of respiratory health endpoints demonstrated that **there was a causal relationship between short-term ozone exposure and respiratory health effects.**	Recent evidence from controlled human exposure, epidemiologic, and animal toxicological studies support and extend the conclusions from the 2013 Ozone ISA that **there is a causal relationship between short-term ozone exposure and respiratory effects.**
Lung function	Controlled human exposure studies of young, healthy adults demonstrate group mean decreases in FEV_1 in the range of 2 to 3% with 6.6-h exposures, while exercising, from concentrations as low as **60 ppb** ozone. The collective body of epidemiologic evidence demonstrate associations between short-term ambient ozone concentrations and decrements in lung function, particularly in children with asthma, children, and adults who work or exercise outdoors.	Controlled human exposure studies of young, healthy adults demonstrate ozone-induced decreases in FEV_1 at concentrations as low as **60 ppb** and the combination of FEV_1 decrements and respiratory symptoms at ozone concentrations **70 ppb** or greater following 6.6-h exposures while exercising. Studies show interindividual variability with some individuals being intrinsically more responsive. Results from recent epidemiologic studies are consistent with evidence from the 2013 Ozone ISA of an association with lung function decrements as low as **33 ppb** (mean 8-h avg ozone concentrations (7:50 a.m.−5:50 p.m.).
Airway responsiveness	A limited number of studies observe increased airway responsiveness in rodents and guinea pigs after being exposed for 72 h to ozone concentrations ranging from less than **300 ppb** up to **1,000 ppb**. As previously reported in the 2006 O_3 AQCD, increased airway responsiveness demonstrated at **80 ppb** in young, healthy adults, and at **50 ppb** in certain strains of rats.	Controlled human exposure studies provide evidence of increased airway responsiveness with exposures as low as **80 ppb**. Baseline airway responsiveness does not appear predictive of changes in lung function following ozone exposure. Recent animal toxicological studies demonstrate increases in airway responsiveness following ozone exposures as low as **800 ppb**. A recent animal toxicological study showed increased airway responsiveness to a greater degree in allergic mice than in naïve mice at **1,000 ppb** for 8 h.

Table IS-4 (Continued): Summary of evidence from epidemiologic, controlled human exposure, and animal toxicological studies on the respiratory effects of short-term exposure to ozone.

	Conclusions from 2013 Ozone ISA	Results and Conclusions from 2019 ISA[a]
Pulmonary inflammation, injury and oxidative stress	Epidemiologic studies provide evidence for associations of ambient ozone with mediators of airway inflammation and oxidative stress and indicated that higher antioxidant levels may reduce pulmonary inflammation associated with ozone exposure. Generally, these studies had mean 8-h daily max ozone concentrations less than **66 ppb**. Controlled human exposure studies show ozone-induced inflammatory responses at **60 ppb**, the lowest concentration evaluated.	Controlled human exposure studies demonstrate ozone-induced decreases in pulmonary inflammation at concentrations as low as **60 ppb** after 6.6 h of exposure. Studies show interindividual variability in inflammatory responses with some individuals reproducibly experiencing intrinsically greater responses than average. Animal toxicological studies demonstrate inflammation, injury, and oxidative stress following ozone exposures as low as **300 ppb** for up to 72 h. Epidemiologic studies observe associations with pulmonary inflammation in studies of healthy children (mean 8-h max ozone concentrations as low as **53 ppb)**.
Respiratory symptoms and medication use	The collective body of epidemiologic evidence demonstrate positive associations between short-term exposure to ambient ozone and respiratory symptoms (e.g., cough, wheeze, and shortness of breath) in children with asthma. Generally, these studies had mean 8-h daily max ozone concentrations less than **69 ppb**.	Controlled human exposure studies provide evidence of increased respiratory symptoms following 6.6-h exposures to **70 ppb and greater**. Limited data suggests that lung function responses to ozone in individuals with asthma may depend on baseline lung function and medication use. The large body of epidemiologic evidence from the 2013 Ozone ISA continues to provide the strongest support for these outcomes.
Lung host defenses	Controlled human exposure studies demonstrate the increased expression of cell surface markers and alterations in sputum leukocyte markers related to innate adaptive immunity with short-term ozone exposures of **80−400 ppb**. Animal toxicological studies demonstrate increased susceptibility to infectious disease with short-term ozone exposures as low as **80 ppb.** Altered macrophage function was reported with exposures as low as **100 ppb**. Other effects on the immune system (i.e., adaptive immunity and natural killer cells) are seen with exposures as low as **500 ppb**.	A limited number of recent controlled human exposure studies report results that are consistent with studies evaluated in the 2013 Ozone ISA that demonstrated impaired lung host defense following acute ozone exposure. A limited number of recent animal toxicological studies demonstrate susceptibility to infectious disease at **2,000 ppb** ozone for 3 h. Recent epidemiologic studies of ED visits for respiratory infection provide the strong support for these outcomes.

Table IS-4 (Continued): Summary of evidence from epidemiologic, controlled human exposure, and animal toxicological studies on the respiratory effects of short-term exposure to ozone.

	Conclusions from 2013 Ozone ISA	Results and Conclusions from 2019 ISA[a]
Allergic and asthma related responses	Controlled human exposure studies in atopic individuals with asthma demonstrate increased airway eosinophils, enhanced allergic cytokine production, increased IgE receptors, and enhanced markers of innate immunity and antigen presentation with short-term exposure to **80−400 ppb** ozone, all of which may enhance allergy and/or asthma. Increased airway responsiveness is seen in atopic individuals with asthma at 120−250 ppb ozone. In allergic rodents, enhanced goblet cell metaplasia is seen using exposure concentrations as low as **100 ppb, and enhanced responses to allergen challenge is seen with short-term exposure to 1,000 ppm ozone**.	A limited number of recent controlled human exposure and animal toxicological studies demonstrate enhanced type 2 immune responses following acute ozone exposures as low as **200 ppb** in atopic adults with asthma and **800 ppb** (8 h a day for 3 days) in healthy rodents. Exacerbated bronchoconstriction (airway resistance) and lung injury is seen in allergic rodents at **1,000 ppb**. These results support and expand upon evidence from the 2013 Ozone ISA that ozone enhances allergic and asthma related responses.
Respiratory hospital admissions, ED visits, and physician visits	Consistent, positive associations of ambient ozone concentrations with respiratory hospital admissions and ED visits in the U.S., Europe, and Canada are observed with supporting evidence from single-city studies. Generally, these studies had mean 8-h max ozone concentrations less than **60 ppb**.	Evidence from many recent, large multicity epidemiologic studies provide further support for an association between ozone and ED visits and hospital admissions for asthma; associations are generally strongest in magnitude for children between the ages of 5 and 18 years in studies with mean 8-h max ozone concentrations between **31 and 54 ppb**. Additional epidemiologic evidence for associations between ozone and hospital admissions and ED visits for combinations of respiratory diseases (**31** to **50** ppb as the study mean daily 8-h max), ED visits for COPD (**33** to **55 ppb** as the study mean daily 1-h max), and ED visits for respiratory infection (**33** to **55 ppb** as the study mean daily 1-h max).
Respiratory mortality	Multicity time-series studies and a multicontinent study consistently demonstrated associations between ambient ozone concentrations and respiratory-related mortality across the U.S., Europe, and Canada with supporting evidence from single-city studies. Generally, these studies had mean 8-h max ozone concentrations less than **63 ppb**.	Recent epidemiologic evidence for respiratory mortality is limited, but there remains evidence of consistent, positive associations, specifically in the summer months, with mean daily 8-h max ozone concentrations between **8.7 and 63 ppb.** When recent evidence is considered in the context of the larger number of studies evaluated in the 2013 Ozone ISA, there remains consistent evidence of an association between short-term ozone exposure and respiratory mortality.

[a]Conclusions from the 2019 ISA include evidence from recent studies integrated with evidence included in previous Ozone ISAs and AQCDs.

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Evidence from epidemiologic studies of healthy populations is generally coherent with experimental evidence, with most of the evidence coming from panel studies that were previously evaluated in the 2013 Ozone ISA (U.S. EPA, 2013b). Several panel studies of healthy children reported decreases in FEV_1 and increases in markers of pulmonary inflammation associated with increases in short-term ozone exposure. While there is coherence between epidemiologic and experimental evidence of ozone-induced lung function decrements and pulmonary inflammation, respiratory symptoms were not associated with ozone exposure in a limited number of epidemiologic studies. However, these studies generally relied on parent-reported outcomes that may have resulted in under- or over-reporting of respiratory symptoms.

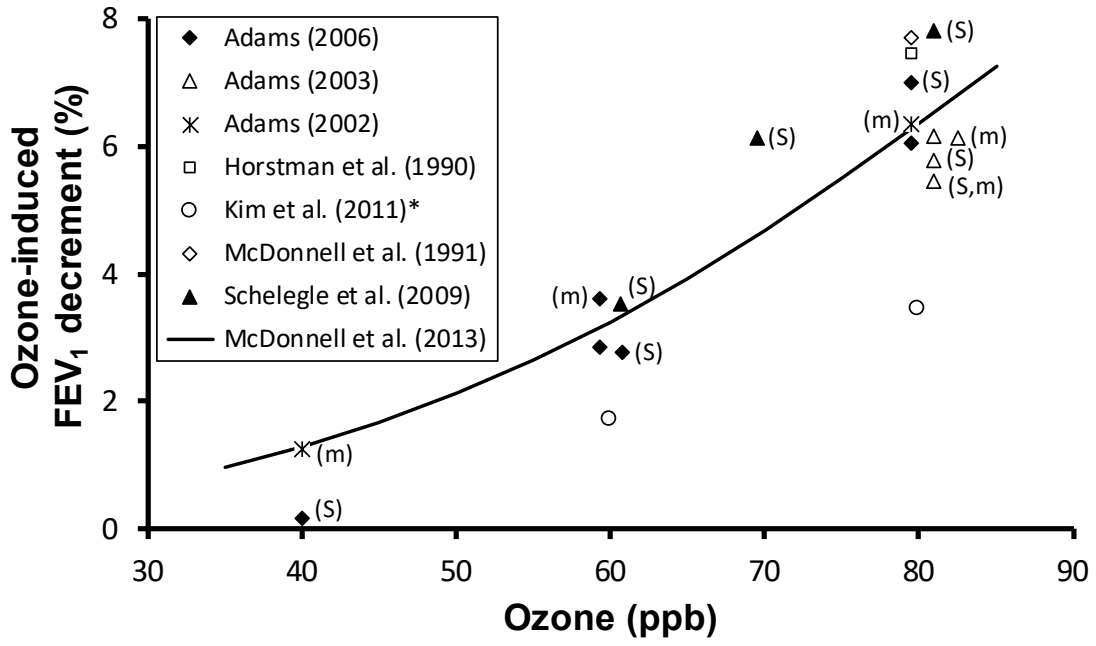
Evidence from numerous recent, large, multicity epidemiologic studies conducted in the U.S. among people of all ages also expands upon evidence from the 2013 Ozone ISA (U.S. EPA, 2013b) to further support an association between ozone exposure and ED visits and hospital admissions for asthma (Section 3.1.5.1 and Section 3.1.5.2). Reported associations were generally highest for children between the ages of 5 and 18 at mean daily 8-hour concentrations of 31−54 ppb. Additionally, consistent, positive associations were reported across models implementing measured and modeled ozone concentrations. A large body of evidence from the 2013 Ozone ISA (U.S. EPA, 2013b) reported ozone associations with markers of asthma exacerbation (e.g., respiratory symptoms, medication use, lung function) that support the ozone-related increases in asthma hospital admissions and ED visits observed in recent studies. Few recent epidemiologic studies in the U.S. or Canada have examined respiratory symptoms and medication use, lung function, and subclinical effects in people with asthma. Recent experimental studies in animals, along with similar studies summarized in the 2013 Ozone ISA (U.S. EPA, 2013b), provide coherence with and biological plausibility for the epidemiologic evidence of asthma exacerbation, indicating respiratory tract inflammation, oxidative stress, injury, allergic skewing, goblet cell metaplasia, and upregulation of mucus synthesis and storage in allergic mice exposed to ozone (Section 3.1.5.4, Section 3.1.5.5, and Section 3.1.5.6).

In addition to epidemiologic evidence of asthma exacerbation, a number of recent epidemiologic studies continue to provide evidence of an association of ozone concentrations with hospital admissions and ED visits for combined respiratory diseases (Section 3.1.8), ED visits for respiratory infection (Section 3.1.7.1), and ED visits for COPD (Section 3.1.6.1.1). Recent epidemiologic evidence for respiratory mortality is limited, but there remains evidence of consistent, positive associations, specifically in the summer months (Section 3.1.9). A limited number of recent controlled human exposure and animal toxicological studies are consistent with studies evaluated in the 2013 Ozone ISA (U.S. EPA, 2013b) that demonstrate altered immunity and impaired lung host defense following acute ozone exposure (Section 3.1.7.3). These findings support the epidemiologic evidence of an association between ozone concentrations and respiratory infection. Additionally, results from recent animal toxicological studies provide new evidence that chronic inflammation enhances sensitivity to ozone exposure, providing coherence for ozone-related increases in ED visits for COPD (Section 3.1.6.1.2).

Copollutant analyses were limited in epidemiologic studies evaluated in the 2013 Ozone ISA, but they did not indicate that associations between ozone concentrations and respiratory effects were confounded by copollutants or aeroallergens. Copollutant analyses have been more prevalent in recent studies and continue to suggest that observed associations are independent of coexposures to correlated pollutants or aeroallergens (Section 3.1.10.1 and Section 3.1.10.2). Despite expanded copollutant analyses in recent studies, determining the independent effects of ozone in epidemiologic studies is complicated by the high copollutant correlations observed in some studies and the possibility for effect estimates to be overestimated for the better measured pollutant in copollutant models (Section 2.5). Nonetheless, the consistency of associations observed across studies with different copollutant correlations, the generally robust associations observed in copollutant models, and evidence from controlled human exposure studies demonstrating respiratory effects in response to ozone exposure in the absence of other pollutants, provide compelling evidence for the independent effect of short-term ozone exposure on respiratory symptoms.

Epidemiologic studies have included analyses to inform our understanding of the lag structure (Section 3.1.10.3) for associations between short-term exposure to ozone and respiratory effects. The largest evidence base for evaluating the lag structure of associations comes from studies of ozone exposure and hospital admissions or ED visits for asthma. The strongest single-day associations were generally observed with ozone concentrations on the same day as the outcome, but positive associations were present across a range of lags, extending as far as 6 days prior to the health outcome of interest. This range indicates that ozone may elicit both immediate and prolonged respiratory effects.

Several controlled human exposure studies provided evidence on the C-R relationship for FEV_1 decrements in young healthy adults exposed during moderate exercise for 6.6. hours to ozone concentrations between 40 and 120 ppb. The lack of any studies at lower ozone concentrations and the small decrements observed at 40 ppb preclude characterization of the C-R relationship at lower concentrations. A model-predicted C-R function is described in a recent study presenting a mechanistic model based on these [and other controlled human exposure data; McDonnell et al. (2013); Figure IS-1; Section 3.1.4.1.1].



Note: All studies used constant exposure concentrations in a chamber unless designated as stepwise (S) and/or facemask (m) exposures. All responses at and above 70 ppb (targeted concentration) were statistically significant. Adams (2006) found statistically significant responses to square-wave chamber exposures at 60 ppb based on the analysis of Brown et al. (2008) and Kim et al. (2011). During each hour of the exposures, subjects were engaged in moderate quasi-continuous exercise (20 L/minute per m^2 BSA) for 50 minutes and rest for 10 minutes. Following the 3rd hour, subjects had an additional 35-minute rest period for lunch. The data at 60 and 80 ppb have been offset for illustrative purposes. McDonnell et al. (2013) illustrates the predicted FEV_1 decrements using Model 3 coefficients at 6.6 hours as a function of ozone concentration for a 23.8-year-old with a BMI of 23.1 kg/m^2.

*80 ppb data for 30 health subjects were collected as part of the Kim et al. (2011) study, but only published in Figure 5 of McDonnell et al. (2012).

Source: Adapted from Figure 6-1 of 2013 Ozone ISA (U.S. EPA, 2013b). Studies appearing in the figure legend are: Adams (2006), Adams (2003), Adams (2002), Folinsbee et al. (1988), Horstman et al. (1990), Kim et al. (2011), McDonnell et al. (2013), McDonnell et al. (1991), and Schelegle et al. (2009).

Figure IS-2 Cross-study comparisons of mean ozone-induced forced expiratory volume in 1 second (FEV_1) decrements in young healthy adults following 6.6 hours of exposure to ozone.

Epidemiologic studies examining the shape of the relationship between ambient air concentrations and the studied health outcome and/or the presence of a threshold in this relationship have been inconsistent (Section 3.1.10.4). While most studies assume a no-threshold, log-linear C-R shape, a limited number of studies have used more flexible models to test this assumption. Results from some of these studies indicate approximately linear associations between ozone concentrations and hospital admissions for asthma, while others indicate the presence of a threshold ranging from 20 to 40 ppb 8-hour max ozone concentrations.

Most epidemiologic studies that examine the relationship between short-term concentrations of ozone in ambient air and health effects rely primarily on a 1-hour max, 8-hour max, or 24-hour avg

averaging times. Epidemiologic time-series and panel studies evaluated in the 2013 Ozone ISA do not provide any evidence to indicate that any one averaging time is more consistently or strongly associated with respiratory-related health effects (U.S. EPA, 2013b). Recent epidemiologic studies examining respiratory effects continue to show evidence of positive associations for each of these averaging times (see Figure 3-4, Figure 3-5, Figure 3-6, and Figure 3-7). For example, Darrow et al. (2011), as detailed in the 2013 Ozone ISA, demonstrated a similar pattern of associations between short-term ozone exposure and respiratory-related ED visits for 1-hour max, 8-hour max, and 24-hour avg exposure metrics (Section 3.1.10.3.2). Similarly, a recent panel study focusing on respiratory symptoms in children reported positive associations when using both a 1-hour max and 8-hour max averaging time [Lewis et al. (2013); Section 3.1.5.3.2]. The combination of evidence from studies evaluated in the 2013 Ozone ISA, along with the results across recent studies that demonstrate positive associations using either a 1-hour max, 8-hour max, or 24-hour avg averaging time, further supports the conclusion that no one averaging time is more consistently or strongly associated with respiratory effects and that each of these averaging times could be surrogates for the exposure conditions that elicit respiratory health effects.

The evaluation of the lag structure of associations is an important consideration when examining the relationship between short-term ozone exposure and respiratory effects. With respect to ozone exposure, epidemiologic studies often examine associations between short-term exposure and health effects over a series of single-day lags, multiday lags, or by selecting lags a priori. For respiratory health effects, when examining more overt effects, such as respiratory-related hospital admissions and ED visits (i.e., asthma, COPD, and all respiratory outcomes), epidemiologic studies reported strongest associations occurring within the first few days of exposure (i.e., in the range of 0 to 3 days). The effects of ozone exposure on subclinical respiratory endpoints, including lung function, respiratory symptoms, and markers of airway inflammation, similarly occur at lags of 0 and 1 day. This finding is consistent with the evidence from controlled human exposure and experimental animal studies of respiratory effects occurring relatively soon after ozone exposures.

In summary, recent studies evaluated since the completion of the 2013 Ozone ISA (U.S. EPA, 2013b) support and expand upon the strong body of evidence indicating a "causal relationship" between short-term ozone exposure and respiratory effects. Controlled human exposure studies demonstrate ozone-induced FEV_1 decrements and respiratory tract inflammation at concentrations as low as 60 ppb after 6.6 hours of exposure with exercise among young, healthy adults. The combination of lung function decrements and respiratory symptoms has been observed following 70 ppb and greater ozone concentrations following 6.6-hour exposures combined with exercise. Epidemiologic studies continue to provide evidence that increased ozone concentrations are associated with a range of respiratory effects, including asthma exacerbation, COPD exacerbation, respiratory infection, and hospital admissions and ED visits for combined respiratory diseases. A large body of animal toxicological studies demonstrate ozone-induced changes in lung function measures, inflammation, increased airway responsiveness, and impaired lung host defense. Additionally, mouse models indicate enhanced ozone-induced inflammation, oxidative stress, injury, allergic skewing, goblet cell metaplasia, and upregulation of mucus synthesis and

storage in allergic mice compared with naïve mice. These toxicological results provide further information on the potential mechanistic pathways that underlie downstream respiratory effects. They also provide continued support for the biological plausibility of the observed epidemiologic results. Thus, the recent evidence integrated across disciplines, along with the total body of evidence evaluated in previous assessments, is sufficient to conclude that there **is a "causal relationship" between short-term ozone exposure and respiratory effects.**

IS.4.3.2 Long-Term Exposure and Respiratory Effects

The 2013 Ozone ISA concluded that there was "likely to be a causal relationship" between long-term exposure to ozone and respiratory health effects (U.S. EPA, 2013b). The epidemiologic evidence for a relationship between long-term ozone exposure and respiratory effects in the 2013 Ozone ISA was provided by epidemiologic studies that typically evaluated the association between the annual average of daily ozone concentrations and new-onset asthma, respiratory symptoms in children with asthma, and respiratory mortality. Notably, associations of long-term ozone concentrations with new-onset asthma in children and increased respiratory symptoms in individuals with asthma were primarily observed in studies that examined interactions between ozone and exercise or different genetic variants. The evidence relating new-onset asthma to long-term ozone exposure was supported by toxicological studies of allergic airways disease in infant monkeys exposed to biweekly cycles of alternating filtered air and ozone (i.e., 9 consecutive days of filtered air and 5 consecutive days of 0.5 ppm ozone, 8 hours/day). This evidence from a nonhuman primate study of ozone-induced changes in the airways provided biological plausibility for early-life exposure to ozone contributing to asthma development in children. Generally, the consistent evidence from epidemiologic and animal toxicological studies formed the basis of the conclusions that there is "likely to be a causal relationship" between long-term exposure to ambient ozone and respiratory effects. Results from a limited number of epidemiologic studies examining potential copollutant confounding suggested that the reported associations were robust to adjustment for other pollutants, including $PM_{2.5}$. Building upon the evidence from the 2013 Ozone ISA, more recent epidemiologic evidence, combined with toxicological studies in rodents and nonhuman primates, provides coherence and biological plausibility to support that there is a "likely to be causal relationship" between long-term exposure to ozone and respiratory effects.

Recent studies continue to examine the relationship between long-term exposure to ozone and respiratory effects. Key evidence supporting the causality determination is presented in Table IS-5. A limited number of recent epidemiologic studies provide generally consistent evidence that long-term ozone exposure is associated with the development of asthma in children (Section 3.2.4.1.1). In addition to investigating the development of asthma, epidemiologic studies have evaluated the relationship between ozone exposure and asthma severity (Section 3.2.4.5). Like the studies described in the 2013 Ozone ISA (U.S. EPA, 2013b), recent studies provide evidence of consistent positive associations between long-term exposure to ozone and hospital admissions and ED visits for asthma and prevalence of

bronchitic symptoms in children with asthma. Notably, some uncertainty remains about the validity of the results from studies examining long-term ozone exposure and hospital admissions and ED visits for asthma, because most of these studies do not adjust for short-term ozone concentrations, despite the causal relationship between short-term exposure and asthma exacerbation (Section 3.1.4.2).

Table IS-5 Summary of evidence from epidemiologic and animal toxicological studies on the respiratory effects associated with long-term ozone exposure.

	Conclusions from 2013 Ozone ISA	Conclusions from 2019 ISA[a]
Respiratory effects	Epidemiologic evidence, combined with toxicological studies in rodents and nonhuman primates, provided biologically plausible evidence that there **is likely to be a causal relationship between long-term exposure to ozone and respiratory effects.**	Epidemiologic evidence, combined with toxicological studies in rodents and nonhuman primates, continue to provide biologically plausible evidence for respiratory effects due to long-term ozone exposure. **Overall, the collective evidence is sufficient to conclude that there is a likely to be causal relationship between long-term ozone exposure and respiratory effects.**
New onset asthma	Animal toxicological studies provided evidence that perinatal exposure to ozone compromises airway growth and development in infant monkeys (**500 ppb;** 6 h a day, 5 days a week for 20 weeks). Animal toxicological studies also demonstrate increased airway responsiveness, allergic airways responses, and persistent effects on the immune system, which may lead to the development of asthma. There is evidence that different genetic variants (HMOX, GST, ARG), in combination with ozone exposure, are related to new-onset asthma. These associations were observed when subjects living in areas where the mean annual 8-h daily max ozone concentration was **55.2 ppb**, compared with those who lived in areas with a mean of **38.4 ppb**.	Recent epidemiologic studies provide generally consistent evidence for associations of long-term ozone exposure with the development of asthma in children. Associations observed in locations with mean annual concentrations of **32.1 ppb** in one study that reported study mean concentrations (community-specific annual average concentrations ranged from **26** to **76 ppb**). Recent animal toxicological studies demonstrate effects on airway development in rodents (**500 ppb**; 6 h a day for 3−22 weeks) and build on and expand the evidence for long-term ozone exposure-induced effects that may lead to asthma development.
Asthma hospital admissions	Epidemiologic studies provided evidence that long-term ozone exposure is related to increased hospital admissions in children and adults, and first childhood asthma hospital admissions in a linear concentration-response relationship. Generally, these studies had mean annual 8-h daily max ozone concentrations less than **41 ppb**	Long-term exposure is associated with hospital admissions and ED visits for asthma in study locations with mean annual ozone concentrations between **30.6** and **47.7 ppb**, although uncertainties remain because most studies do not adjust for short-term ozone concentrations.

Table IS-5 (Continued): Summary of evidence from epidemiologic and animal toxicological studies on the respiratory effects associated on long-term ozone exposure.

	Conclusions from 2013 Ozone ISA	Conclusions from 2019 ISA[a]
Pulmonary structure and function	Evidence for pulmonary function effects was inconsistent, with some epidemiologic studies observing positive associations (mean annual 8-h daily max ozone concentrations less than **65 ppb**). Results from toxicological studies indicated that long-term exposure of adult monkeys and rodents (**>120 ppb**; 6 h a day, 5 days a week for 20 weeks) can result in irreversible morphological changes in the lung, which in turn can influence pulmonary function.	Recent animal toxicological studies provide evidence that postnatal ozone exposure may affect processes in the developing lung, including impaired alveolar morphogenesis, a key step in lung development, in infant monkeys (**500 ppb**; 6 h a day for 3−22 weeks). Notably, the impairments in alveolar morphogenesis were reversible (reversibility of the other effects was not studied). A limited number of recent epidemiologic studies continue to provide inconsistent support for an association between long-term ozone exposure and lung function development in children.
Pulmonary inflammation, injury and oxidative stress	Several epidemiologic studies (mean 8-h max ozone concentrations less than **69 ppb**) and animal toxicological studies (as low as **500 ppb**) added to existing evidence of ozone-induced inflammation and injury.	Recent experimental studies in animals provide evidence that postnatal ozone exposure may affect the developing lung (**500 ppb**). Results from studies of neonatal rodents demonstrate ozone-induced changes in injury and inflammatory and oxidative stress responses during lung development (**1,000 ppb**).
Lung host defenses	Evidence demonstrated a decreased ability to respond to pathogenic signals in infant monkeys exposed to **500 ppb** ozone and an increase in severity of post-influenza alveolitis in rodents exposed to **500 ppb**.	A recent study demonstrates decreased ability to respond to pathogenic signals in infant monkeys exposed to **500 ppb**.
Allergic responses	Evidence demonstrated a positive association between allergic response and ozone exposure, but the magnitude of the association varied across studies; exposure to ozone may increase total IgE in adult asthmatics. Allergic indicators in infant monkeys and adult rodents were increased by exposure to ozone concentrations of **500 ppb**.	Cross-sectional epidemiologic studies provide generally consistent evidence that ozone concentrations (mean annual concentration less than **51.5 ppb**) are associated with hay fever/rhinitis and serum-markers of allergic response, although uncertainties related to study design and potential confounding by pollen remain. Recent animal toxicological studies continue to provide evidence for ozone-induced airway eosinophilia in infant monkeys (**100 ppb**; 0.33 h per day for 5 days per week for 2 weeks and once weekly for 12 weeks).
Development of COPD	Animal toxicological studies provided evidence that long-term ozone exposure could lead to persistent inflammation and interstitial remodeling in adult rodents and monkeys, potentially contributing to the development of chronic lung disease such as COPD. The 2013 Ozone ISA did not evaluate any epidemiologic studies that examined the relationship between long-term exposure to ozone and the development of COPD.	One recent epidemiologic study provides evidence of an association between long-term ozone concentrations and incident COPD hospitalizations (mean annual concentrations **39.3 ppb**). Recent animal toxicological studies provide consistent evidence that subchronic ozone exposure (**500−1,000 ppb**) can lead to airway injury and inflammation. In adult animals, these changes may underlie the progression and development of chronic lung disease and provide biological plausibility for ozone-induced development of COPD.

Table IS-5 (Continued): Summary of evidence from epidemiologic and animal toxicological studies on the respiratory effects associated on long-term ozone exposure.

	Conclusions from 2013 Ozone ISA	Conclusions from 2019 ISA[a]
Respiratory mortality	A single study demonstrated that exposure to ozone (long-term mean ozone less than **104 ppb**) elevated the risk of death from respiratory causes. This effect was robust to the inclusion of $PM_{2.5}$ in a copollutant model.	Recent epidemiologic studies provide some evidence of an association with respiratory mortality, but the evidence is not consistent (mean annual ozone concentrations **25.9−57.5 ppb**). New evidence from one study reports an association with COPD mortality.

[a]Conclusions from the 2019 ISA include evidence from recent studies integrated with evidence included in previous Ozone ISAs and AQCDs.

In support of evidence from recent epidemiologic studies, a number of recent animal toxicological studies expand the evidence base for long-term ozone exposure-induced effects leading to asthma development (Section 3.2.4.1.2). Specifically, both older and more recent long-term ozone exposure studies in nonhuman primates show that postnatal ozone exposure can compromise airway growth and development, promote the development of an allergic phenotype, and cause persistent alterations to the immune system (Section 3.2.4.6.2). In addition, findings that ozone exposure enhances injury, inflammation, and allergic responses in allergic rodents provide biological plausibility for the relationship between ozone exposure and the exacerbation of allergic asthma.

In addition to studies of asthma, several new or expanded lines of evidence from epidemiologic and animal toxicological studies published since the completion of the 2013 Ozone ISA provide evidence of associations between long-term ozone exposure and the development of COPD (Section 3.2.4.3) and allergic responses (Section 3.2.4.6). A recently available epidemiologic study provides limited evidence that long-term ozone exposure is associated with incident COPD hospitalizations in adults with asthma. This finding is supported by recent animal toxicological studies that provide consistent evidence of airway injury and inflammation resulting from subchronic ozone exposures. These results are coherent with animal toxicological studies reviewed in the 2013 Ozone ISA, which demonstrated that chronic ozone exposure damages distal airways and proximal alveoli, resulting in persistent inflammation and lung tissue remodeling that leads to irreversible changes including fibrotic- and emphysematous-like changes in the lung. Respiratory tract inflammation and morphologic and immune system-related changes may underlie the progression and development of chronic lung disease like COPD.

A larger body of epidemiologic studies also supports an association between long-term ozone exposure and allergic responses, including hay fever/rhinitis and serum allergen-specific IgE. While recent studies demonstrate generally consistent results, potential confounding by pollen exposure remains an uncertainty. However, there is supporting evidence from animal toxicological studies demonstrating enhanced allergic responses in allergic rodents (Section 3.2.4.6.2). In addition, animal toxicological studies reviewed in the short-term exposure section show type 2 immune responses in nasal airways of rodents exposed repeatedly to ozone, indicating that ozone exposure can trigger allergic responses

(Section 3.1.4.4.2). These findings are characteristic of induced nonatopic asthma and rhinitis and provide biological plausibility for the observed epidemiologic associations with hay fever/rhinitis.

Taken together, previous and more recent animal toxicological studies of long-term exposure to ozone provide biological plausibility for the associations reported in the recent epidemiologic studies. Specifically, there is strong evidence of ozone-induced inflammation, injury, and oxidative stress in adult animals. These effects represent initial events through which ozone may lead to a number of downstream respiratory effects, including altered morphology in the lower respiratory tract and the development of COPD. Furthermore, there is evidence of a range of ozone-induced effects on lung development in neonatal rodents and infant monkeys, including altered airway architecture, airway sensory nerve innervation, airway cell death pathways, increased serotonin-positive airway cells, and immunomodulation. An infant monkey model of allergic airway disease also demonstrated effects on lung development, including compromised airway growth, impaired alveolar morphogenesis, airway smooth muscle hyperreactivity, an enhanced allergic phenotype, priming of responses to oxidant stress, and persistent effects on the immune system. These various upstream effects provide a plausible pathway through which ozone may act on downstream events. These events include altered immune function leading to altered host defense and allergic responses, as well as morphologic changes leading to the development of asthma. A more thorough discussion of the biological pathways that potentially underlie respiratory health effects resulting from long-term exposure to ozone can be found in Section 3.2.3.

Recent epidemiologic studies provide some evidence that long-term ozone exposure is associated with respiratory mortality, but the evidence is not consistent across studies (Section 3.2.4.9). A recent nationwide study in the U.S. reported associations between ozone and the underlying causes of respiratory mortality, including COPD. This finding is supported by the new lines of evidence from animal toxicological and epidemiologic studies on the development of COPD, as discussed previously. Results from epidemiologic studies of ozone-related respiratory mortality in populations outside the U.S are inconsistent.

A notable source of uncertainty across the reviewed epidemiologic studies is the lack of examination of potential copollutant confounding. A limited number of studies that include results from copollutant models suggest that ozone associations may be attenuated but still positive after adjustment for NO_2 or $PM_{2.5}$. However, the few studies that include copollutant models examine different outcomes, making it difficult to draw strong conclusions about the nature of potential copollutant confounding for any given outcome. Importantly, in addition to studies that explicitly address potential copollutant confounding through modeling adjustments, many studies report modest copollutant correlations, suggesting that strong confounding due to copollutants is unlikely. Another source of uncertainty common to epidemiologic studies of air pollution is the potential for exposure measurement error. The majority of recent epidemiologic studies of long-term ozone exposure use concentrations from fixed-site monitors as exposure surrogates. Exposure measurement error relating to exposure assignment from fixed-site monitors has the potential to bias effect estimates in either direction, although it is more

common that effect estimates are underestimated, and the magnitude of the bias is likely small relative to the magnitude of the effect estimate, given that ozone concentrations do not vary over space as much as other criteria pollutants, such as NO_2 or SO_2 (Section 2.3.1.1).

Strong coherence from animal toxicological studies supports the observed epidemiologic associations related to respiratory morbidity. Experimental evidence also provides biologically plausible pathways through which long-term ozone exposure may lead to respiratory effects. **Overall, the collective evidence is sufficient to conclude that there is "likely to be a causal relationship" between long-term ozone exposure and respiratory effects.**

IS.4.3.3 Short-Term Exposure and Metabolic Effects

Metabolic syndrome is a term used to describe a collection of risk factors that include high blood pressure (elevated systolic and/or diastolic blood pressure), dyslipidemia (elevated triglycerides and low levels of high-density lipoprotein [HDL] cholesterol), obesity (central obesity), and increased fasting blood glucose (Alberti et al., 2009). The presence of these risk factors may predispose someone to an increased risk of type 2 diabetes and cardiovascular disease. Diagnosis of metabolic syndrome is based on the presence of three of these five risk factors (Alberti et al., 2009). The metabolic effects reviewed in this ISA include metabolic syndrome, diabetes, metabolic disease mortality, and indicators of metabolic syndrome. Indicators of metabolic syndrome include alterations in glucose and insulin homeostasis, peripheral inflammation, liver function, neuroendocrine signaling, and serum lipids, among other endpoints.

The evidence was not sufficient to evaluate metabolic effects as a separate health effect category in the 2013 Ozone ISA. As a result, there were no causality determinations for metabolic effects in the 2013 Ozone ISA (U.S. EPA, 2013b). Since the completion of the 2013 Ozone ISA, the number of studies examining short-term ozone exposure and metabolic effects has expanded substantially (Table IS-6). Results from animal toxicological studies of metabolic effects demonstrate that short-term ozone exposure impairs glucose and insulin homeostasis (e.g., glucose intolerance, hyperglycemia, dyslipidemia of triglycerides, glucagon concentration, altered blood pressure, impaired β-cell function, increased hepatic gluconeogenesis, and neuroendocrine activation contributing to altered metabolic function) after inhalation exposure to 0.25 to 1 ppm ozone. Controlled human exposure to ozone in intermittently exercising subjects, for 2 hours at an exposure of 0.3 ppm ozone or fresh air with 15 minute on/off exercise in a controlled chamber, confirms activation of the neuroendocrine stress response, and shows the formation of ketone bodies, a biomarker of diabetes (Section 5.1.5). Previous epidemiologic studies provide inconsistent evidence for elevated HbA1c (a biomarker of diabetes and an indicator of the degree of glycemic control in diabetics), increased triglycerides, altered serum cholesterol, increased HOMA-IR, and fasting glucose level instability associated with short-term ozone concentrations ranging from 19.4−64.4 ppb (mean 24-hour avg across study locations; Section 5.1.3).

Recent studies of short-term ozone exposure and metabolic effects compared associations between different age groups. One epidemiologic study observed increased risk among older adults (e.g., 75−84 years and 85+ years) compared with other age groups (<65 years) for hospital admissions for diabetic coma (Section 5.1.7.1) with an average ozone concentration of 64.4 ppb. In addition, an animal toxicological study demonstrated greater metabolic effects (i.e., increased triglycerides and serum insulin) in aged animals.

The strongest evidence for metabolic effects of short-term ozone exposure is provided by animal toxicological studies that show impaired glucose tolerance, increased triglycerides, fasting hyperglycemia, and increased hepatic gluconeogenesis in various strains of rodents in multiple laboratories (Section 5.1.3.3). Biological plausibility for an effect of short-term ozone exposure on metabolic effects is indicated by results from controlled human exposure studies and animal toxicological studies showing that ozone activates sensory nerves and triggers the central neuroendocrine stress response, which includes increased corticosterone, cortisol, or epinephrine production. Additionally, a recent controlled human exposure study reported that short-term ozone exposure increases ketone body formation, a biomarker of diabetes. Ketone body formation begins when ozone acts as a sensory and pulmonary irritant and activates sensory nerves in the respiratory tract that induce downstream effects on the autonomic nervous system. Evidence demonstrates that the hypothalamus, pituitary, and adrenals are activated by ozone exposure, and removal of the adrenal pathway (i.e., adrenalectomy or pharmacologically) can block the induction of metabolic syndrome in rodents exposed to ozone. In combination with limited epidemiologic and controlled human exposure evidence, the expanding animal toxicological studies show robust evidence of short-term ozone exposure contributing to activation of neuroendocrine pathways that lead to impairment of glucose and insulin homeostasis, decreased glucagon, impaired pancreatic β-cell function, and dyslipidemia. **Overall, the collective evidence is sufficient to conclude that there is "likely to be a causal relationship" between short-term ozone exposure and metabolic effects.**

Table IS-6 Summary of evidence from epidemiologic, controlled human exposure, and animal toxicological studies on the metabolic effects of short-term exposure to ozone.

	Results and Conclusions from 2019 ISA
Metabolic effects	Recent evidence from controlled human exposure, epidemiologic, and animal toxicological studies support a **likely to be causal relationship between short-term ozone exposure and metabolic effects.**
Altered metabolic function	Animal toxicological studies demonstrate impaired glucose intolerance, hyperglycemia, dyslipidemia of triglycerides, altered glucagon concentrations, altered blood pressure, impaired β-cell function, increased hepatic gluconeogenesis, and neuroendocrine activations, all of which contribute to altered metabolic function after inhalation exposure to ozone at concentrations from 0.25 to 1 ppm (6 h a day for 2 days). A controlled human exposure study also shows activation of the neuroendocrine system (2 h at 0.3 ppm ozone or fresh air exposure with 15 min on/off exercise in a controlled chamber).
Diabetes biomarkers and precursors	A controlled human exposure study shows increased ketone body formation (2 h at 0.3 ppm ozone or fresh air exposure with 15 min on/off exercise in a controlled chamber), a biomarker of diabetes. A limited number of epidemiologic studies provide some evidence for some biomarkers of diabetes and other precursors to diabetes (increased triglycerides, altered serum cholesterol, increased HOMA-IR, fasting glucose level instability) in an exposure range from 19.4−64.4 ppb mean 24-h avg across study locations although evidence is inconsistent across studies. An animal toxicological study shows increased HOMA-IR after ozone exposure (0.8 ppm ozone).

IS.4.3.4 Long-Term Exposure and Metabolic Effects

In the 2013 Ozone ISA, evidence was insufficient to evaluate metabolic effects as a separate health effect category. Therefore, no causality determinations for metabolic effects were made in that document (U.S. EPA, 2013b). Since then, the epidemiologic and experimental literature investigating long-term ozone exposure and outcomes related to metabolic syndrome has expanded substantially (Table IS-7). Positive associations between long-term exposure to ozone and diabetes-related mortality were observed in recent evaluations of well-established cohorts in the U.S. (mean daily ozone concentration across study locations 38.2 ppb) and Canada (mean annual average ozone concentration across study locations 39.6 ppb). The mortality results are supported by epidemiologic and experimental studies reporting effects on glucose homeostasis and serum lipids, as well as other indicators of metabolic function (e.g., peripheral inflammation and neuroendocrine stress response). Findings from an epidemiologic study of metabolic disease in 33 communities in China demonstrate increases in metabolic syndrome associated with a mean increased ozone concentration of 25.1 ppb. Additionally, in prospective cohort studies in the U.S. and Europe, increased incidence of type 2 diabetes is observed in association with long-term ozone exposure (mean annual average ozone concentration across study locations 37.5−49.4 ppb).

Recent studies examined the potential for copollutant confounding by evaluating copollutant models that included $PM_{2.5}$, PM_{10}, and NO_2 (Section 5.2.9). The limited number of recent studies provide some evidence that the metabolic effects associated with long-term ozone exposure are independent of coexposure to correlated copollutants.

Epidemiologic studies evaluating long-term ozone exposure and metabolic effects did not show stronger associations in older adults compared with other age groups. For example, a longitudinal cohort study of diabetes incidence reported increased risk estimates for those under 50 years old, but not for subjects aged 50 to 60 years, or those over 60 years (mean exposure 49.4 ppb). Similarly, a cohort study of black women reported increased hazard ratios for all age groups evaluated, with the greatest risk observed for women under 40 years in age, intermediate risk for women aged 40−54, and the lowest risk for women over 55 years old with a mean exposure of 37.5 ppb of ozone. Conversely, a recent study that examined the effect of age on health outcomes in rodents showed that senescent or aged animals were more sensitive to ozone-dependent serum insulin changes. In the same study, young adult rodents exposed to ozone did not have significant changes in serum insulin with ozone exposure.

Animal toxicological studies address some of the uncertainty in the epidemiologic evidence related to the independent effect of ozone exposure by providing evidence of direct effects on metabolic function. The animal toxicological studies showed evidence that long-term ozone exposure resulted in impaired insulin signaling, glucose intolerance, hyperglycemia, and insulin resistance (Section 5.2.3.1). In addition, these pathophysiological changes were often accompanied by increased inflammatory markers in peripheral tissues and the activation of the neuroendocrine stress response (Section 7.2.1.5). Importantly, short-term ozone exposure has been shown to contribute to the development of metabolic syndrome in animals, which is coherent with the evidence that long-term ozone exposure leads to development or worsening of metabolic syndrome or its risk factors. **Overall, the collective evidence is sufficient to conclude that there is "likely to be a causal relationship" between long-term ozone exposure and metabolic effects**.

Table IS-7 Summary of evidence from epidemiologic and animal toxicological studies on the metabolic effects associated on long-term ozone exposure.

	Results and Conclusions from 2019 ISA
Metabolic effects	Recent evidence from controlled human exposure, epidemiologic, and animal toxicological studies support that there is **likely to be a causal relationship between long-term ozone exposure and metabolic effects**.
Altered metabolic function	Epidemiologic and experimental animal studies report effects on glucose homeostasis and serum lipids, as well as other indicators of metabolic function (e.g., peripheral inflammation and neuroendocrine activation). Animal toxicological studies provide evidence that long-term ozone exposure results in impaired insulin signaling, and induced glucose intolerance, hyperglycemia, and insulin resistance (0.25 to 1 ppm ozone; 6 h a day, 2 days a week for 13 weeks).
Metabolic syndrome and diabetes	Epidemiologic evidence for increased incidence of type 2 diabetes is associated with long-term ozone concentrations of 37.5−49.4 ppb (mean annual average ozone concentration across study locations) in prospective cohort studies in the U.S. and Europe.
Diabetes mortality	Epidemiologic studies report positive associations between long-term exposure to ozone and diabetes-related mortality in well-established cohorts in the U.S.(38.2 ppb; mean annual average ozone concentration across study locations) and Canada (39.6 ppb, mean annual average ozone concentration across study locations).

IS.4.3.5 Short-Term Exposure and Cardiovascular Effects

The 2013 Ozone ISA concluded that there is a "likely to be causal" relationship between relevant short-term exposures and cardiovascular effects, but it also identified important uncertainties (U.S. EPA, 2013b). The available animal toxicological studies demonstrated ozone-induced impaired vascular and cardiac function, as well as changes in heart rate (HR) and heart rate variability (HRV), while the controlled human exposure studies provided some evidence, though limited coherence with the animal studies. The epidemiologic evidence, while reporting associations between short-term ozone exposure and cardiovascular mortality, did not observe associations between short-term ozone exposure and cardiovascular morbidity. This lack of coherence between the results investigating associations with cardiovascular morbidity and cardiovascular mortality was recognized as a complication in interpreting the overall evidence for ozone-induced cardiovascular effects.

More recent animal toxicological studies published since the 2013 Ozone ISA provide generally consistent evidence for impaired heart function and endothelial dysfunction, but limited evidence for indicators of arrhythmia, HRV, and markers of oxidative stress and inflammation in response to ozone exposure. Additional controlled human exposure studies have been published in recent years, although they show little evidence for ozone-induced effects on cardiovascular endpoints. Specifically, some recent studies do not indicate an effect of ozone on cardiac function, ST segment, endothelial dysfunction, or

HR, while other recent studies provide little evidence that ozone exposure can result in changes in blood pressure, indicators of arrhythmia, HRV, markers of coagulation, and inflammatory markers. The number of epidemiologic studies evaluating short-term ozone concentrations and cardiovascular effects has grown somewhat, but overall, remains limited and continues to provide little, if any, evidence for associations with heart failure, heart attack, arrhythmia and cardiac arrest, or stroke. Recent epidemiologic evidence for short-term ozone exposure and cardiovascular mortality is limited to one multicity study, but the collective body of evidence spanning multicity studies evaluated in the 2013 Ozone ISA provides evidence of consistent positive associations. Overall, many of the same limitations and uncertainties that existed in the body of evidence in the 2013 Ozone ISA continue to exist. However, the number of controlled human exposure studies evaluating short-term ozone exposure and cardiovascular endpoints has grown, and now includes studies at concentrations closer to those likely to be encountered in U.S. ambient air. When evaluated in the context of the studies available for the 2013 Ozone ISA, the controlled human exposure study evidence, overall, is less consistent and less indicative of a relationship (Table IS-8).

Table IS-8 Summary of evidence from epidemiologic, controlled human exposure, and animal toxicological studies on the cardiovascular effects of short-term ozone exposure.

	Conclusions from 2013 Ozone ISA	Conclusions from 2019 ISA
Cardiovascular effects	Evidence from animal toxicological studies demonstrated ozone-induced impaired vascular and cardiac function, as well as changes in HR and HRV. This evidence was supported from a limited number of controlled human exposure studies in healthy adults demonstrating changes in HRV, as well as in blood markers associated with an increase in coagulation. There was limited or no evidence from epidemiologic studies for short-term ozone exposure and cardiovascular morbidity, such as effects related to HF, IHD, and MI, arrhythmia and cardiac arrest, or thromboembolic disease. There was consistent evidence from epidemiologic studies reporting positive associations between short-term ozone exposure and cardiovascular-related mortality. Overall, there **is likely to be a causal relationship between long-term exposure to ozone and respiratory effects.**	Recent animal toxicological studies continue to provide evidence for impaired heart function and endothelial dysfunction, with limited evidence for indicators of arrhythmia, HRV, and markers of oxidative stress and inflammation in response to ozone exposure. Recent controlled human exposure studies provide little evidence for ozone-induced effects on a number of cardiovascular endpoints. No effect of ozone was reported for indicators of cardiac function, IHD, endothelial dysfunction, or changes in HR. There is limited and inconsistent evidence for changes in cardiac electrophysiology, HRV, blood pressure, markers of coagulation, and inflammatory markers. Epidemiologic studies remain few and continue to provide little, if any, evidence for associations with HF, IHD, and MI, arrhythmia and cardiac arrest, or stroke. Overall, the evidence is **suggestive of, but not sufficient to infer, a causal relationship.**

Table IS-8 (Continued): Summary of evidence from epidemiologic, controlled human exposure, and animal toxicological studies on the cardiovascular effects of short-term ozone exposure.

	Conclusions from 2013 Ozone ISA	Conclusions from 2019 ISA
Heart failure, impaired heart function	A limited number of animal toxicological studies demonstrated ozone-induced cardiovascular effects, including decreased cardiac function. Epidemiologic studies generally did not observe associations between short-term ozone exposure and cardiovascular morbidity; studies of cardiovascular-related hospital admissions and ED visits did not find consistent evidence of a relationship with ozone exposure.	Multiple animal toxicological studies report some indicators of impaired cardiac function following short-term ozone exposure (~200−300 ppb for 3−4 h). However, a recent controlled human exposure study (100 and 200 ppb for 3 h) reported no changes in measures of cardiac function. There is a limited number of recent studies of hospital admissions and ED visits that analyzed associations with heart failure, and they continue to report inconsistent associations with short-term exposure to ozone.
Ischemic heart disease	Animal toxicological studies, although few, demonstrated ozone-induced cardiovascular effects, including enhanced ischemia/reperfusion (I/R) injury. Epidemiologic studies generally did not observe associations between short-term ozone exposure and cardiovascular morbidity; studies of cardiovascular-related hospital admissions and ED visits did not find consistent evidence of a relationship with ozone exposure.	An animal toxicological study in SH rats demonstrates ST segment depression following an 800 but not 200 ppb exposure to ozone for 4 h. However, no such changes are observed in the single controlled human exposure study (70 and 120 ppb for 3 h). Recent epidemiologic studies consistently report null or weak positive effect estimates in analyses of MI, including for STEMI and NSTEMI.
Cardiac and endothelial dysfunction	Animal toxicological studies, although limited in number, demonstrated ozone-induced cardiovascular effects, including vascular disease and injury.	Recent animal toxicological studies demonstrate generally consistent evidence for impaired cardiac and endothelial function in rodents following short-term ozone exposure of 0.4−1.0 ppm for 4 h. However, coherence with controlled human exposure and epidemiologic studies is lacking.
Cardiac electrophysiology, arrhythmia, cardiac arrest	Animal toxicological studies, although few, demonstrated ozone-induced cardiovascular effects, including disrupted NO-induced vascular reactivity. Epidemiologic studies reported generally positive associations for hospital admissions or ED visits due to arrythmia or dysrhythmia.	Recent animal toxicological studies demonstrate limited evidence for changes in indicators of conduction abnormalities (800 but not 200 ppb for 3−4 h). Multiple controlled human exposure studies report little effect of short-term ozone exposure on conduction abnormalities (70 and 120 ppb for 2−3 h). Increases in out-of-hospital cardiac arrests associated with 8-h max or 24-h avg increases in ozone concentrations were reported by a few case-crossover studies; however, analyses of other endpoints (e.g., dysrhythmia, arrhythmia, or atrial fibrillation) generally report null results.

Table IS-8 (Continued): Summary of evidence from epidemiologic, controlled human exposure, and animal toxicological studies on the cardiovascular effects of short-term ozone exposure.

	Conclusions from 2013 Ozone ISA	Conclusions from 2019 ISA
Blood pressure changes and hypertension	A limited number of epidemiologic studies reported inconsistent associations with measures of blood pressure. Two studies observed increases in DBP associated with ozone concentration, but the association was attenuated to the null after adjusting for $PM_{2.5}$ concentrations.	Recent animal toxicological studies demonstrate inconsistent effects of ozone-induced effects on changes in blood pressure (300 and 500 ppb for 3−8 h). Multiple controlled human exposure studies report no evidence of an ozone-induced effect on blood pressure (120−700 ppb for 1−3 h), while a single controlled human exposure study reported a decrease in DBP. Few epidemiologic panel studies evaluated blood pressure, and the results were inconsistent.
Heart rate and heart rate variability	Animal toxicological studies, although few, demonstrated ozone-induced cardiovascular effects, including increased HRV. Controlled human exposure studies provided some coherence with the evidence from animal toxicological studies, by demonstrating increases and decreases in HRV following relatively low (**120 ppb** during rest) and high (**300 ppb** with exercise) ozone exposures, respectively.	Evidence is inconsistent for changes in HR in animals (~200−800 ppb for 3−8 h) and lacking for changes in HR in healthy adults from multiple controlled human exposure studies (70−300 ppb for 1−4 h). With respect to HRV, there is limited evidence for changes in animal toxicological (200−800 ppb for 3−4 h) and controlled human exposure (70−300 ppb for 1−4 h) studies. Similarly, recent epidemiologic panel studies have reported inconsistent associations between short-term exposure to ozone and both HR and HRV.
Coagulation and thrombosis	A controlled human exposure study demonstrated changes in markers of coagulation following short-term ozone exposure. Specifically, there were decreases in PAI-1 and plasminogen levels and a trend toward an increase in tPA. There was very limited animal toxicological evidence that short-term exposure to ozone could result in an increase in factors related to coagulation. Epidemiologic studies observed inconsistent results for coagulation biomarkers such as PAI-1, fibrinogen, and vWF.	Recent animal toxicological studies provide limited evidence for changes in factors that may promote coagulation (0.25−1.0 ppm for 4 h). Similarly, there is limited additional evidence from recent controlled human exposure studies that short-term ozone exposure can result in changes to markers of coagulation that may promote thrombosis (100−300 ppb for 1−2 h). Epidemiologic studies continue to observe inconsistent associations with changes in biomarkers of coagulation.
Systemic inflammation and oxidative stress	Controlled human exposure studies demonstrated ozone-induced effects on blood biomarkers of systemic inflammation and oxidative stress.	There is inconsistent evidence from recent animal toxicological studies for an increase in markers associated with systemic inflammation and oxidative stress (300−800 ppb for 2−24 h) and some evidence for increases in markers of systemic inflammation from CHE studies (100−300 ppb for 0.5−4 h). Additionally, the newly available epidemiologic panel study did not observe an association between short-term ozone concentrations and myeloperoxidase.
Stroke	A limited number of epidemiologic studies observed inconsistent associations with stroke.	Inconsistent results were observed in several recent epidemiologic studies that analyzed hospital admissions and ED visits for stroke and stroke subtypes.

Table IS-8 (Continued): Summary of evidence from epidemiologic, controlled human exposure, and animal toxicological studies on the cardiovascular effects of short-term ozone exposure.

	Conclusions from 2013 Ozone ISA	Conclusions from 2019 ISA
Cardiovascular hospital admissions and ED visits	With few exceptions, studies of ozone concentrations and cardiovascular hospital admissions and ED visits for all CVD diagnoses combined did not report positive associations.	Recent studies that reported a risk ratio for combined cardiovascular disease outcomes show a similar inconsistent pattern to those studies included in the 2013 Ozone ISA.
Cardiovascular mortality	Multicity epidemiologic studies observed positive associations for cardiovascular mortality in all-year and summer/warm season analyses. Lack of coherence with epidemiologic studies of cardiovascular morbidity remains an important uncertainty.	A recent multicity study is consistent with the evidence examining cardiovascular mortality evaluated in the 2013 Ozone ISA.

[a]Conclusions from the 2019 ISA include evidence from recent studies integrated with evidence included in previous Ozone ISAs and AQCDs.

When considered as a whole, the evidence is "suggestive of, but not sufficient to infer, a causal relationship" between short-term exposure to ozone and cardiovascular effects. This causality determination represents a change from the conclusion in the 2013 Ozone ISA. This change is largely because the number of controlled human exposure studies showing little evidence of ozone-induced cardiovascular effects has grown substantially, while the epidemiologic evidence for ozone effects on endpoints other than mortality continues to be limited. Consequently, the plausibility for a relationship between short-term ozone exposure to cardiovascular health effects is weaker than it was in the previous review, leading to the revised causality determination.

IS.4.3.6 Short-Term Exposure and Total Mortality

Recent multicity epidemiologic studies conducted in the U.S. and Canada continue to provide evidence of consistent, positive associations between short-term ozone exposure and total mortality in both all-year and summer/warm season analyses across different averaging times (i.e., maximum daily 1-hour max, max daily 8-hour avg, 8-hour avg, and 24-hour avg) (Table IS-9). The limited assessment of cause-specific mortality (e.g., respiratory mortality [Section 3.1.9], cardiovascular mortality [Section 4.1.14]) in recent studies is consistent with the pattern of positive associations reported for studies evaluated in the 2013 Ozone ISA. Lastly, most of the recent multicity studies examined associations between short-term ozone exposure and mortality using ozone data collected before the year 2000, with only Di et al. (2017) including more recent ozone concentration data.

Recent studies continue to assess the influence of important potential confounders on the ozone-mortality relationship, including copollutants, temporal/seasonal trends, and weather covariates. Overall, these studies report that associations remain relatively unchanged across the different approaches

used to control for each confounder. The assessment of potential copollutant confounding in recent studies demonstrates that associations between short-term ozone concentrations and mortality remain positive in copollutant models with PM_{10} or NO_2. Importantly, the issues surrounding the assessment of potential copollutant confounding that complicate interpretation (as detailed in the 2013 Ozone ISA) persist, specifically within studies that relied on different PM sampling schedules, such as every 3rd- and 6th-day PM sampling (U.S. EPA, 2013b).

Building upon the 2013 Ozone ISA, there remains strong evidence for respiratory effects due to short-term ozone exposure (Appendix 3) that is consistent within and across disciplines and which provides coherence and biological plausibility for the positive respiratory mortality associations reported across epidemiologic studies. Although there remains epidemiologic evidence for ozone-induced cardiovascular mortality and animal toxicological evidence of cardiovascular effects, a large number of recent controlled human exposure studies are not consistent with the evidence presented in the 2013 Ozone ISA from controlled human exposure studies showing cardiovascular effects. The limited experimental evidence, in combination with the lack of coherence between experimental and epidemiologic studies of cardiovascular morbidity, does not allow for an understanding of potential biological pathways leading to cardiovascular mortality (Appendix 4) or other causes of mortality.

Overall, the recent multicity studies conducted in the U.S. and Canada provide additional support for the consistent, positive associations with total mortality reported across multicity studies evaluated in the 2006 Ozone AQCD (U.S. EPA, 2006a) and 2013 Ozone ISA (U.S. EPA, 2013b). These results are supported by studies that further examine uncertainties in the ozone-mortality relationship, such as potential confounding by copollutants and other variables, modification by temperature, and the C-R relationship and whether a threshold exists. Although there continues to be strong evidence from studies of respiratory morbidity to support respiratory mortality, there remains relatively limited biological plausibility and coherence within and across disciplines to support the epidemiologic evidence for cardiovascular mortality, the largest contributor to total mortality. Collectively, evidence is "suggestive of, but not sufficient to infer, a causal relationship" between short-term ozone exposure and total mortality.

Table IS-9 Summary of evidence from epidemiologic studies on the association of short-term ozone exposure with mortality.

	Conclusions from 2013 Ozone ISA	Results and Conclusions from 2019 ISA[a]
Mortality	Consistent, positive associations were reported across multicity and multicontinent studies in combination with strong evidence from studies of respiratory morbidity. There was limited evidence from studies of cardiovascular morbidity, providing coherence and biological plausibility**. Evidence demonstrated that there was a likely to be causal relationship between short-term ozone exposure and mortality.**	Recent multicity studies continue to provide evidence of consistent, positive associations, which is supported by strong evidence from studies of respiratory morbidity, providing coherence and biological plausibility. Recent studies of cardiovascular morbidity do not provide coherence between experimental and epidemiologic studies, and therefore, biological plausibility for cardiovascular mortality is absent. **Evidence is suggestive of, but not sufficient to infer, a causal relationship between short-term ozone exposure and mortality.**
Epidemiologic evidence	Multicity and multicontinent studies provided evidence of consistent positive associations for total (nonaccidental), respiratory, and cardiovascular mortality.	Recent multicity studies continue to provide evidence of consistent, positive associations with total (nonaccidental), respiratory, and cardiovascular mortality, but the cause-specific mortality evidence is limited to one recent multicity study.
Copollutant confounding	Ozone-mortality associations remained positive and relatively unchanged in copollutant models with PM and $PM_{2.5}$ components, but analyses of $PM_{2.5}$ components are limited by the every-3rd and 6th-day sampling schedule.	Recent multicity studies have conducted a limited assessment of potential copollutant confounding, but report that ozone-mortality associations remain positive and relatively unchanged in copollutant models with PM_{10} and NO_2, the only pollutants assessed.
Biological plausibility	The strong and consistent evidence within and across scientific disciplines for respiratory morbidity provided coherence and biological plausibility for respiratory mortality. For cardiovascular mortality, controlled human exposure and animal toxicological studies provided initial evidence supporting a biologically plausible mechanism by which short-term ozone exposure could lead to cardiovascular mortality, but there was inconsistency in results between experimental and epidemiologic studies of cardiovascular morbidity.	There continues to be strong and consistent evidence within and across disciplines for respiratory morbidity, which provides coherence and biological plausibility for respiratory mortality. Although there remains evidence of cardiovascular mortality, recent controlled human exposure studies do not report evidence of cardiovascular effects in response to short-term ozone exposure, indicating a lack of coherence between experimental and epidemiologic studies and providing limited evidence of a biologically plausible pathway to cardiovascular mortality or to other causes of mortality.

[a]Conclusions from the 2019 ISA include evidence from recent studies integrated with evidence included in previous Ozone ISAs and AQCDs.

IS.4.3.7 Other Health Endpoints

The evidence for the other health endpoints not discussed in previous sections, including long-term ozone exposure and cardiovascular effects and mortality, and short- and long-term ozone exposure and reproductive effects, nervous system effects, and cancer, is limited or inconsistent, resulting in causality determinations of either "suggestive of, but not sufficient to infer, a causal relationship" or "inadequate to infer the presence or absence of a causal relationship." The evidence for these health effects is summarized here, with more details of the evidence that formed the basis for these conclusions in Appendix 4, Appendix 6, and Appendix 7.

IS.4.3.7.1 Long-Term Ozone Exposure and Cardiovascular Effects

Collectively, **the body of evidence for long-term ozone exposure and cardiovascular effects is "suggestive of, but not sufficient to infer, a causal relationship".** Recent animal toxicological and epidemiologic studies add to the body of evidence that formed the basis of the conclusions in the 2013 Ozone ISA for cardiovascular health effects. This body of evidence is limited, however, with some experimental and observational evidence for subclinical cardiovascular health effects and little evidence for associations with outcomes such as IHD or MI, HF, or stroke. The strongest evidence for the association between long-term ozone exposure and cardiovascular health outcomes continues to come from animal toxicological studies of impaired cardiac contractility and epidemiologic studies of blood pressure changes and hypertension and cardiovascular mortality. Recent epidemiologic studies observed positive associations with changes in blood pressure or hypertension, but animal toxicological studies do not report effects of ozone on blood pressure changes. In conclusion, the results observed across both recent and older experimental and observational studies conducted in various locations provide limited evidence for an association between long-term ozone exposure and cardiovascular health effects.

IS.4.3.7.2 Ozone Exposure and Reproductive Effects

Overall, the evidence is "suggestive of, but not sufficient to infer, a causal relationship" between ozone exposure and (1) male and female reproduction and fertility and (2) pregnancy and birth outcomes. Separate conclusions are made for these groups of reproductive effects because they are likely to have different etiologies and critical exposure windows over different lifestages. The 2013 Ozone ISA concluded that the evidence was "suggestive of a causal relationship" between ozone exposure and the inclusive category for all reproductive and developmental outcomes.

The strongest evidence in the 2013 Ozone ISA for effects on reproduction and fertility came from epidemiologic and animal toxicological studies of sperm. Recent studies of sperm quality are consistent with this evidence but remain limited. Uncertainties that contribute to the determination include a lack of

evaluation of copollutant confounding or multiple potential sensitive windows of exposure, and the generally small sample size of studies in human subjects.

The strongest evidence in the 2013 Ozone ISA for effects on pregnancy and reproduction came from epidemiologic studies of birth weight. Recent studies of birth weight are consistent with this evidence but remain limited. There are several well-designed, well-conducted studies that indicate an association between ozone and poorer birth outcomes, particularly for outcomes of continuous birth weight and preterm birth. In particular, studies of preterm birth that examine exposures in the first and second trimesters show fairly consistent positive associations (increased ozone exposures associated with increased odds of preterm birth). In addition, some animal toxicological studies demonstrate decreased birth weight and changes in uterine blood flow. Epidemiologic studies of continuous birth weight and preterm birth did not generally adjust for potential copollutant confounding, although studies that did appeared to show limited impacts. There is also inconsistency across exposure windows for associations with continuous birth weight. Also, the magnitude of effect estimates varies.

IS.4.3.7.3 Short-Term Ozone Exposure and Nervous System Effects

Overall, the evidence is "suggestive of, but not sufficient to infer, a causal relationship" between short-term exposure to ozone and nervous system effects. The 2013 Ozone ISA concluded that the evidence was "suggestive of a causal relationship" between short-term ozone exposure and nervous system effects. The strongest evidence supporting this causality determination came from experimental animal studies of CNS structure and function. Most of the recent experimental animal studies indicate that short-term exposure to ozone induces oxidative stress and inflammation in the central nervous system (Section 7.2.1.3). In some cases, these effects are associated with changes in brain morphology and effects on neurotransmitters. In some instances, the effects of short-term ozone exposure on the nervous system were exacerbated in aged animals. No epidemiologic studies of short-term ozone exposure and nervous system effects were reviewed in the 2013 Ozone ISA, and the epidemiologic evidence remains limited. Recent epidemiologic evidence consists only of a study reporting an association between short-term ozone exposure and depressive symptoms, and several studies of hospital admissions or ED visits for symptoms related to a range of nervous system diseases or mental disorders (e.g., multiple sclerosis, Alzheimer's disease, Parkinson's disease, depression, psychiatric disorders). These findings for depressive symptoms are coherent with experimental animal studies showing depression-like behaviors in rodents. Biological plausibility of these effects is supported by multiple toxicological studies in laboratory animals showing inflammation and morphological changes in the brain following short-term ozone exposure (Section 7.2.1.2).

IS.4.3.7.4 Long-Term Ozone Exposure and Nervous System Effects

Overall, the evidence is "suggestive of, but not sufficient to infer, a causal relationship" between long-term ozone exposure and nervous system effects. This conclusion is consistent with that of the 2013 Ozone ISA. The strongest evidence supporting the causality determination for long-term ozone exposure and nervous system effects from the 2013 Ozone ISA came from animal toxicological studies demonstrating effects on CNS structure and function, with several studies indicating the potential for neurodegenerative effects similar to Alzheimer's or Parkinson's diseases in a rat model. The body of evidence has grown since the 2013 Ozone ISA. Recent epidemiologic studies have examined nervous system effects, including cognitive effects, depression, neurodegenerative disease, and autism. Although the epidemiologic evidence remains limited, the strongest evidence is for effects on cognition in adults. Recent experimental animal studies continue to provide coherence for these effects. Several recent animal toxicological studies report increased markers of oxidative stress and inflammation, including lipid peroxidation, microglial activation, and cell death following long-term exposure to ozone. There was some evidence to indicate that aged and young populations may have increased sensitivity to ozone exposure. Uncertainties that contribute to the causality determination include the limited number of epidemiologic studies, the lack of consistency across the available studies of Alzheimer's and Parkinson's disease, and the limited evaluation of copollutant confounding in these studies. In addition, the evidence supporting the biological plausibility of the associations with autism or ASD in epidemiologic studies is limited.

IS.4.3.7.5 Long-Term Ozone Exposure and Cancer

The evidence describing the relationship between exposure to ozone and cancer remains inadequate to determine whether a causal relationship exists. In the 2013 Ozone ISA, very few studies were available to assess the relationship between long-term ozone exposure and cancer. The few available epidemiologic and animal toxicological studies indicated that ozone exposure may contribute to DNA damage. However, given the overall lack of studies, the 2013 Ozone ISA concluded that the evidence was inadequate to determine whether a causal relationship existed between long-term ozone exposure and cancer. More recent studies provide some additional animal toxicological evidence of DNA damage. In addition, several, but not all, recent cohort and case-control studies have observed positive associations between long-term ozone exposure and lung cancer incidence or mortality. Several of the studies evaluating lung cancer mortality were conducted in populations that had already been diagnosed with cancer in a different organ system. Associations between ozone exposure and other types of cancer were generally null. Given the limited evidence base, the lack of an evaluation of copollutant confounding in epidemiologic studies reporting associations, and the evaluation of study populations that had already been diagnosed with cancer in several of the epidemiologic studies, the evidence is not sufficient to draw a conclusion regarding causality.

IS.4.3.7.6 Long-Term Ozone Exposure and Mortality

Collectively, this body of evidence is "suggestive of, but not sufficient to infer, a causal relationship" between long-term ozone exposure and total mortality. Recent epidemiologic studies add to the limited body of evidence that formed the basis of the conclusions of in 2013 Ozone ISA for total mortality. This body of evidence is generally inconsistent, with some U.S. and Canadian cohorts reporting modest positive associations between long-term ozone exposure and total mortality, while other recent studies conducted in the U.S, Europe, and Asia reporting null or negative associations. The strongest evidence for the association between long-term ozone exposure and total (nonaccidental) mortality continues to come from analyses of patients with pre-existing disease from the Medicare cohort and from recent evidence demonstrating positive associations with cardiovascular mortality. The evidence from the assessment of ozone-related respiratory disease, with more limited evidence from cardiovascular and metabolic morbidity, provides some biological plausibility for mortality due to long-term ozone exposures. In conclusion, the inconsistent associations observed across both recent and older cohort and cross-sectional studies conducted in various locations provide limited evidence for an association between long-term ozone exposure and mortality.

IS.4.4 At-Risk Populations

Interindividual variation in human responses to ambient air pollution exposure can result in some groups or lifestages being at increased risk for health effects. The NAAQS are intended to protect public health with an adequate margin of safety. In so doing, protection is provided for both the population as a whole and those potentially at increased risk for health effects in response to exposure to a criteria air pollutant [e.g., ozone; see Preamble to the ISAs; (U.S. EPA, 2015)]. There is interindividual variation in both physiological responses, as well as exposure to ambient air pollution. The scientific literature has used a variety of terms to identify factors and subsequently populations or lifestages that may be at increased risk of an air pollutant-related health effect, including *susceptible, vulnerable, sensitive, at risk,* and *response-modifying factor* [Vinikoor-Imler et al. (2014); see Preamble to the ISAs; (U.S. EPA, 2015)]. Acknowledging the inconsistency in definitions for these terms across the scientific literature and the lack of a consensus on terminology in the scientific community, "at-risk" is the all-encompassing term used in ISAs for groups with specific factors that increase the risk of an air pollutant (e.g., ozone)-related health effect in a population, as initially detailed in the 2013 Ozone ISA (U.S. EPA, 2013b). Therefore, this ISA takes an inclusive and all-encompassing approach and focuses on identifying those populations or lifestages potentially "at risk" of an ozone-related health effect.

As discussed in the Preamble to the ISAs (U.S. EPA, 2015), the risk of health effects from exposure to ozone may be modified as a result of intrinsic (e.g., pre-existing disease, genetic factors) or extrinsic factors (e.g., sociodemographic or behavioral factors), differences in internal dose (e.g., due to variability in ventilation rates or exercise behaviors), or differences in exposure to air pollutant

concentrations (e.g., more time spent in areas with higher ambient concentrations). Some factors may lead to a reduction in risk and are recognized during the evaluation process, but for identifying those populations or lifestages at greater risk to inform decisions on the NAAQS, the focus in this ISA is on characterizing those factors that may increase risk. While a combination of factors (e.g., residential location and socioeconomic status [SES]) may increase the risk of ozone-related health effects in portions of the population, information on the interaction among factors remains limited. Thus, this ISA characterizes the individual factors that potentially result in increased risk for ozone-related health effects [see Preamble to the ISAs; (U.S. EPA, 2015)].

IS.4.4.1 Approach to Evaluating and Characterizing the Evidence for At-Risk Factors

The ISA takes a pragmatic approach to identifying and evaluating factors that may increase the risk of a population or specific lifestage to an ambient air ozone-related health effect, described in detail in the Preamble to the ISAs (U.S. EPA, 2015) and illustrated in Table IS-10. Briefly, in contrast to the overall evaluation of ozone exposures and health effects presented in Appendix 3−Appendix 7, this section specifically aims to summarize the consideration of evidence for populations and lifestages potentially at increased risk of an ozone-related health effect. While Appendix 3−Appendix 7 include a discussion of some populations and lifestages in order to explicitly characterize the causal nature between ozone exposure and health effects based on the body of evidence (e.g., children, individuals with asthma), this section focuses on summarizing evidence that can inform the identification of such populations and lifestages. In addition, the populations and lifestages explicitly considered in this ISA include those with pre-existing asthma, children, older adults, and outdoor workers, for which there was adequate evidence of increased risk in the 2013 Ozone ISA.

The evidence evaluated in this section includes relevant studies discussed in Appendix 3−Appendix 7 of this ISA and builds on the evidence presented in the 2013 Ozone ISA (U.S. EPA, 2013b). Based on the approach developed in previous ISAs (U.S. EPA, 2016, 2013a, b), recent evidence is integrated across scientific disciplines and health effects, and where available, with information on exposure and dosimetry. In evaluating factors and population groups, greater emphasis is placed on the evidence for those health outcomes for which a "causal" or "likely to be causal" relationship is concluded in Appendix 3−Appendix 7 of this ISA.

As discussed in the Preamble to the ISAs (U.S. EPA, 2015), consideration of at-risk populations includes evidence from epidemiologic, controlled human exposure, and animal toxicological studies, in addition to relevant exposure-related information. *Regarding epidemiologic studies, the evaluation focuses on those studies that include stratified analyses to compare populations or lifestages exposed to similar air pollutant concentrations within the same study design along with consideration of the strengths and limitations of each study*. Other epidemiologic studies that do not stratify results but instead examine a specific population or lifestage can provide supporting evidence for the pattern of associations

observed in studies that formally examine effect measure modification. Similar to the characterization of evidence in Appendix 3–Appendix 7, the greatest emphasis is placed on patterns or trends in results across studies. Experimental studies in human subjects or animal models that focus on factors, such as genetic background or health status, are evaluated because they provide coherence and biological plausibility of effects observed in epidemiologic studies. Also evaluated are studies examining whether factors may result in differential exposure to ozone and subsequent increased risk of ozone-related health effects. Conclusions are made with respect to whether a specific factor increases the risk of an ozone-related health effect based on the characterization of evidence using the framework detailed in Table IS-10.

Table IS-10 Characterization of evidence for factors potentially increasing the risk for ozone-related health effects.

Classification	Health Effects
Adequate evidence	There is substantial, consistent evidence within a discipline to conclude that a factor results in a population or lifestage being at increased risk of air pollutant-related health effect(s) relative to some reference population or lifestage. Where applicable, this evidence includes coherence across disciplines. Evidence includes multiple high-quality studies.
Suggestive evidence	The collective evidence suggests that a factor results in a population or lifestage being at increased risk of air pollutant-related health effect(s) relative to some reference population or lifestage, but the evidence is limited due to some inconsistency within a discipline or, where applicable, a lack of coherence across disciplines.
Inadequate evidence	The collective evidence is inadequate to determine whether a factor results in a population or lifestage being at increased risk of air pollutant-related health effect(s) relative to some reference population or lifestage. The available studies are of insufficient quantity, quality, consistency, and/or statistical power to permit a conclusion to be drawn.
Evidence of no effect	There is substantial, consistent evidence within a discipline to conclude that a factor does not result in a population or lifestage being at increased risk of air pollutant-related health effect(s) relative to some reference population or lifestage. Where applicable, the evidence includes coherence across disciplines. Evidence includes multiple high-quality studies.

IS.4.4.2 Summary of At-Risk Populations

The 2013 Ozone ISA (U.S. EPA, 2013b) concluded that there was adequate evidence to classify individuals with pre-existing asthma, children and older adults, individuals with reduced intake of certain nutrients (i.e., vitamins C and E), and outdoor workers as populations at increased risk to the health effects of ozone. These conclusions were based on the consistency in findings across studies as well as evidence of coherence in results from different scientific disciplines. Recent studies provide additional evidence that individuals with pre-existing asthma and children are at increased risk of the effects of

ozone. There is relatively little recent evidence for older adults, individuals with reduced intake of certain nutrients, and outdoor workers, and the evidence presented in the 2013 Ozone ISA is adequate to classify them as at-risk populations.

Recent, large multicity epidemiologic studies conducted in the U.S. expand upon evidence from the 2013 Ozone ISA to provide further support the relationship between ozone and ED visits and hospital admissions for asthma among individuals with pre-existing asthma (Table IS-11; Section IS.4.4.3.1).

Generally, studies comparing age groups also reported higher magnitude associations for respiratory hospital admissions and ED visits among children (Section IS.4.4.4.1) than for adults. In addition, recent evidence from studies of nonhuman primates demonstrate ozone-induced respiratory effects and support the biological plausibility of associations between long-term exposure to ozone and the development of asthma in children observed in epidemiologic studies. Specifically, these experimental studies indicate that early-life ozone exposure can cause structural and functional changes that could potentially contribute to airway obstruction and increased airway responsiveness. Also, children have higher exposure and dose due to increased time spent outdoors and ventilation rate, and childrens' respiratory systems are also still undergoing lung growth.

The majority of evidence for older adults being at increased risk of health effects related to ozone exposure comes from studies of short-term ozone exposure and mortality evaluated in the 2013 Ozone ISA (Section IS.4.4.4.2).

Table IS-11 Summary of evidence for populations at increased risk to the health effects of ozone.

	Conclusions from 2013 Ozone ISA	Conclusions from 2019 ISA
Adequate evidence		
Pre-existing asthma	Collective evidence from controlled human exposure studies is supported by toxicological studies. Some, but not all, epidemiologic studies report greater risk of health effects among individuals with asthma.	Evidence from controlled human exposure and animal toxicological studies provide biological plausibility for the associations observed in epidemiologic studies of short-term ozone exposure and asthma exacerbation. Results from experimental studies in humans demonstrate that ozone exposures lead to increased respiratory symptoms, lung function decrements, increased airway responsiveness, and increased lung inflammation in individuals with asthma.

Table IS-11 (Continued): Summary of evidence for populations at increased risk to the health effects of ozone.

	Conclusions from 2013 Ozone ISA	Conclusions from 2019 ISA
Children	Controlled human exposure and toxicological studies provide evidence of increased risk from ozone exposure for younger ages, which is coherent with findings from epidemiologic studies that report larger associations for respiratory ED visits and hospital admissions for children than adults.	Recent, large multicity epidemiologic studies conducted in the U.S. expand upon previous evidence and support an association between ozone and ED visits and hospital admissions for asthma, which are strongest in children between the ages of 5 and 18; animal toxicological studies in infant monkeys indicate that early-life ozone exposure can cause structural and functional changes that could potentially contribute to airway obstruction and increased airway responsiveness.
Older adults	Epidemiologic studies report consistent positive associations between short-term ozone exposure and mortality in older adults.	Controlled human exposure studies demonstrate changes in FEV_1 and FVC among older adults at a relatively light activity level and brief duration of ozone exposure, though these responses are not greater than in other age groups; evidence from studies of metabolic effects is inconsistent.
Outdoor workers	Strong evidence from 2006 Ozone AQCD, which demonstrated increased exposure, dose, and ultimately risk of ozone-related health effects in this population supports that there is adequate evidence to indicate that increased exposure to ozone through outdoor work increases the risk of ozone-related health effects.	No recent information has been evaluated that would inform or change prior conclusions.
Genetic factors	Multiple genetic variants have been observed in epidemiologic and controlled human exposure studies to affect the risk of ozone-related respiratory outcomes and support is provided by animal toxicological studies of genetic factors.	No recent information has been evaluated that would inform or change prior conclusions.
Diet	Individuals with reduced intake of vitamins E and C are at risk for ozone-related health effects based on substantial, consistent evidence both within and among disciplines.	No recent information has been evaluated that would inform or change prior conclusions.
Suggestive evidence		
Sex	Evidence for increased risk for ozone-related health effects present for females in some studies and males in other studies; some indication that females are increased risk of ozone-related respiratory hospital admissions and ED visits.	No recent information has been evaluated that would inform or change prior conclusions.
Pre-existing obesity	Multiple epidemiologic, controlled human exposure, and toxicological studies report increased ozone-related respiratory health effects among obese individuals.	Recent animal toxicological studies expand upon previous evidence and continue to indicate that, compared to lean mice, obese mice exhibit enhanced airway responsiveness and pulmonary inflammation in response to acute ozone exposures.

Table IS-11 (Continued): Summary of evidence for populations at increased risk to the health effects of ozone.

	Conclusions from 2013 Ozone ISA	Conclusions from 2019 ISA
SES	Most studies report that individuals with low SES and those living in neighborhoods with low SES are more at risk for ozone-related respiratory hospital admissions and ED visits; inconsistent results for mortality and reproductive outcomes.	No recent information has been evaluated that would inform or change prior conclusions.
Inadequate evidence		
Race/ethnicity	A limited number of studies indicate that there may be race-related increase in risk of ozone-related health effects for some outcomes.	No recent information has been evaluated that would inform or change prior conclusions.
Pre-existing COPD	Epidemiologic studies indicate that persons with COPD may have increased risk of ozone-related cardiovascular effects, but little information is available on whether COPD leads to an increased risk of ozone-induced respiratory effects.	Small number of recent studies provided inadequate evidence to determine whether COPD results in an increased risk of ozone-related health effects.
Pre-existing CVD	Most short-term exposure studies did not report increased ozone-related cardiovascular morbidity for individuals with pre-existing CVD. Limited number of studies examined whether CVD modifies the association between ozone and respiratory effects. Some evidence that CVD increases risk of ozone-related mortality.	Some studies provide evidence that cardiovascular disease exacerbates the respiratory effects of ozone exposure; a limited number of recent epidemiologic cohort studies observed increased risk estimates for incident diabetes among those with pre-existing hypertension or among subjects that had some pre-existing condition (MI, COPD, hypertension, or hyperlipidemia) compared to those without pre-existing disease.
Pre-existing diabetes	There are a limited number of epidemiologic studies and lack of controlled human exposure studies or toxicological studies to determine whether pre-existing diabetes modifies ozone effects on health.	A limited number of recent studies provides some evidence that individuals with pre-existing metabolic disease may be at greater risk of mortality associated with long-term ozone exposure.
Smoking	There are a limited number of studies and insufficient coherence for differences in ozone-related health effects by smoking status.	No recent information has been evaluated that would inform or change prior conclusions.

IS.4.4.3 Pre-existing Disease

Individuals with some pre-existing diseases may be considered at greater risk of an air pollution-related health effect because they may be in a compromised biological state that can vary depending on the disease and severity. The 2013 Ozone ISA (U.S. EPA, 2013b) concluded that there was adequate evidence that those with pre-existing respiratory disease, specifically asthma, were at greater risk for the health effects associated with exposure to ozone, but that evidence was inadequate to determine whether those with COPD, cardiovascular disease, or diabetes were at increased risk of ozone-related health effects. Of the recent epidemiologic studies evaluating effect measure modification by pre-existing disease or condition, most focused on asthma, COPD, or cardiovascular disease. Table IS-12 presents the prevalence of these diseases according to the Centers for Disease Control and Prevention's (CDC's) National Center for Health Statistics (Blackwell et al., 2014), including the proportion of adults with a current diagnosis categorized by age and geographic region. The large proportions of the U.S. population affected by many chronic diseases, including various respiratory and cardiovascular diseases, indicates the potential public health impact, and thus, the importance of determining whether identifying populations that may be at increased risk for ozone-related health effects.

Table IS-12 Prevalence of respiratory diseases, cardiovascular diseases, diabetes, and obesity among adults by age and region in the U.S. in 2012.

Chronic Disease/Condition	Adults (18+) N (in thousands)	Age (%)[a] 18−44	45−64	65−74	75+	Region (%)[b] North-east	Midwest	South	West
All (N, in thousands)	234,921	111,034	82,038	23,760	18,089	42,760	53,378	85,578	53,205
Selected respiratory diseases									
Asthma[c]	18,719	8.1	8.4	7.8	6.0	9.2	8.1	7.3	7.8
COPD—chronic bronchitis	8,658	2.5	4.7	4.9	5.2	3.2	4.4	3.9	2.4
COPD—emphysema	4,108	0.3	2.3	4.7	4.7	1.3	2.0	1.9	1.0
Selected cardiovascular diseases/conditions									
All heart disease	26,561	3.8	12.1	24.4	36.9	10.0	11.6	11.6	9.3
Coronary heart disease	15,281	0.9	7.1	16.2	25.8	5.3	6.5	7.0	5.1
Hypertension	59,830	8.3	33.7	52.3	59.2	21.4	24.1	26.6	21.5
Stroke	6,370	0.6	2.8	6.3	10.7	1.8	2.5	3.0	2.5
Metabolic disorders/conditions									
Diabetes	21,391	2.4	12.7	21.1	19.8	7.6	8.4	10.0	7.3
Obesity (BMI ≥30 kg/m^2)	64,117	26	33.7	29.7	18	25.1	29.9	29.9	25.2
Overweight (BMI 25−30 kg/m^2)	78,455	31.4	36.8	40.7	38.6	34.3	34.1	34.2	35.3

BMI = body mass index; COPD = chronic obstructive pulmonary disease.

[a]Percentage of individual adults within each age group with disease, based on N (at the top of each age column).

[b]Percentage of individual adults (18+) within each geographic region with disease, based on N (at the top of each region column).

[c]Asthma prevalence is reported for "still has asthma."

Source: Blackwell et al. (2014); National Center for Health Statistics: Data from Tables 1−4, 7, 8, 28, and 29 of the Centers for Disease Control and Prevention report.

IS.4.4.3.1 Pre-existing Asthma

Asthma is the leading chronic illness affecting children. Approximately 8.0% of adults and 9.3% of children (age <18 years) in the U.S. currently have asthma (Blackwell et al., 2014; Bloom et al., 2013). Regarding consideration of those with asthma potentially being at increased risk for an ozone-related health effect, it is important to note that individuals with asthma, and children in general, tend to have a higher degree of oronasal breathing, which can result in greater penetration of ozone into the lower respiratory tract.

The 2013 Ozone ISA concluded that there is adequate evidence that individuals with asthma are at increased risk of health effects related to ozone exposure based on a number of controlled human exposure, epidemiologic, and animal toxicological studies. Consistent with this evidence, recent, large multicity epidemiologic studies conducted in the U.S. expand upon evidence from the 2013 Ozone ISA to provide further support for an association between ozone and ED visits and hospital admissions for asthma. Hospital admission and ED visit studies that presented age-stratified results reported the strongest associations in children between the ages of 5 and 15 years. Additionally, associations were observed across a range of ambient ozone concentrations and were consistent in models where exposure was assigned using either measured or modeled ozone concentrations. While there is a lack of recent epidemiologic studies conducted in the U.S. or Canada that have examined respiratory symptoms and medication use, lung function, and subclinical effects in people with asthma, a large body of evidence from the 2013 Ozone ISA (U.S. EPA, 2013b) reported ozone associations with these less severe indicators of asthma exacerbation that provide support for the ozone-related increases in asthma hospital admissions and ED visits observed in recent studies.

Evidence from controlled human exposure and animal toxicological studies provide biological plausibility for the associations observed in epidemiologic studies of short-term ozone exposure and asthma exacerbation. Results from experimental studies in humans demonstrate that ozone exposures lead to increased respiratory symptoms, decrements in lung function, increased airway responsiveness, and increased lung inflammation in individuals with asthma. However, observed responses across the range of endpoints did not generally differ due to the presence of asthma. Animal toxicological studies similarly found that ozone exposures altered lung function measures, increased airway responsiveness, and increased pulmonary inflammation and bronchoconstriction in allergic animals. In contrast to controlled human exposure studies, there was some evidence from studies of rodents that the observed respiratory effects were enhanced in allergic animals compared to naïve animals.

Overall, recent evidence expands upon evidence available in the 2013 Ozone ISA and is adequate to conclude that individuals with pre-existing asthma are at greater risk of ozone-related health effects based on the substantial and consistent evidence within epidemiologic studies and the coherence with toxicological studies.

IS.4.4.3.2 Pre-existing Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) comprises chronic bronchitis and emphysema and affects approximately 6.8 million adults in the U.S. (Table IS-12). In the U.S., over 4% of adults report having chronic bronchitis and almost 2% report having emphysema (Pleis et al., 2009). Chronic lower respiratory disease, including COPD, was ranked as the third leading cause of death in the U.S. in 2011 (Hoyert and Xu, 2012). Given that people with COPD have compromised respiratory function and underlying respiratory tract inflammation, it is plausible that they could be at increased risk for an array of ozone-related health effects.

Epidemiologic studies evaluated in the 2013 Ozone ISA indicate that individuals with COPD may have increased risk of ozone-related cardiovascular effects, but little information was available on whether COPD leads to an increased risk of ozone-induced respiratory effects. A limited number of recent epidemiologic studies provide inconsistent evidence that individuals with pre-existing COPD could be at greater risk for respiratory health effects associations with ozone exposure. Overall, a limited number of recent studies add to the scarce evidence available in the 2013 Ozone ISA and, collectively, is inadequate to conclude whether or not individuals with pre-existing COPD are at greater risk of ozone-related health effects.

IS.4.4.3.3 Pre-existing Obesity

Obesity, defined as a BMI of 30 kg/m^2 or greater, is an issue of increasing importance in the U.S., with self-reported obesity at 39.8% of the general population in 2016, up from 26.7% in 2009 (Hales et al., 2017). BMI may affect ozone-related health effects through multiple avenues, including systematic inflammation, increased pre-existing disease, and poor diet. Increased risk of air pollution-related health effects has been observed among obese individuals compared with nonobese individuals (U.S. EPA, 2009). The 2013 Ozone ISA concluded that there was suggestive evidence for increased ozone-related respiratory health effects among obese individuals. This conclusion was based on evidence from controlled human exposure studies and epidemiologic studies reporting greater lung function decrements in obese compared to nonobese individuals, as well as enhanced pulmonary inflammation in genetically and dietarily obese mice (U.S. EPA, 2013b).

Recent animal toxicological studies expand the body of evidence evaluated in the 2013 Ozone ISA and continue to indicate that, compared with lean mice, obese mice exhibit enhanced airway responsiveness and pulmonary inflammation in response to acute ozone exposures. In contrast, a recent controlled human exposure study reported evidence of ozone-related increases in pulmonary inflammation in both obese and normal-weight adult women during exercise, but inflammatory responses did not differ between the groups. Overall, recent studies contribute some additional support to the evidence available in the 2013 Ozone ISA and there is suggestive evidence indicating that individuals with pre-existing obesity are at potentially increased risk of ozone-related health effects based on the

limited evidence within epidemiologic studies and some coherence from controlled human exposure and animal toxicological studies.

IS.4.4.3.4 Pre-existing Metabolic Syndrome

Metabolic syndrome is a term used to describe a collection of risk factors that include high blood pressure, dyslipidemia (elevated triglycerides and low levels of high-density lipoprotein [HDL] cholesterol), obesity (particularly central obesity), and increased fasting blood glucose (Alberti et al., 2009). The presence of these risk factors may predispose an individual to an increased risk of type 2 diabetes and cardiovascular disease. In the 2013 Ozone ISA, a limited number of epidemiologic studies provided inadequate evidence to indicate whether individuals with metabolic syndrome (generally indicated by a diabetes diagnosis) were at an increased risk of ozone-related health effects compared to those without diabetes.

In recent studies of a diabetes-prone mouse model, subacute ozone exposure increased airway inflammation and proinflammatory genes in lung tissue (Section 3.1.6.2). In contrast, an epidemiologic panel study observed a negative association between increased ozone exposure and pulmonary inflammation in adults with type 2 diabetes mellitus. This inverse association may be explained by negative correlations with copollutants that demonstrated strong positive associations with pulmonary inflammation in the same population. Overall, a limited number of recent studies add to the small body of evidence available in the 2013 Ozone ISA and, collectively, the evidence is inadequate to conclude that individuals with pre-existing metabolic disease are at greater risk of ozone-related health effects.

IS.4.4.3.5 Pre-existing Cardiovascular Disease

Cardiovascular disease has become increasingly prevalent in the U.S., with about 12% of adults aged 45−64 years reporting a diagnosis of heart disease (Table IS-12). This number doubles to 24% among adults aged 65−74 years and is even higher for adults aged 75 years and older. A high prevalence of other cardiovascular-related conditions has also been observed, such as hypertension which is prevalent among more than 50% of older adults. In the 2013 Ozone ISA, most epidemiologic studies evaluating short-term ozone exposure did not report increased risk of cardiovascular morbidity for individuals with or without pre-existing cardiovascular disease. There was some evidence from a limited number of epidemiologic studies that those with pre-existing cardiovascular disease were at greater risk of ozone-related mortality compared with those without pre-existing cardiovascular disease. Overall, the 2013 Ozone ISA concluded that the evidence was inadequate to classify pre-existing cardiovascular disease as a potential at-risk factor for ozone-related health effects.

Several recent studies evaluated respiratory effects of acute ozone exposure (0.2−1 ppm, 3−6 hours) in rodents with cardiovascular disease. Some of the studies provide evidence that

cardiovascular disease exacerbates the respiratory effects of ozone exposure. Injury, inflammation, oxidative stress, lung function changes, and increased airway responsiveness were documented in animals with cardiovascular disease in response to ozone exposure. Acute ozone exposure in animal models of hypertension resulted in enhanced injury, inflammation, and airway responsiveness compared with healthy animals. A limited number of recent epidemiologic cohort studies evaluated the potential for pre-existing cardiovascular disease to modify associations between long-term ozone exposure and metabolic effects. These studies observed increased risk estimates for incident diabetes among those with pre-existing hypertension or among subjects that had some pre-existing condition (MI, COPD, hypertension, or hyperlipidemia) compared with those without pre-existing disease. Overall, a limited number of recent studies add to the evidence available in the 2013 Ozone ISA and, collectively, are inadequate to conclude whether individuals with pre-existing metabolic disease are at greater risk of ozone-related health effects.

IS.4.4.4 Lifestage

Lifestage refers to a distinguishable time frame in an individual's life characterized by unique and relatively stable behavioral and/or physiological characteristics that are associated with development and growth (U.S. EPA, 2014). Differential health effects of ozone across lifestages could be due to several factors. With regard to children, the human respiratory system is not fully developed until 18−20 years of age; therefore, it is biologically plausible for children to have increased intrinsic risk for respiratory effects if exposures are sufficient to contribute to potential perturbations in normal lung development. Moreover, children in general may experience higher exposure to ozone than adults based on more time spent outdoors while exercising during afternoon hours when ozone concentrations may be highest. The ventilation rates also vary between children and adults, particularly during moderate/heavy activity. Children have higher ventilation rates relative to their lung volume, which tends to increase the dose normalized to lung surface area. Older adults, typically considered those 65 years of age or greater, have weakened immune function, impaired healing, decrements in pulmonary and cardiovascular function, and greater prevalence of chronic disease [Table IS-12; Blackwell et al. (2014)], which may contribute to, or worsen, health effects related to ozone exposure. Also, exposure or internal dose of ozone may differ across lifestages due to varying ventilation rates, increased oronasal breathing at rest, and time-activity patterns.

For decades, children, especially those with asthma, and older adults have been identified as populations at increased risk of health effects related to ozone exposure (U.S. EPA, 2013b, 2006a, 1996a). Long-standing evidence from controlled human exposure studies demonstrated that children have greater spirometric responses to ozone compared with middle-aged or older adults (U.S. EPA, 1996a). In addition, epidemiologic studies reported larger associations for respiratory hospital admissions and ED visits for children than for adults, and animal toxicological studies demonstrated ozone-induced health effects in immature animals, including infant monkeys, although the effects were not consistently greater

in young animals than adult animals (U.S. EPA, 2013b). Compared with other age groups, there is evidence for an increased risk of mortality associated with ozone exposure among older adults (U.S. EPA, 2013b, 2006a). The 2013 Ozone ISA concluded that there was adequate evidence that children and older adults are at increased risk of ozone-related health effects.

IS.4.4.4.1 Children

Recent, large multicity epidemiologic studies conducted in the U.S. expand on evidence from the 2013 Ozone ISA and provide further support for an association between short-term ozone exposure and ED visits and hospital admissions for asthma. Hospital admission and ED visit studies that presented age-stratified results reported the strongest associations in children between the ages of 5 and 18 years. The evidence relating new-onset asthma to long-term ozone exposure is supported by toxicological studies in infant monkeys, which indicate that postnatal ozone exposures can lead to the development of asthma. This nonhuman primate evidence of ozone-induced respiratory effects supported the biological plausibility of associations between long-term exposure to ozone and the development of asthma in children observed in epidemiologic studies. Specifically, these experimental studies indicate that early-life ozone exposure can cause structural and functional changes that could potentially contribute to airway obstruction and increased airway responsiveness.

Overall, recent evidence expands upon evidence available in the 2013 Ozone ISA and is adequate to conclude that children are at greater risk of ozone-related health effects based on the substantial and consistent evidence within epidemiologic studies and the coherence with animal toxicological studies.

IS.4.4.4.2 Older Adults

Collectively, the majority of evidence for older adults being at increased risk of health effects related to ozone exposure comes from studies of short-term ozone exposure and mortality. Many of these were evaluated in the 2013 Ozone ISA. As reported in the 1996 and 2006 Ozone AQCDs (U.S. EPA, 2006a, 1996a), decrements in lung function and increases in respiratory symptoms in response to ozone exposure decreased with increasing age. However, whether inflammatory responses persisted with increasing age remained unstudied at the time of the 2013 Ozone ISA (U.S. EPA, 2013b). Two recent controlled human exposure studies demonstrate inflammatory responses in older adults, but it is not possible to quantify inflammatory response as a function of age because of differences in experimental protocols (i.e., duration of exposure to ozone, ozone concentration, activity level, and post-exposure time of sputum collection). A recent controlled human exposure study also demonstrates changes in FEV_1 and FVC among adults aged 55−70 years at a relatively light activity level and brief duration of exposure, but a statistically significant interaction with age was not observed. This is generally consistent with studies evaluated in previous assessments that showed lung function decrements declining with age, but still

being present in adults 50−60 years of age. This recent study was conducted at a lower ozone delivery rate, which is more representative of that likely to occur in the ambient environment and shows small lung function decrements occurring in adults of age group ranging up to 70 years. These recent studies demonstrate that inflammatory responses and lung function changes following ozone exposure can occur in older adults, but do not indicate greater responses in older adults than other age groups.

Overall, recent studies add little to the evidence available in the 2013 Ozone ISA. This evidence is adequate to conclude that older adults are at greater risk of ozone-related health effects.

IS.5 Evaluation of Welfare Effects of Ozone

The scientific evidence for welfare effects of ozone is largely for effects on vegetation and ecosystems and effects on climate. Appendix 8 presents the most policy-relevant information related to this review of the NAAQS for ecological effects of ozone. Appendix 9 presents the most policy-relevant information related to this review of the NAAQS for effects on climate. The framework for causal determinations [see Preamble (U.S. EPA, 2015)] has been applied to the body of scientific evidence to examine effects attributed to ozone exposure. Conclusions from the 2013 Ozone ISA and key findings that inform the current causality determinations for welfare effects of ozone are summarized in Table IS-13.

Table IS-13 Summary of evidence for welfare effects of ozone.

Endpoint	Conclusions from 2013 Ozone ISA	Conclusions from Current Draft ISA
Visible foliar injury Section 8.2	**Causal relationship** Visible foliar injury from ozone exposure was well characterized and documented over several decades of research prior to the 2013 Ozone ISA on sensitive tree, shrub, herbaceous, and crop species in the U.S. Some sensitive species that show visible injury identified in field surveys are verified in controlled exposure settings. Ozone concentrations are high enough to induce visible symptoms in sensitive vegetation.	**Causal relationship** Studies published since the 2013 Ozone ISA strengthen previous conclusions that there is strong evidence that ozone causes foliar injury in a variety of plant species. The use of bioindicators to detect phytotoxic levels of ozone is a longstanding and effective methodology and is supported by more information on sensitive species.

Table IS-13 (Continued): Summary of evidence for welfare effects of ozone.

Endpoint	Conclusions from 2013 Ozone ISA	Conclusions from Current Draft ISA
Reduced vegetation growth Section 8.3	**Causal relationship** Studies added to the evidence from the 2006 AQCD and earlier assessments and indicated that ozone reduced growth of vegetation. Studies from the Aspen FACE experiment showed reduction in total biomass in aspen, paper birch, and sugar maple, findings which were overall consistent with OTC studies in previous NAAQS reviews. Meta-analysis showed ambient ozone concentrations (approx. 40 ppb avg across all hours of exposure) decreased annual total biomass growth of forest species by an average of 7% with potentially greater exposures with elevated ozone. Studies also demonstrated that ozone alters biomass allocation, generally reducing C allocated to roots.	**Causal relationship** New evidence from controlled exposure experiments and illustration of potential impacts using models built with empirical data strengthen previous conclusions that ozone reduces plant growth and biomass. Additional studies find that ozone significantly changes patterns of carbon allocation below and aboveground.
Reduced plant reproduction Section 8.4	**No separate causality determination; included with plant growth** Evidence from studies that ozone alters reproduction in herbaceous and woody plant species adds to evidence from the 2006 AQCD (primarily in herbaceous and crop species) for ozone effects on metrics of plant reproduction.	**Causal relationship** A new meta-analysis published since the 2013 Ozone ISA provides strong and consistent evidence for negative effects of ozone on plant reproduction. For all exposure categories evaluated, including the lowest exposure category of <40 ppb, between one and eight metrics of reproduction significantly decreased. In addition, more evidence is available that plant reproductive tissues are directly affected by ozone exposure.
Increased tree mortality Section 8.4.3	**Causality not assessed** Evidence built on observations from the 2006 Ozone AQCD of decline of conifer forests over time observed in several regions affected by elevated ozone along with other factors (Valley of Mexico, southern France, Carpathian Mountains). At the Aspen FACE site, there was reduced growth and increased mortality of a sensitive aspen clone.	**Likely to be a causal relationship** In a new large-scale multivariate analysis evaluating tree mortality over a 15-year period ozone significantly increased tree mortality in 7 out of 10 plant functional types in the eastern and central U.S. An Aspen FACE study shows that sensitive aspen genotypes have increased mortality compared to tolerant genotypes.
Reduced yield and quality of agricultural crops Section 8.5	**Causal relationship** Detrimental effects of ozone on crop production were recognized since the 1960s. There are well-documented yield losses in a variety of agricultural crops with increasing ozone concentration. Ozone also decreased crop quality. Modeling studies at large geographic scales showed ozone generally reduced crop yield but impacts vary across regions and species.	**Causal relationship** Greenhouse, OTC, FACE, and modeling studies published since the 2013 Ozone ISA strengthen previous conclusions that ozone reduces yield in major U.S. crops including wheat, soybean, and other nonsoy legumes. Advances in characterization of ozone effects on U.S. crop yield include further geographic and temporal refinement of ozone sensitivity. For soybean, there are updated exposure-response curves.

Table IS-13 (Continued): Summary of evidence for welfare effects of ozone.

Endpoint	Conclusions from 2013 Ozone ISA	Conclusions from Current Draft ISA
Altered herbivore growth and reproduction Section 8.6	**Causality not assessed** A meta-analysis of 16 studies found that elevated ozone decreased development time and increased pupal mass in insect herbivores. Other field and laboratory studies reported species-level and community-level responses in insects yet the directionality of response to ozone was mixed. This is congruent with findings from the 2006 AQCD and 1996 AQCD, where statistically significant effects on herbivorous insects were observed, but did not provide any consistent pattern of response across growth, reproduction, and mortality endpoints.	**Likely to be a causal relationship** There is a large body of evidence showing altered growth and reproduction in insect herbivores. More research has since been published on a range of species and at varying levels of ozone exposure although there is no clear trend in the directionality of response for most metrics. The most commonly measured responses are fecundity, development time, and growth.
Alteration of plant-insect signaling Section 8.7	**Causality not assessed** A few experimental and modeling studies reported altered chemical signaling in insect-plant interactions due to ozone exposure. The effect of ozone on chemical signaling is an emerging area of study that may result in further elucidation of effects with more empirical data.	**Likely to be a causal relationship** Laboratory, greenhouse, OTC, and Finnish FACE experiments expand the evidence for altered/degraded emissions of chemical signals from plants and reduced detection of volatile plant signaling compounds by insects, including pollinators, in the presence of ozone. Affected plant-insect interactions include plant defense against herbivory and insect attraction to plants. New evidence includes consistent effects in multiple insect species.
Reduced productivity in terrestrial ecosystems Section 8.8.1	**Causal relationship** Studies from long-term FACE experiments provided evidence of the association of ozone exposure and reduced productivity at the ecosystem scale. Results across different ecosystem models were consistent with the FACE experimental evidence. Models consistently found that ozone exposure negatively impacted indicators of ecosystem productivity. Studies at the leaf and plant scales show that ozone decreased photosynthesis and plant growth, providing coherence and plausibility for reported decreases in ecosystem productivity. Magnitude of response varied among plant communities.	**Causal relationship** Modeling studies and controlled exposure experiments (including Aspen FACE), published since the 2013 Ozone ISA strengthen previous conclusions. Much of the research is confirmatory, with some work providing new mechanistic insight into the effects of ozone on productivity and creating a more nuanced understanding of how these effects vary among species, communities, and environmental conditions.
Reduced carbon sequestration in terrestrial ecosystems Section 8.8	**Likely to be a causal relationship** Studies add to the strong and consistent evidence in the 2006 AQCD that ozone decreases plant photosynthesis. Most assessments of the effects of ozone on terrestrial C are from model simulations.	**Likely to be a causal relationship** Several new model simulations strengthen previous conclusions from the 2013 Ozone ISA by providing further support for regional and global scale decreases in terrestrial C sequestration from ozone pollution; however, these relationships are spatially and temporally dependent. One empirical study from the Aspen FACE experiment adds to the evidence base for reduced ecosystem C content.

Table IS-13 (Continued): Summary of evidence for welfare effects of ozone.

Endpoint	Conclusions from 2013 Ozone ISA	Conclusions from Current Draft ISA
Alteration of belowground biogeochemical cycles Section 8.9	**Causal relationship** It has been documented since the 2006 Ozone AQCD that while belowground roots and soil organisms are not exposed directly to ozone, belowground processes could be affected by ozone through alterations in the quality and quantity of carbon supply to the soils from photosynthates and litterfall. The 2013 Ozone ISA presented evidence that ozone was found to alter multiple belowground endpoints including root growth, soil food web structure, soil decomposer activities, soil respiration, soil carbon turnover, soil water cycling, and soil nutrient cycling.	**Causal relationship** New evidence confirms conclusions from the 2013 Ozone ISA on effects on soil decomposition, soil carbon, and soil nitrogen. The direction and magnitude of these changes often depends on the species, site, and time of exposure.
Alteration of terrestrial community composition Section 8.10	**Likely to be a causal relationship** The body of evidence is for effects on community composition shifts in terrestrial plant communities. For broadleaf forests, the ozone-tolerant aspen clone was the dominant clone at the Aspen FACE site. In grasslands, evidence generally showed shifts from grass-legume mix to grass species. A shift in community composition of bacteria and fungi was observed in both natural and agricultural systems, although no general pattern could be discerned.	**Causal relationship** Recent evidence builds upon the conclusions of the 2013 Ozone ISA by strengthening the understanding of effects of ozone on forest and grassland communities and confirming that effects upon soil microbial communities are diverse. New observational and experimental studies of ozone effects on tree species extend to regional forest composition in the eastern U.S. In grasslands, new studies are consistent with previous research that ozone shifts grassland community composition.
Alteration of ecosystem water cycling Section 8.11	**Likely to be a causal relationship** Ozone can affect water use in plants and ecosystems through several mechanisms including damage to stomatal functioning and loss of leaf area. Several field and modeling studies showed an association of ozone exposure and the alteration of water use and cycling in vegetation and ecosystems. Direction of response varied among studies.	**Likely to be a causal relationship** New evidence is consistent with the findings in the 2013 Ozone ISA. New evidence identifies a relationship between ozone and wood anatomy associated with water transport. Additional studies add to the evidence base for decreased root growth and density. New empirical and modeling studies continue to show reduced sensitivity of stomatal closing in response to ozone. There are a few studies that scale-up these changes to effects on ecosystem scales including a study linking ozone effects on tree growth and water use to ecosystem stream flow in six watersheds in eastern U.S. forests and from Aspen FACE.
Radiative forcing (RF) Section 9.2	**Causal relationship** The 2013 Ozone ISA reported an RF of 0.35 W/m^2 from tropospheric ozone from preindustrial times to the present (1750 to 2005) based on multimodel studies as reported in the AR4 IPCC assessment.	**Causal relationship** New evidence is consistent with the findings in the 2013 Ozone ISA. The most recent IPCC assessment, AR5, reports tropospheric ozone RF as 0.40 (0.20 to 0.60) W/m^2, which is within range of previous assessments (i.e., AR4). There have also been a few individual modeling studies of tropospheric ozone RF since AR5 which reinforce the AR5 estimates and the causal relationship between tropospheric ozone and RF.

Table IS-13 (Continued): Summary of evidence for welfare effects of ozone.

Endpoint	Conclusions from 2013 Ozone ISA	Conclusions from Current Draft ISA
Temperature, precipitation and related climate variables Section 9.3	**Likely to be a Causal Relationship** The increase of tropospheric ozone abundance has contributed an estimated 0.1−0.3°C warming to the global climate since 1750 based on studies included in the AR4 IPCC assessment.	**Likely to be a Causal Relationship** Consistent with previous estimates, the effect of tropospheric ozone on global surface temperature continues to be estimated at roughly 0.1−0.3°C since preindustrial times, with larger effects regionally. In addition to temperature, ozone changes have impacts on other climate metrics such as precipitation and atmospheric circulation patterns. Current limitations in climate modeling tools, variation across models, and the need for more comprehensive observational data on these effects represent sources of uncertainty in quantifying the precise magnitude of climate responses to ozone changes, particularly at regional scales.

IS.5.1 Ecological Effects

The evidence for ozone effects on vegetation and ecosystems is best understood in the context of some general concepts within ecology. Ecosystems[1] are inherently complex and inter-connected. Ecosystem structure may be described by a variety of measurements used to assess ozone response at different levels of biological organization [i.e., suborganismal, organism, population,[2] community[3]; Suter et al. (2005)]. For example, ozone effects on sensitive species at the whole-plant scale of biological organization (i.e., reduced growth and biomass, reduced plant reproduction, decreased yield) cascade up to effects on population and community structure and ecosystem function (Figure IS-3). "Function" refers to the suite of processes and interactions among the ecosystem components that involve energy or matter. Examples include water dynamics and the flux of trace gases from processes such as photosynthesis, decomposition, or carbon cycling. Ecosystem changes are often considered undesirable if important structural or functional components of the ecosystems are altered following pollutant exposure (U.S. EPA, 2013a, 1998). Methods to assess effects of ozone on ecological structure and function range from indoor controlled environment laboratory and greenhouse studies to field observational studies where

[1] A functional unit consisting of living organisms (biota), their nonliving environment and the interactions within and between them (Team et al., 2014).

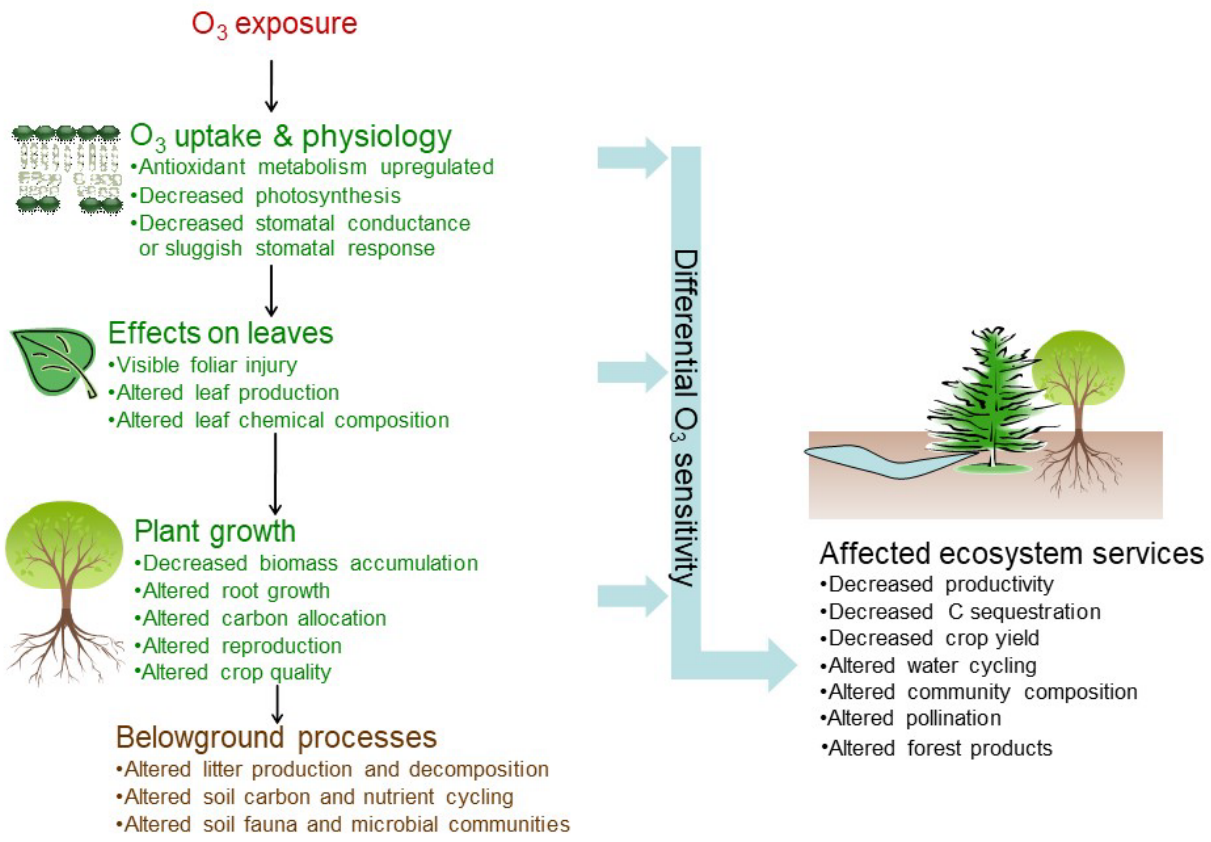
[2] An ecological population consists of interbreeding groups of individuals of the same species that occupy a defined geographic space. Metrics to assess response in ecological populations include changes over time in abundance or density (number of individuals in a defined area), age or sex structure, and production or sustainable rates of harvest (Barnthouse et al., 2008).

[3] Interacting populations of different species occupying a common spatial area form a community (Barnthouse et al., 2008). Community level attributes affected by pollutants include species richness, species abundance, composition, evenness, dominance of one species over another, or size (area) of the community (U.S. EPA, 2013a).

biological changes are measured in uncontrolled situations with high natural variability (U.S. EPA, 2015). Free-air carbon dioxide/ozone enrichment (FACE) systems are a more natural way of estimating ozone effects on aboveground and belowground processes. Research conducted at the SoyFACE facility in Illinois (to study responses in soybean fields) and the Aspen FACE (in operation from 1998 to 2011) system in Wisconsin (to study responses in broadleaf forest) have contributed a substantial body of robust evidence that supports the characterization of ozone effects at multiple scales. Experimental methodologies and approaches are summarized in Section 8.1.2.

Ozone effects on ecosystems are also inter-connected to human health and well-being. The term "ecosystem services" refers to a concept that ecosystems provide benefits to people, directly or indirectly (Costanza et al., 2017), and these benefits are socially and economically valuable goods and services deserving of protection, restoration, and enhancement (Boyd and Banzhaf, 2007). The concept of ecosystem services recognizes that human well-being and survival are not independent of the rest of nature and that humans are an integral and inter-dependent part of the biosphere. Preservation of ecosystem structure and function contributes to the sustainability of ecosystem services that benefit human welfare and society. Ecosystem services affected by ozone include productivity, carbon sequestration, crop yield, water cycling, community composition, pollination, and production of forest commodities (Figure IS-3).

Tropospheric ozone affects terrestrial ecosystems across the entire continuum of biological organization from the cellular and subcellular level to the individual organism up to ecosystem level processes and services (Figure IS-3). For ozone, the majority of evidence for ecological effects is for vegetation. Damage to terrestrial ecosystems caused by ozone is largely a function of uptake of ozone into the leaf via stomata (gas exchange openings on leaves). Subsequent reactions with plant tissues produce reactive oxygen species that affect cellular function (Section 8.1.3 and Figure 8-2). Reduced photosynthesis, altered carbon allocation, and impaired stomatal function lead to observable responses in plants. Observed vegetation responses to ozone include visible foliar injury (Section IS.5.1.1); and whole-plant level responses (Section IS.5.1.2) including reduction in aboveground and belowground growth, altered reproduction, and decreased yield. Plant-fauna linkages affected by ozone include herbivores that feed on ozone-damaged plants and interactions mediated by volatile plant signaling compounds (Section IS.5.1.3). Ozone can result in broad changes in ecosystems such as productivity and carbon sequestration (Section IS.5.1.4), belowground processes (Section IS.5.1.5), terrestrial community composition (Section IS.5.1.6), and water cycling (Section IS.5.1.7). Effects of ozone exposure on aboveground and belowground ecosystem components, across trophic levels, and on carbon allocation at multiple scales of biological organization are described for forests (Section IS.5.1.8.1) and grasslands (Section IS.5.1.8.2).



Source: Adapted from U.S. EPA (2013b).

Figure IS-3 Illustrative diagram of ozone effects cascading up through scales of biological organization from the cellular level to plants and ecosystems.

IS.5.1.1 Visible Foliar Injury

In the 2013 Ozone ISA the evidence was sufficient to conclude a causal relationship between ozone exposure and visible foliar injury on sensitive vegetation across the U.S. Visible foliar injury (Figure IS-4) resulting from exposure to ozone has been well characterized and documented in over six decades of research on many tree, shrub, herbaceous, and crop species using both long-term field studies and laboratory approaches (U.S. EPA, 2013b, 2006a, 1996b, 1986, 1978; NAPCA, 1970; Richards et al., 1958). Recent experimental evidence continues to show a consistent association between visible injury and ozone exposure (Section 8.2). In a recent global-scale synthesis documenting foliar injury from ozone

exposure in the field, across gradients, or in controlled ozone experiments, at least 179 of the identified plant species have populations in the U.S. (Table 8-4). The use of sensitive species as biological indicators to detect phytotoxic levels of ozone is a longstanding and effective methodology. More recently, ozone-sensitive species planted in ozone gardens serve as a source of data on plant responses and as an educational outreach tool. Although visible injury is a bioindicator of the presence of phytotoxic concentrations of ozone in ambient air, it is not always a reliable predictor of other negative effects on vegetation (e.g., growth, reproduction), and foliar injury can vary considerably between and within taxonomic groups (U.S. EPA, 2013b). Since the 2013 Ozone ISA, new sensitive species showing visible foliar injury continue to be identified and the role of modifying factors such as soil moisture and time of day in visible foliar injury symptoms are further characterized (Section 8.2 and Section 8.12). New information is consistent with the conclusions of the 2013 Ozone ISA that **the body of evidence is sufficient to infer a "causal relationship" between ozone exposure and visible foliar injury.**

Note: Tulip poplar (*Liriodendron tulipifera*) on the left and black cherry (*Prunus serotina*) on the right.
Source: USDA Plants Database. Forest Service Forest Inventory and Analysis Program.

Figure IS-4 Representative ozone foliar injury in two common tree species in the U.S.

IS.5.1.2 Whole-Plant Effects

The phytotoxicity of tropospheric ozone has been documented for over 50 years in a variety of plant species (U.S. EPA, 2013b, 2006a, 1996b, 1986, 1978). Ozone-induced oxidative damage at the biochemical and leaf-level (Figure IS-3) lead to changes in photosynthesis and carbon allocation which scale up to reduced growth and impaired reproduction in individual plants. Plant growth is assessed by

quantification of biomass, and analysis of patterns in carbon allocation to aboveground and belowground plant parts. Direct exposure of reproductive tissues to ozone or indirect effects due to injury of vegetative tissues results in fewer total available resources to invest in flowers or seeds. In plants cultivated for agricultural production, damage due to ozone is assessed as reduced crop yield and quality. The evidence indicates causal relationships between ozone and plant growth, plant reproduction, and crop yield, and a likely to be causal relationship between ozone and tree mortality. Such relationships indicate detrimental effects of ozone at the individual-organism scale of biological organization.

In the 2013 Ozone ISA the evidence was sufficient to conclude a causal relationship between ozone exposure and reduced growth of native woody and herbaceous vegetation. As reported in previous assessments, ozone has long been known to cause decreases in growth which is documented in many species including herbaceous plants, grasses, shrubs, and trees (U.S. EPA, 2013b, 2006a, 1996b, 1986, 1978). In an analysis conducted in the 2013 Ozone ISA, effects on growth from the Aspen FACE site closely agreed with exposure-response functions based on data from earlier OTC experiments (U.S. EPA, 2013b). New controlled exposure experiments consistently demonstrate reduced plant growth, and models built with empirical data illustrate potential larger-scale impacts (Section 8.3). In support of findings in the 2013 Ozone ISA and prior AQCDs, a recent international synthesis of studies published over the past five decades documenting reductions in biomass due to ozone exposure. At least 69 plant species that have populations in the U.S. (Table 8-7). In addition to reduced growth, numerous studies from different ecosystems find ozone significantly changes patterns of carbon allocation below and aboveground. New evidence from Aspen FACE for effects on growth and biomass of vegetation includes shifts in wood anatomy (e.g., vessel size and density) and altered distribution of roots across the soil profile following long-term exposure to elevated ozone. Biomass allocation within an individual plant is relevant to whole plant growth and function. New studies provide context for scaling up long-known detrimental effects of ozone on photosynthesis and growth on numerous plant species to changes at the community and ecosystem level (Section 8.3.3). New information is consistent with the conclusions of the 2013 Ozone ISA that the **evidence is sufficient to infer a "causal relationship" between ozone exposure and reduced vegetation growth.**

Ozone effects on metrics of plant reproduction (e.g., flower number, fruit number, fruit weight, seed number, rate of seed germination) in multiple experimental settings (e.g., in vitro, whole plants in the laboratory, whole plants and/or reproductive structures in the green house, and whole plant communities in the field) reported in the 2006 Ozone AQCD, the 2013 Ozone ISA, and this ISA clearly show ozone reduces plant reproduction [Section 8.4; U.S. EPA (2013b, 2006a)]. A qualitative review in the 2006 Ozone AQCD showed that plant reproductive organs may be particularly sensitive to ozone injury (Black et al., 2000). The biological mechanisms underlying ozone's effect on plant reproduction are twofold. They include both direct negative effects on reproductive tissues and indirect negative effects that result from decreased photosynthesis and other whole-plant physiological changes. Since the 2013 Ozone ISA, a quantitative meta-analysis of >100 independent studies of crop and noncrop species (published from 1968 to 2010) showed statistically significant and sometimes large decreases in reproduction (Leisner and

Ainsworth, 2012). Two metrics of plant reproduction, fruit number and fruit weight, show greater reductions under increased ozone when combined across species for ozone concentrations that span 40 to >100 ppb; other metrics do not show such reductions or do so across a narrower range of ozone concentrations. In addition, there is more recent evidence that plant reproductive tissues are directly affected by ozone exposure. There are a few new studies on the effects of ozone on phenology (i.e., timing of germination and flowering), and similar to previously reviewed studies, they have less consistent results than the studies on plant reproduction. In the 2013 Ozone ISA, plant reproduction was considered with plant growth. Increased research and synthesis on ozone effects on plant reproduction (Table 8-9) warrants a separate causality category and evidence is now **sufficient to infer a "causal relationship" between ozone exposure and reduced plant reproduction**.

Multiple studies from different research groups show the co-occurrence of ozone exposure and increased mortality of trees (Section 8.4.3 and Table 8-10). Evidence for plants other than trees is currently lacking. Studies linking ozone and tree mortality are consistent with known and well-established individual plant-level mechanisms that explain ozone phytotoxicity, including variation in sensitivity and tolerance based on age class, genotype, and species. Increased mortality is also consistent with effects at higher levels of biological organization, including changes in vegetation cover and altered community composition (Section 8.10). Since the 2013 Ozone ISA, a large-scale empirical analysis was conducted of factors contributing to annual mortality of trees using over three decades of Forest Inventory and Analysis data. This U.S. Forest Service data showed a significant positive correlation between 8-hour max ozone concentration and tree mortality. Ozone significantly increased tree mortality in 7 out of 10 plant functional types in the eastern and central U.S. (Dietze and Moorcroft, 2011). Experimentally, elevated ozone exposure has been shown to increase mortality in sensitive aspen genotypes (Moran and Kubiske, 2013). This evidence is considered with studies from the 2006 AQCD and 2013 Ozone ISA where decline of conifer forests under ozone exposure was continually observed in several regions [Valley of Mexico, southern France, Carpathian Mountains; U.S. EPA (2013b, 2006a)]. Previous evidence and new evidence evaluated here **is sufficient to infer a "likely to be causal relationship" between ozone exposure and tree mortality.**

In the 2013 Ozone ISA, the evidence was sufficient to conclude a causal relationship between ozone exposure and reduced yield and quality of agricultural crops. The detrimental effect of ozone on crop production has been recognized since the 1960s, and a large body of research has subsequently characterized decreases in yield and quality of a variety of agricultural and forage crops (U.S. EPA, 2013b, 2006a, 1996b, 1986, 1978). The 1986 Ozone AQCD and 1996 Ozone AQCDs reported new OTC experiments on growth and yield, including U.S. EPA's National Crop Loss Assessment Network (NCLAN), that served at the basis for exposure-response functions for agricultural crop species (U.S. EPA, 1996b, 1986). As in noncrop plants, the concentrations at which damage is observed vary from species to species and sometimes between genotypes of the same species.

There is a considerable amount of new research on major U.S. crops, especially soybean, wheat, and other nonsoy legumes at concentrations of ozone occurring in the environment (Section 8.5). For soybean, further refinement of exposure-response curves and analysis of yield data identified a critical level of 32 ppb (7-hour seasonal mean) at which a 5% loss can occur (Osborne et al., 2016). At SoyFACE, a linear decrease in yield at the rate of 37 to 39 kg per hectare per ppb ozone exposure over 40 ppb (AOT40) was observed across two growing seasons (Betzelberger et al., 2012). Meta-analyses published since the 2013 Ozone ISA provide further supporting evidence that current levels of ambient ozone decrease wheat growth and yield and affect reproductive and developmental plant traits important to agricultural and horticultural production (Section 8.5). Recent advances in characterizing ozone's effects on U.S. crop yield include further geographic and temporal refinement of ozone sensitivity and national-scale estimates of crop losses attributable to ozone. Previous research highlighted in the 2013 Ozone ISA and previous AQCDs show ozone effects on crop yield and crop quality (U.S. EPA, 2013b, 2006a, 1996a, 1986, 1978). New information is consistent with the conclusions of the 2013 Ozone ISA that **the body of evidence is sufficient to infer a "causal relationship" between ozone exposure and reduced yield and quality of agricultural crops.**

IS.5.1.3 Effects on Plant-Fauna Interactions

In addition to detrimental effects on plants, elevated ozone can alter ecological interactions between plants and other species, including (1) herbivores consuming ozone-exposed vegetation, (2) pollinators and seed dispersers, and (3) predators and parasitoids of insect herbivores. Many of these interactions are mediated through volatile plant signaling compounds (VPSCs), which plants use to signal to other community members (Section 8.7). Elevated tropospheric ozone has been shown to alter the production, emission, dispersion, and lifespan of VPSCs thereby reducing the effectiveness of these signals. VPSCs play an important role in attracting pollinators, and their alteration can affect the crucial ecosystem service of pollination of wild plants and crops. Ozone exposure also modifies chemistry and nutrient content of leaves (U.S. EPA, 2013b), which may affect the physiology and behavior of herbivores (Section 8.6).

Previous ozone assessments have evaluated studies examining ozone-insect-plant interactions and found information on a wide range of insect species studied in the orders Coleoptera (weevils, beetles), Hemioptera (aphids), and Lepidoptera [moths, butterflies; U.S. EPA (2013b, 2006a, 1996b)]. The majority of studies focused on growth and reproduction while fewer studies considered herbivore survival and population- and community-level responses to ozone. Although statistically significant effects were frequently observed, they did not provide any consistent pattern of response across growth, reproduction, and mortality endpoints. Research has since been published on additional species and at varying levels of ozone exposure, although there is no clear trend in the directionality of response for most effects (Section 8.6). The most commonly measured responses are fecundity, development time, growth, and feeding preferences (Table 8-14). The strongest evidence of ozone effects is from herbivorous insects

with limited evidence from vertebrate feeding studies. Changes in nutrient content and leaf chemistry following ozone exposure likely account for observed effects in herbivores. The body of evidence is **sufficient to infer a "likely to be causal relationship" between ozone exposure and alteration of herbivore growth and reproduction.**

In the 2013 Ozone ISA, a few experimental and modeling studies reported altered insect-plant interactions that are mediated through chemical signaling (U.S. EPA, 2013b). New empirical research from laboratory, greenhouse, OTC, and FACE experiments expand the evidence for altered/degraded emissions of chemical signals from plants and reduced detection of volatile plant signaling compounds by insects, including pollinators, in the presence of ozone (Section 8.7 and Table 8-17). New evidence includes consistent effects in multiple insect species, although this research has examined only a small fraction of the total number of chemical signaling responses potentially affected by ozone. Elevated ozone (≥50 ppb) degrades some plant VPSCs, changing the floral scent composition and reducing floral scent dispersion. Preference studies in a few insect species show reduced pollinator attraction, decreased plant host detection, and altered plant host preference in the presence of elevated, yet environmentally relevant ozone concentrations. Exposure to elevated ozone had variable effects on VPSCs emissions and on the stability of individual volatile compounds with potentially important ecological implications for plant-insect signaling involved in defense against herbivory. To attract predators and parasitoids that target phytophagous insects, plants emit more VPSCs. Parasitoid-host attraction was either reduced, enhanced, or unaffected by elevated ozone. **The body of evidence is sufficient to infer a "likely to be causal relationship" between ozone exposure and alteration of plant-insect signaling.**

IS.5.1.4 Reduced Productivity and Carbon Sequestration

The evidence in the 2013 Ozone ISA was sufficient to conclude a causal relationship between ozone exposure and reduced plant productivity (U.S. EPA, 2013b). Studies at the leaf and plant scale show that ozone decreases plant growth, providing biological plausibility for decreases in ecosystem productivity. Evidence of decreased ecosystem productivity from ozone exposure comes from many different experiments with different study designs in a variety of ecosystems: OTC experiments; long-term, ecosystem-manipulation, chamberless exposure experiments (Aspen FACE, SoyFACE, FinnishFACE); empirical models using eddy covariance measures; forest productivity models parameterized with empirical physiological and tree life history data; and various well-studied ecosystem models and scenario analysis (Section 8.8.1). New information is consistent with the conclusions of the 2013 Ozone ISA that **the body of evidence is sufficient to infer a "causal relationship" between ozone exposure and reduced productivity in terrestrial ecosystems.**

The evidence in the 2013 Ozone ISA was sufficient to conclude a likely causal relationship between ozone exposure and decreased terrestrial carbon sequestration (U.S. EPA, 2013b). Ozone-mediated changes in plant carbon budgets result in less carbon available for allocation to various

pools: reproductive organs, leaves, stems, storage, and roots as well as maintenance, defense, and repair. Changes in allocation (Section 8.8.3) can scale up to population- and ecosystem-level effects, including changes in soil biogeochemical cycling (Section 8.9), increased tree mortality (Section 8.4.3), shifts in community composition (Section 8.10), changes to species interactions (Section 8.6), declines in ecosystem productivity and carbon sequestration (Section 8.8), and alteration of ecosystem water cycling (Section 8.11). The relationship between ozone exposure and terrestrial C sequestration is difficult to measure at the landscape scale. Most of the evidence regarding this relationship is from model simulations, although this endpoint was also examined in a long-term manipulative chamberless ecosystem experiment (Aspen FACE). For example, experiments at Aspen FACE found ozone exposure caused a 10% decrease in cumulative (Net Primary Production) and an associated 9% decrease in ecosystem C storage, although the effects of ozone gradually disappeared towards the end of the 10-year exposure (Talhelm et al., 2014; Zak et al., 2011) possibly due to loss of ozone-sensitive individuals and lower ozone exposures in the last 3 years. Additional studies at this research site suggests that the effects of ozone on plant productivity will be paralleled by large and meaningful decrease in soil C, but the experimental observations reviewed did not find a direct link between ozone, NPP, and soil C pools. It is likely that stand age and development and disturbance regimes are complicating factors in the partitioning of ecosystem-level effects of ozone exposure on carbon sequestration. Even with these limitations, the results from the Aspen FACE experiment and the model simulations provide further evidence that is consistent with the conclusions of the 2013 Ozone ISA that **the body of evidence is sufficient to infer a "likely to be causal relationship" between ozone exposure and reduced carbon sequestration in ecosystems**.

IS.5.1.5 Belowground Processes/Biogeochemical Cycles

In the 2013 Ozone ISA, the evidence was sufficient to conclude that there is a causal relationship between ozone exposure and the alteration of belowground biogeochemical cycles (U.S. EPA, 2013b). It has been documented since the 2006 Ozone AQCD (U.S. EPA, 2006a) that while belowground roots and soil organisms are not exposed directly to ozone, below-ground processes can be affected by ozone through alterations in the quality and quantity of carbon supply to the soils from photosynthates and litterfall (Andersen, 2003). The 2013 Ozone ISA presented evidence that ozone was found to alter multiple belowground endpoints including root growth, soil food web structure, soil decomposer activities, soil respiration, soil carbon turnover, soil water cycling, and soil nutrient cycling. The new evidence since the 2013 Ozone ISA (U.S. EPA, 2013b) included in this assessment confirms ozone effects on soil decomposition (Section 8.9.1), soil carbon (Section 8.9.2), and soil nitrogen (Section 8.9.3), although the direction and magnitude of these changes often depends on the species, site, and length of exposure. As in the 2013 Ozone ISA, the evidence is **sufficient to conclude that there is a "causal relationship" between ozone exposure and the alteration of belowground biogeochemical cycles.**

IS.5.1.6 Terrestrial Community Composition

In the 2013 Ozone ISA, the evidence was sufficient to conclude that there is a likely causal relationship between ozone exposure and the alteration of community composition of some ecosystems, including conifer forests, broadleaf forests, and grasslands, and altered fungal and bacterial communities in the soil in both natural and agricultural systems (U.S. EPA, 2013b). Ozone effects on individual plants can alter the larger plant community as well as the belowground community of microbes and invertebrates, which depend on plants as carbon sources. Ozone may alter community composition by having uneven effects on co-occurring species, decreasing the abundance of sensitive species and giving tolerant species a competitive advantage. Key new studies (Wang et al., 2016; Gustafson et al., 2013) model ozone effects on regional forest composition in the eastern U.S. Additionally, a global-scale synthesis of decades of research on an array of ozone effects on plants confirms that some plant families (e.g., Myrtaceae, Salicaceae, and Onograceae) are more susceptible to ozone damage than others (Bergmann et al., 2017). This lends biological plausibility to a mechanism by which elevated ozone alters terrestrial community composition by inhibiting or removing ozone-sensitive plant species or genotypes, which alters competitive interaction to favor the growth or abundance of ozone-tolerant species or genotypes. In grasslands, previous evidence included multiple studies from multiple research groups to show that elevated ozone shifts the balance among grasses, forbs, and legumes (Section 8.10.1.2). There are new studies with findings consistent with earlier research (Section 8.10), including new studies from European grasslands that found exposure-response relationships between ozone and community composition. The 2013 Ozone ISA presented multiple lines of evidence that elevated ozone alters terrestrial community composition, and recent evidence strengthens our understanding of the effects of ozone upon plant communities, while confirming that the effects of ozone on soil microbial communities are diverse (Table 8-20). The evidence is **sufficient to conclude that there is a "causal relationship" between ozone exposure and the alteration of community composition of some ecosystems.**

IS.5.1.7 Ecosystem Water Cycling

In the 2013 Ozone ISA, the evidence was sufficient to conclude a likely causal relationship between ozone exposure and the alteration of ecosystem water cycling (U.S. EPA, 2013b). Ozone can affect water use in plants and ecosystems through several mechanisms, including damage to stomatal functioning and loss of leaf area, which may affect plant and stand evapotranspiration and lead, in turn, to possible effects on hydrological cycling. Although the 2013 Ozone ISA found no clear universal consensus on leaf-level stomatal conductance response to ozone exposure, many studies reported incomplete stomatal closure and loss of stomatal control in several plant species, which result in increased plant water loss [Section 9.4.5; U.S. EPA (2013b)]. Additionally, ozone has been found to alter plant water use through decreasing leaf area index, accelerating leaf senescence, and by causing changes in branch architecture, which can significantly affect stand-level water cycling. There is mounting biologically relevant, statistically significant, and coherent evidence from multiple studies of various

types about the mechanisms of ozone effects on plant water use and ecosystem water cycling (reduced leaf area, reduced leaf longevity, changes in root and branch biomass and architecture, changes in vessel anatomy, stomatal dysfunction, reduced sap flow; [Section 8.11]). Additionally, there are a few strong studies that scale up these changes to effects on ecosystem scales and show significant effects. The most compelling evidence is from six watersheds in eastern forests and from Aspen FACE (Kostiainen et al., 2014; Sun et al., 2012). This new information adds to the evidence base in the 2013 Ozone ISA and supports the conclusion that **the body of evidence is sufficient to infer a "likely to be causal relationship" between ozone exposure and the alteration of ecosystem water cycling**.

IS.5.1.8 Integration of Ozone Effects in Ecosystems

IS.5.1.8.1 Forests

The effects of ozone exposure on U.S. forests have been an active area of research for over 50 years; evaluation of the role of ozone in forest health declines in the mixed conifer forest of the San Bernardino Mountains began in the early 1960s (Miller and McBride, 1999). Since that time, studies have confirmed variation in sensitivity to ozone exposure in trees and plants based on age class, genotype, and species (U.S. EPA, 2013b, 2006a, 1996b). There has been strong and consistent evidence from multiple studies that ozone-induced oxidative damage leads to declines in photosynthesis and carbon gain, which scale up to reduced growth in individual plants [Section 8.3; U.S. EPA (2013b, 2006a, 1996b). For example, studies from the Aspen FACE experiment have shown that ozone caused reduction in total biomass in quaking aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*), and sugar maple [*Acer saccharum*; U.S. EPA (2013b)]. These findings were overall consistent with open top chamber studies that established ozone exposure-response relationships on growth in a number of native U.S. tree species detailed in previous NAAQS reviews (U.S. EPA, 2013b); these species include aspen, black cherry (*Prunus serotina*), tulip poplar (*Liriodendron tulipifera*), white pine (*Pinus strobus*), and ponderosa pine (*Pinus ponderosa*). In addition to overall reductions in growth, there is evidence that ozone changes plant growth patterns by significantly reducing root growth in some tree species. New information reviewed in the current document support earlier conclusions that ozone reduces photosynthesis, growth, and carbon allocation in a number of plant species found in forest ecosystems.

In addition to declines in root carbon allocation, results from Aspen FACE and other experimental studies reviewed in the 2013 Ozone ISA consistently found that ozone exposure reduced litter production and altered leaf chemistry in trees (U.S. EPA, 2013b). These direct effects of ozone on plants may lead to changes in soil properties and processes in forests, but these changes are dependent on species and genotype of community members, and potentially on other factors like the stage of stand development.

Ozone effects on tree water use can also scale up to significant and measurable effects on ecosystem water cycling in forests. Ozone-mediated impairment of stomatal function in plants has been documented for decades (Keller and Häsler, 1984), although impairment seems to be species specific. Studies continue to show reduced sensitivity of stomatal closing in response to various factors (light, vapor pressure deficit, temperature, soil moisture) when exposed to ozone ("sluggish stomata") in a number of species. A recent meta-analysis of ozone effects on stomatal response in 68 species (including trees, crops, and grassland) found that trees were the most adversely affected, with 73% showing an altered stomatal response. In this synthesis, four tree species exhibited sluggish stomata and 13 showed stomatal opening in response to ozone (Mills et al., 2016; Mills et al., 2013). Ozone exposure has also been linked to decreased water use efficiency and changes in sap flow (Mclaughlin et al., 2007a; Mclaughlin et al., 2007b) and to reduced late-season stream flow in eastern forest ecosystems (Sun et al., 2012).

Differences between species in ozone sensitivity leads to significant changes in forest community composition, as ozone sensitive trees decline and are replaced by less sensitive ones (Section 8.10.1.1). Species-specific responses to ozone in terms of plant growth reductions and biomass allocation are a possible mechanism for these community shifts. In a model simulation of long-term effects of ozone on a typical forest in the southeastern U.S. involving different tree species with varying ozone sensitivity, Wang et al. (2016) found that ozone significantly altered forest community composition and decreased plant biodiversity. Models using Aspen FACE data confirm that ozone effects on tree biomass and productivity scale to affect community composition at the genotype and species level (Moran and Kubiske, 2013). In simulations using Aspen FACE data of northern forests at the landscape level over centuries, elevated ozone altered species abundance and the speed of replacement and succession (Gustafson et al., 2013). Multiple studies from different research groups show the co-occurrence of ozone exposure and increased mortality of trees (Section 8.4.3). In a Bayesian empirical model built with field measurement data from the U.S. Forest Service's Forest Inventory and Analysis program, ozone significantly increased tree mortality in 7 out of 10 plant functional types in the eastern and central U.S. (Dietze and Moorcroft, 2011).

IS.5.1.8.2 Grasslands

In grassland ecosystems, herbaceous plants and grasses in particular are the dominant vegetation rather than shrubs or trees. There is a wide range of sensitivity to ozone in grassland plant communities. For example, studies going back to the 1996 Ozone AQCD show varying ozone sensitivity within the genus *Trifolium* (clover) and general shifts in community biomass that favors grass species (U.S. EPA, 1996a). Evidence reviewed in the 2013 Ozone ISA from a large-scale ozone fumigation experiment in grasslands demonstrated ozone decreases gross primary productivity in these systems (Volk et al., 2011). Experiments reviewed in the 2013 Ozone ISA and previous AQCDs and the current Ozone ISA generally show ozone associated with biomass loss, and a decrease in nutritive quality of forage species. Further,

ozone responses differed across species of grassland plants (Volk et al., 2006). Ozone effects on seed production, germination, and flower number and date of peak flowering have been demonstrated in representative grassland species (Section 8.4).

In grasslands, ozone effects on biodiversity or species composition may result from competitive interactions among plants in mixed-species communities. Studies from mesocosm, OTC, and FACE experiments generally show a shift in the biomass from grass-legume mixtures over time, in favor of grass species. There are also new studies from European grasslands that found exposure-response relationships for community composition (Section IS.5.1.9) that included some species that also grow in the U.S. In the 2013 Ozone ISA, a review of ozone sensitive plant communities [identified as sensitive if they had six or more species that exhibited significant ozone-caused changes in biomass in peer-reviewed controlled experiments; Mills et al. (2007)] found that the largest number of these sensitive communities were associated with grassland ecosystems (U.S. EPA, 2013b). Among grassland ecosystems, alpine grassland, subalpine grassland, woodland fringe, and dry grassland were identified as the most ozone-sensitive communities. Ozone effects on grassland ecosystems extend belowground to the associated soil microbial communities (Section 8.10.2), which show changes in proportions of bacteria or fungi in response to elevated ozone and to fauna that feed on grassland vegetation.

IS.5.1.9 Exposure-Response Relationships

For over 40 years, controlled ozone exposure experiments have yielded a wealth of information on exposure-response relationships. Ozone exposure response has been demonstrated in many tree and herbaceous species, including crops (U.S. EPA, 2013b, 2006a, 1996b, 1986, 1978). As described in Section IS.3.2, various indices have been used to quantify ozone exposure in plants, including threshold-weighted (e.g., SUM06) and continuous sigmoid-weighted (e.g., W126) functions. Weighting of cumulative indices takes into account the greater effects of ozone on vegetation with elevated ozone concentrations. As ozone concentrations increase, plant defense mechanisms are overwhelmed and the capacity of the plant to detoxify reactive oxygen species is compromised (U.S. EPA, 2013b). For decades, it has also been well characterized that plant sensitivity varies by time of day and development stage. Growth responses vary depending on the growth stage of the plant. Furthermore, the time of highest ozone concentrations may not occur at the time of maximum plant uptake. Weighting of hourly concentrations and the diurnal and seasonal time window of exposure are the most important variables in a cumulative exposure index (U.S. EPA, 2013b). For vegetation, quantifying exposure with indices that accumulate the ozone hourly concentrations and preferentially weight the higher concentrations improves the explanatory power of exposure for effects on growth and yield, compared with using indices based on mean and peak exposure values.

None of the information on the effects of ozone on vegetation published since the 2013 Ozone ISA has modified conclusions on quantitative exposure-response relationships. Since the 2013 Ozone

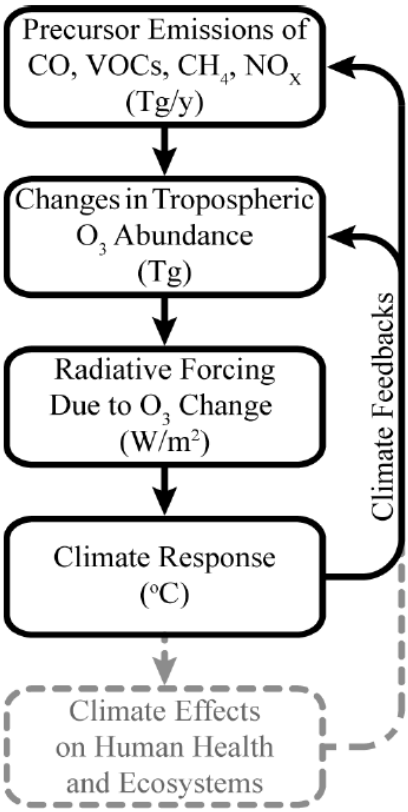
ISA, there have been a few new experimental studies that add more exposure-response relationship information to the large historical database available on U.S. plants (Section 8.13.2). In a new experimental study, Betzelberger et al. (2012) studied seven cultivars of soybean at the SoyFACE experiment in Illinois. They found that the cultivars showed similar responses in a range of ozone exposures expressed as AOT40 (Section IS.3.2). These results support conclusions of previous studies (Betzelberger et al., 2010) and the 2013 Ozone ISA that sensitivity of current soybean genotypes is not different from early genotypes; therefore, soybean response functions developed in the NCLAN program remain valid. A study by Neufeld et al. (2018) provided information on foliar injury response on two varieties of cutleaf coneflower (*Rudbeckia laciniata*). For example, one variety had statistically detectable foliar injury when the 24 hour W126 index reached 23 ppm hour (12-hour AOT40 = 12 ppm hour). Although recent U.S. exposure-response studies in experimental systems are limited, U.S. and international syntheses have highlighted response function information (e.g., biomass growth, foliar injury, yield) for grassland and other plant species that occur in the U.S. (see Section 8.13.2). For example, in a synthesis of previously published studies, linear relationships of biomass growth in response to ozone were found using AOT40 for 87 grassland species that occur in Europe (van Goethem et al., 2013). Seventeen of these species are native to the U.S. and 65 additional species have been introduced to the U.S. and may have significant ecological, horticultural, or agricultural value (USDA, 2015). This study has the most significant amount of new exposure response information for plants in the U.S.

IS.5.2 Effects on Climate

Changes in the abundance of tropospheric ozone perturb the radiative balance of the atmosphere by interacting with incoming solar radiation and outgoing longwave radiation. This effect is quantified by the radiative forcing metric. Radiative forcing is the perturbation in net radiative flux at the tropopause (or top of the atmosphere) caused by a change in radiatively active forcing agent(s) after stratospheric temperatures have readjusted to radiative equilibrium (stratospherically adjusted radiative forcing). Through this effect on the Earth's radiation balance, tropospheric ozone plays a major role in the climate system, and increases in its ozone abundance contribute to climate change (Myhre et al., 2013).

For ozone effects on climate (Appendix 9), there are inter-connections to human health and ecosystems. As discussed in the 2013 Ozone ISA, the Earth's atmosphere-ocean system responds to changes in radiative forcing with a climate response, including a change in near-surface air temperature with associated impacts on precipitation and atmospheric circulation patterns. This climate response causes downstream climate-related health and ecosystem effects, such as the combined health effects of both climate (e.g., heat waves) and ozone air quality or redistribution of diseases or ecosystem characteristics. Feedbacks from both the direct climate response and such downstream effects can, in turn, affect the abundance of tropospheric ozone and ozone precursors through multiple mechanisms (Figure IS-5). Variations in climate can potentially alter the conditions that lead to the formation,

transport, and persistence of ozone in the troposphere (Appendix 1), as well as increased vulnerability of plants and ecosystems. The degree to which climate and weather alter the effects of ozone is context and species specific because damage to terrestrial ecosystems caused by ozone is largely a function of plant uptake. Factors that modify the effects of ozone in ecosystems, including carbon dioxide, weather, and climate are discussed in Section 8.12.



Source: U.S. EPA (2013b).

Figure IS-5 Schematic illustrating the effects of tropospheric ozone on climate; including the relationship between precursor emissions, tropospheric ozone abundance, radiative forcing, climate response and climate impacts.

Characterization of ozone impacts on radiative forcing (Section 9.2) builds on the findings in the 2013 Ozone ISA and draws heavily on the IPCC Assessment Reports. In the 2013 Ozone ISA, the evidence was sufficient to conclude a causal relationship between tropospheric ozone and radiative forcing (U.S. EPA, 2013b). The 2013 Ozone ISA reported a radiative forcing (RF) of 0.35 W/m^2 from the change in tropospheric ozone abundance from preindustrial times to the present (1750 to 2005) based on

multimodel studies (Forster et al., 2007). The most recent IPCC assessment, AR5, reports tropospheric ozone RF as 0.40 (0.20 to 0.60) W/m^2 (Myhre et al., 2013), which is within range of previous assessments (i.e., AR4). There have also been a few individual studies of tropospheric ozone RF (Section 9.2) since AR5 , including the study of tropospheric ozone RF based on the Coupled Model Intercomparison Project Phase 6 (CMIP6) data set, and the Atmospheric Chemistry and Climate Model Intercomparison Project (ACCMIP) multimodel study of tropospheric chemistry, all of which reinforce the AR5 estimates and continues to support **a "causal relationship" between tropospheric ozone and RF.**

In the 2013 Ozone ISA, the evidence was sufficient to conclude a likely to be causal relationship, via radiative forcing, between tropospheric ozone and climate change (now referred to as "temperature, precipitation, and related climate variables"; the revised title for this causality statement provides a more accurate reflection of the available evidence) between tropospheric ozone and climate change (U.S. EPA, 2013b). New studies reviewed in Section 9.3 are consistent with previous estimates and the effect of tropospheric ozone on global surface temperature continues to be estimated at roughly 0.1−0.3°C since preindustrial times (Xie et al., 2016; Myhre et al., 2013), with larger effects regionally. In addition to temperature, ozone changes affect other climate metrics such as precipitation and atmospheric circulation patterns (Macintosh et al., 2016; Allen et al., 2012; Shindell et al., 2012). All of this evidence reinforces **a "likely to be causal relationship" between temperature, precipitation and related climate variables**.

IS.6 Key Aspects of Health And Welfare Effects Evidence

There is extensive scientific evidence that demonstrates health and welfare effects from exposure to ozone. In assessing the older and more recent evidence, the U.S. EPA characterizes the key strengths and remaining limitations of this evidence. In the process of assessing the evidence across studies and scientific disciplines and ultimately forming causality determinations, the U.S. EPA takes into consideration multiple aspects that build upon the Hill criteria (Hill, 1965) and include, but are not limited to, consistency in findings, coherence of findings, and evidence of biological plausibility [see U.S. EPA (2015)]. As documented by the extensive evaluation of evidence throughout the subsequent appendices to this ISA, the U.S. EPA carefully considers uncertainties in the evidence, and the extent to which recent studies have addressed or reduced uncertainties from previous assessments, as well as the strengths of the evidence. Uncertainties considered in the epidemiologic evidence, for example, include potential confounding by copollutants or covarying factors and exposure error. The U.S. EPA evaluates many other important considerations (not uncertainties) such as coherence of evidence from animal and human studies, heterogeneity of risk estimates, and the shape of the concentration-response relationships. All aspects are considered along with the degree to which chance, confounding, and other biases affect interpretation of the scientific evidence in the process of drawing scientific conclusions and making causality determinations. Uncertainties do not necessarily change the fundamental conclusions of the literature base. In fact, some conclusions may be robust to such uncertainties. Where there is clear

evidence linking ozone with health and welfare effects with or despite minimal remaining uncertainties, the U.S. EPA makes a determination of a causal or likely to be causal relationship.

IS.6.1 Health Effects Evidence: Key Findings

A large body of scientific evidence spanning many decades clearly demonstrates there are health effects related to both short- and long-term ozone exposure (Figure IS-6). The strongest evidence supports a relationship between ozone exposure and respiratory health effects. The collective body of evidence for each health outcome category evaluated in this ISA is systematically considered and assessed, including the inherent strengths, limitations, and uncertainties in the overall body of evidence, resulting in the causality determinations detailed in Table IS-1. Through identification of the strengths and limitations in the evidence, this ISA may help in the prioritization of research efforts to support future ozone NAAQS reviews.

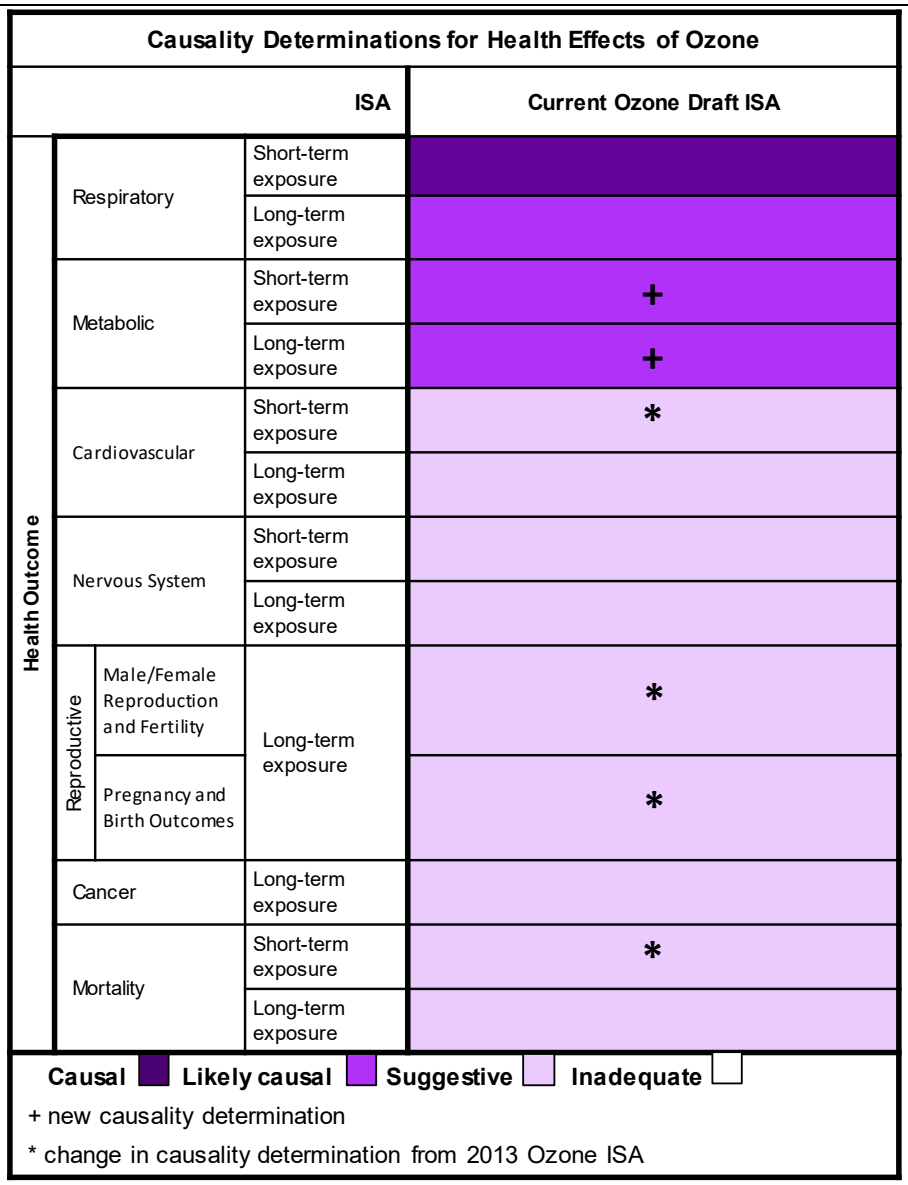


Figure IS-6 Causality determinations for health effects of short- and long-term exposure to ozone.

An inherent strength of the evidence integration in this ISA is the extensive amount (in both breadth and depth) of available evidence resulting from decades of scientific research that describes the relationship between both short- and long-term ozone exposure and health effects. The breadth of the enormous database is illustrated by the different scientific disciplines that provide evidence (e.g., controlled human exposure, epidemiologic, animal toxicological studies), the range of health outcomes examined (e.g., respiratory, cardiovascular, metabolic, reproductive, and nervous system effects, as well as cancer and mortality), and the large number of studies within several of these outcome

categories. The depth of the literature base is exemplified by the examination of effects that range from biomarkers of exposure, to subclinical effects, to overt clinical effects, and even mortality. Depth is further demonstrated through the variety of the study designs used across the scientific disciplines and exposure duration periods.

In this ISA, modern systematic review methodologies are applied to identify and characterize this expansive evidence base (see Appendix 10 for details). The evidence is effectively integrated from (1) different scientific disciplines, (2) a variety of study designs within the same scientific discipline, and (3) a span of different health endpoints within a health effect category. Finally, a formal framework is systematically applied to draw conclusions about the causal nature of the relationship between ozone exposure and health effects (U.S. EPA, 2015).

A first step in integrating evidence for a health effect category is to consider the biological plausibility of health responses observed in association with ozone exposure. The process for characterizing biological plausibility is described in Section IS.4.2. Recent studies in humans and animals expand on findings from prior assessments (U.S. EPA, 2013b, 2006a, 1996a) to further understand plausible pathways that may underlie the observed respiratory health effects related to short-term exposure to ozone (Figure 3-1). Consistent evidence for several respiratory endpoints within a large number of animal toxicological, controlled human exposure, and epidemiologic studies, as well as coherent evidence across these studies contribute to a large degree of certainty in assessing the relationship between short-term ozone exposure and this health effect category. Furthermore, uncertainty is addressed by epidemiologic studies that examine potential copollutant confounding, examine different model specifications, and account for potential confounders.

Older and more recent studies also provide evidence for biologically plausible pathways that may underlie respiratory effects related to long-term ozone exposure, and metabolic effects related to both short- and long-term exposure. Epidemiologic studies of long-term ozone exposure and respiratory effects are supported by numerous animal toxicological studies examining related endpoints. This coherence reduces some of the uncertainty related to the independence of the ozone effect, though there are some remaining uncertainties for these health effects. For example, there are still relatively few studies evaluating the effect of ozone exposure on metabolic effects in human populations (i.e., controlled human exposure or epidemiologic studies).

With regard to short-term ozone exposure and cardiovascular health effects, there is some evidence for biologically plausible pathways for the worsening of IHD or HF, the development of heart attack or stroke, and cardiovascular-related ED visits and hospital admission (Figure 4-1). However, the evidence mainly comes from animal toxicological studies, is generally not supported by controlled human exposure studies, and is limited for epidemiologic studies. While there is some epidemiologic evidence that short-term ozone concentrations are associated with total mortality, the evidence of plausible steps that could lead to death (e.g., IHD, HF) are lacking in epidemiologic studies that examined these types of endpoints (e.g., hospital admissions for IHD or HF). Furthermore, controlled human exposure studies in

healthy adults generally do not show that short-term ozone exposure leads to the types of intermediate health effects (e.g., impaired vascular function, changes in ECG measures) that could lead to IHD or stroke. Most of the studies supporting the biological plausibility of epidemiologic studies of mortality are from animal studies that are not generally supported by studies in humans.

Older and recent studies examining short- or long-term ozone exposure and several other health effects (i.e., nervous system effects, reproductive effects, cancer) are few or report inconsistent evidence of an association with the health effect of interest. For these health effects, there is often limited coherence across studies from different scientific disciplines, and limited evidence for biologically plausible pathways by which effects could occur. Other sources of uncertainty, such as limited assessment of potential copollutant confounding, are inherent in these evidence bases.

There is strong and consistent animal toxicological evidence linking short- and long-term ozone exposure with respiratory, cardiovascular, and metabolic health effects. However, several uncertainties should be considered when evaluating and synthesizing evidence from these studies. Experimental studies are often conducted at ozone concentrations higher than those observed in ambient air (i.e., 250 to >1,000 ppb) to evoke a response within a reasonable study length. These studies are informative and the conduct of studies at these concentrations is commonly used for identifying potential human hazards. There are also substantial differences in exposure concentrations and exposure durations between animal toxicological and controlled human exposure studies. For example, animal toxicological studies generally expose rodents to 250 to >1,000 ppb, while controlled human exposure studies generally expose humans to 60 to 300 ppb. Additionally, a number of animal toxicological studies were performed in rodent disease models, while controlled human exposure studies generally are conducted in healthy individuals. This difference could explain some of the inconsistencies across studies from these scientific disciplines. Controlled human exposure studies do not typically include unhealthy or diseased individuals for ethical reasons; therefore, this represents an important uncertainty to consider in interpreting the results of these studies. Additional animal toxicological studies conducted at lower concentrations could help to reduce this uncertainty. Finally, in addition to exposure concentration and disease status differences in physiology (e.g., rodents are obligate nose breathers), differences in the duration and timing of exposure (e.g., rodents are exposed during the day, during their resting cycle, while humans are exposed during the day when they are normally active), and differences in the temperature at which the exposure was conducted, may contribute to the lack of coherence between results of animal and human studies. Dosimetric studies of animals and humans might inform understanding of the potential role of such differences.

Controlled human exposure studies provide the strongest evidence for the effects of short-term ozone exposure on respiratory effects. There are, however, several limitations of controlled human exposure studies. These include the study of generally healthy individuals and the measurement of relatively minor health effects (or indices of health effects) for ethical reasons (unhealthy or very sick people are rarely studied). Therefore, individuals that may be at greater risk are not included in controlled

human exposure studies. However, controlled human exposure studies offer several strengths for studying human health effects from ozone exposure. The experimental nature of controlled human exposure studies allows them to virtually eliminate the chance, bias, and other potential confounding factors inherent in observational epidemiologic studies. In addition, controlled human exposure studies are not susceptible to some of the uncertainties commonly attributed to animal toxicological studies, such as the need to extrapolate between animal models and humans, and the use of relatively high ozone concentrations compared with concentrations typically encountered in ambient air.

Though susceptible to chance, bias, and other potential confounding due to their observational nature, epidemiologic studies have the benefit of evaluating real-world exposure scenarios and can include populations that cannot typically be included in controlled human exposure studies, such as children, pregnant women, and individuals with pre-existing disease. In addition, innovations in epidemiologic study designs and methods have substantially reduced the role of chance, bias, and other potential confounders in well-designed, well-conducted epidemiologic studies. Many epidemiologic studies have been conducted in diverse geographic locations, encompassing different population demographics, and using a variety of exposure assignment techniques. They continue to report consistent, positive associations between short-term ozone exposure and health effects. When combined with coherent evidence from experimental studies, the epidemiologic evidence can support and strengthen determinations of the causal nature of the relationship between health effects and exposure to ozone at relevant ambient air concentrations.

The most common source of uncertainty in epidemiologic studies of ozone is exposure measurement error. The majority of recent epidemiologic studies of long-term ozone exposure use concentrations from fixed-site monitors as exposure surrogates. Some recent epidemiologic studies incorporate new ozone exposure assignment methods that integrate several sources of available data (i.e., satellite observations, CTM predictions, and ambient monitors) into a spatiotemporal model. These hybrid methods are well validated by ozone monitors in areas with moderate to high population density, and they better allow for the inclusion of populations from less urban areas, where monitor density is lower. Relatively low spatial variability of ozone (compared with UFP, CO, NO_2, or SO_2) in most locations increases confidence in application of these methods for predicting ozone exposure.

Additionally, the populations included in epidemiologic studies have long-term, variable, and uncharacterized exposures to ozone and other ambient pollutants. Epidemiologic studies evaluate the relationship between health effects and specific ozone concentrations during a defined study period. The generally consistent and coherent associations observed in these epidemiologic studies contribute to the causality determinations and the causal nature of the effect of ozone exposure on health effects, However, they do not provide information about which averaging times or exposure metrics may be eliciting the health effects under study.

Each of the exposure assignment methods used in short- and long-term ozone exposure epidemiologic studies have inherent strengths and limitations, and exposure measurement errors

associated with those methods contribute bias and uncertainty to health effect estimates. For short-term exposure studies, exposure measurement error generally leads to underestimation and reduced precision of the association between short-term ozone concentrations and health effects. For long-term exposure studies, exposure measurement error can bias effect estimates in either direction, although it is more common that effect estimates are underestimated. Underestimation of health effect associations in short- and long-term ozone exposure studies implies that true health effect associations are even larger than what is reported in epidemiologic studies. The magnitude of bias in the effect estimate is likely small for ozone, because ozone concentrations do not vary over space as much as other criteria pollutants, such as NO_X or SO_2 (Section 2.6).

Copollutant analyses were limited in epidemiologic studies evaluated in the 2013 Ozone ISA but indicated that associations between ozone concentrations and health effects were not confounded by copollutants or aeroallergens (U.S. EPA, 2013b). Copollutant analyses are more prevalent in recent studies and continue to suggest that observed associations are independent of coexposures to correlated pollutants or aeroallergens. Despite expanded copollutant analyses in recent studies, determining the independent effects of ozone in epidemiologic studies is complicated by the high copollutant correlations observed in some studies, and the possibility for effect estimates to be overestimated for the better measured pollutant in copollutant models (Section 2.5). That said, some studies report modest copollutant correlations, which suggests that strong confounding due to copollutants is unlikely. In addition, evidence from copollutant models is available for a small subset of all the pollutants that co-occur with ozone in the air. Nonetheless, the consistency of associations observed across studies with different copollutant correlations, the generally robust associations observed in copollutant models, and evidence from other scientific discipline generally provide compelling evidence for an independent effect of ozone exposure on human health and reduce the uncertainties associated with potential copollutant confounding.

The 2013 Ozone ISA noted that multicity epidemiologic studies, particularly examining short-term ozone exposure and mortality, reported evidence of heterogeneity in the magnitude and precision of risk estimates across cities. There are few recent multicity studies of short-term ozone exposure and health effects that could allow an evaluation of such heterogeneity; thus, the uncertainty identified in the 2013 Ozone ISA remains.

Examination of the concentration-response (C-R) relationship has primarily been conducted in studies of short-term ozone exposure and respiratory health effects or mortality, with some more recent studies characterizing this relationship for long-term ozone exposure and mortality. Across recent studies that used a variety of statistical methods to examine potential deviations from linearity, evidence continues to support a linear C-R relationship, but with less certainty in the shape of the curve at lower concentrations (i.e., below 30−40 ppb). In addition, some studies evaluate the potential for a population-level threshold, below which health effects would unlikely be observed. Generally, these studies conclude that if a population-level threshold exists, it would occur at lower concentrations (i.e., below 30−40 ppb) where there is less certainty in the ozone-health effect relationship due to few

observations at these lower concentrations. Similar to the uncertainty mentioned previously, the populations included in epidemiologic studies have long-term, variable, and uncharacterized exposures to ozone and other ambient pollutants. Epidemiologic studies evaluate the C-R relationship between health effects and specific ozone concentrations during a defined study period. The generally consistent C-R relationships observed in these epidemiologic studies do not indicate which averaging times or exposure metrics may be eliciting the health effects under study.

IS.6.2 Welfare Effects Evidence: Key Findings

The collective body of evidence for each welfare endpoint evaluated in this ISA was carefully considered and assessed, including the inherent strengths, limitations, and uncertainties in the overall body of evidence, resulting in the causality determinations for ecological effects detailed in Table IS-2 and effects on climate in Table IS-3.

IS.6.2.1 Ecological Effects

A large body of scientific evidence spanning more than 60 years clearly demonstrates there are effects on vegetation and ecosystems attributed to ozone exposure resulting from anthropogenic activities (U.S. EPA, 2013b, 2006a, 1996b, 1986, 1978; NAPCA, 1970; Richards et al., 1958). There is high certainty in ozone effects on impairment to leaf physiology as mechanisms for cascading effects at higher levels of biological organization (e.g., plant growth, ecosystem productivity; Section 8.1.3; Figure IS-7). The overwhelming strength of many of the studies is that they consist of controlled ozone exposure to plants, plots of forests, and crop fields to eliminate any confounding factors (Section 8.12). For example, for ozone effects on plants, there are robust exposure response functions (i.e., from carefully controlled experimental conditions, involving multiple concentrations and based on multiple studies) for about a dozen important tree species and a dozen major commodity crop species.

The use of visible foliar injury to identify phytotoxic levels of ozone is an established and widely used methodology. However, foliar injury is not always a reliable indicator of other negative effects on vegetation (e.g., growth, reproduction), and there is a lack of quantitative exposure-response information that takes into account the important role of soil moisture in foliar injury. As documented in the 2013 Ozone ISA (Table IS-13) and retained in the current Ozone ISA (Figure IS-7), there are causal relationships between ozone exposure and visible foliar injury at the individual-organism level, and causal relationships between ozone exposure and reduced plant growth and crop yield from the individual to population levels. Since the 2013 Ozone ISA (U.S. EPA, 2013b), a meta-analysis of existing literature on plant reproductive metrics and new research support a causal relationship between ozone exposure and reduced plant reproduction. In the previous ISA, plant reproduction was considered within the broader

category of growth but the current body of evidence for this endpoint warrants a separate causality category.

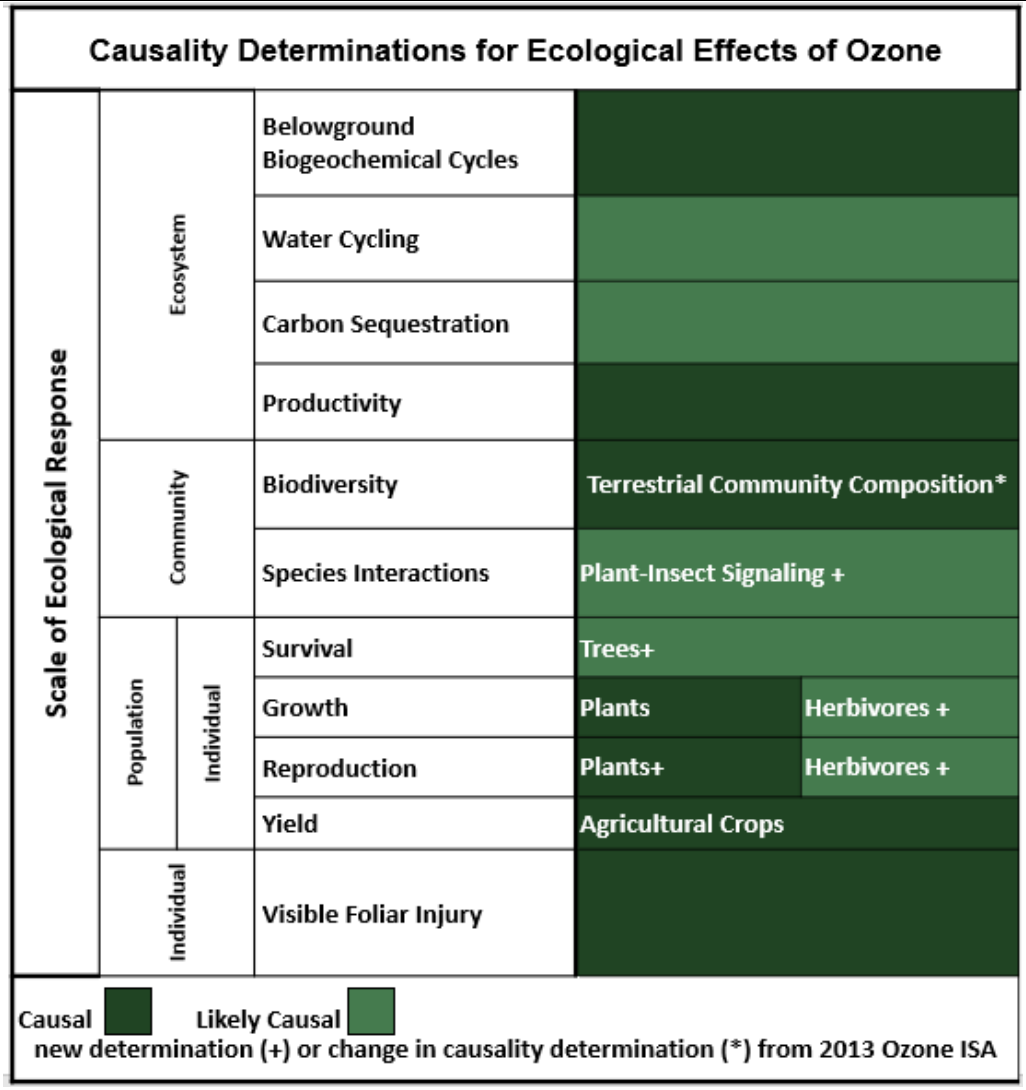


Figure IS-7 Causality determinations for ozone across biological scales of organization and taxonomic groups.

While the effect of ozone on vegetation is well established in general, there are some knowledge gaps regarding precisely which species are sensitive and what exposures elicit adverse responses for many species. Currently there are over 40,000 plants and lichens occurring in the U.S. as documented by the USDA PLANTS database (USDA, 2015). It not feasible to know what the effects are on all U.S. species and what the ecological consequences of the differential sensitivities are of these species. However, there

have been many important trees, crops, and other plants studied to indicate the potential array of ecological effects in the U.S. The exposure-response relationships for a subset of individual plants are discussed in Section 8.13. Within and between these species there is a range of sensitivities, and it is difficult to identify the representativeness of these relationships within the wider population of plants that occur in the U.S. There are also uncertainties about how plant responses change with age and size. The technique of meta-analysis is one approach that can be used to consolidate and extract a summary of significant responses from a selection of previously published studies. These studies can show causal links of ecological endpoints to ozone exposure; they are robust enough to overcome individual variation and are useful for looking at trends in plant response across, for example, geographic locations, environmental conditions, plant functional groups, and ecosystems.

The majority of evidence for ecological effects of ozone is for vegetation. Fewer studies examine plant-ozone-insect interactions. There are multiple, statistically significant findings showing ozone effects on fecundity and growth in insect herbivores. However, no consistent directionality of response is observed across the literature, and uncertainties remain in regard to different plant consumption methods across species and the exposure conditions associated with particular severities of effects. There is also variation in study designs and endpoints used to assess ozone responses. Most responses observed in insects appear to be indirect (i.e., mediated through ozone effects on vegetation, although direct effects of ozone exposure on insects could also play a role). New research in chemical ecology has provided clear evidence of ozone modification of VPSCs and behavioral responses of insects to these modified chemical signatures; however, most of these studies have been carried out in laboratory conditions rather than in natural environments. Characterization of airborne pollutant effects on chemical signaling in ecosystems is an emerging area of research with information available on a relatively small number of insect species and plant-insect associations and knowledge gaps in the mechanisms and consequences of modulation of VPSCs by ozone.

There are some uncertainties in characterizing how ozone damage to leaves and individual plant species scale up to ecological communities and ecosystem processes. Although scaling ozone effects to the ecosystem level remains a challenge, there is a large body of knowledge of how ecosystems work through ecological observations and models that simulate processes at multiple scales. The models scale up and attempt to capture interactive effects of multiple stressors in ecosystems in the field. Studies of ozone effects beyond the plant scale use a combination of empirical studies and statistical modeling, or large controlled exposure ecosystem experiments, or field observations along ozone gradients. Interactive effects in natural ecosystems with multiple stressors (e.g., drought, disease) are difficult to study, but can be addressed through different statistical methods. For example, multivariate models and mechanistic models have been used for studying ozone with other environmental factors [e.g., Dietze and Moorcroft (2011)] and for scaling up ozone effects on tree growth and water use to ecosystem stream flow [e.g., Sun et al. (2012)]. Another approach is to use meta-analysis techniques to examine trends across large geographic areas or at higher biological levels of organization (e.g., plant functional groups, forest types).

More research on ecosystem-level responses will strengthen understanding of scaling across different levels of biological organization.

In general, the most promising approaches to scaling ozone effects at the ecosystem level include evaluation of ecological response using a suite of parameters and exposure-response functions, both empirical and modeled. The quantitative uncertainty of empirically observed variables in ecology is determined by the use of statistics. In general, ecological endpoints affected by ozone were reported in the ISA if they were statistically significant. In addition, models of chemical and ecological processes provide representations of biological interactions through mathematical expressions. The models used can be complex, including many interacting variables. Each of the input variables in a model has some uncertainty. Models can also be evaluated on the basis of the mechanistic understanding of how ecological systems work and how ozone effects may propagate through ecological systems.

IS.6.2.2 Effects on Climate

Ozone is an important greenhouse gas, and increases in its abundance have affected the Earth's climate. Over the last century, global average surface air temperature has increased by approximately 1.0°C, and emissions of greenhouse gases are the dominant cause (Wuebbles et al., 2017; IPCC, 2013). There are many other aspects of the global climate system that are changing in addition to this warming, including melting glaciers, reductions in snow cover and sea ice, sea level rise, ocean acidification, and increases in the frequency or intensity of many types of extreme weather events (Wuebbles et al., 2017). The magnitude of future climate change, globally and regionally, and in terms of both temperature increases and these other types of associated impacts, will depend primarily on the amount of greenhouse gases emitted globally (Wuebbles et al., 2017; IPCC, 2013). The most recent IPCC report, AR5, which is a comprehensive assessment of the peer-reviewed literature, reported tropospheric ozone RF as 0.40 (0.20 to 0.60) W/m^2 (Myhre et al., 2013). In the 2013 Ozone ISA, there was a causal relationship between tropospheric ozone and RF and a likely to be causal relationship between tropospheric ozone and climate change (U.S. EPA, 2013b). None of the new studies indicate a change to either causality determination (Figure IS-8).

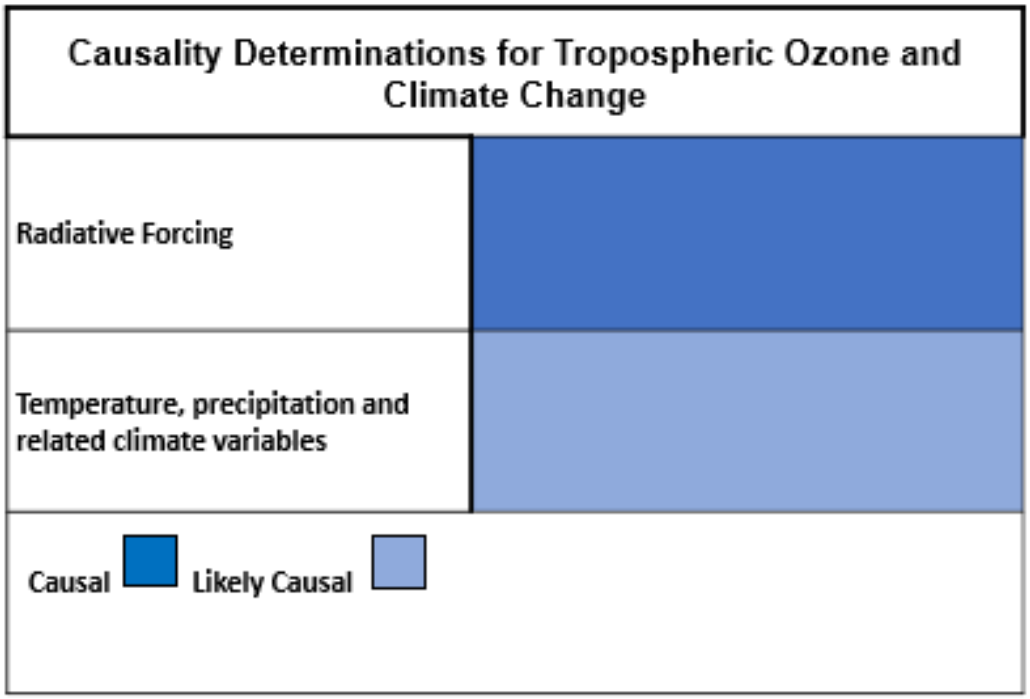


Figure IS-8 Causality determinations for tropospheric ozone and climate change.

While the warming effect of tropospheric ozone in the climate system is well established in general, precisely quantifying changes in surface temperature due to tropospheric ozone changes, along with related climate effects, requires complex climate simulations, including important feedbacks and interactions. Current limitations in climate modeling tools, variation across models, and the need for more comprehensive observational data on these effects represent sources of uncertainty in quantifying the precise magnitude of climate responses to ozone changes, particularly at regional scales (Myhre et al., 2013). Some new research has explored certain additional aspects of the climate response to ozone RF beyond global and regional temperature change. Specifically, ozone changes are understood to affect other climate metrics, such as precipitation and atmospheric circulation patterns, and new evidence has continued to support and further quantify this understanding. Various uncertainties render the precise magnitude of the overall effect of tropospheric ozone on climate more uncertain than that of the well mixed GHGs (Myhre et al., 2013). These include the remaining uncertainties in the magnitude of RF estimated to be attributed to tropospheric ozone.

IS.7 References for Integrative Synthesis

Adams, WC. (2002). Comparison of chamber and face-mask 6.6-hour exposures to ozone on pulmonary function and symptoms responses. Inhal Toxicol 14: 745-764. http://dx.doi.org/10.1080/08958370290084610.

Adams, WC. (2003). Comparison of chamber and face mask 6.6-hour exposure to 0.08 ppm ozone via square-wave and triangular profiles on pulmonary responses. Inhal Toxicol 15: 265-281. http://dx.doi.org/10.1080/08958370390168283.

Adams, WC. (2006). Comparison of chamber 6.6-h exposures to 0.04-0.08 ppm ozone via square-wave and triangular profiles on pulmonary responses. Inhal Toxicol 18: 127-136. http://dx.doi.org/10.1080/08958370500306107.

Alberti, KG; Eckel, RH; Grundy, SM; Zimmet, PZ; Cleeman, JI; Donato, KA; Fruchart, JC; James, WP; Loria, CM; Smith, SC. (2009). Harmonizing the metabolic syndrome: A joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 120: 1640-1645. http://dx.doi.org/10.1161/CIRCULATIONAHA.109.192644.

Allen, RJ; Sherwood, SC; Norris, JR; Zender, CS. (2012). Recent Northern Hemisphere tropical expansion primarily driven by black carbon and tropospheric ozone. Nature 485: 350-354. http://dx.doi.org/10.1038/nature11097.

Andersen, CP. (2003). Source-sink balance and carbon allocation below ground in plants exposed to ozone. New Phytol 157: 213-228. http://dx.doi.org/10.1046/j.1469-8137.2003.00674.x.

Barnthouse, LW; Munns, RM, Jr; Sorensen, MT. (2008). Population-level ecological risk assessment. Pensacola, FL: Taylor & Francis. http://www.crcpress.com/product/isbn/9781420053326.

Bergmann, E; Bender, J; Weigel, HJ. (2017). Impact of tropospheric ozone on terrestrial biodiversity: A literature analysis to identify ozone sensitive taxa. J Appl Bot Food Qual 90: 83-105. http://dx.doi.org/10.5073/JABFQ.2017.090.012.

Betzelberger, A; Yendrek, CR; Sun, J; Leisner, CP; Nelson, RL; Ort, DR; Ainsworth, EA. (2012). Ozone exposure response for U.S. soybean cultivars: Linear reductions in photosynthetic potential, biomass, and yield. Plant Physiol 160: 1827-1839. http://dx.doi.org/10.1104/pp.112.205591.

Betzelberger, AM; Gillespie, KM; Mcgrath, JM; Koester, RP; Nelson, RL; Ainsworth, EA. (2010). Effects of chronic elevated ozone concentration on antioxidant capacity, photosynthesis and seed yield of 10 soybean cultivars. Plant Cell Environ 33: 1569-1581. http://dx.doi.org/10.1111/j.1365-3040.2010.02165.x.

Black, VJ; Black, CR; Roberts, JA; Stewart, CA. (2000). Tansley Review No. 115: Impact of ozone on the reproductive development of plants. New Phytol 147: 421-447. http://dx.doi.org/10.1046/j.1469-8137.2000.00721.x.

Blackwell, DL; Lucas, JW; Clarke, TC. (2014). Summary health statistics for U.S. adults: National Health Interview Survey, 2012. (Vital and Health Statistics: Series 10, No. 260). Hyattsville, MD: National Center for Health Statistics. http://www.cdc.gov/nchs/data/series/sr_10/sr10_260.pdf.

Bloom, B; Jones, LI; Freeman, G. (2013). Summary health statistics for U.S. children: National Health Interview Survey, 2012. (Vital and Health Statistics: Series 10, No. 258). Hyattsville, MD: National Center for Health Statistics. http://www.cdc.gov/nchs/data/series/sr_10/sr10_258.pdf.

Boyd, J; Banzhaf, S. (2007). What are ecosystem services? The need for standardized environmental accounting units. Ecol Econ 63: 616-626.

Brown, JS; Bateson, TF; Mcdonnell, WF. (2008). Effects of exposure to 0.06 ppm ozone on FEV1 in humans: A secondary analysis of existing data. Environ Health Perspect 116: 1023-1026. http://dx.doi.org/10.1289/ehp.11396.

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

Costanza, R; De Groot, R; Braat, L; Kubiszewski, I; Fioramonti, L; Sutton, P; Farber, S; Grasso, M. (2017). Twenty years of ecosystem services: How far have we come and how far do we still need to go? Ecosyst Serv 28: 1-16. http://dx.doi.org/10.1016/j.ecoser.2017.09.008.

Darrow, LA; Klein, M; Sarnat, JA; Mulholland, JA; Strickland, MJ; Sarnat, SE; Russell, AG; Tolbert, PE. (2011). The use of alternative pollutant metrics in time-series studies of ambient air pollution and respiratory emergency department visits. J Expo Sci Environ Epidemiol 21: 10-19. http://dx.doi.org/10.1038/jes.2009.49.

Di, Q; Dai, L; Wang, Y; Zanobetti, A; Choirat, C; Schwartz, JD; Dominici, F. (2017). Association of short-term exposure to air pollution with mortality in older adults. JAMA 318: 2446-2456. http://dx.doi.org/10.1001/jama.2017.17923.

Dietze, MC; Moorcroft, PR. (2011). Tree mortality in the eastern and central United States: Patterns and drivers. Global Change Biol 17: 3312-3326. http://dx.doi.org/10.1111/j.1365-2486.2011.02477.x.

Folinsbee, LJ; Mcdonnell, WF; Horstman, DH. (1988). Pulmonary function and symptom responses after 6.6-hour exposure to 0.12 ppm ozone with moderate exercise. J Air Waste Manag Assoc 38: 28-35. http://dx.doi.org/10.1080/08940630.1988.10466349.

Forster, P; Ramaswamy, V; Artaxo, P; Berntsen, T; Betts, R; Fahey, DW; Haywood, J; Lean, J; Lowe, DC; Myhre, G; Nganga, J; Prinn, R; Raga, G; Schultz, M; Van Dorland, R. (2007). Changes in atmospheric constituents and in radiative forcing. In Climate change 2007: The physical science basis Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (pp. 129-234). Cambridge, United Kingdom: Cambridge University Press. http://www.ipcc.ch/pdf/assessment-report/ar4/wg1/ar4-wg1-chapter2.pdf.

Gustafson, EJ; Kubiske, ME; Sturtevant, BR; Miranda, BR. (2013). Scaling Aspen-FACE experimental results to century and landscape scales. Landsc Ecol 28: 1785-1800. http://dx.doi.org/10.1007/s10980-013-9921-x.

Hales, CM; Carroll, MD; Fryar, CD; Ogden, CL. (2017). Prevalence of Obesity Among Adults and Youth: United States, 20152016. NCHS Data Brief 288.

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

Horstman, DH; Folinsbee, LJ; Ives, PJ; Abdul-Salaam, S; Mcdonnell, WF. (1990). Ozone concentration and pulmonary response relationships for 6.6-hour exposures with five hours of moderate exercise to 0.08, 0.10, and 0.12 ppm. Am J Respir Crit Care Med 142: 1158-1163. http://dx.doi.org/10.1164/ajrccm/142.5.1158.

Hoyert, DL; Xu, J. (2012). Deaths: Preliminary data for 2011. Natl Vital Stat Rep 61: 1-51.

IPCC (Intergovernmental Panel on Climate Change). (2013). Climate change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change. In TF Stocker; D Qin; GK Plattner; MMB Tignor; SK Allen; J Boschung; A Nauels; Y Xia; V Bex; PM Midgley (Eds.). Cambridge, UK: Cambridge University Press. http://www.ipcc.ch/report/ar5/wg1/.

Jaffe, DA; Cooper, OR; Fiore, AM; Henderson, BH; Tonnesen, GS; Russell, AG; Henze, DK; Langford, AO; Lin, M; Moore, T. (2018). Scientific assessment of background ozone over the US: Implications for air quality management. 6. http://dx.doi.org/10.1525/elementa.309.

Keller, T; Häsler, R. (1984). The influence of a fall fumigation with ozone on the stomatal behavior of spruce and fir. Oecologia 64: 284-286. http://dx.doi.org/10.1007/BF00376884.

Kim, CS; Alexis, NE; Rappold, AG; Kehrl, H; Hazucha, MJ; Lay, JC; Schmitt, MT; Case, M; Devlin, RB; Peden, DB; Diaz-Sanchez, D. (2011). Lung function and inflammatory responses in healthy young adults exposed to 0.06 ppm ozone for 6.6 hours. Am J Respir Crit Care Med 183: 1215-1221. http://dx.doi.org/10.1164/rccm.201011-1813OC.

Kostiainen, K; Saranpaa, P; Lundqvist, SO; Kubiske, ME; Vapaavuori, E. (2014). Wood properties of Populus and Betula in long-term exposure to elevated CO2 and O-3. Plant Cell Environ 37: 1452-1463. http://dx.doi.org/10.1111/pce.12261.

Lefohn, AS; Laurence, JA; Kohut, RJ. (1988). A comparison of indices that describe the relationship between exposure to ozone and reduction in the yield of agricultural crops. Atmos Environ 22: 1229-1240. http://dx.doi.org/10.1016/0004-6981(88)90353-8.

Lefohn, AS; Runeckles, VC. (1987). Establishing standards to protect vegetation - ozone exposure/dose considerations. Atmos Environ 21: 561-568. http://dx.doi.org/10.1016/0004-6981(87)90038-2.

Leisner, CP; Ainsworth, EA. (2012). Quantifying the effects of ozone on plant reproductive growth and development. Global Change Biol 18: 606-616. http://dx.doi.org/10.1111/j.1365-2486.2011.02535.x.

Lewis, TC; Robins, TG; Mentz, GB; Zhang, X; Mukherjee, B; Lin, X; Keeler, GJ; Dvonch, JT; Yip, FY; O'Neill, MS; Parker, EA; Israel, BA; Max, PT; Reyes, A; Committee, CAAACS. (2013). Air pollution and respiratory symptoms among children with asthma: Vulnerability by corticosteroid use and residence area. Sci Total Environ 448: 48-55. http://dx.doi.org/10.1016/j.scitotenv.2012.11.070.

Macintosh, CR; Allan, RP; Baker, LH; Bellouin, N; Collins, W; Mousavi, Z; Shine, KP. (2016). Contrasting fast precipitation responses to tropospheric and stratospheric ozone forcing. Geophys Res Lett 43: 1263-1271. http://dx.doi.org/10.1002/2015GL067231.

Mautz, WJ; Bufalino, C. (1989). Breathing pattern and metabolic rate responses of rats exposed to ozone. Respir Physiol Neurobiol 76: 69-77.

McCorry, LK. (2007). Physiology of the autonomic nervous system [Review]. Am J Pharm Educ 71: 78.

McDonnell, WF; Kehrl, HR; Abdul-Salaam, S; Ives, PJ; Folinsbee, LJ; Devlin, RB; O'Neil, JJ; Horstman, DH. (1991). Respiratory response of humans exposed to low levels of ozone for 6.6 hours. Arch Environ Occup Health 46: 145-150. http://dx.doi.org/10.1080/00039896.1991.9937441.

McDonnell, WF; Stewart, PW; Smith, MV. (2013). Ozone exposure-response model for lung function changes: An alternate variability structure. Inhal Toxicol 25: 348-353. http://dx.doi.org/10.3109/08958378.2013.790523.

McDonnell, WF; Stewart, PW; Smith, MV; Kim, CS; Schelegle, ES. (2012). Prediction of lung function response for populations exposed to a wide range of ozone conditions. Inhal Toxicol 24: 619-633. http://dx.doi.org/10.3109/08958378.2012.705919.

Mclaughlin, SB; Nosal, M; Wullschleger, SD; Sun, G. (2007a). Interactive effects of ozone and climate on tree growth and water use in a southern Appalachian forest in the USA. New Phytol 174: 109-124. http://dx.doi.org/10.1111/j.1469-8137.2007.02018.x.

Mclaughlin, SB; Wullschleger, SD; Sun, G; Nosal, M. (2007b). Interactive effects of ozone and climate on water use, soil moisture content and streamflow in a southern Appalachian forest in the USA. New Phytol 174: 125-136. http://dx.doi.org/10.1111/j.1469-8137.2007.01970.x.

Miller, PR; McBride, JR. (1999). Assessment of ecological risks and implications for policy and management in the San Bernardino Mountains. In PR Miller; JR McBride (Eds.), Oxidant air pollution impacts in the montane forests of southern California: A case study of the San Bernardino Mountains (pp. 397-404). New York, NY: Springer. http://dx.doi.org/10.1007/978-1-4612-1436-6_19.

Mills, G; Harmens, H; Wagg, S; Sharps, K; Hayes, F; Fowler, D; Sutton, M; Davies, B. (2016). Ozone impacts on vegetation in a nitrogen enriched and changing climate. Environ Pollut 208: 898-908. http://dx.doi.org/10.1016/j.envpol.2015.09.038.

Mills, G; Hayes, F; Jones, MLM; Cinderby, S. (2007). Identifying ozone-sensitive communities of (semi-)natural vegetation suitable for mapping exceedance of critical levels. Environ Pollut 146: 736-743. http://dx.doi.org/10.1016/j.envpol.2006.04.005.

Mills, G; Wagg, S; Harmens, H. (2013). Ozone pollution: Impacts on ecosystem services and biodiversity. Bangor, UK: NERC/Centre for Ecology & Hydrology. http://nora.nerc.ac.uk/id/eprint/502675/.

Moran, EV; Kubiske, ME. (2013). Can elevated CO2 and ozone shift the genetic composition of aspen (Populus tremuloides) stands? New Phytol 198: 466-475. http://dx.doi.org/10.1111/nph.12153.

Myhre, G; Shindell, D; Bréon, FM; Collins, W; Fuglestvedt, J; Huang, J; Koch, D; Lamarque, JF; Lee, D; Mendoza, B; Nakajima, T; Robock, A; Stephens, G; Takemura, T; Zhang, H. (2013). Anthropogenic and natural radiative forcing. In TF Stocker; D Qin; GK Plattner; MMB Tignor; SK Allen; J Boschung; A Nauels; Y Xia; V Bex; PM Midgley (Eds.), Climate change 2013: The physical science basis Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change (pp. 659-740). Cambridge, UK: Cambridge University Press. http://www.ipcc.ch/report/ar5/wg1/.

NAPCA (National Air Pollution Control Administration). (1970). Air quality criteria for photochemical oxidants [EPA Report]. (AP-63). Washington, DC: U.S. Department of Health, Education, and Welfare. http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=9100E2Z7.txt.

Neufeld, HS; Johnson, J; Kohut, R. (2018). Comparative ozone responses of cutleaf coneflowers (Rudbeckia laciniata var. digitata, var. ampla) from Rocky Mountain and Great Smoky Mountains National Parks, USA. Sci Total Environ 610-611: 591-601. http://dx.doi.org/10.1016/j.scitotenv.2017.08.046.

NRC (National Research Council). (2009). Science and decisions: Advancing risk assessment. Washington, DC: The National Academies Press. http://dx.doi.org/10.17226/12209.

OECD (Organisation for Economic Co-operation and Development). (2016). Users handbook supplement to the guidance document for developing and assessing AOPs. (ENV/JM/MONO(2016)12). http://dx.doi.org/10.1787/5jlv1m9d1g32-en.

OMB (U.S. Office of Management and Budget). (2004). Final information quality bulletin for peer review. (Memorandum M-05-03). Washington, DC: US Office of Management and Budget (OMB). http://www.whitehouse.gov/sites/default/files/omb/assets/omb/memoranda/fy2005/m05-03.pdf.

Osborne, SA; Mills, G; Hayes, F; Ainsworth, EA; Büker, P; Emberson, L. (2016). Has the sensitivity of soybean cultivars to ozone pollution increased with time? An analysis of published dose-response data. Global Change Biol 22: 3097-3111. http://dx.doi.org/10.1111/gcb.13318.

Pleis, JR; Lucas, JW; Ward, BW. (2009). Summary health statistics for U.S. adults: National Health Interview Survey, 2008. (Vital and Health Statistics: Series 10, No. 242). Hyattsville, MD: National Center for Health Statistics. http://www.cdc.gov/nchs/data/series/sr_10/sr10_242.pdf.

Richards, BL; Middleton, JT; Hewitt, WB. (1958). Air pollution with relation to agronomic crops: V: Oxidant stipple of grape. J Am Soc Agron 50: 559-561. http://dx.doi.org/10.2134/agronj1958.00021962005000090019x.

Schelegle, ES; Morales, CA; Walby, WF; Marion, S; Allen, RP. (2009). 6.6-hour inhalation of ozone concentrations from 60 to 87 parts per billion in healthy humans. Am J Respir Crit Care Med 180: 265-272. http://dx.doi.org/10.1164/rccm.200809-1484OC.

Shindell, D; Kuylenstierna, JC; Vignati, E; van Dingenen, R; Amann, M; Klimont, Z; Anenberg, SC; Muller, N; Janssens-Maenhout, G; Raes, F; Schwartz, J; Faluvegi, G; Pozzoli, L; Kupiainen, K; Höglund-Isaksson, L; Emberson, L; Streets, D; Ramanathan, V; Hicks, K; Oanh, NT; Milly, G; Williams, M; Demkine, V; Fowler, D. (2012). Simultaneously mitigating near-term climate change and improving human health and food security. Science 335: 183-189. http://dx.doi.org/10.1126/science.1210026.

Sun, G; Mclaughlin, SB; Porter, JH; Uddling, J; Mulholland, PJ; Adams, MB; Pederson, N. (2012). Interactive influences of ozone and climate on streamflow of forested watersheds. Global Change Biol 18: 3395-3409. http://dx.doi.org/10.1111/j.1365-2486.2012.02787.x.

Suter, GW; Norton, SB; Fairbrother, A. (2005). Individuals versus organisms versus populations in the definition of ecological assessment endpoints. Integr Environ Assess Manag 1: 397-400. http://dx.doi.org/10.1002/ieam.5630010409.

Talhelm, AF; Pregitzer, KS; Kubiske, ME; Zak, DR; Campany, CE; Burton, AJ; Dickson, RE; Hendrey, GR; Isebrands, JG; Lewin, KF; Nagy, J; Karnosky, DF. (2014). Elevated carbon dioxide and ozone alter productivity and ecosystem carbon content in northern temperate forests. Global Change Biol 20: 2492-2504. http://dx.doi.org/10.1111/gcb.12564.

Team, CW; Pachauri, RK; Meyer, LA. (2014). Annex II: Glossary [Mach, K.J., S. Planton and C. von Stechow (eds.)]. In T Core Writing; RK Pachauri; LA Meyer (Eds.), Climate Change 2014: Synthesis Report Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (pp. 117-130). Geneva, Switzerland: IPCC. https://www.ipcc.ch/site/assets/uploads/2018/02/AR5_SYR_FINAL_Annexes.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (1978). Air quality criteria for ozone and other photochemical oxidants [EPA Report]. (EPA/600/8-78/004). Washington, DC. http://nepis.epa.gov/exe/ZyPURL.cgi?Dockey=200089CW.txt.

U.S. EPA (U.S. Environmental Protection Agency). (1986). Air quality criteria for ozone and other photochemical oxidants [EPA Report]. (EPA-600/8-84-020aF - EPA-600/8-84-020eF). Research Triangle Park, NC. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=PB87142949.

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

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

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

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

U.S. EPA (U.S. Environmental Protection Agency). (2006b). Air Quality Criteria for Ozone and Related Photochemical Oxidants. Volume 1 of 3. (EPA/600/R-05/004AF; EPA600R05004AF). Research Triangle Park, NC. National Center for Environmental Assessment.
: Environmental Protection Agency General. http://nepis.epa.gov/exe/ZyPURL.cgi?Dockey=P100CJZN.txt.

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

U.S. EPA (U.S. Environmental Protection Agency). (2013a). Integrated science assessment for lead [EPA Report]. (EPA/600/R-10/075F). Research Triangle Park, NC: U.S. Environmental Protection Agency, National Center for Environmental Assessment. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=255721.

U.S. EPA (U.S. Environmental Protection Agency). (2013b). Integrated science assessment for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-10/076F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment-RTP Division. http://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=247492.

U.S. EPA (U.S. Environmental Protection Agency). (2014). Integrated review plan for the primary national ambient air quality standards for nitrogen dioxide [EPA Report]. (EPA-452/R-14/003). Research Triangle Park, NC: U.S. Environmental Protection Agency, National Center for Environmental Assessment. http://www.epa.gov/ttn/naaqs/standards/nox/data/201406finalirpprimaryno2.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2015). Preamble to the integrated science assessments [EPA Report]. (EPA/600/R-15/067). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, RTP Division. https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=310244.

U.S. EPA (U.S. Environmental Protection Agency). (2016). Integrated science assessment for oxides of nitrogen-Health Criteria (final report) [EPA Report]. (EPA/600/R-15/068). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment. http://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=526855.

USDA (U.S. Department of Agriculture). (2015). Natural Resources Conservation Service PLANTS database. Available online at http://plants.usda.gov/java/ (accessed

van Goethem, TM; Azevedo, LB; van Zelm, R; Hayes, F; Ashmore, MR; Huijbregts, MA. (2013). Plant species sensitivity distributions for ozone exposure. Environ Pollut 178: 1-6. http://dx.doi.org/10.1016/j.envpol.2013.02.023.

Vinikoor-Imler, LC; Owens, EO; Nichols, JL; Ross, M; Brown, JS; Sacks, JD. (2014). Evaluating potential response-modifying factors for associations between ozone and health outcomes: A weight-of-evidence approach [Review]. Environ Health Perspect 122: 1166-1176. http://dx.doi.org/10.1289/ehp.1307541.

Volk, M; Bungener, P; Contat, F; Montani, M; Fuhrer, J. (2006). Grassland yield declined by a quarter in 5 years of free-air ozone fumigation. Global Change Biol 12: 74-83. http://dx.doi.org/10.1111/j.1365-2486.2005.01083.x.

Volk, M; Obrist, D; Novak, K; Giger, R; Bassin, S; Fuhrer, J. (2011). Subalpine grassland carbon dioxide fluxes indicate substantial carbon losses under increased nitrogen deposition, but not at elevated ozone concentration. Global Change Biol 17: 366-376. http://dx.doi.org/10.1111/j.1365-2486.2010.02228.x.

Wang, B, in; Shugart, HH; Shuman, JK; Lerdau, MT. (2016). Forests and ozone: productivity, carbon storage, and feedbacks. Sci Rep 6: 22133. http://dx.doi.org/10.1038/srep22133.

Watkinson, WP; Campen, MJ; Wichers, LB; Nolan, JP; Costa, DL. (2003). Cardiac and thermoregulatory responses to inhaled pollutants in healthy and compromised rodents: Modulation via interaction with environmental factors. Environ Res 92: 35-47. http://dx.doi.org/10.1016/S0013-9351(02)00023-3.

Wuebbles, DJ; Fahey, DW; Hibbard, KA; Dokken, DJ; Stewart, BC; Maycock, TK. (2017). USGCRP, 2017: Climate science special report: Fourth national climate assessment, Volume I. Washington, DC: U.S. Global Change Research Program. https://science2017.globalchange.gov/.

Xie, B; Zhang, H; Wang, Z; Zhao, S; Fu, Q. (2016). A modeling study of effective radiative forcing and climate response due to tropospheric ozone. Adv Atmos Sci 33: 819-828. http://dx.doi.org/10.1007/s00376-016-5193-0.

Zak, DR; Pregitzer, KS; Kubiske, ME; Burton, AJ. (2011). Forest productivity under elevated CO2 and O3: positive feedbacks to soil N cycling sustain decade-long net primary productivity enhancement by CO2. Ecol Lett 14: 1220-1226. http://dx.doi.org/10.1111/j.1461-0248.2011.01692.x.

APPENDIX 1 ATMOSPHERIC SOURCE, CHEMISTRY, METEOROLOGY, TRENDS, AND BACKGROUND OZONE

- The sources of ground-level ozone vary widely, including emissions due to human activities within the U.S. and internationally, natural and biological processes, and dynamics within Earth's atmosphere. Uncertainties in the rates of precursor emissions for the sources of background ozone are typically high, due to the difficulties associated with characterizing and quantifying such variables as biological variability in vegetation species across U.S. regions, and the effects of meteorological variability on sources sensitive to temperature, moisture, and atmospheric circulation patterns.
- Understanding concerning the production of ozone during wintertime in the Intermountain West and the depletion of ozone on coastlines continues to advance. Local emissions related to oil and gas production combined with strong atmospheric inversions (cold pools) explain the unusually high concentrations of ground-level ozone in Western mountain basins during winter. Photochemical mechanisms that include halogen radical-based heterogeneous reactions in sea salt particles have been found to account for previously unexplained reduced concentrations in surface ozone along urban areas on U.S. maritime coastlines.
- Large-, regional-, and local-scale atmospheric circulation patterns influence both observed U.S. background ozone and the local production of ground-level ozone. Interannual (e.g., the El Niño-Southern Oscillation [ENSO] cycle) and multidecadal (e.g., the Pacific Decadal Oscillation [PDO] and Atlantic Multidecadal Oscillation [AMO]) climate variability is especially important for USB ozone or other measures of background ozone in the U.S. because these climate patterns affect long-range transport of international pollution, the frequency of deep stratospheric intrusion, stagnation events, and wildfire activity.
- U.S. anthropogenic emissions of ozone precursors have declined over the past two decades. For example, U.S. NO_X emissions decreased by 48% between 2002 and 2014. As precursor concentrations decrease, the U.S. ambient ozone concentration distribution is compressing (i.e., 95th percentile concentrations are decreasing at the same time 5th percentile concentrations are increasing), consistent with chemistry expected after reductions in NO_X.
- U.S. background ozone continues to account for a large fraction of ambient ozone concentrations as a result of stratospheric exchange, international transport, wildfires, lightning, global methane emissions, and natural biogenic and geogenic precursor emissions. New results concerning U.S. background ozone are (1) a wider range of concentration estimates, and poorer agreement between models have been observed than were reported in the 2013 Ozone ISA, with a range of uncertainty of ~10 ppb for seasonal average concentrations, (2) U.S. background concentrations are uncorrelated with local ground-level concentrations above ~60 ppb, and (3) an increasing trend of U.S. background concentration at high elevation western U.S. sites before approximately 2010 now shows signs of slowing or even reversing, probably due to decreasing East Asian precursor emissions.

1.1 Overview

This Appendix reviews scientific advances in atmospheric ozone research relevant to this review of the NAAQS for ozone and other photochemical oxidants and the related air quality criteria. The primary focus is on new evidence concerning the contributions of ozone from natural and non-U.S. sources. Ozone is one of a group of photochemical oxidants formed by atmospheric photochemical reactions of hydrocarbons with nitrogen oxides in the presence of sunlight. Photochemical oxidants were defined in the 1970 Air Quality Criteria Document as compounds found in the atmosphere that oxidize a reference material such as potassium iodide that is not oxidized by atmospheric oxygen (NAPCA, 1970). Other photochemical oxidants formed by photochemical reactions of hydrocarbons and nitrogen oxides include nitrogen dioxide (NO_2), peroxyacetyl nitrate (PAN), hydrogen peroxide, nitrous acid, and organic peroxides. Close agreement between ozone measurements and the photochemical oxidant measurements upon which the early NAAQS was based indicated that the contribution of these other oxidant species was very small (NAPCA, 1970). Shortly after, measurements of photochemical oxidants as a class of pollutant species became increasingly rare, and in 1979 ozone became the NAAQS indicator for air pollutant photochemical oxidants. Ozone is the only photochemical oxidant other than NO_2 that is routinely monitored. The current state of scientific understanding concerning NO_2 is addressed in the 2016 ISA for the Oxides of Nitrogen—Health Based Criteria (U.S. EPA, 2016b). Data for other photochemical oxidants are generally derived from a few special field studies. Extensive national scale data on temporal or geospatial concentration patterns of these other oxidants are scarce. Moreover, few studies of the health and welfare impacts of other photochemical oxidants beyond ozone have been identified by literature searches conducted for other recent ozone assessments (U.S. EPA, 2013, 2006a). For these reasons, discussion of photochemical oxidants in this document focuses on ozone.

Material in this Appendix is based primarily on a systematic literature review of sources, chemistry, estimation methods, and concentration trends of ozone from natural and non-U.S. sources following procedures described in Appendix 10. For context, brief summaries of up-to-date trends in U.S. anthropogenic ozone precursor emissions and ozone concentration trends from available U.S. EPA databases are also included. In addition, winter ozone, halogen chemistry, satellite measurements, and chemical transport modeling are identified as research areas in which progress had been made since publication of the 2013 Ozone ISA (U.S. EPA, 2013). For these topics, brief summaries of the most relevant developments are also provided.

The most commonly used ozone metrics for assessing the impacts on human health and ecosystems, and the performance of atmospheric models are evaluated (Section 1.2), followed by a discussion of new advances in our understanding of ozone sources and emissions (Section 1.3), atmospheric chemistry (Section 1.4), the influence of meteorology and climate change on ozone concentrations (Section 1.5), and measurements and modeling (Section 1.6). The Appendix also includes a summary of ambient ozone concentrations throughout the U.S. through 2017, as well as an assessment of concentration trends (Section 1.7). Section 1.8 discusses the latest developments in understanding

background ozone and how it contributes to ambient concentrations. It includes background estimation methods, as well as estimates of the contribution of background ozone to ambient ozone concentrations. This section is followed by an appendix summary (Section 1.9).

1.2 Metrics and Definitions

1.2.1 Ozone Metrics

Several different averaging times are commonly used in ozone metrics. Each has had a role in NAAQS and been used in studies of human health, ecological effects, and atmospheric model evaluation. The choice of metric in a study depends on the study purpose.

1.2.1.1 Ambient Air Concentration Metrics

Ozone concentration metrics are generally based on measurements or estimates expressed as a volume-volume *mixing ratio*, with units of parts per million (ppm) or parts per billion (ppb). Technically, ppm and ppb are not concentration units, which are defined as moles per unit volume and depend on temperature and pressure. This distinction is generally acknowledged in the atmospheric science literature. In contrast, the term *mixing ratio* is rarely used in the literature on health and vegetation effects but is instead usually substituted with the term *concentration*, understood to be more broadly interpreted as the amount of a substance in a fluid without distinguishing units. For this reason, the term *concentration* is generally used instead of *mixing ratio* in this document to maintain consistency with its use in the health and ecological effects literature. *Mixing ratio* is still used in the more technical discussions of atmospheric sources and chemistry in Section 1.3 and Section 1.4.

- The daily maximum 1-hour average (MDA1), daily maximum 8-hour average (MDA8), and daily 24-hour average concentrations (DA24) are among the most widely used short-term air quality metrics in epidemiologic studies.
- Seasonal and monthly averages of MDA1, MDA8, and DA24 are used for long-term metrics in epidemiologic studies. Hourly ozone concentrations and longer term averages of these metrics are also used for atmospheric model evaluation (Dennis et al., 2010).
- Design values are used by the U.S. EPA to designate and classify nonattainment areas, as well as to assess progress towards meeting the NAAQS. A design value is a statistic that describes the air quality status of a given location relative to a particular NAAQS. The design values for the ozone NAAQS are the 3-year average of the annual 4th-highest MDA8 ozone concentrations.

1.2.1.2 Ecosystem Exposure Metrics

For ecosystem exposure, cumulative exposure indicators are frequently used that extend over longer time periods, such as growing season or year (U.S. EPA, 2013). The W126, SUM06, and AOTx exposure indices are metrics used for ecosystem exposure. Further details on these exposure indices are provided in the 2013 Ozone ISA (U.S. EPA, 2013) and Section 8.13.1.

- The W126 exposure metric is a sigmoidally weighted sum of all hourly ozone concentrations observed during a specified day and seasonal time window. The sigmoidal weighting of hourly ozone concentration is given by $W_C = 1/(1 + 4{,}403e^{-126C})$, where C is the hourly ozone concentration in ppm.
- SUM06 is the sum of all hourly concentrations greater than or equal to 60 ppb observed during a specified daily and seasonal time window.
- AOTx is the sum of differences between hourly ozone concentrations greater than a specified threshold during a specified daily and seasonal time window. For example, AOT40 is the sum of differences between hourly concentrations above 40 ppb.

1.2.2 Background Ozone Definitions

Use of the term *background ozone* varies within the air pollution research community. The most widely used definitions and applications are described in this section. The term has generally been used to describe ozone levels that would exist in the absence of anthropogenic emissions within a particular area and has been broadly applied to every geospatial scale: local, regional, national, continental, or global. For instance, on a local scale, ozone that originates from precursor emissions outside of a locality's municipal boundaries could be considered background ozone in that locality. Similarly, on a national scale, background ozone could be defined as ozone that is not formed from anthropogenic emissions within national boundaries.

1.2.2.1 U.S. Background Ozone

In this document, the term *U.S. background* (USB) is used to assess background ozone. The USB concentration is defined as the ozone concentration that would occur if all U.S. anthropogenic ozone precursor emissions were removed.

- This definition helps distinguish the ozone that can be controlled by precursor emissions reductions within the U.S. from ozone originating from natural and foreign precursor sources that cannot be controlled by U.S. regulations.
- The distinction between U.S. anthropogenic and USB sources is not always straightforward, with ambiguities or debate regarding U.S. anthropogenic methane (Fiore et al., 2014), U.S. anthropogenic emissions that have recirculated globally (McDonald-Buller et al., 2011),

international shipping and aviation (U.S. EPA, 2015), prescribed fires (U.S. EPA, 2015), and soil emissions (Rasool et al., 2016) (see Section 1.3.2.1).

- As defined here, USB is a model construct that cannot be measured using ambient monitoring data. This approach is consistent with the 2006 Ozone Air Quality Criteria Document (AQCD) (U.S. EPA, 2006a) and the 2013 Ozone ISA (U.S. EPA, 2013), which also used modeled estimates of USB. The 2006 Ozone AQCD (U.S. EPA, 2006a) concluded that background ozone concentrations could not be determined exclusively from ozone measurements at relatively remote monitoring sites because of long-range transport of ozone originating from U.S. anthropogenic precursors even at the most remote monitoring locations. Reliance on atmospheric modeling for USB concentrations estimates continued in the 2013 Ozone ISA (U.S. EPA, 2013). In earlier assessments, ozone estimates were based on measurements at monitoring sites with low concentrations that appeared to be isolated from anthropogenic sources (Altshuller and Lefohn, 1996; Trainer et al., 1993).

1.2.2.2 Apportionment-Based U.S. Background (USB)

Modeling approaches for estimating USB can be classified as either source-sensitivity or source-apportionment approaches (see Section 1.8.1). USB was originally estimated using source-sensitivity approaches. *Apportionment-based USB* (USB_{AB}) has been defined as the amount of ozone formed from sources other than U.S. anthropogenic sources as estimated via an apportionment technique (Dolwick et al., 2015).

- The distinction between USB and USB_{AB} is important because apportionment techniques for estimating USB_{AB} are designed to realistically treat nonlinear and nonadditive interactions of USB and U.S. anthropogenic emissions that affect both production and destruction of ozone. In contrast, source-sensitivity modeling approaches originally used for estimating USB are not designed to address these interactions.
- USB and USB_{AB} are not the same quantity estimated with different approaches but are actually estimates of conceptually different quantities. While USB is an estimate of ozone concentrations that could be achieved if U.S. anthropogenic sources were eliminated, USB_{AB} is an estimate of how much ozone can be attributed to background sources when those anthropogenic sources are present. Differences in modeling approaches used to estimate USB and USB_{AB} are described in Section 1.8.1.

1.2.2.3 U.S. Background (USB) Averaging Time

The averaging time of a USB estimate is intended to match the averaging time of the total ozone concentration measured. For example, it would be inappropriate to estimate the USB contribution to a MDA8 ozone concentration (see Section 1.2.1.1) using a seasonal mean USB estimate. This is because meteorological conditions under which high anthropogenic ozone concentrations are produced differ from those under which high USB ozone concentrations are produced (see Section 1.3 and Section 1.5.1).

- Estimates of USB on days with high MDA8 concentrations are more relevant for understanding USB contributions on those days than are seasonal mean USB estimates.

- Seasonal mean USB is more relevant for understanding source contributions to long-term concentrations.
- As discussed by Jaffe et al. (2018) and in Section 1.8.1, USB MDA8 estimates on specific days are more uncertain than USB seasonal mean estimates, because of considerable daily variation influenced by season, meteorology, and elevation.

1.2.2.4 Other Background Ozone Definitions

Other definitions besides USB have been used in previous U.S. EPA science assessments. Although USB is emphasized in this document, research results based on *North American background* (NAB) and *natural background* are also included. These terms were also widely used in the 2013 ozone ISA (U.S. EPA, 2013) and in earlier ozone assessments.

- NAB has been defined as the ozone concentration that would occur in the U.S. in the absence of anthropogenic emissions in continental North America (U.S. EPA, 2013). NAB has also been referred to as policy-relevant background (PRB) in earlier publications (U.S. EPA, 2007).
- *Emissions-influenced background* (EIB) has been defined as another measure of background ozone estimated from source apportionment modeling approaches while including chemical interactions with anthropogenic emissions (Lefohn et al., 2012).
- *Natural background* ozone is defined as the ozone concentrations that would occur if all anthropogenic emissions were removed worldwide. Processes that contribute to natural background ozone include ozone transport from the stratosphere and ozone formed from precursor emissions originating from wildfires, lightning, natural methane sources, plants, and other natural VOC and NO_X emissions (see Section 1.3).

1.2.2.5 Baseline Ozone

Baseline ozone is an alternative metric to USB or NAB that has been defined as the measured ozone concentration at rural or remote sites that have not been influenced by recent, local emissions (Jaffe et al., 2018). In contrast to USB, baseline ozone is by definition directly measured.

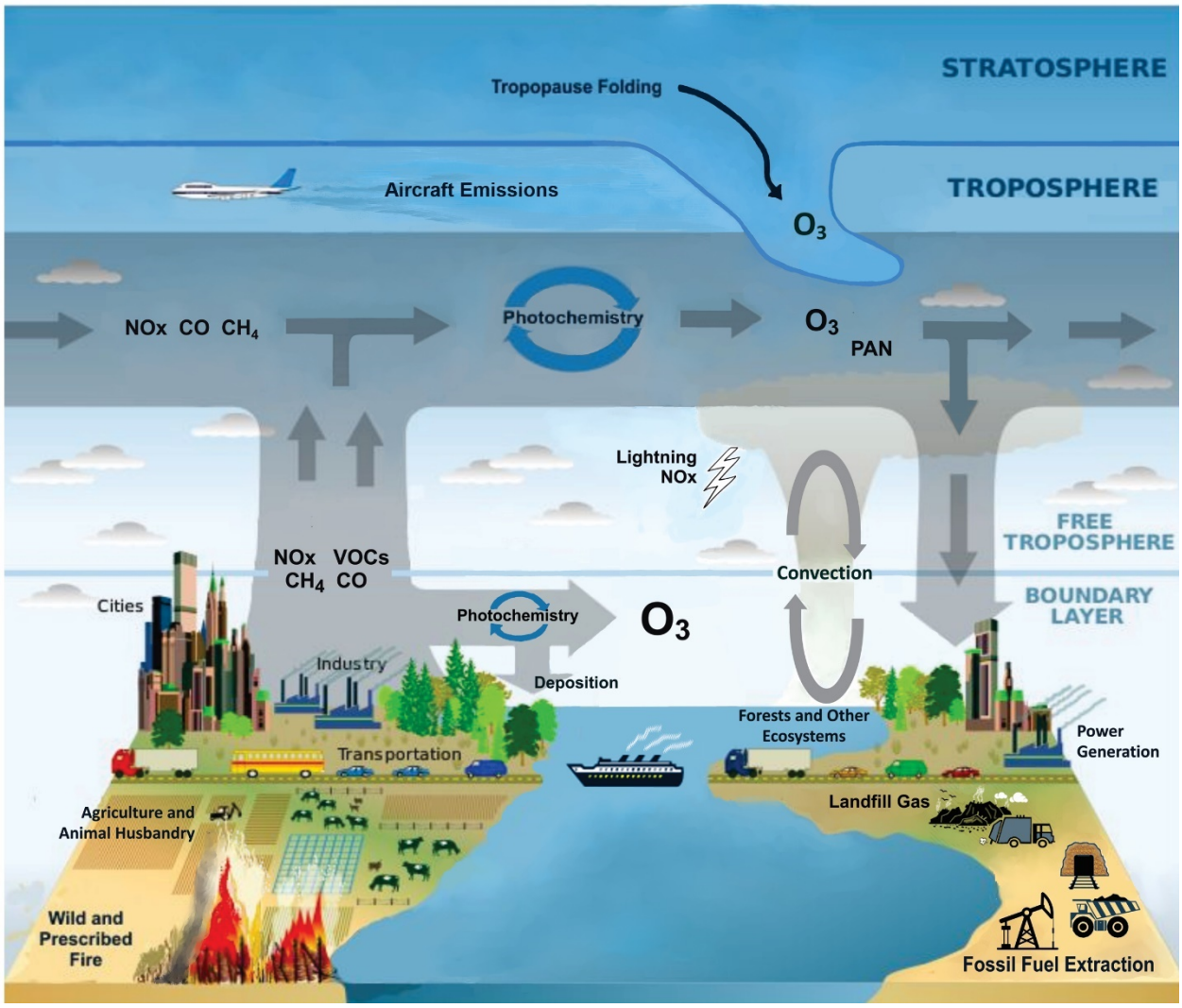
- Baseline measurements are typically from monitors in locations that are minimally influenced by local anthropogenic sources, and samples used as baseline measurements are limited to those monitored during meteorological conditions consistent with the relative absence of local contamination.
- Baseline ozone can include the ozone produced from U.S. emissions that circle the globe and may also include effects of same-state emissions. An example of the latter would be ozone from U.S. emissions near the West Coast or Gulf Coast that is transported over the Pacific Ocean or Gulf of Mexico, respectively, and then transported back onshore.
- In some cases, sources that impact baseline ozone may not similarly impact ozone in populated locations. For instance, baseline ozone measured on a mountaintop may include stratospheric influences that are not representative of contributions in nearby lower elevation locations.

- There are several reasons why baseline ozone measurements cannot be used as a proxy to estimate USB ozone levels in urban areas. As previously described, baseline ozone can include contributions from U.S. emissions. Additionally, baseline ozone monitors can be very distant from urban sites, and ozone measured at the baseline site can be destroyed through surface deposition or chemical reactions during transport from the baseline site to a downwind monitor. In addition, atmospheric conditions may not favor transport of baseline ozone from the monitor location to populated areas at lower elevations.
- Another reason why baseline ozone measurements cannot be used as a proxy for USB ozone levels in urban areas is that meteorological conditions that favor mixing from the free troposphere to ground level have strong ventilation and are not conducive to photochemical ozone episodes that produce the highest urban ozone concentrations (see Section 1.5.1). Stratospheric intrusion events are an exception (see Section 1.3.2).
- While baseline ozone measurements cannot be used directly to estimate USB ozone, baseline ozone data are useful for evaluating the CTMs that are used to provide model estimates of USB ozone.

1.3 Sources of U.S. Ozone and Its Precursors

U.S. tropospheric ozone (i.e., ozone that may have harmful health and environmental impacts) is classified in this assessment as either being derived from U.S. anthropogenic sources or background (USB). Anthropogenic ozone within the U.S. is further defined as the product of photochemical reactions of precursors derived from human activities. USB ozone, as defined in Section 1.2.2.1, has a broader, more complex array of sources. These include natural precursor sources as well as precursors transported from across U.S. borders from both nearby and distant locations within the Northern Hemisphere. Ozone derived from the stratosphere and from the reaction of internationally-transported precursors in the upper troposphere can be drawn down into the lower troposphere through atmospheric dynamics (i.e., vertical movement of large air masses between the stratosphere and the troposphere). Figure 1-1 illustrates the complexities associated with attributing measured ground-level ozone to particular sources.

The main focus of this section is recent scientific findings concerning the sources of USB ozone. To provide context for this discussion, updated information on U.S. anthropogenic ozone precursor emissions and trends in those emissions is included.



Source: Adapted from CCSP (2003).

Figure 1-1 Major atmospheric processes and precursor sources contributing to ambient ozone.

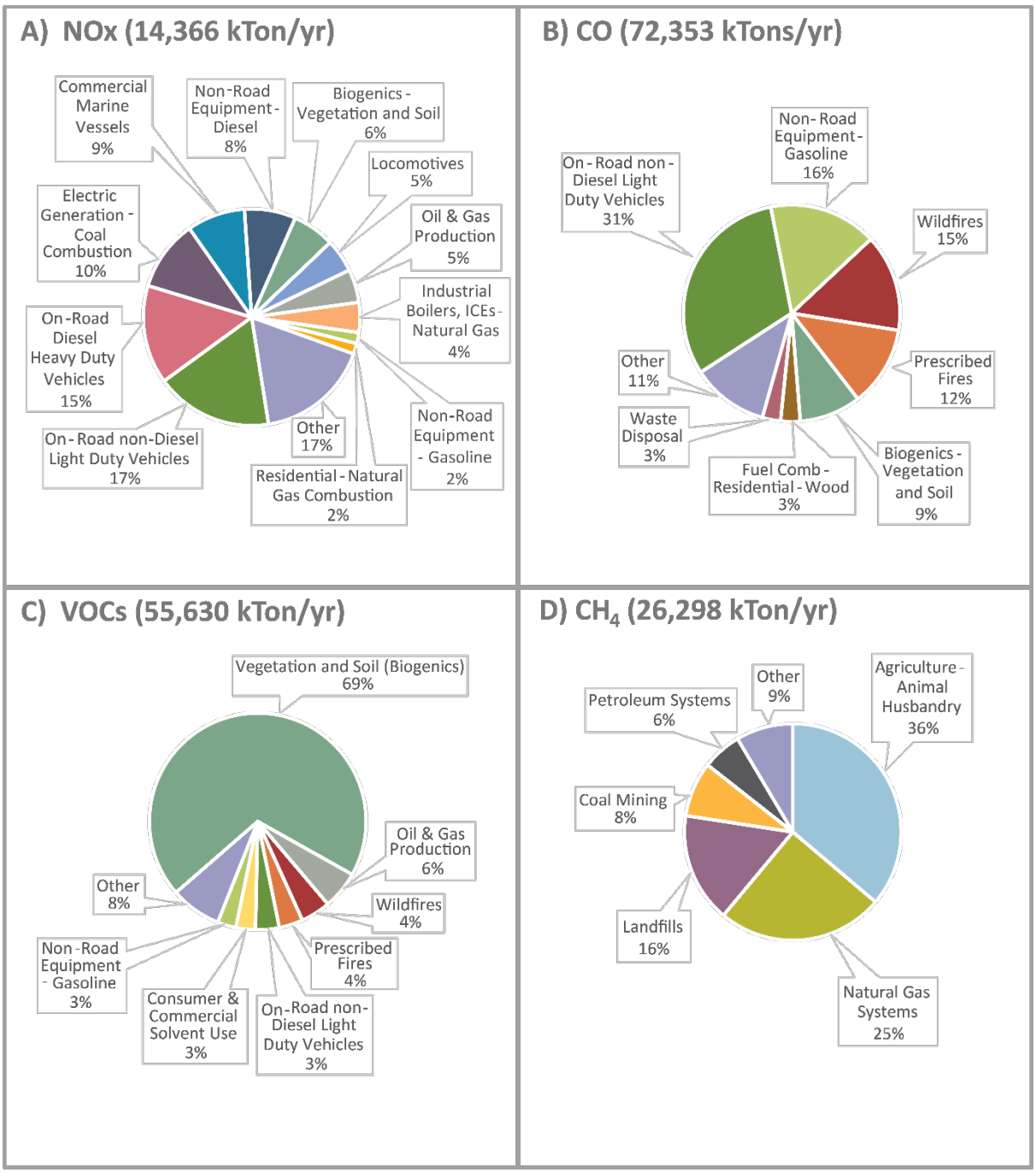
1.3.1 Precursor Sources

Ozone formed in the troposphere is, primarily, the product of photochemical reactions between nitrogen oxides (NO_X) and carbon-containing compounds including carbon monoxide (CO), methane (CH_4), and volatile organic compounds (VOCs). This section summarizes current estimates of U.S. anthropogenic precursor emissions by source type. Following this summary is a discussion of recent findings concerning global/international and natural precursor emissions sources.

1.3.1.1 Ozone Precursor Emissions: Anthropogenic Sources and Trends in the U.S.

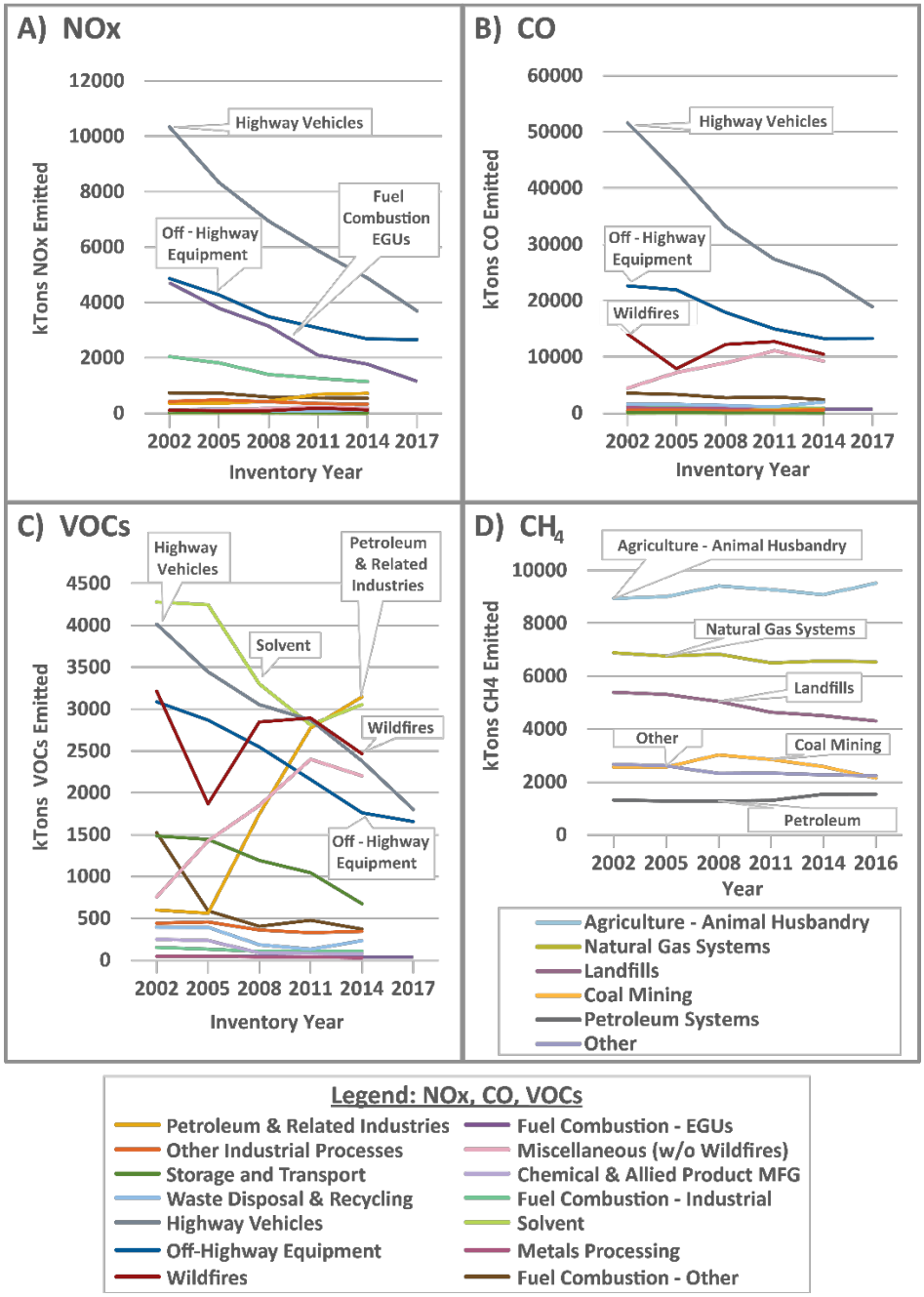
Figure 1-2 provides a visual summary of annual emissions by the largest U.S. sources of anthropogenic NO_X, CO, VOCs, and methane. These estimates are taken from the publicly available versions of the U.S. EPA National Emissions Inventory [2014 NEI, Version 2; U.S. EPA (2018a)] and of the U.S. Inventory of Greenhouse Gases and Sinks (U.S. EPA, 2016c). Emissions of each precursor are shown as a function of source type.

The U.S. EPA maintains a database, beginning with 1970, that provides information about criteria pollutant (or precursor) emissions trends for a set of aggregate categories that account for major, or "Tier 1,"source types (U.S. EPA, 2019b). National emissions estimates for these categories are derived from the NEI and are included in the trends dataset when an updated version of the inventory has been finalized for public release. The 2014 NEI is the most recent inventory available to the general public, with the 2017 NEI currently in development (due for public release in 2020). However, annual emissions estimates for some of the relevant ozone precursors are currently available for mobile sources and the electric utility sector. Mobile source emissions are calculated for the NEI using the U.S. EPA Motor Vehicle Emission Simulator (MOVES) model (U.S. EPA, 2011). These values are available for inclusion in the Tier 1 Trends dataset in advance of the release of the official 2017 NEI. Electric utilities continuously monitor and provide quarterly reports of emissions of NO_X and SO_X to U.S. EPA's Clean Air Markets Program, as required under the Clean Air Act. These data (U.S. EPA, 2019a) were used to provide an estimate of 2017 emissions from the electric utility sector for the trends dataset. Figure 1-3, showing U.S. Tier 1 precursor emissions trends since 2002, includes estimated NO_X emissions by electric utilities, as described, for 2017. Emissions of NO_X, CO, and VOCs, as estimated by the MOVES model, for 2017 are likewise included. Emissions by all other source categories are given through 2014, as taken from the 2014 NEI.



Sources: A)−C) 2014 U.S. EPA National Emissions Inventory, Version 2 (U.S. EPA, 2018a) and; D) 2016 U.S. Inventory of Greenhouse Gases (U.S. EPA, 2016c).

Figure 1-2 Relative ozone precursor emissions by U.S. sector: A) nitrogen oxides (NO_X). B) carbon monoxide (CO). C) volatile organic compounds (VOCs). Biogenic VOCs, which can be important in the production of ozone in urban areas, is included for context. D) methane (CH_4).



Sources: A)−C) U.S. EPA National Emissions Trends (U.S. EPA, 2019b) and; D) the 2016 U.S. Inventory of Greenhouse Gases (U.S. EPA, 2016c).

Figure 1-3 U.S. anthropogenic ozone precursor emission trends. Sources shown generate 90% or more of known emissions, excluding biogenic sources, for the indicated precursor: A) nitrogen oxides (NO_X), B) carbon monoxide (CO), C) volatile organic compounds (VOCs), D) methane (CH_4). Not shown: "Other" NO_X, CO, and VOC emissions categories that, together, account for less than 10% of total emissions for each precursor.

1.3.1.1.1 U.S. Anthropogenic NO_X

Anthropogenic NO_X sources at local and regional scales within the U.S. have been recently discussed in detail in the ISAs devoted to ecological effects of NO_X, SO_X and PM (U.S. EPA, 2018b) and to the health effects of NO_X (U.S. EPA, 2016b).

- *Emissions of NO_X within the U.S. decreased by 47% between 2002 and 2014*. Figure 1-2 summarizes the main NO_X emissions source categories included in the 2014 NEI (U.S. EPA, 2017). Highway vehicles are the largest source category of NO_X emissions nationwide, contributing 1 Tg N/year to total NO_X emissions nationwide. Off-highway vehicles, electricity generating units (EGUs), other forms of stationary fuel combustion, and industrial processes each contribute between 0.4 and 0.7 Tg N/year to nationwide NO_X emissions. Figure 1-3 shows the steep decline in U.S. NO_X emissions, primarily due to on-road vehicle emissions changes, between 2002 and 2014. Estimated Tier 1 emissions of NO_X have decreased by 47% between 2002 and 2014 (Figure 1-3).

1.3.1.1.2 U.S. Anthropogenic Carbon Monoxide

The Integrated Science Assessment for Carbon Monoxide (U.S. EPA, 2010) describes the sources of anthropogenic carbon monoxide (CO) as primarily on- and off-road mobile emissions, followed by prescribed burning. Wildfires and soils emit much of the remaining total.

- *Overall, emissions at the national scale, between 2002 and 2014, have declined by approximately 30%.* The 2014 NEI reports on-road mobile emissions at 31% of total U.S. CO emissions; off-road at 16%; wildfires at 15%; prescribed fires at 12%; and soil emissions at 9% (U.S. EPA, 2017). The values reported for wildfires and soils are uncertain to a much larger degree than for the other sources. Estimated Tier 1 emissions of CO have declined by 36% between 2002 and 2014 (Figure 1-3).

1.3.1.1.3 U.S. Anthropogenic and Biogenic Volatile Organic Compounds (VOCs)

The NEI includes estimates of biogenic along with anthropogenic VOC emissions. Biogenic sources contribute substantially more to the U.S. emissions inventory than anthropogenic sources, can play an important role urban ozone formation and are, therefore, included for context in this section. As described in the 2013 Ozone ISA, VOCs that are important for the photochemical formation of ozone include alkanes, alkenes, aromatic hydrocarbons, carbonyl compounds, alcohols, organic peroxides, and halogenated organic compounds. These compounds range widely in photochemical reactivity and, consequently, atmospheric lifetimes. For example, isoprene has an atmospheric lifetime of approximately an hour, whereas methane has an atmospheric lifetime of about a decade. In urban areas, compounds representing all classes of VOCs and CO are important for ozone formation. In nonurban vegetated areas, biogenic VOCs emitted from vegetation tend to dominate the VOC budget.

- U.S. industrial and related VOC emissions have increased by approximately ~20% since 2012, while other anthropogenic emissions have declined over the same period. At the national scale, emissions by biogenic sources dominate the U.S. inventory at 71%. These emissions are spatially heterogeneous, having a greater effect on VOC concentrations in certain U.S. locations. Wildfires emit 4% with the remaining 25% attributed to anthropogenic sources in 2014 (U.S. EPA, 2017). Figure 1-3 shows the trends in Tier 1 emissions (i.e., not including biogenic VOCs) between 2002 and 2014. Overall, VOC emissions by Tier 1 sources have declined by 17% over that period.

1.3.1.1.4 U.S. Anthropogenic Methane

Methane, a major precursor for ozone at the global scale, is not included in the U.S. NEI. Methane emissions are, however, reported in U.S. Inventory of Greenhouse Gases and Sinks (U.S. EPA, 2016c). The U.S. GHG Inventory and the NEI are not directly comparable because of differences in source classifications, methods, and underlying assumptions. However, Figure 1-2 provides methane trends as reported in the U.S. Greenhouse Gas Emissions inventory for the 2002−2016 time frame.

- *Overall, total U.S. anthropogenic methane emissions decreased between 1990 and 2015*. Recent studies indicate that total U.S. anthropogenic methane emissions decreased by 16% between 1990 and 2015 (NASEM, 2018). However, the methane trends differed between the individual source categories. The U.S. GHG inventory indicates that agriculture and natural gas systems are the largest U.S. sources of methane. Emissions from landfills and coal mining have trended downwards since the 2005−2008 time period. The agricultural emissions trend varied between 2002 and 2014, but has shown a notable increase since 2014. Petroleum systems were constant between 2002 and 2011, increased between 2011 and 2014, then remained constant between 2014 and 2016. From 2002 to 2016, the inventory showed little change (−5%), in overall annual estimated emissions.

1.3.1.2 Global and International Sources of Anthropogenic Ozone Precursors

Quantifying the emissions from sources that contribute to USB ozone represents a substantial scientific challenge. As mentioned in Section 1.2.2.1, in the case of emissions from international sources (i.e., anthropogenic and wildfire emissions from other countries, international shipping and aviation), and of long-lived chemical precursors such as methane, identifying the specific sources and quantifying their contributions to USB ozone is difficult under most circumstances. In some cases, satellites can capture images of intact or partially intact emissions plumes of some precursors making it possible, using back-trajectory modeling tools, to track these plumes to their origins. But, in most cases atmospheric mixing and transport processes obscure the origins of those international emissions that can be detected by remote sensing. In the case of chemical species that are stabilized by the low temperatures in the upper troposphere, such as PAN, recirculation within the global atmosphere further confuses emissions accounting.

1.3.1.2.1 Global Methane

The 2013 Ozone ISA (U.S. EPA, 2013) reported an estimate by Zhang et al. (2011) of the effect of anthropogenic methane emissions on global annual mean ozone concentrations at ground level of ~4−5 ppb. North American emissions of methane were described as uncertain, but were considered to be a small fraction of total anthropogenic input. Before the last assessment, ozone production derived from methane oxidation was shown to be most prominent in regions with frequent vertical mixing and in locations with NO_X-saturated chemistry, such as southern California and the New York-New Jersey region (Fiore et al., 2008). In the same study, surface ozone was close to twice as sensitive to methane in the planetary boundary layer (i.e., below about 2.5 km) than to methane in the free troposphere (Fiore et al., 2008). Model studies also indicate that the sensitivity of global tropospheric ozone to methane is about 0.11−0.16 Tg ozone per Tg CH_4/year (Zhang et al., 2016; Fiore et al., 2008).

- *The methane concentrations over the U.S. are influenced by global methane sources.* The atmospheric methane abundance over the U.S. is influenced by global methane sources because of the residence time for methane (NASEM, 2018). The atmospheric residence time for methane is about a decade, allowing methane to be relatively homogeneously distributed around the globe (NASEM, 2018). Therefore, the U.S. methane budget cannot be considered in isolation from the global methane budget.
- The U.S. contributes approximately 20% to total methane in the atmosphere of the Northern Hemisphere, and about 10% of total global methane emissions in recent years. For the 2003 to 2012 period, half of the total global methane emissions were attributed to Africa, South America, and Southeast Asia combined, while the U.S. accounted for about one-tenth of the total global emissions (Saunois et al., 2016).
- *Main global anthropogenic methane sources include agriculture and waste, fossil fuels, and biomass and biofuel burning.* An ensemble of studies attribute about 34% of the global anthropogenic methane to agriculture and waste, 19% to fossil fuels (coal mining and oil and gas industry), and 6% to biomass and biofuel burning between 2003 and 2012 (Saunois et al., 2016). The remaining total global methane emissions (i.e., about 41% of the total global methane emissions) are generated by natural sources. These studies also estimated that global anthropogenic methane emissions are about 328 Tg CH_4/year using top-down inventories (Saunois et al., 2016). Top-down inventories use atmospheric observations within an atmospheric inverse-modeling framework. Model results indicate that the global anthropogenic emissions of methane decreased by about 15% between 1980 and 2010 (Zhang et al., 2016). However, it should be noted that methane emission estimates are highly uncertain due to measurement and model uncertainties and not fully understanding the methane sources and sinks.
- *Recent studies show that global mean methane concentrations are well over twice that of the preindustrial period.* The global mean methane concentration has nearly tripled between preindustrial time and December 2017. Studies show that methane concentrations rose sharply throughout the 20th century, then leveled off for a period of time beginning around 2000 (NASEM, 2018). Studies also reveal a sustained increase in atmospheric methane levels in the 1980s (by an average of 12 ± 6 ppb/year), a slowdown in growth in the 1990s (6 ± 8 ppb/year), and a general stabilization from 1999 to 2006 (Kirschke et al., 2013). Between 2007 and 2010, methane levels resumed rising (Kirschke et al., 2013).
- *In recent years, the total global mean methane concentration has increased annually by about 3.5 ppb.* Between 2003 and 2012, the global mean methane concentration is estimated to have

increased at a rate of 3.5 + 0.2 ppb/year (Saunois et al., 2016). Some recent studies suggest that the methane increases were mainly due to increases in fossil fuel production (e.g., coal and oil and gas industry) and agricultural emissions, while other studies point to large uncertainties in natural emissions (Van Dingenen et al., 2018). Modeling studies also suggest that natural sources contribute to the inter-annual variability of methane, while anthropogenic emissions, mainly emitted in the Northern Hemisphere, have played a major role in the increase of methane observed since 2005 (Bader et al., 2017).

- *Global tropospheric ozone levels are enhanced when methane* increases. Studies suggest that increases in global methane since the 1800s have yielded higher levels of global tropospheric ozone (and ground-level ozone) worldwide (NASEM, 2018). Studies indicate that there is an approximately linear relationship between anthropogenic methane emissions and tropospheric ozone, such that for every teragram per year decrease in methane emissions, ozone could decrease by 11 ppt to 15 ppt (Fiore et al., 2008).
- *Global methane abundance contributes to rising U.S. surface ozone during all months*. Based on a set of transient chemistry-climate model simulations between 2005 and 2100, the global methane abundance contributes to rising surface ozone during all months, with the largest influence during cooler months when the ozone lifetime is longer (Rieder et al., 2018; Clifton et al., 2014). These simulations indicate that the sensitivity of the ozone mixing ratio to potential changes in global methane abundance is about 7−16 ppb over the northeastern U.S. and by about 12−19 ppb over the intermountain western U.S. at the end of the 21st century (Clifton et al., 2014).

1.3.1.2.2 International Emissions of Ozone Precursors

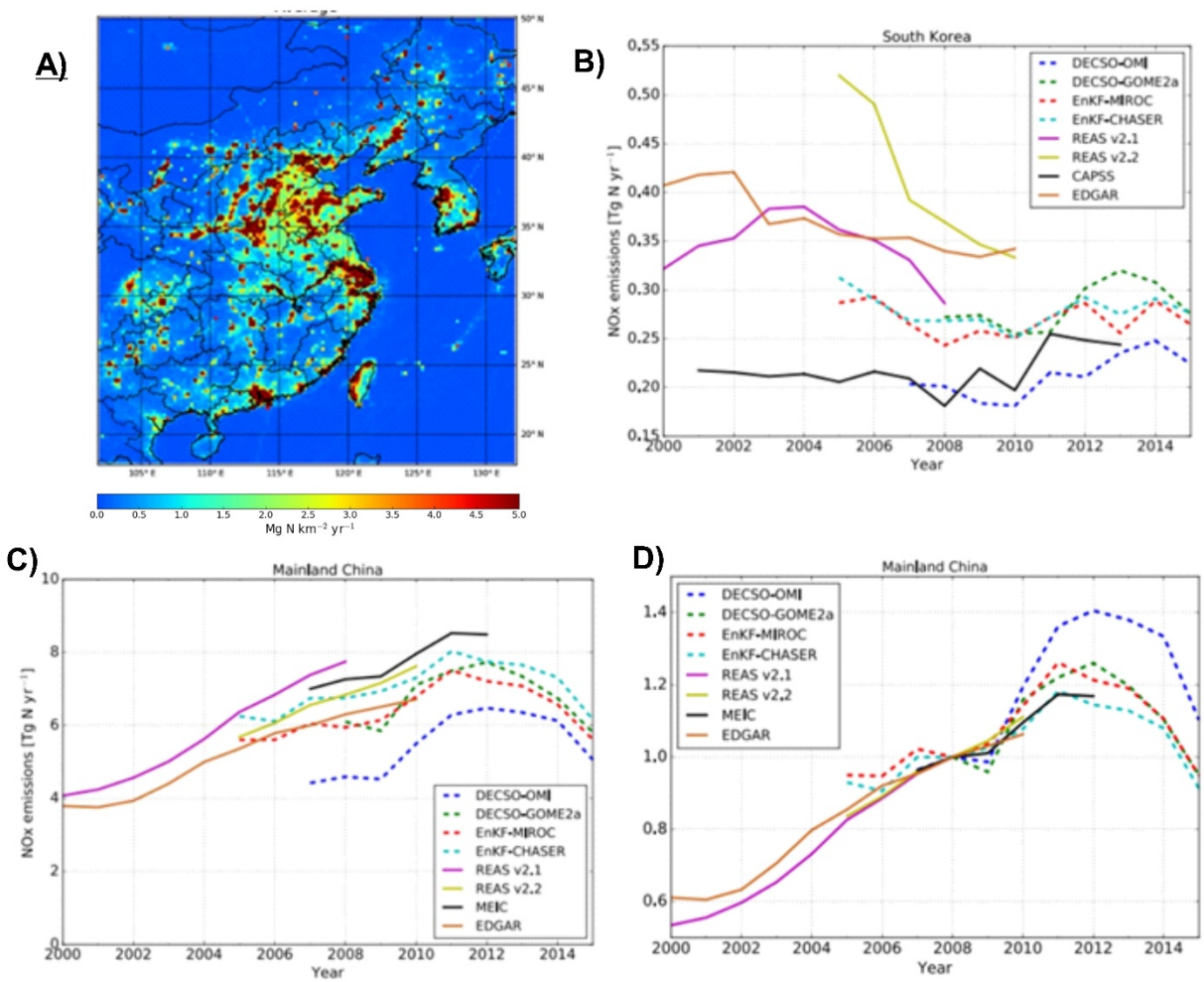
Ozone precursor emissions by countries that are "upwind" of the U.S. can contribute to U.S. ozone. As described in earlier assessments (U.S. EPA, 2013, 2006a, b), under certain atmospheric conditions, precursors emitted by large cities and other sources can be lofted above the boundary layer into the high-altitude zone referred to as the "free troposphere." (see Figure 1-1). NO_X and ozone have significantly longer atmospheric residence times in this colder atmospheric zone due to slower rates of reaction than they have near Earth's surface (Rastigejev et al., 2010). Furthermore, NO_X can react to form reservoir species (i.e., species that can remain stable over very long distances) at these altitudes. These reservoir species include PAN and similar compounds that become unstable at the warmer temperatures of the lower troposphere, regenerating reactive NO_X.

Large-scale atmospheric flows in the free troposphere can transport these pollutants and their reaction products (i.e., ozone precursors and ozone formed within the plume) across continents and oceans. Plumes from these international sources experience shear processes and dilution during advection downwind. However, distinctive, coherent plumes have been observed by aircraft, sondes, and satellites for a week or more. Downward mixing from the upper troposphere by way of other meteorological processes, such as convective mixing, can then bring ozone down into the boundary layer.

International sources of ozone precursors do vary in significance for USB ozone, depending on their relationship with the continental U.S. with respect to atmospheric dynamics and long-range circulation patterns. Asia, as described in previous ISAs (U.S. EPA, 2013, 2006a, b), has been an

important source of ozone precursors. Emitted due west of the continental U.S., across the Pacific Ocean, Asian precursors have been identified as contributing to USB ozone in the western states, and in the central and eastern U.S. under particular atmospheric transport conditions. Ozone precursor emissions from China and other Asian countries have been estimated to have consistently grown in the 1990−2010 period (Hoesly et al., 2018). However, within the past decade, trends in NO_X and CO emissions from China, the largest source in Asia, have begun to level off, then decline.

- *Satellite-derived NO_X inventories for China show a rapid decline in emissions beginning in 2012.* Inventories based on bottom-up accounting of emissions using activity values and emissions factors can be time consuming to develop. Emissions estimates for Asia are not currently available beyond 2012. However, inventories derived from inverse modeling constrained by satellite observations can be produced in near real time and are available for assessing Asian NO_X emissions rates. Ding et al. (2017) compared emissions estimates from four conventional bottom-up inventories (Emissions Database for Global Atmospheric Research [EDGAR], Multiresolution Emissions Inventory for China [MEIC], Regional Emissions Inventory in Asia, Versions 2.1 and 2.2 [REAS 2.1 and REAS 2.2]) to four satellite-derived inventories (DECSO-OMI, DECSO-GOME2a, EnKF-MIROC, EnKF-CHASER) for the domain shown in Figure 1-4, Panel A. While differences are present in the time-series results among all of the inventories, a clear trend in emissions from China is present across the ensemble (see Figure 1-4). Deviations in the temporal behavior of the various satellite-derived emissions are shown in Panel D of Figure 1-4, in which emissions estimates from all of the inventories have been normalized to their 2008 values. Chinese NO_X emissions climbed annually until approximately 2012 before leveling off and then declining. In contrast, there is very little agreement among the conventional NO_X inventories for South Korea. South Korean satellite-derived emissions estimates also differ significantly but demonstrate the same increasing, then decreasing trend between 2010 and 2015, as shown in Panel B of Figure 1-4.

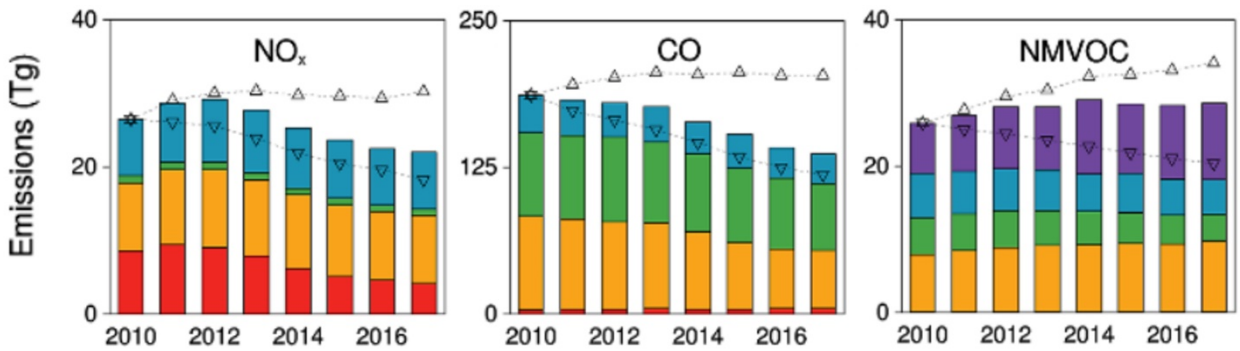


Source: Adapted from Ding et al. (2017). Permission pending.

Figure 1-4 Asian anthropogenic ozone precursor emission trends. A) The study domain, indicating annual NO_X flux rates by location, B) Annual NO_X emissions from eight inventories over South Korea, C) Annual NO_X emissions from eight inventories over China, and D) Temporal deviations among eight NO_X emissions inventories, when normalized with respect to 2008 emissions.

- *Stringent air quality standards implemented in 2013 within China have markedly reduced national emissions.* Zheng et al. (2018) applied the bottom-up inventory model underlying the Multi-resolution Emission Inventory for China (MEIC) to estimate anthropogenic emissions for 31 Chinese provinces. Figure 1-5 shows these estimates, aggregated to provide annual national emissions values. The results of this accounting indicate that China's emissions of NO_X and CO have declined by 17 and 27%, respectively, while nonmethane VOCs grew by approximately 5 Tg/year between 2010 and 2017. Zheng et al. (2018) analyzed this inventory using index decomposition analysis to identify the drivers behind these changes. The results of this analysis

indicated that stringent controls on power plant emissions were responsible for declines in NO_X. Improvements in combustion efficiency and oxygen blast furnace gas recycling in the industrial sector accounted for reductions in CO emissions.



Note: Red = power sector emissions; yellow = industrial emissions; green = residential emissions; blue = transportation emissions; purple = solvent use.

Source: Adapted from Zheng et al. (2018). Permission pending.

Figure 1-5 Anthropogenic ozone precursor emission trends derived using the MEIC emissions model. Lines marked with inverted triangles show the projected emissions trajectory, assuming activity levels were held constant at 2010 levels; upright triangles indicate projected trajectories assuming pollution controls were held constant at 2010 levels.

1.3.1.3 Natural Ozone Precursor Emissions

Ozone attributed to natural sources is formed through photochemical reactions involving natural emissions of ozone precursors from vegetation, microbes, animals, burning biomass (e.g., forest fires), and lightning.

1.3.1.3.1 Biogenic Nitrogen Oxide Emissions: Fertilized Soils

Biogenic sources of NO_X were not discussed in either the 2013 Ozone ISA or the ISA for the Oxides of Nitrogen—Health Criteria (U.S. EPA, 2016b, 2013). The topic was briefly mentioned in the ISA for Oxides of Nitrogen, Oxides of Sulfur, and Particulate Matter—Ecological Criteria (2nd external review draft) (U.S. EPA, 2018b). Microbial nitrification ($NH_4^+ \rightarrow NO_3^-$) and denitrification ($NO_3^- \rightarrow N_2$) processes in soils produce NO, contributing to local and regional atmospheric NO_X concentrations. Soil

NO emissions rates can be high enough to affect local and regional ozone concentrations under certain circumstances (Vinken et al., 2014). However, these rates are highly uncertain, being sensitive to biotic, abiotic, and anthropogenic factors and their interactions, such as climate, soil moisture and temperatures, and soil N content that can be altered by the addition of ammonium or nitrate fertilizers (Hall et al., 2018). Short, intense NO_X pulses following agricultural fertilization activities and precipitation events have been detected by satellite (Vinken et al., 2014). Hickman et al. (2017) found a nonlinear response in NO soil emissions, as a function of increasing fertilizer application and crop species, but high spatial variability among flux-rates led to significant uncertainty in the nature of the functional relationship. Soil moisture, conversely, substantially reduces NO emissions, leading to added uncertainty due to inhomogeneities in moisture content at the field scale (Hall et al., 2018).

Biogenic emissions of NO_X are estimated to contribute only a small part to national NO_X emissions, about 7.5% or 0.3 Tg N/year of the national total for all NO_X of 4.0 Tg N/year nationwide based on values reported in the 2014 NEI (U.S. EPA, 2018b, 2017). This estimate was computed based on 2014 meteorology data from the Weather Research and Forecasting (WRF) model Version 3.8 (WRF 3.8), using the Biogenic Emission Inventory System, Version 3.61 (BEIS 3.61) model, based on land use and vegetation data (U.S. EPA, 2016a). However the fraction of total soil NO_X due to fertilizer applications versus from natural soils are not reported separately. U.S. EPA (2018b) estimated fertilizer application contributes ~10−20% of global NO_X emissions. Further details on estimating biogenic NO_X emissions are given in the NEI Technical Support Document (U.S. EPA, 2016a).

1.3.1.3.2 Biogenic Volatile Organic Compounds (VOCs)

Vegetation emits substantial quantities of VOCs, such as terpenoid compounds (isoprene, 2-methyl-3-buten-2-ol, monoterpenes), compounds in the hexanal family, alkenes, aldehydes, organic acids, alcohols, ketones, and alkanes. Biogenic VOCs contribute to the mix of reactive organic precursors in polluted areas, such as urban settings with high concentrations of NO_X. As described in the 2013 Ozone ISA (U.S. EPA, 2013), vegetation is a major source of highly reactive, relatively low molecular weight organic compounds that contribute to the production of tropospheric ozone. Biogenic VOCs are particularly important precursors in the southeastern U.S. because of that region's warm climate and diversity of vegetation.

As discussed in the 2013 Ozone ISA, satellite measurements of formaldehyde (HCHO), produced by the oxidation of isoprene and other VOCs, have been used to estimate biogenic VOC emissions attributed to isoprene. Satellite-based and model techniques capture the spatial variability of biogenic isoprene emissions in the U.S. reasonably well, with ~40% uncertainty in satellite-derived isoprene emissions, which is similar to the ~50% error associated with model-based techniques (U.S. EPA, 2013).

- Biogenic VOCs fall into three major classes, with the smallest (isoprene) comprising one-third of all emissions, followed by increasingly large and complex compounds. According to the 2014

NEI (U.S. EPA, 2017), the major chemicals emitted by plants are isoprene (30%) and other terpenoid and sesquiterpenoid compounds (25%), with the remainder consisting of assorted oxygenated compounds and hydrocarbons. These specific estimates of biogenic emissions of VOCs were provided by the Biogenic Emissions Inventory System (BEIS) model Version 3.61 with data from the Biogenic Emissions Landuse Database (BELD) Version 4.1 and annual meteorological data. However, other emissions models are available, such as the Model of Emissions of Gases and Aerosols from Nature (MEGAN), which can also be used to develop emissions inputs for global and regional modeling efforts.

- VOC emissions from biogenic sources are estimated to be substantially larger than from anthropogenic sources at the global and national scales. The annual rate of VOC emissions from biogenic sources reported in the 2014 NEI v2 is ~39 MT/year. By comparison, VOC emissions from anthropogenic sources in the 2014 NEI v2 were ~17 MT/year. (Note: wildfire-derived VOC emissions [~2 MT/year] are counted as anthropogenic in the NEI. The effects of wildfire emissions on USB ozone are discussed in Section 1.3.1.3.3). Anthropogenic VOCs make up a larger fraction of VOCs in certain urban areas such as Los Angeles.

- Differences in vegetation-related biogenic VOC emissions as a function of species, meteorology, and geographic location introduce significant uncertainty in emissions estimates. Insufficient measurement data and modeling limitations, as summarized in the 2013 Ozone ISA (U.S. EPA, 2013), contribute to significant uncertainty in estimates of natural emissions. Uncertainty estimates can range from about 50% for isoprene under midday summer conditions in some locations to about a factor of ten for some compounds and landscapes (Guenther et al., 2000). Most biogenic VOC emissions occur during the warmer seasons because of their dependence on temperature and incident sunlight, but sesquiterpene emissions occur year-round. The BEIS and MEGAN models have been shown to predict spatially similar emissions, but modeling results can differ between them by about a factor of two, specifically for isoprene (Carlton and Baker, 2011), which is the most abundant biogenic VOC globally and nationally.

- Recent modeling studies provide a range of estimates of the contribution of biogenic VOCs to ozone mixing ratios, including max daily 8-hour avg (MDA8) concentrations. For instance, Huang et al. (2013b), cited in Jaffe et al. (2018), ran the multiscale Sulfur Transport and Deposition Modeling system at a 60-km grid scale for a time period in the summer of 2008, turned off biogenic emissions relative to a base case simulation, and found that biogenic emissions have slight negative impacts over most regions in Nevada, Idaho, Washington, and Oregon, due to the NO_X sensitive regime in those areas, and an estimated contribution of up to 15 ppb to MDA8 ozone from biogenic emissions over Northern California and the California Central Valley. In a study by Zare et al. (2014), a Danish Eulerian Hemispheric Model (DEHM) simulation for the year 2006 indicated that biogenic VOCs enhanced the average ozone mixing ratio by about 11% over the land areas of the Northern Hemisphere relative to a base case simulation for which BVOC were not turned off. In additional sensitivity simulations, Zare et al. (2014) turned off all natural emissions of VOCs, NO_X, NH_3, SO_2, CH_4, PM, CO and sea salt collectively and individually relative to a base case simulation. Zare et al. (2014) acknowledged that the sum of the individual sensitivities can be different from the results of the collective zero-out simulation for the various regions of the world due to nonlinearity in the processes of ozone formation chemistry. The discrepancies are different from region to region because of different atmospheric chemical regimes in each individual region. In a modeling simulation over the continental U. S. (CONUS domain) from May to September 2011, Zhang et al. (2017) used the Comprehensive Air Quality Model with Extensions (CAMx) Ozone Source Apportionment Tool (OSAT) with BEIS. The CAMx OSAT algorithm attributes ozone production to VOCs only when ozone forms under VOC-limited conditions. Zhang et al. (2017) found that domain-wide, biogenic VOCs can contribute on average 10−19% to regional ozone formation, with higher

contributions in the western U.S. and lower contributions in the southeastern U.S. Ozone formation in the southeast is typically NO_X-limited due to intense BVOC emissions in that region of the U.S. Hence, CAMx OSAT attributes most of the ozone in this region to NO_X rather than VOCs.

- Model emissions estimates of isoprene are sensitive to estimates of photosynthetically active radiation (PAR) and details concerning land use and species mapping. Isoprene makes up the largest fraction of vegetation emissions. Hu et al. (2015) found that the GEOS-Chem atmospheric model with the MEGAN v2.1 biogenic inventory reproduced isoprene observations at a site in the U.S. upper Midwest to within model uncertainty given improved land cover and temperature estimates. One of the key uncertainties for modeling biogenic VOC emissions comes from the estimation of PAR reaching the vegetation canopy. Zhang et al. (2017) found that using satellite retrievals instead of modeled PAR reduced BEIS and MEGAN estimates of isoprene by an average of 3−4% and 9−12%, respectively, but the simulations still overestimate observed ground-level isoprene concentrations by a factor of 1.1 for BEIS and 2.6 for MEGAN. The satellite retrievals in this study covered most of the continental U.S. Another key limitation for modeling biogenic VOC emissions comes from a lack of complete and up-to-date land use and species mapping information. Bash et al. (2016), using BEIS 3.61 with an updated canopy model formulation and improved land use and vegetation representation, found better agreement between CMAQ isoprene and monoterpene estimates compared with observations in northern California.

- Confidence in estimates of the contribution of biogenic VOC emissions to ground-level ozone remains low. Biogenic VOCs are well-understood contributors to ozone formation. However, uncertainties in the emissions of biogenic VOCs, limitations in the tools employed by photochemical models, complete and up-to-date land use and species mapping information, and a lack of a complete and detailed scientific understanding of biogenic VOC oxidation chemistry make it difficult to accurately apportion the fraction of ozone due to anthropogenic VOCs versus biogenic VOCs.

1.3.1.3.3 Landscape Fires

Landscape fires, including prescribed burning and wildfires, are complex sources of VOCs, methane, CO, and NO_X. Emissions from wildfire, in particular, are episodic but can have a significant downwind impact on ozone concentrations in populated areas. New observations and modeling study results identifying wildfire effects on observed ozone concentrations corroborate and extend the evidence presented in the 2013 Ozone ISA (U.S. EPA, 2013).

- *Wildfires contribute a few parts per billion (ppb) to seasonal mean ozone values in the U.S., but episodic contributions may be as high as 30 ppb*. Wildfire emissions and their subsequent photochemistry have highly variable impacts on ozone. Jaffe et al. (2018) and Jaffe and Wigder (2012) concluded, on the basis of their synthesis of the results of more than 100 recent scientific studies related to USB or other measures of background ozone, that wildfires contribute up to a few ppb of ozone to seasonal mean surface concentrations in the continental U.S. However, on an episodic basis, numerous studies demonstrate that wildfires may contribute up to 30 ppb to MDA8 at specific times and surface locations. They noted that ozone production generally increases up to 5 days downwind of emissions following a wildfire event. Ozone production measured by the ratio $\Delta O_3/\Delta CO$ was highly variable, and typically higher when emissions were transported and mixed with air from NO_X-rich urban areas. A global modeling study (Mao et al.,

2013) further supports the estimate of biomass burning's contribution to mean tropospheric ozone concentrations given by Jaffe et al. (2018), although as discussed below, modeled estimates of ozone production from fires remain highly uncertain.

- *In-plume photochemistry converts NO_X to PAN, a reservoir species for NO_X, increasing its capacity for affecting ozone concentrations far downwind of a fire*. Lagrangian plume models and box models have also been employed to better understand wildfire smoke plume chemistry and have generally corroborated observational studies showing rapid in-plume NO_X sequestration into PAN, which provides a reservoir of reactive nitrogen with a long lifetime in the free troposphere. Additional observational evidence of significant PAN production in wildfire smoke has emerged (Fischer et al., 2018; Busilacchio et al., 2016).

- *Eulerian photochemical modeling remains highly uncertain in its estimates of the impact of fire emissions on ozone due to insufficient information on fuels, meteorological conditions influencing smoke production, as well as existing model grid scales and the sufficiency of the photochemical mechanisms available*. Jaffe et al. (2018) discussed current methodologies of isolating the effects on ozone from biomass burning. Such modeling continues to have high uncertainty, arising from wide variation in the production of NO_X and VOC among different fires. These variations in precursor emissions arise from physical and chemical differences between fuel types, moisture content, and meteorological conditions. Capturing wildfire dynamical processes, such as plume heights, in models is also an area of active model development and improvement. Jaffe et al. (2018) observed that reductions in the uncertainty in the estimates of USB ozone from fire emissions will require developing or improving models that integrate the results of intensive field studies with evaluation and comparison of Eulerian, Lagrangian, and statistical models.

- *Statistical models based on observational data have also been used to identify the effects of fire emissions on downwind MDA8 ozone concentrations*. Statistical models of observed ozone data, combined with colocated particulate matter measurements and satellite data from the NOAA Hazard Mapping system, have shown some capability of identifying days with surface smoke impacts and in estimating the amount of added ozone from wildfire smoke above what would be expected on a typical smoke-free day under similar conditions. Jaffe et al. (2018) described a few instances of such statistical model applications in the western U.S. where they identified a few days with MDA8 ozone greater than 70 ppb that were impacted by ozone from wildfire smoke. Newer studies have used statistical models to attribute the amount of ozone from biomass burning. Liu et al. (2017b) used 10 monitoring sites in Kansas to apportion the contribution of MDA8 ozone from prescribed range/pasture burning on elevated ozone days in April between 2001 and 2016. On days exceeding 70 ppb, they found the average ozone attributed to biomass burning was 21 ± 9 ppb. Additionally, Lindaas et al. (2017), using surface monitoring data in Colorado, estimated a contribution of up to 10 ppb ozone on each day. In a more comprehensive analysis, including colocated $PM_{2.5}$ measurements and nearby temperature measurements, Gong et al. (2017) estimated a fire emissions impact on mean MDA8 ozone of 3−36 ppb (88% of the monitors, within a 95% confidence interval). They also looked at the frequency of smoke-impacted days when ozone monitors exceeded 70 ppb and found that the percentage of impacted days ranged widely, but sites with the highest number of 70 ppb exceedance days generally had fewer than 20% of days that were smoke-impacted. Brey and Fischer (2016) looked at all smoke impact days but did not separate wild and prescribed fire from anthropogenic biomass burning sources. The more recent study by Gong et al. (2017) suggested that the Brey and Fischer (2016) analysis overestimates the effect of fire emissions on ozone production, especially in coastal areas because they did not include a number of additional meteorological variables, such as air mass transport patterns, in their statistical model.

- *Satellite-based detection of fire emissions continues to be a work in progress.* Many studies employing satellite data to estimate NO_X and other trace gases emitted by wildfires have been

conducted (Schreier et al., 2015; Tanimoto et al., 2015; Mebust and Cohen, 2014; Schreier et al., 2014; Ross et al., 2013; Worden et al., 2013; Mebust et al., 2011; Tereszchuk et al., 2011). A recent study assessed emission coefficients of NO_X via OMI NO_2 tropospheric column densities and MODIS fire radiative energy for three fuel land types and reported markedly lower estimates then previous estimates. These lower estimates are potentially due to underlying satellite retrieval inputs (Mebust et al., 2011). Also, researchers assessed the potential of the TES instrument to characterize fire-derived PAN in the free troposphere over North America in summer (Fischer et al., 2018), but validation of the data was incomplete.

1.3.1.3.4 Lightning NO_X

Nitrogen oxide is produced when lightning causes the dissociation of N_2 into nitrogen radicals that subsequently react with molecular oxygen. The 2013 Ozone ISA (U.S. EPA, 2013) discussed the highly uncertain U.S. estimates provided by Fang et al. (2010) for lightning-generated NO_X (LNO_X) of ~0.6 MT for July 2004, or ~ 40% of the anthropogenic emissions for the same period. However, Fang et al. (2010) also estimated that ~98% is formed in the free troposphere, limiting the direct effect on local, ground-level ozone. Contributions to the surface NO_X burden are low because most of this NO_X is oxidized to NO_Z species, including nitric acid (HNO_3), and nitrous acid (HONO), peroxyacetyl nitrate (PAN), peroxymethacrylic nitrate (MPAN), and peroxylpropionyl nitrate (PPN), during downward transport into the planetary boundary layer. The remaining 2% of LNO_X is formed within the planetary boundary layer. The 2013 Ozone ISA (U.S. EPA, 2013) also described the indirect effect that lightning has on USB or NAB ozone by initiating wildfires.

- *Eighty percent of NO_X is generated by lightning in the upper troposphere, where it can have a longer atmospheric residence time than NO_X derived from ground sources*. Although the LNO_X source is significantly smaller than combustion-derived NO_X, it is produced in the upper troposphere where the atmospheric lifetimes of NO_X and ozone are long (Murray et al., 2012). Monks et al. (2015), in their synthesis of several studies, reported that LNO_X is responsible for more than 80% of upper tropospheric NO_X and can also affect surface ozone levels through its role in the determining the OH/HO_2 ratio.
- *LNO_X shortens the atmospheric lifetime of CH_4.* As previously discussed, methane is an important ozone precursor. By producing sudden bursts of excess OH, methane is removed from the atmosphere to form methyl-peroxy radical. The methyl-peroxy radical, once formed, reacts immediately to form ozone (Monks et al., 2015).

1.3.2 Stratosphere-Troposphere Exchange Processes

1.3.2.1 Tropopause Folding

Tropospheric ozone derived from stratosphere-troposphere dynamics was described in detail in the 2013 Ozone ISA (U.S. EPA, 2013). Stratospheric air rich in ozone can be transported into the

troposphere under certain meteorological circumstances, with maximum contributions at midlatitudes during the late winter and early spring. In a process known as "tropopause folding," deep stratospheric intrusions of ozone-rich air can occur; they form only episodically but have the ability to quickly and directly reach the surface (U.S. EPA, 2013). The descent of stratospheric ozone into the troposphere is along isentropic surfaces, and these intrusions are often observed as "filaments" or "ribbons" in water vapor satellite imagery or identified by meteorological data (e.g., relative humidity, potential vorticity) or chemical (e.g., beryllium 7) tracers (U.S. EPA, 2013).

The 2013 Ozone ISA (U.S. EPA, 2013) discussed the potential role of deep convection, another form of stratosphere-troposphere exchange, as a mechanism for transporting stratospheric ozone into the upper troposphere. The 2013 Ozone ISA noted the study of Tang et al. (2011), which through modeling estimated that deep convection penetrating the tropopause increases the stratospheric-to-troposphere ozone flux by 19% annually in the Northern Hemisphere, with greatest impacts occurring in the summer months (49% in June). While the 2013 Ozone ISA (U.S. EPA, 2013) highlighted studies showing the influence of stratospheric-tropospheric exchange to surface ozone, it did not estimate STE's impact on USB ozone.

- *Deep stratospheric intrusions are common in the western U.S., impacting high elevation locations during the springtime.* The incidence of tropopause folds is greatest in the early part (late winter and spring) of the year when synoptic-scale midlatitude cyclones are most active, occurring near upper level frontal zones where Rossby wave breaking is prevalent (Langford et al., 2017; Skerlak et al., 2015; U.S. EPA, 2013; Lin et al., 2012a).
- *Stratospheric intrusions can be observed with lidar and other tools*. Langford et al. (2015) used lidar measurements and modeling results to estimate stratospheric influence of up to 30 ppb during high surface ozone events around the Las Vegas, Nevada area during the 2013 Las Vegas Ozone Study (LVOS). Meteorological variables and ozone data from the high resolution NASA MERRA-2 reanalysis data set were used to identify stratospheric intrusion events over Colorado that occurred during the spring of 2012 (Knowland et al., 2017).
- *Stratospheric intrusions can be simulated with global chemistry models, although uncertainties remain*. The GEOS-Chem and GFDL-AM3 global chemistry models (Zhang et al., 2014; Lin et al., 2012a) have successfully simulated springtime deep stratospheric events affecting high-elevation sites in the western U.S. The GEOS-Chem simulations showed consistent springtime contributions of stratospheric ozone between 8.8 and 9.4 ppb, with contributions of up to 15 ppb during intrusion events. The AM3 model estimated contributions from stratospheric ozone ranging from 17 to 40 ppb during the high surface ozone events from a model simulation of the spring of 2010. However, AM3 is thought to overestimate ozone contributions from the stratosphere (Lin et al., 2012b).
- *Stratospheric intrusions can lead to spikes in hourly and daily ozone concentrations, or smaller increases over several days.* Deep stratospheric intrusions have been shown to directly reach the ground surface, albeit infrequently. Intrusions often extend into the mid troposphere over longer timescales (up to 2 weeks) and may mix downward and affect surface ozone concentrations (Stohl et al., 2000). For example, the influence of stratospheric intrusions have been seen at populated areas like Boulder, CO (Langford et al., 2009), which showed ozone concentrations as high as 100 ppb in 1-minute data. Stratospheric intrusions can lead to ozone spikes seen in hourly

and daily data (Langford et al., 2009) or to smaller ozone increases over several days (Lin et al., 2012a).

- *Quantifying the contribution of STE to surface ozone remains challenging and is a source of uncertainty in estimating USB ozone*. Stratosphere-troposphere exchange of ozone has been observed using ground measurements, in situ aircraft or balloon measurements; through remote sensing (lidar, satellite); identified with reanalysis data; and modeled via chemical transport and global chemistry models. However, STE's contribution to USB ozone remains hard to quantify. As previously mentioned, the AM3 global chemistry model (Lin et al., 2012b) has been used to estimate the stratospheric ozone contribution from deep intrusion events to be between 17 and 40 ppb at high surface ozone sites during springtime in the western U.S. Stratospheric intrusion events reaching the surface have less influence on surface ozone during the summer months when total ground-level ozone concentrations tend to be highest.

1.3.2.2 Deep Convective Mixing

Since the previous assessment, studies of the dynamics within thunderstorm anvil clouds has revealed that deep convection can entrain stratospheric ozone and draw it down into the upper troposphere. The Deep Convective Cloud and Chemistry (DC3) (Barth et al., 2015) aircraft field campaign over the central U.S. in May and June of 2012 identified this process using in situ measurements (Huntrieser et al., 2016; Pan et al., 2014). Pan et al. (2014) observed in situ ozone mixing ratios as high as 150 ppb in the upper troposphere adjacent to the storm cloud edge. They postulated that these high concentrations could be the result of the dynamical response to tropospheric air overshooting the tropopause, with stratospheric air being mixed down into the upper troposphere and wrapping around the cloud edges of the thunderstorm outflow. The high ozone concentrations found at the storm edges were anticorrelated with mixing ratios of measured CO, indicating the stratosphere as the source of the ozone-enriched air. The study found ozone enhancement in the upper troposphere near storm cloud edges on numerous flight sample cases that indicated the prevalence of the deep convection stratospheric-tropospheric exchange (STE) mechanism during the 2012 field campaign. Although the studies of Pan et al. (2014) and Huntrieser et al. (2016) provided observed data of deep convection leading to the downward flux of stratospheric air into the troposphere, the authors did not estimate the contribution deep convection made to USB or other measures of background ozone at the surface.

1.4 Ozone Photochemistry

The general photochemistry of tropospheric ozone is well understood and described in detail in previous U.S. EPA integrated science assessments and criteria documents (U.S. EPA, 2013, 2006a) and textbooks (Seinfeld and Pandis, 2006; Finlayson-Pitts and Pitts, 2000). Ozone is a product of the oxidation of carbonaceous precursor gases in the presence of NO_X. The involvement of NO_X as an oxidant ensures rapid ozone formation in the presence of solar radiation. This mechanism differs greatly from the chemistry of stratospheric ozone formation or of ozone formed by lightning. The former requires the hard

solar ultraviolet radiation present above the troposphere and the latter requires an electrical discharge at a voltage sufficient to ionize molecular nitrogen.

Recent developments in ground-level ozone chemistry include observations and studies concerning unexpectedly high ozone concentrations observed during winter in western U.S. mountain basins, and new work concerning the role of marine halogen chemistry in depleting marine and coastal ozone concentrations. Chemistry and emissions associated with these processes are not included in all models, adding to uncertainty in the evaluation of USB ozone at sites impacted by ozone that has been transported over marine environments.

1.4.1 Winter Ozone in Western Intermountain Basins

Ordinarily, ozone is a spring/summer/fall pollutant with the highest annual MDA8 levels typically occurring on hot, sunny, stagnant days associated with summer weather conditions. As first described in the 2013 Ozone ISA (U.S. EPA, 2013), high ozone levels during winter conditions have been observed in two western U.S. intermountain basins with relatively high levels of anthropogenic precursor emissions from oil and gas activity: Utah's Uinta Basin (UB) and Wyoming's Upper Green River Basin (UGRB). These high ozone episodes date back to at least the winter of 2005 in the UGRB and the winter of 2009 in the UB (Helmig et al., 2014; Schnell et al., 2009).

- *High wintertime ozone events continue to occur in the Uinta and Upper Green River Basins.* Winter ozone levels in the UB and UGRB have been measured as high as 150 ppb (1-hour avg) or greater (Helmig et al., 2014; Rappenglueck et al., 2014). For comparison, max 1- and 8-hour ozone levels in the winter of 2013 in the UB exceeded that of summer ozone levels of the Los Angeles basin (Helmig et al., 2014), a location that has historically experienced some of the highest summertime ozone episodes in the U.S. In the winter of 2008, the UGRB observed MDA8 values above 75 ppb 14 times (Schnell et al., 2009), and in the winter of 2013 the UB experienced 39 days with MDA8 values greater than 75 ppb at individual monitoring stations (Helmig et al., 2014).
- Wintertime mountain basin high ozone episodes occur on cold winter days with low wind speeds, clear skies, substantial snow cover, extremely shallow boundary layers driven by strong temperature inversions, and substantial ozone precursor emissions activity from the oil and gas sector. Wintertime inversions with low winds are sometimes referred to as "cold pool events," or more specifically, "valley cold pool events" which are defined as an inversion below the maximum crest height of the surrounding mountains coupled with average wind speeds beneath the inversion top that are less than 5 m/second (Ahmadov et al., 2015). These inversions, which trap and concentrate local anthropogenic precursor emissions, can last several days or longer until advection or turbulent mixing breaks them up (Ahmadov et al., 2015). During these events, the strong inversion isolates the local air mass from overlying layers of the atmosphere (no mixing). Therefore, there is little to no influence from upwind emissions sources. Large sources of local precursor emissions drive the ozone episodes during these cold pool events. High ozone episodes during valley cold pool events have not been observed in areas without oil and gas sector activity. Snow cover enhances the strength and persistence of the surface inversion layer and contributes to ozone formation photochemistry by enhanced photolysis rates (due to the high albedo of the

snow surface) (Ahmadov et al., 2015; Field et al., 2015; Edwards et al., 2014; Rappenglueck et al., 2014; Warneke et al., 2014). The relatively snow-free conditions during the winter of 2012 in the UB were not accompanied by high ozone events, but the cold pool conditions during the snow-covered winter of 2013 resulted in a number of days where MDA8 values measured above 75 ppb (Ahmadov et al., 2015).

- The chemistry driving wintertime ozone episodes seems to be different from the chemistry driving summertime ozone episodes in terms of radical sources, as seen in measurements and by modeling studies. Ozone production involves the hydroxyl (OH) radical, which in summer is primarily formed from the photolysis of pre-existing ozone and subsequent reaction of one of the products of this reaction, the electronically excited state atomic oxygen ($O[^1D]$), with water vapor. There is typically less solar radiation and water vapor during the winter, which is why Edwards et al. (2014) saw a 15- to 60-fold decrease in OH production from this pathway (relative to summer) when modeling the UB high ozone episodes for the winter of 2013. Edwards et al. (2014) found that the dominant source of radicals was from the photolysis of carbonyl compounds associated with the high VOC emissions from oil and gas activity in the basin during these episodes. (Zhou et al.) conducted photochemical box model simulations using the Master Chemical Mechanism v3.3 and found similar sensitivity results to that of Edwards et al. (2014) for the UB wintertime ozone episodes. Like Edwards et al. (2014), Ahmadov et al. (2015) suggested that VOC photochemistry is an important source of radicals, including those formed from primary and secondary formaldehyde photolysis, as well as from photolysis of dicarbonyls and hydroxy ketones. Sensitivity studies show the ozone formation regime during the 2013 episodes in the UB was VOC-limited (Ahmadov et al., 2015). In the UGRB, Rappenglueck et al. (2014) found that the dominant source of OH production for the winter of 2011 was nitrous acid (HONO) photolysis with minor pathways of production from alkene ozonolysis and formaldehyde photolysis. Rappenglueck et al. (2014) suggested the HONO is formed through nitric acid (HNO_3) produced during the atmospheric oxidation of NO_X deposited onto the snow surface where it undergoes photo-enhanced heterogeneous conversion to HONO as well as combustion-related emissions of HONO. However, Edwards et al. (2014) found that HONO was not present in high concentrations and, therefore, could not be a major source of OH production during winter ozone episodes in the UB. Oil and gas extraction is the only major source of anthropogenic emissions in the remotely located UGRB. These emissions include NO_X from compressors and drill rigs and methane and nonmethane hydrocarbons (VOCs) from wellhead production equipment (Rappenglueck et al., 2014).

- *Oil and gas sector impacts on ambient ozone levels extend beyond wintertime ozone episodes.* Recent occurrences of high wintertime ozone episodes and initial investigations into the anthropogenic emissions and the chemistry driving these events indicate the importance of future research to accurately quantify the role of increasing oil and gas sector emissions on ambient ozone in the western U.S. Modeling studies summarized by Ahmadov et al. (2015) indicated enhancements of 5−10 ppb to summertime 8-hour ozone concentrations that are attributed to oil and gas extraction activity in various locations across the U.S. Cheadle et al. (2017) analyzed precursor species measurements and meteorology data including back trajectories in the northern Front Range in Colorado to estimate ambient ozone enhancement attributable to nearby oil and gas activity and found that on specific summer days oil- and gas-related precursor emissions could contribute locally up to 30 ppb ozone.

1.4.2 Halogen Chemistry

Multiphase processes have been associated with the release of reactive halogen species from marine aerosol particles. The atmospheric chemistry of halogens involves compounds containing chlorine, bromine, or iodine which can react among themselves and with other species and can be important for tropospheric ozone destruction (U.S. EPA, 2013).

- *Additional studies have further resolved the influences of halogen chemistry on ozone mixing ratios.* Ozone mixing ratios and deposition velocities over the ocean vary with atmospheric turbulence and seawater chemical composition. The sea-to-air movement of chemical species containing halogens like bromine, iodine, and chlorine affects the ozone photochemistry in the atmosphere above the oceans. For example, photolysis and oxidation of halogen-bearing species can release iodine and bromine, which can catalytically react with ozone to reduce ozone levels over the ocean (Sarwar et al., 2015). Tuite et al. (2018) measured iodine monoxide (IO) during periods when low ozone (<25 ppb) air masses originating over the Gulf of Mexico flowed onshore near Galveston, TX. Tuite et al. (2018) compared these measurements to a CAMx model simulation which incorporated halogen chemistry and concluded that iodine is the most influential halogen in the Texas gulf coast area. The analyses of their measurements and model simulations indicate iodine chemistry played a role in keeping ozone mixing ratios low in the relatively clean offshore air that flowed onshore during the study period (Tuite et al., 2018).
- *Ozone is sometimes overpredicted along marine coastlines in photochemical model simulations*. Incorporating marine halogen chemistry into modeling studies improved agreement with observed ozone in some circumstances. Sarwar et al. (2015) incorporated enhanced ozone deposition and marine halogen chemistry involving photolysis of higher iodine oxides into a photochemical model (hemispheric CMAQ) simulation and found that including these reactions improves ozone model performance by reducing ozone levels to better compare to observations near marine environments in the Northern Hemisphere. Sarwar et al. (2015) found enhanced deposition reduces mean summer-time surface ozone by ~3% over marine regions in the Northern Hemisphere. Halogen chemistry without the photochemical reactions of higher iodine oxides reduces surface ozone by ∼15% whereas simulations with the photochemical reactions of higher iodine oxides indicate ozone reductions of ~48%. Over most terrestrial regions near the coast, ozone mixing ratios are reduced by 2−4 ppb due to halogen chemistry without the photolysis of higher iodine oxides. Gantt et al. (2017) incorporated the same detailed iodide-mediated ozone deposition and marine halogen chemistry as Sarwar et al. (2015) to a finer (CMAQ) domain over the continental U.S. as well as a parameterized version of the marine halogen chemistry to preserve computational time. The parameterized version was applied as a first-order ozone loss rate over oceanic grid cells as a function of atmospheric pressure. Gantt et al. (2017) did this for the lateral boundary conditions generated by the hemispheric model feeding the regional scale model as well as for the regional model simulation over the continental U.S. domain and compared the results to ambient air measurements. Including the marine halogen processes in the model improved overpredictions of surface ozone along the coast and over the open ocean, achieving reductions in bias of 2−3 ppb for the majority of the sites along the Gulf and Atlantic coasts, but exacerbated underpredictions of high surface ozone in some near-coast areas like California's Central Valley and the urban areas of Washington D.C. and New York City (Gantt et al., 2017). Many previous modeling studies which characterize background ozone did not include a complete treatment of marine halogen chemistry (Emery et al., 2012; Zhang et al., 2011) and therefore may overestimate background ozone transported over marine environments.

- *Halogen marine chemistry can play a role in coastal urban air quality.* The ocean is a natural source of halogenated compounds which when released to the atmosphere can undergo photolysis and oxidation to release reactive chlorine, bromine, and iodine radicals. In marine environments near coastal cities with polluted urban air, gas-phase chlorine emissions (Cl_2 and HOCl) and chloride from sea salt can increase ozone mixing ratios by releasing NO_2 from photolysis of nitryl ($ClNO_2$) as well as through the oxidation of VOCs by chlorine radicals. In a 4-km photochemical model simulation incorporating marine halogen chemistry over Los Angeles, Muñiz-Unamunzaga et al. (2018) saw improved regional/coastal air quality predictions compared with measurements. Some earlier $ClNO_2$ modeling papers showed that $ClNO_2$ can increase ozone formation in winter by up to 13 ppb and in summer by up to 6.6 ppb, although typical ozone increases were generally below 2 ppb (Sarwar et al., 2012; Simon et al., 2009). While photolysis of $ClNO_2$ can lead to the formation of ozone, Muñiz-Unamunzaga et al. (2018) found that the chemistry of chlorine-, bromine-, and iodine-containing compounds together have a net reduction effect on surface ozone concentrations, with the reduction being larger near the coast and smaller farther inland. In terms of the impact of halogen chemistry on NO_X, which is important as a precursor to ozone, the effect of halogen chemistry on NO_2 varies by emission source distribution; however, Muñiz-Unamunzaga et al. (2018) saw that NO_2 concentrations generally increased over nonurbanized areas and the ocean and decreased in downtown Los Angeles when halogen chemistry was incorporated into the model.

- Halogen chemistry depletes ground-level ozone directly by reaction with bromine and iodine radicals and indirectly by changing the budget and balance of important atmospheric oxidants like NO_X and HO_X (Muñiz-Unamunzaga et al., 2018; Stone et al., 2018). The research of Muñiz-Unamunzaga et al. (2018) supports the finding that in polluted coastal areas like the megacity of Los Angeles, halogen chemistry can shift the NO_X partitioning to NO, while in nonpolluted areas with high concentrations of halogens, the reaction between XO and NO (where X = I or Br) shifts the balance to NO_2. Muñiz-Unamunzaga et al. (2018) saw a decrease in HO_2 due to halogen chemistry with a small increase in diurnal mean OH concentration for an overall decrease in HO_X radicals. Likewise, Stone et al. (2018) saw a substantial decrease in HO_2 with the inclusion of halogen chemistry and a marginal increase in OH concentrations at certain locations but an overall decrease in OH and HO_X globally.

1.5 Inter-annual Variability and Longer Term Trends in Meteorological Effects on Anthropogenic and U.S. Background (USB) Ozone

In addition to the quantities of ozone precursors emitted into the atmosphere by human activities and natural sources, temperature, wind patterns, cloud cover, and precipitation also very important variables in the production of atmospheric ozone (Nolte et al., 2018). The 2013 Ozone ISA highlighted the importance of meteorology on ozone formation (i.e., temperature dependence, the magnitude of solar radiation, and the mixing/transport of ozone and its precursors). Meteorology is, therefore, an important factor in the formation and transport of USB ozone. The 2013 Ozone ISA explained that multiyear trends in U.S. ozone concentrations are influenced by the number of synoptic-scale and mesoscale stagnation events, which vary from year-to-year, often making it difficult to evaluate the progress and effectiveness of emissions reduction programs. Since the 2013 Ozone ISA, additional studies have looked into the role of meteorological effects on ozone and the year-to-year trends in ozone concentrations. Large-, regional-,

and local-scale atmospheric circulation patterns have been shown to influence both observed U.S. background ozone and the local production of ground-level ozone. More recently, Nolte et al. (2018) described emerging, robust evidence that the effects of climate warming on meteorology are negatively affecting ground-level ozone concentrations.

Large-scale meteorology patterns influence USB ozone in several ways, including the likelihood of the occurrence of deep stratospheric intrusions events in the western U.S., the transport of Asian pollution to the U.S., and regional temperature and precipitation patterns which can influence the frequency and distribution of wildfires emissions of VOC precursors from vegetation and combustion-derived NO_X. During localized stagnation events conducive to ozone production, ground-level ozone concentrations can be further influenced by regional and large-scale meteorology patterns or by regional-scale background ozone aloft being mixed down to the surface at urban sites.

Large-scale meteorology patterns help create the local-scale conditions that are conducive to photochemical production of the ground-level. Example conditions include stagnation events associated with high temperatures and high ozone concentrations versus cool, wet meteorological conditions associated with lower ambient ozone concentrations.

Large-scale atmospheric circulation patterns are also subject to variability on annual and decadal scales, which is reflected in the patterns of regional- and local-scale U.S. ground-level ozone concentrations.

1.5.1 Meteorological Effects on Ozone Concentrations at the Ground Level

Meteorology at the regional and local scales establishes the chemical conditions that govern the formation of ozone. Meteorological variables of importance at these scales include temperature, relative humidity, wind speed, and precipitation. Synoptic-scale circulation (i.e., meteorological processes at scales on the order of 1,000 km) are particularly important in determining ozone formation at regional and local scales.

- *Ozone was found to be strongly correlated to meteorology over the Intermountain West.* Reddy and Pfister (2016) found that surface ozone in the western U.S. is well correlated with the 500 millibar (mb) pressure level height. The study showed that the July mean max 8-hour ozone increased when the mean July 500 mb height also increased. Over the western U.S., increases in the 500 mb level are often associated with weather (clear skies, low wind) that is conducive to ozone formation. By using the 500 mb height variable to detrend and correct for the influence of meteorology, the study found that July max 8-hour ozone has steadily decreased from 1995 to 2013 in the Wasatch Front area surrounding Salt Lake City, UT. Over the same time period, a general increase in July max 8-hour ozone was found along the Front Range (Denver area) of Colorado. The study hypothesizes that ozone increases in Denver areas may be the result of emissions associated with population growth and/or emissions from the increased activity of nearby oil and gas development.

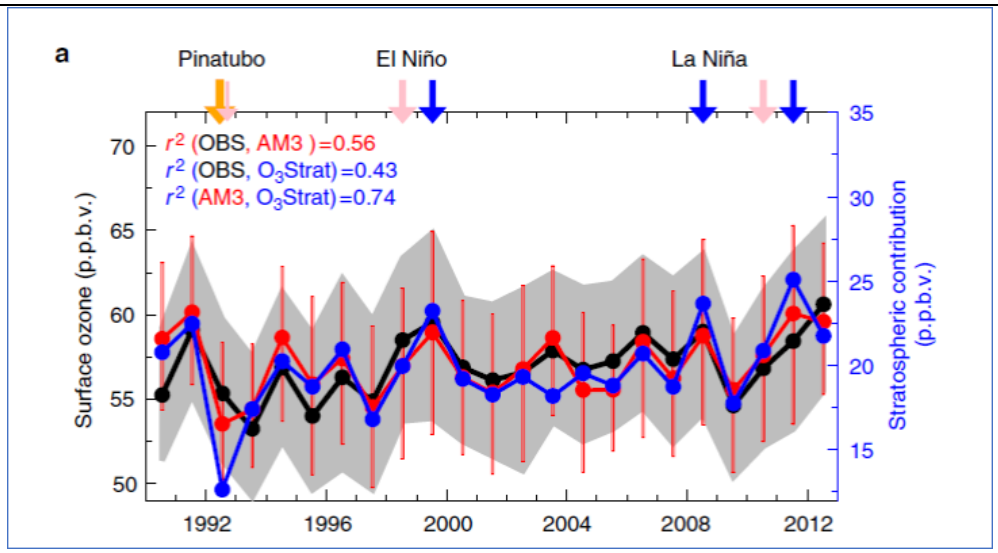
- *Summertime ozone in the eastern U.S. and Midwest are affected by synoptic-scale meteorology patterns.* Shen et al. (2015) quantified the sensitivity of max daily 8-hour ozone in the eastern U.S. and Midwest to regional meteorology patterns. They found that ozone over the eastern U.S. and Midwest is sensitive to the location of the polar jet stream and the location of the Bermuda High pressure system. Lower surface ozone concentrations occur more frequently when the polar jet stream is situated over the Midwest and eastern U.S. When the location of the Bermuda High shifts eastward, clean marine air masses, which are less conducive to ozone formation, are able to reach the eastern U.S. A westward shift of the Bermuda High prevents marine air from reaching the continent and promotes conditions conducive to ozone formation (e.g., stagnation, clear skies, higher temperatures). Overall, the study finds observed ozone trend (from 1980−2012) in the eastern U.S. is decreasing, supporting the work of (Cooper et al., 2012).
- *The effects of local precursor emissions controls can be masked by meteorological variability.* By using empirical methods to detrend and account for meteorological variability on ozone, Henneman et al. (2015) showed that the overall mean max daily 8-hour ozone had decreased by 4% between 2000 and 2012 in Atlanta, GA.

1.5.2 Inter-annual and Multidecadal Climate Variability

The 2013 Ozone ISA did not discuss trends in meteorology associated with periodic variations in winds and sea-surface temperatures. Nevertheless, natural variability induced by large-scale climatological cycles has the ability to influence synoptic-scale patterns important for surface ozone. Inter-annual (e.g., the El Niño-Southern Oscillation [ENSO] cycle) and multidecadal (e.g., the Pacific Decadal Oscillation [PDO]) climate variability is especially important for USB ozone or other measures of background ozone in the U.S. because these climate patterns affect long-range transport of international pollution, the frequency of deep stratospheric intrusion, stagnation events, and wildfire activity.

- *The frequency of stratospheric intrusion events is linked to natural climate variability.* Lin et al. (2015) showed the frequency and inter-annual variability of springtime stratospheric intrusion events due to deep tropopause folds in the western U.S. are linked to the ENSO cycle and the subsequent location of the polar jet stream. Under La Niña conditions, the polar jet is often set up over the western U.S. and leads to more frequent springtime stratospheric intrusion events reaching surface locations compared with the same season following an El Niño (see Figure 1-6). Recent work by Albers et al. (2018) highlighted the importance of wintertime buildup of ozone in the lower portion of the stratosphere on the inter-annual variability of stratospheric intrusions in the western U.S.
- *Long-Range Transport of Asian pollution is sensitive to ENSO and the PDO.* Lin et al. (2014) found that the influence of Asian pollution and ozone measurements at surface observations in Hawaii are tied to inter-annual (via ENSO) and multidecadal (via the PDO) climate variability and notes that these climate patterns are likely important for transporting international emissions to the western U.S.
- *Deep stratospheric intrusions are sensitive to the ENSO cycle and the Northern Annular Node (NAM) inter-annual oscillation.* An increase in upper tropospheric ozone is often associated with El Niño events (Langford, 1999). However, this increase in upper level ozone rarely reaches the surface. During La Niña, the upper level jet is typically positioned over the western U.S. where

deep stratospheric intrusions more frequently reach the surface (Lin et al., 2015). Thus, in the western U.S., La Niña years are more likely to see an increase in stratospheric-influenced background ozone during springtime/early summer than El Niño years. Albers et al. (2018) tied the strength of springtime ozone intrusion events to the NAM inter-annual oscillation.



Note: The black trace provides the observed median daily 8-hour ozone. The red trace is the Geophysical Fluid Dynamics Laboratory's (GFDL) AM3 model estimate of ozone, and the blue trace is the AM3 model estimated stratospheric contribution.

Source: Permission pending Lin et al. (2015).

Figure 1-6 Model-estimated April−May stratospheric ozone contributions and observed surface ozone concentrations between 1990 and 2013 at 22 high-elevation sites in the western U.S.

- *The Atlantic Multidecadal Oscillation (AMO) and ENSO influences ozone levels in the eastern U.S.* (Shen et al., 2017) found that the warm phase of the AMO drives warmer, drier, and stagnant weather in the eastern and midwestern U.S. and that a shift from the cold to warm phase of the AMO can increase max daily 8-hour ozone between 1 and 5 ppb in this region.
- *Inter-annual variability of wintertime ozone in the Intermountain U.S. has been tied to the phase of the Arctic Oscillation.* The Arctic Oscillation (AO) refers to an atmospheric circulation pattern over the mid to high latitudes of the Northern Hemisphere and can have a strong influence on weather and climate in the U.S., especially during winter. (Zhou et al.) found that year-to-year variability of wintertime ozone concentrations in the intermountain West were correlated to the AO, where a negative phase of AO is associated with higher wintertime ozone and vice versa. Within oil and gas basins of the intermountain West, the colder surface temperatures associated with the negative AO, along with consistent snow cover, can lead to elevated wintertime daily maximum 8-hour ozone above 70 ppb. For example, wintertime 8-hour ozone concentrations reached greater than 100 ppb in the Uinta Basin in Utah during the 2013 negative phase of the AO. In contrast, the positive AO winters of 2012 and 2014, which lacked snow cover and had

warmer surface temperatures, saw much lower ozone concentrations, at daily maximum 8-hour levels well below 70 ppb.

- *Variability in climate can influence the activity of wildfires in the western U.S.* The frequency and distribution of fire activity in the western U.S. is influenced by temperature and precipitation patterns associated with climate variability (Abatzoglou et al., 2016).

1.6 Measurements and Modeling

1.6.1 Advances in Ozone Measurement Methods

This section provides a concise overview of methods used in monitoring networks and advances in remote sensing using satellite-based technology for ozone and ozone precursor measurements. While there is growing literature on low-cost sensors for ozone measurement, they have not been widely applied in studies of atmospheric concentration distributions, human exposure, or health impacts, so studies about them will not be reviewed here.

1.6.1.1 Network Monitoring Methods

A new Federal Reference Method (FRM) for ozone measurement was established in 2015 (40 CFR Part 50 Appendix D). The new ozone FRM is based on the detection of chemiluminescence from the reaction of ozone with nitric oxide (NO). It was adopted because instruments based on chemiluminescence from the reaction of ozone with ethylene were no longer commercially available. Further discussion of chemiluminescence and UV measurements of ozone are presented in the 2013 Ozone ISA (U.S. EPA, 2013). Almost all State and Local Air Monitoring Stations (SLAMS) that report data to the U.S. EPA's Air Quality System (AQS) database use the Federal Equivalence Method (FEM) based on UV absorption.

1.6.1.2 Satellite-Based Remote Sensing Methods

Satellite instruments used to retrieve data on trace gases provide a routine and systematic data set, with the measurements used to provide important column observations of ozone and ozone precursors at scales that range from regional to global.

- Satellite-based remote sensing methods measure the total ozone column rather than ppm or ppb in the atmosphere, and mathematical methods to derive tropospheric or surface ozone concentrations are needed. Thus, there is more uncertainty in surface estimates derived from satellite-based measurements than from monitoring network measurements.

- While the vertical sensitivity of space-based measurements to ozone and its precursors is variable and depends on the method (Martin, 2008), various satellite data sets have been shown in recent modeling studies to provide useful observational constraints for tropospheric ozone (Emili et al., 2014) or both tropospheric ozone and NO_2 (Huang et al., 2014).
- Satellite observations also provide useful measurements to characterize processes that can contribute to USB ozone, such as stratospheric transport/intrusions (Lin et al., 2012a). They can improve the characterization of USB ozone precursor emissions such as LNO_X, VOCs from biogenic sources (Mebust and Cohen, 2014; Mebust et al., 2011), or the vertical lofting of emissions into the upper troposphere [e.g., CO:ozone ratios Kim et al. (2013); Voulgarakis et al. (2011)].
- Because the measurements are consistent over time, the observed trends, seasonal or inter-annual, provide quantitative information that can be used to test the representation of processes relevant to USB ozone in models. For example, year-to-year climate variability (Ziemke et al., 2010) affects the distribution of upper tropospheric ozone or CO, and day-to-day temperature variations affect the emission and chemistry of biogenic VOC and NO_X emissions.
- Because ground-level concentration estimates from satellites can have substantially greater uncertainty than total column ozone measurements, these technologies are most suitable for investigating trends in total column ozone or in the upper troposphere (Gaudel et al., 2018). Currently, satellite-based estimates of ground-level ozone concentrations require considerable supplemental information and/or assumptions about atmospheric characteristics and conditions.
- While the use of satellite-based remote sensing methods is becoming more widespread in each of these applications, it is useful to understand the strengths, limitations, and appropriate use of satellite measurements for estimating ozone and ozone precursors in the atmosphere. Satellite-based spectrometers provide measurements of backscattered sunlight and thermal radiation in ultraviolet (UV), visible (VIS), and infrared (IR) spectral ranges at various spectral resolutions and spatial sampling rates. These satellite-based radiance measurements can detect and quantify tropospheric aerosols and several trace gases, including ozone and ozone precursors. Space-based retrieval of ozone and other trace gases from instruments aboard satellite platforms must account for variability of the radiance measured (e.g., solar spectrum, albedo, IR emissivity, and skin temperature), the path of light through the atmosphere (e.g., Rayleigh scattering, clouds, temperature gradients for thermal IR), and the vertical profile of the absorbing species. Whenever the above factors are not well characterized, a priori assumptions can affect the retrieval products to varying degrees (Duncan et al., 2014; Martin, 2008).
- The quantitative findings of a satellite study must be evaluated in the context of the uncertainty of the underlying satellite data set and associated analysis methods. Factors to consider include, but are not limited to, the maturity of the underlying satellite retrieval algorithm and data product for a particular type of satellite observation, the robustness of validation efforts (short term vs. long term) of algorithms and data products, the length of the study, and a clear description of the data quality flag used to screen the quality of the satellite data. Of all these factors, validation of the algorithm and data products is often the most difficult to accomplish because of the paucity of critical geophysical measurements (e.g., tropospheric column ozone, NO_2, CO, HCHO, partial column amounts, or profiles) that are spatially and temporally consistent with the satellite measurement concentrations. Operational networks, such as the Pandora Global Network and Total Carbon Column Observing Network (TCCON) (Wunch et al., 2011) are emerging to support these efforts.
- Reprocessing of geophysical data products from calibrated radiance data continues to develop as input assumptions (Russell et al., 2011) and techniques improve (Zoogman et al., 2014; Cuesta et

al., 2013; Natraj et al., 2011). This will allow satellite products to be used in characterizing USB ozone in the free troposphere versus the boundary layer on a routine basis.

1.6.2 Advances in Regional Chemical Transport Modeling

The 2013 Ozone ISA provided an overview of chemical transport models (CTMs), including the relevant processes, numerical approaches, relevant spatial scales, and methods for evaluation (U.S. EPA, 2013). Since the previous review, numerous improvements have been developed including (1) the addition of a halogen chemistry mechanism (Gantt et al., 2017); (2) better representation of land cover and near-surface meteorology (Ran et al., 2016), dry deposition and stomatal uptake (Rydsaa et al., 2016), stratosphere-troposphere exchange (Phoenix et al., 2017), and biogenic emissions (Bash et al., 2016); and (3) better integration of meteorological models and CTMs (Xing et al., 2017). The 2013 Ozone ISA identified uncertainties in the fate of nitrogen oxides and oxidant chemistry in remote areas, which have been reduced by advances in biogenic VOC chemistry (Lee et al., 2014; Xie et al., 2013) and new analyses of nitrogen oxide lifetime in the atmosphere (Li et al., 2018). The 2013 Ozone ISA (U.S. EPA, 2013) also identified errors introduced by the coupling of regional- and global-scale models, which has since been improved by the development of more systematic techniques (Henderson et al., 2014), development of hemispheric scale CMAQ (Mathur et al., 2017), and improvement of horizontal resolution in global models (Huang et al., 2013a). This section summarizes recent efforts to evaluate the performance of these more advanced models for simulating ozone over the U.S.

- The accuracy of model estimates of ozone concentration, when compared to observations, varies depending on location, time, and averaging metric. The most straightforward form of model evaluation is to compare the simulated ozone concentrations from different models with the ambient measurements. The Air Quality Model Evaluation International Initiative included simulations over North America from four different research groups. The hourly ozone was compared with 200 surface observation sites and the normalized mean bias was reported to range from −22 to 2.4% (Im et al., 2015). The most recently published evaluation of the CMAQ model finds that hourly ozone concentrations in all seasons (Appel et al., 2017) are underestimated, but that the bias varies spatially. An evaluation of the WRF-Chem model using the 1-hour max ozone concentrations reported normalized mean bias of −15% at urban locations (Yahya et al., 2015). A meta-analysis examining 6 peer-reviewed journal articles published from 2006−2012 also found that the average ozone concentration is usually simulated with lower mean bias than the 1-hour max ozone concentration. Simon et al. (2012) reported that the average ozone concentration is usually simulated with mean bias between 1 and 7 ppb, while the 1-hour max ozone concentration mean bias ranged between 4 and 12 ppb (25th−75th percentile of reported studies). The normalized mean error for hourly ozone ranged between 21 and 47 ppb, while the normalized mean error for the 1-hour max ozone concentration ranged between 19 and 22 ppb (25th−75th percentile of reported studies). Because the estimated model error varies considerably, it is important to evaluate the model results using observations and statistical metrics relevant to the application of interest.
- Differences between model chemical parameterizations can introduce a variance in simulated ozone concentrations of 5%. The accuracy of the ozone simulation depends on the accuracy of the simulation of many inter-connected physical, chemical, and biological systems. Many studies

have examined each of these processes to further improve chemical transport modeling. An intercomparison of just the chemistry models that participated in AQMEII, using identical meteorological conditions, chemical boundary conditions, photolysis rates, and biogenic and anthropogenic emissions, found on average 5% variability due to differences in the chemistry parameterization, with larger differences for the modeled NO_X:VOC ratio, suggesting greater variability in the model's estimate of the sensitivity to emission changes (Knote et al., 2015).

- Limitations in meteorological process simulations can introduce errors. A study by Ryu et al. (2018) attributed up to 40% of the ozone bias to errors in the simulation of clouds, noting that photolysis reactions and biogenic VOC emissions both depend on sunlight. The simulation of atmospheric mixing near the surface, namely the planetary boundary layer, is also relevant to estimating the daily peak ozone, and an intercomparison of different approaches did not yield a single model that performed best (Cuchiara et al., 2014).
- Uncertainty in emissions leads to uncertainty in simulated ozone concentrations. Ozone simulations can be improved with more accurate estimates of the magnitude and timing of biogenic and anthropogenic emissions (Travis et al., 2016; Ahmadov et al., 2015), although the importance of errors in estimated emissions depends on the relative availability of NO_X or VOCs (Kota et al., 2015).
- Models are able to capture the spatial and temporal features of ozone trends but tend to underestimate the magnitude of the trend. Another important aspect to model evaluation is the determination of whether the model can correctly simulate the trends in concentrations and attribute those trends to changes in emissions and weather (Foley et al., 2015). A 21-year hemispheric CMAQ simulation (Xing et al., 2015) captured the decline in ozone concentrations over the U.S. due to precursor emission reductions over the period 1990−2010, but underestimated the magnitude (observed: −1.1% change per year, simulated: −0.64% change per year), although the change in NO_2 was more accurately simulated (observed: −2.3% change per year, simulated: −2.2% change per year). During the 2000−2010 period, the model captured the observed downward trend in the Southwest and Midwest but underestimated the trends in other regions (Astitha et al., 2017). A study using the CAMx model over the South Coast Air Basin in California (Karamchandani et al., 2017) showed an improvement over previous results but still generally underestimated the reduction in ozone in response to emission reductions over the years in that area. With more coarse spatial resolution, global-scale models have also been used to examine trends over the U.S. (Lin et al., 2017; Strode et al., 2015). The global simulations are evaluated using more remote monitoring stations designed to capture regional trends, and the evaluation demonstrates that the models are able to capture the spatial and seasonal differences in the ozone trends, but underestimate the magnitude of the decrease in ozone attributed to emission reductions over the eastern U.S.

1.7 Ambient Air Concentrations and Trends

This section investigates spatiotemporal variability in ambient ozone concentrations. Ambient ozone data reported in this section were obtained from AQS using data obtained from the State and Local Air Monitoring Stations (SLAMS) network for ozone. The SLAMS network was described in detail in the 2013 Ozone ISA (U.S. EPA, 2013), and there have been no major changes. The number of monitors has increased slightly to more than 1,300, and subsets of monitors are also part of the Photochemical Assessment Monitoring Stations (PAMS) network and the National Core (NCore) network for

multipollutant measurements, also described in the 2013 Ozone ISA (U.S. EPA, 2013). Most ozone monitors report hourly average concentrations with a required precision of 1 ppb and minimum detection limit of 5 ppb or less. Data are available as reported (1-hour avg), or further summarized as: (1) the average of the hourly observations over a 24-hour period (DA24), (2) the maximum hourly observation occurring in a 24-hour period (MDA1), and (3) the max 8-hour running average of the hourly observations occurring in a 24-hour period (MDA8).

Analyses in this section are based on data from 2015−2017 using either (1) a year-round data set, with data only from those monitors that report year-round or (2) a warm-season data set with data from all monitors that report data from May to September. Table 1-1 and Table 1-2 provide summary statistics generated from the year-round and warm-season data sets, respectively, using SLAMS network monitoring data from 2015−2017. Monitoring site locations corresponding to the warm-season and year-round data sets are shown in Figure 1-7. The year-round data set includes data from considerably fewer monitors than the warm-season data set, and year-round monitors are more concentrated in the southern half of the U.S. because of monitoring requirements in these areas. States are required to monitor for ozone for varying lengths of time during the year depending on which months are likely to see elevated ozone levels from at least May to September. The warm-season data set was used to examine the majority of ozone season data while providing a consistent time frame for comparison across states. All available monitoring data including data from year-round monitors were also included in the warm-season data set after removing observations outside the 5-month window. The data in Table 1-1 and Table 1-2 show a strong seasonal pattern of ozone concentration.

Table 1-1 Nationwide distributions of ozone concentrations (ppb) from the year-round data set 2015−2017.

Time Period[a]	N Sites	N Obs	Mean	SD	Min	1	5	10	25	50	75	90	95	98	99	Max	Max Site ID
1-h max (MDA1)																	
Year round	809	830,984	45	14	0	17	25	29	36	44	53	63	69	78	85	163	060710005
Winter	761	196,858	37	9	0	13	21	26	32	38	43	48	51	55	59	132	490472003
Spring	792	207,700	50	11	0	25	32	36	42	49	56	63	68	74	79	134	201730010
Summer	789	206,617	51	16	0	20	26	30	40	50	60	71	79	89	97	163	060710005
Autumn	792	204,603	43	13	0	17	25	29	35	42	50	59	66	75	82	152	060370016
Time Period[a]	**N Sites**	**N Obs**	**Mean**	**SD**	**Min**	**1**	**5**	**10**	**25**	**50**	**75**	**90**	**95**	**98**	**99**	**Max**	**Max Site ID**
8-h max (MDA8)																	
Year round	804	819,452	41	13	0	14	22	26	32	40	49	57	62	69	74	136	060719004
Winter	756	194,106	34	9	0	10	18	22	28	34	40	44	47	51	54	121	490472003
Spring	784	203,990	46	10	0	22	29	33	39	46	53	59	63	68	71	109	060714003
Summer	782	203,088	46	14	0	18	23	27	35	46	55	64	70	77	83	136	060719004
Autumn	788	201,810	39	11	0	14	21	25	31	38	45	53	59	66	72	112	060370016

Table-1-1 (Continued): Nationwide distributions of ozone concentrations (ppb) from the year round data set 2015−2017.

Time Period[a]	N Sites	N Obs	Mean	SD	Min	1	5	10	25	50	75	90	95	98	99	Max	Max Site ID
24-h avg (DA24)																	
Year round	809	830,984	30	11	0	7	13	17	23	30	38	44	48	53	56	96	490472003
Winter	761	196,858	25	10	0	4	9	12	18	26	32	38	41	44	47	96	490472003
Spring	792	207,700	36	9	0	15	20	24	29	36	42	47	51	55	57	83	090050005
Summer	789	206,617	33	11	0	12	16	19	25	33	41	48	52	57	61	95	060710005
Autumn	792	204,603	27	9	0	8	13	16	21	27	34	40	44	48	52	85	060570005

N sites = number of sites; N Obs = number of observations; SD = standard deviation; Min = minimum; 1, 5, 10, 25, 50, 90, 95, 98, 99 = 1st, 5th, 10th, 25th, 50th, 90th, 95th, 98th, 99th percentiles;. Max = maximum; Max Site ID = U.S. EPA Air Quality System identification number for monitoring site corresponding to observation in max column.

[a]Winter = December−February, spring = March−May, summer = June−August, autumn = September−November.

Table 1-2 Nationwide distributions of ozone concentrations (ppb) from the warm-season data set 2015−2017.

U.S. Region[a]	N Sites	N Obs	Mean	SD	Min	1	5	10	25	50	75	90	95	98	99	Max	Max Site ID
1-h max (MDA1)																	
U.S.	1,279	548,118	50	14	0	21	28	32	40	49	58	67	74	83	90	237	470370011
C	199	87,066	50	12	0	25	31	35	41	49	57	65	70	75	79	140	290190011
ENC	96	45,052	46	12	0	22	28	31	38	45	54	62	68	74	79	119	550790085
NE	195	84,892	48	14	0	22	28	31	38	47	57	67	73	81	87	126	090011123
NW	33	13,709	43	14	1	18	24	27	33	42	51	60	68	76	82	125	410290201
S	150	63,789	46	14	6	19	23	27	36	46	55	64	70	77	84	136	482010024
SE	216	90,523	46	13	0	20	25	29	36	45	54	62	66	72	77	237	470370011
SW	132	54,252	57	11	10	32	40	44	51	57	63	70	75	82	86	123	490495010
W	201	87,817	57	18	2	22	30	35	44	55	67	80	89	100	108	163	060710005
WNC	57	24,018	49	10	6	22	32	36	43	50	55	60	63	67	69	129	300630024
U.S. Region[a]	**N Sites**	**N Obs**	**Mean**	**SD**	**Min**	**1**	**5**	**10**	**25**	**50**	**75**	**90**	**95**	**98**	**99**	**Max**	**Max Site ID**
8-h max (MDA8)																	
U.S.	1,273	541,670	45	13	0	18	24	28	36	45	53	61	66	73	78	136	060719004
C	199	86,314	45	11	0	21	27	31	37	45	52	59	63	68	71	97	170310076
ENC	95	41,476	42	11	0	19	25	28	34	41	49	57	62	67	71	99	550790085

Table 1-2 (Continued): Nationwide distributions of ozone concentrations (ppb) from the warm-season data set 2015−2017.

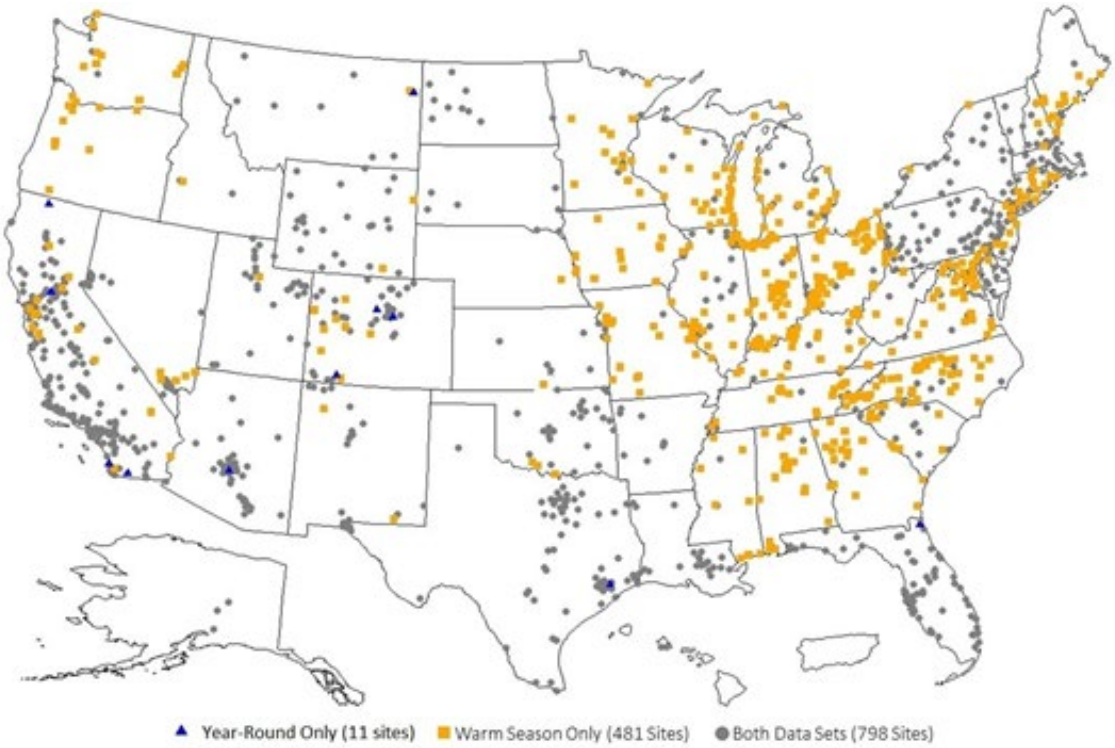
U.S. Region[a]	N Sites	N Obs	Mean	SD	Min	1	5	10	25	50	75	90	95	98	99	Max	Max Site ID
NE	195	84,147	43	12	0	19	25	28	34	42	52	60	65	71	75	101	090070007
NW	33	13,633	39	12	1	15	20	24	30	38	46	55	60	66	71	116	410050004
S	150	63,241	41	13	4	17	20	24	31	41	50	58	62	68	72	109	482010024
SE	216	89,652	41	12	0	17	22	25	32	41	49	56	60	64	67	106	130670003
SW	132	53,842	53	9	8	29	37	41	47	53	58	64	67	71	74	93	080350004
W	198	85,923	51	15	1	20	27	32	40	50	61	71	77	85	91	136	060719004
WNC	56	23,442	46	9	3	19	29	33	40	47	52	57	59	62	64	78	460990008
U.S. Region[a]	**N Sites**	**N Obs**	**Mean**	**SD**	**Min**	**1**	**5**	**10**	**25**	**50**	**75**	**90**	**95**	**98**	**99**	**Max**	**Max Site ID**
24-h avg (DA24)																	
U.S.	1,279	548,118	32	10	0	12	16	19	25	32	39	46	50	55	58	95	060710005
C	199	87,066	31	8	0	14	18	21	25	31	37	42	46	50	52	70	390850007
ENC	96	45,052	31	9	0	13	18	20	25	31	37	43	47	51	54	68	260190003
NE	195	84,892	31	9	0	12	17	20	25	30	37	43	47	52	55	83	090050005
NW	33	13,709	27	9	1	9	13	16	21	27	34	40	44	48	51	80	410050004
S	150	63,789	29	10	2	11	14	16	21	28	36	42	46	50	52	70	481671034
SE	216	90,523	28	9	0	11	14	17	21	27	34	41	45	49	52	68	471550101
SW	132	54,252	41	8	7	20	26	30	35	41	46	51	54	57	59	75	040218001
W	201	87,817	37	12	0	15	20	23	29	36	45	53	58	64	68	95	060710005

Table 1-2 (Continued): Nationwide distributions of ozone concentrations (ppb) from the warm-season data set 2015−2017.

U.S. Region[a]	N Sites	N Obs	Mean	SD	Min	1	5	10	25	50	75	90	95	98	99	Max	Max Site ID
WNC	57	24,018	36	9	2	13	20	24	31	37	42	47	50	53	55	66	560130099

N sites = number of sites; N obs = number of observations; SD = standard deviation; Min = minimum; 1,5,10,25,50,90,95,98,99 = 1st, 5th, 10th, 25th, 50th, 90th, 95th, 98th, 99th percentiles; Max = maximum; Max Site ID = U.S. EPA Air Quality System identification number for monitoring site corresponding to observation in max column.

[a]C = Central (Illinois, Indiana, Kentucky, Missouri, Ohio, Tennessee, West Virginia); ENC = East North Central (Iowa, Minnesota, Michigan, Wisconsin); NE = Northeast (Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont); NW = Northwest (Alaska, Idaho, Oregon, Washington); S = South (Arkansas, Kansas, Louisiana, Mississippi, Oklahoma, Texas); SE = Southeast (Alabama, Florida, Georgia, North Carolina, South Carolina, Virginia); SW = Southwest (Arizona, Colorado, New Mexico, Utah); W = West (California, Hawaii, Nevada), WNC = West North Central (Montana, Nebraska, North Dakota, South Dakota, Wyoming).



Source: U.S. EPA 2018 analysis of Air Quality System network data 2015−2017.

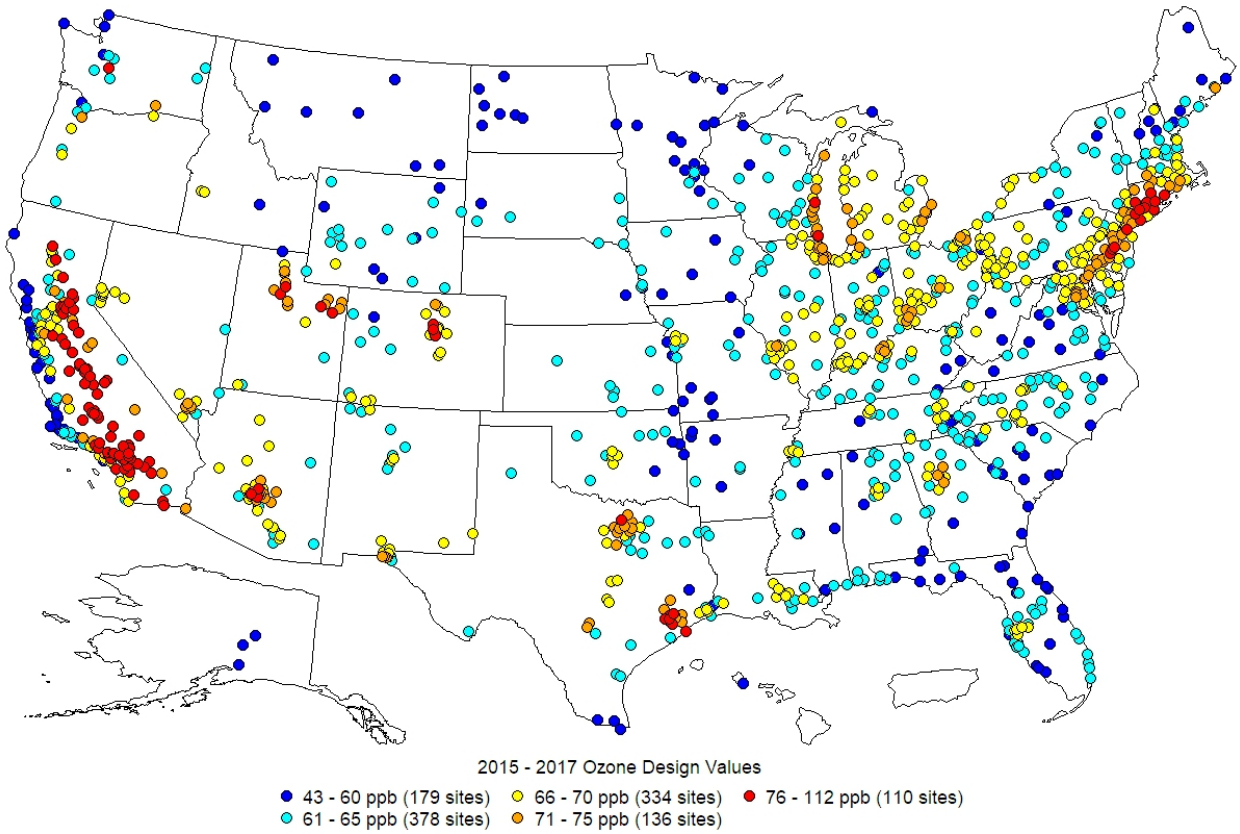
Figure 1-7 Monitor locations for the warm-season and year-round data sets.

- The mean and upper percentiles of the nationwide ozone concentrations are slightly higher in the warm-season data in Table 1-2 than in the year-round data from Table 1-1, and the standard deviation (SD) is similar between the two data sets.
- A strong seasonal pattern in ozone concentrations is evident in the year-round data, with lower MDA8 concentrations in autumn (median = 38 ppb) and winter (median = 34 ppb) and higher concentrations in spring (median = 46 ppb) and summer (median = 46 ppb). Seasonal differences are even greater for upper percentiles. A similar seasonal pattern was reported in the 2013 Ozone ISA (U.S. EPA, 2013).
- For the warm-season data set, the 2015−2017 98th percentile MDA1, MDA8, and DA24 concentrations are 83, 73, and 55 ppb, respectively.

  The median 2015−2017 MDA1, MDA8, and DA24 ozone concentrations for the warm-season data set are 49, 45, and 32 ppb, respectively.
- For the year-round data set, the 2015−2017 98th percentile MDA1, MDA8, and DA24 concentrations are 78, 69, and 53 ppb, respectively, and median 2015−2017 MDA1, MDA8, and DA24 ozone concentrations are 44, 40, and 30 ppb, respectively.

Figure 1-8 through Figure 1-11 summarize ambient ozone concentration patterns and trends. These figures contain data from both warm-season and year-round monitors for all monitors that met the completeness criterion of 75% data capture. The data sets used in Table 1-1 and Table 1-2 are combined, and the data in Figure 1-8 through Figure 1-11 reflect concentration metrics applied to the entire period of monitor operation, rather than the same season across all monitors.

- Figure 1-8 shows the design values, or the 3-year avg of the annual 4th-highest 8-hour daily max (MDA8) ozone concentrations for 2015−2017 (see Section 1.2.1.1). The highest design values (>76 ppb) occur in central and southern California, Arizona, Colorado, Utah, Texas, along the shore of Lake Michigan, and in the Northeast Corridor.



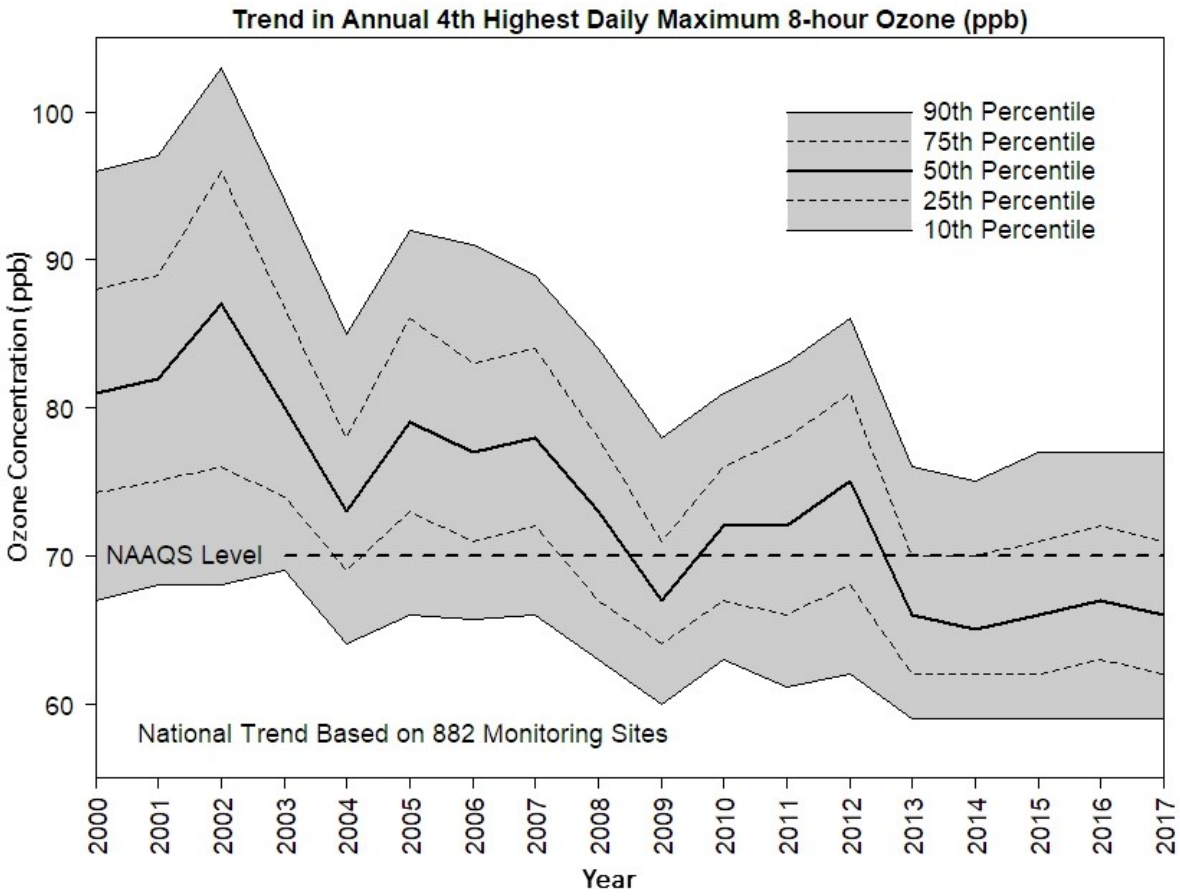
ppb = parts per billion.

Note: Values determined for the entire period of monitor operation for each monitor with 75% or greater data capture. Both warm-season and year-round monitors included.

Source: U.S. EPA 2018 analysis of Air Quality System network data 2015−2017.

Figure 1-8 Individual monitor ozone concentrations in terms of design values for 2015−2017.

- Figure 1-9 shows the decreasing trend in the annual 4th-highest MDA8 ozone concentration from 882 U.S. monitors. The median annual 4th-highest MDA8 ozone concentration across those sites decreased from more than 80 ppb in 2000 to less than 70 ppb in 2017. Other studies also reported a decreasing trend over periods of 15 years or more for 4th-highest MDA8 ozone concentration or other ozone concentration metrics associated with higher concentrations (Lefohn et al., 2017; Simon et al., 2015; Strode et al., 2015).



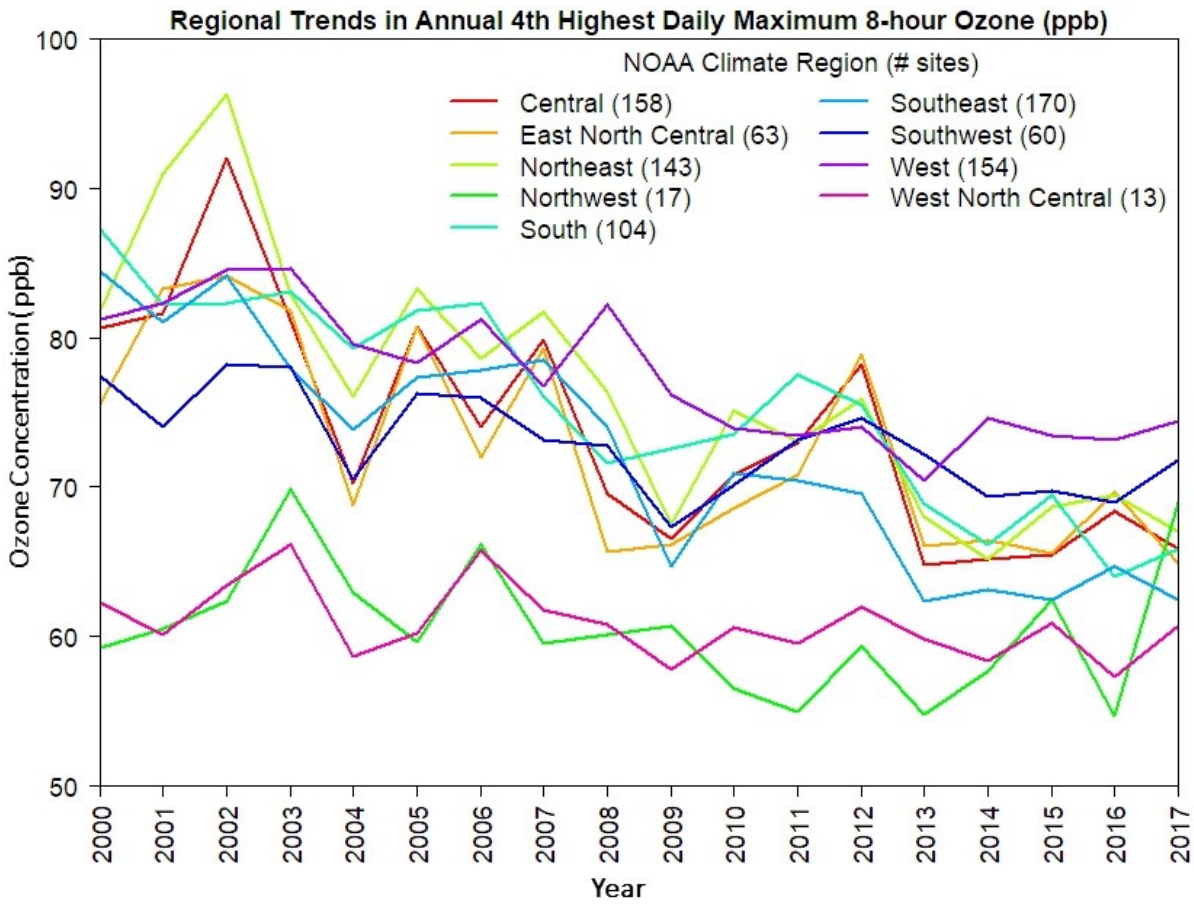
ppb = parts per billion.

Note: Although the trend lines are annual values, the level marked on the figure pertains to the 3-year avg of annual 4th-highest daily max 8-hour concentrations over a consecutive 3-year period, and conclusions cannot be reached regarding exceedances through its comparison to individual years. All monitors with 75% or greater data capture included. Both warm-season and year-round monitors included.

Source: U.S. EPA, National Air Quality: Status and Trends of Key Air Pollutants, https://www.epa.gov/air-trends/ozone-trends, accessed July 2018.

Figure 1-9 National 4th-highest 8-hour daily max ozone trend and distribution across 882 U.S. Ozone monitors 2000−2017 (concentrations in ppb).

- Figure 1-10 shows a regional breakdown of the trend in 4th-highest MDA8 ozone concentrations. Declines are observed in most regions, with the strongest declines in regions that had the greatest 4th-highest MDA8 ozone concentrations.



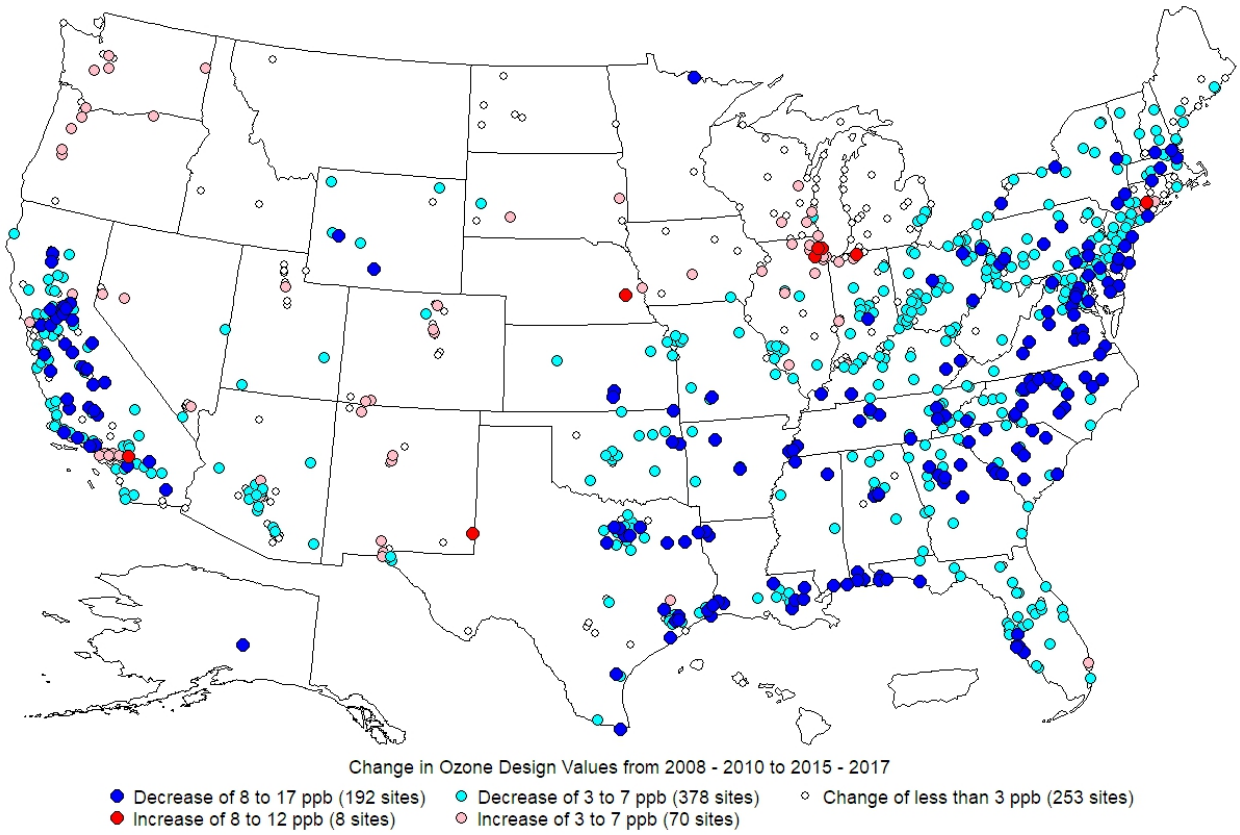
ppb = parts per billion.

Note: All monitors with 75% or greater data capture included, both warm-season and year-round monitors. C = Central (Illinois, Indiana, Kentucky, Missouri, Ohio, Tennessee, West Virginia), ENC = East North Central (Iowa, Minnesota, Michigan, Wisconsin), NE = Northeast (Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont), NW = Northwest (Alaska, Idaho, Oregon, Washington), S = South (Arkansas, Kansas, Louisiana, Mississippi, Oklahoma, Texas), SE = Southeast (Alabama, Florida, Georgia, North Carolina, South Carolina, Virginia), SW = Southwest (Arizona, Colorado, New Mexico, Utah), W = West (California, Hawaii, Nevada), WNC = West North Central (Montana, Nebraska, North Dakota, South Dakota, Wyoming).

Source: U.S. EPA, National Air Quality: Status and Trends of Key Air Pollutants, https://www.epa.gov/air-trends/ozone-trends, accessed July 2018.

Figure 1-10 Trend in mean 4th-highest 8-hour daily max ozone by U.S. region 2000−2017.

- In contrast to the decreasing trend in ozone metrics associated with higher concentrations, 5th percentile ozone concentrations at the lower end of the ozone concentration distribution have exhibited both increasing and decreasing trends in summer, depending on individual monitors, and a generally increasing trend in winter from 1998−2013 (Simon et al., 2015). These observations demonstrate that a compression of the ozone concentration distribution has occurred over this period.
- Figure 1-11 shows the geographic difference in the design values for all U.S. monitors between the 2008−2010 period and the 2015−2017 period. Since the 2008−2010 period was used to designate attainment and nonattainment areas for the 2008 ozone NAAQS, this comparison indicates progress achieved since efforts to meet that standard began.



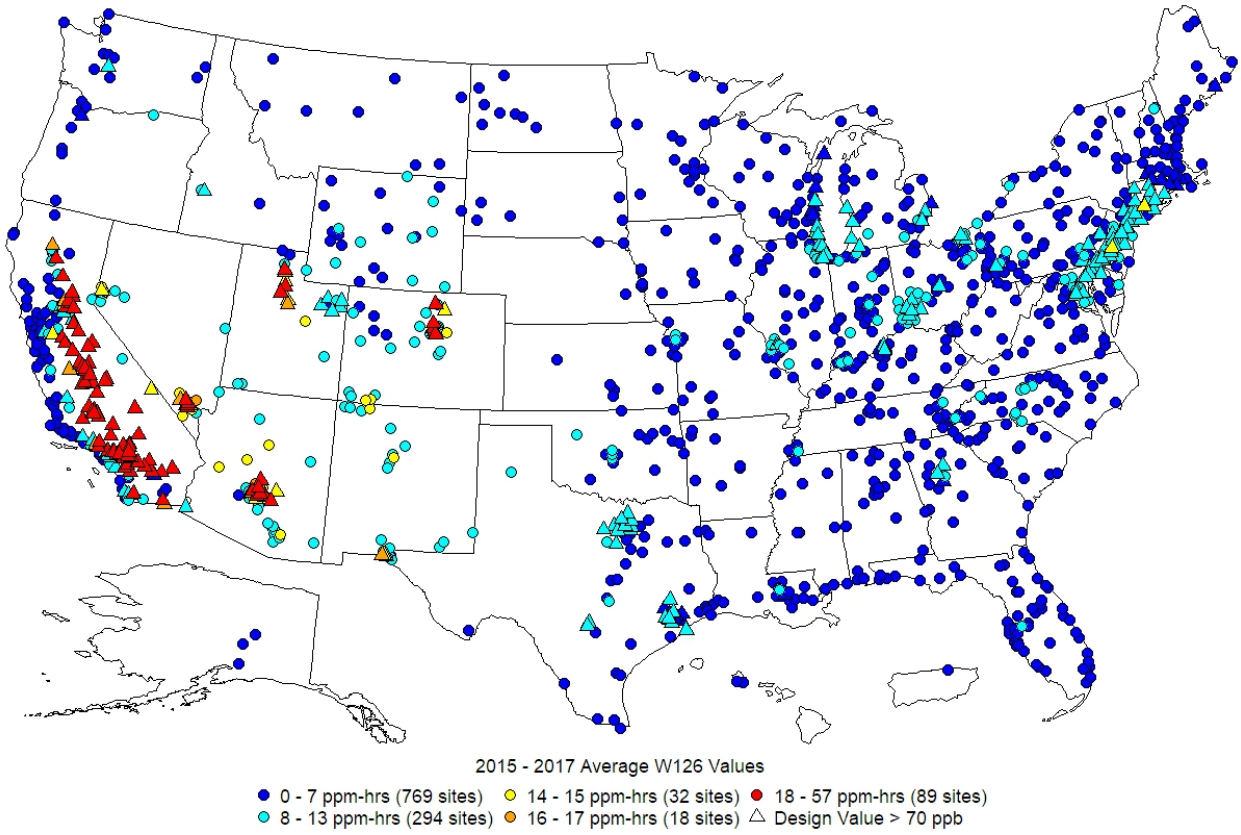
ppb = parts per billion.

Note: All monitors with 75% or greater data capture included, both warm-season and year-round monitors.

Source: U.S. EPA 2018 analysis of Air Quality System network data 2015−2017 and 2008−2010.

Figure 1-11 Individual monitor 3-year avg of the changes in ozone design values from 2008−2010 to 2015−2017.

- Note that Figure 1-11 compares concentrations between two time periods but the differences should not be interpreted as a trend. As discussed in Section 1.5, natural inter-annual variability in synoptic-scale meteorology patterns can influence ozone formation and transport in specific years; therefore, trends must be derived from a longer time series rather than comparisons of two discrete sets of years.
- Diel characteristics of ozone concentration were described in the 2013 Ozone ISA (U.S. EPA, 2013) and have changed little. In urban areas, 1-hour daily max concentrations typically occur in the early afternoon, and the difference between highest and lowest concentration varies considerably by city. There is little difference in diel profiles between weekdays and weekends. In rural areas, there was considerable variability in diel patterns. Diel patterns are described in more detail in the 2013 Ozone ISA (U.S. EPA, 2013).
- Figure 1-12 shows W126 exposure metric values (see Section 1.2.1.2) for network monitoring sites averaged over 2015−2017. The highest W126 values occur in California, Nevada, Arizona, Colorado, and Utah at sites with design values above 70 ppb (Figure 1-8).



ppb = parts per billion.

Note: All monitors with 75% or greater data capture included, both warm-season and year-round monitors.

Source: U.S. EPA 2018 analysis of Air Quality System network data 2015−2017.

Figure 1-12 Individual monitor W126 exposure metric values for 2015−2017.

1.8 U.S. Background Ozone Concentrations

Broadly speaking, USB ozone is used in this document to mean ozone that cannot be reduced by domestic emission controls or other domestic interventions within the U.S. More precise definitions of USB and other definitions of background ozone are thoroughly discussed in Section 1.8.1. Major contributors to USB are stratospheric transport (Section 1.3.2), wildfires (Section 1.3.1.3), natural precursors (Section 1.3.1.3), and international sources [Section 1.3.1.2; Jaffe et al. (2018)]. Quantification of USB ozone on days when MDA8 ozone concentrations exceed 70 ppb is more relevant to understanding USB ozone contributions at the upper end of the distribution than are seasonal mean USB ozone estimates because USB varies daily and is a function of season, meteorology, and elevation (Jaffe et al., 2018). Applications of chemical transport models (CTMs) to estimate USB ozone have found that USB concentrations are relatively constant with increasing total ozone concentration, indicating that days with higher ozone concentrations generally occur because of higher U.S. anthropogenic contributions (Dolwick et al., 2015). Thus, estimates of average percentage USB contributions will generally be higher for seasonal averages than for days at the upper end of the distribution because these longer periods include many days with lower ozone concentrations. Lower USB contributions on days of high ozone concentration can result from meteorological conditions that favor large ozone production from U.S. anthropogenic sources relative to USB sources. The highest ozone concentrations observed in the U.S. have historically occurred during stagnant conditions when an air mass remains stationary over a region abundant in anthropogenic ozone precursor sources (U.S. EPA, 2013, 2006a, 1996). Conversely, the largest USB contributions often occur when the atmosphere is well mixed (see Section 1.5) and transport of USB ozone generated in the stratosphere or during long-range transport of Asian or natural precursors in the upper troposphere more readily occurs (Langford et al., 2015).

Based on these considerations, this section emphasizes USB on days with high ozone concentration as the most relevant for discussing USB ozone, and wherever possible, the focus is on estimates of USB under these conditions because they are most relevant for evaluating the potential for a role of USB ozone in contributing to the highest ozone concentrations. Discussion of seasonal and monthly means of hourly data are also included because longer averaging times are relevant to assessments of health and ecological effects. Seasonal and monthly mean USB ozone estimates are also useful for comparing model data to monitoring data to get a first-order estimate of a model's ability to simulate annual cycles, long-term trends, and inter-annual variability.

1.8.1 Modeling Strategies Applied to Estimate U.S. Background Ozone

As described in Section 1.2.2.1, USB ozone cannot be reliably estimated using ambient monitoring data because monitors can be influenced by U.S. emissions, including both relatively nearby emissions and interstate and hemispheric transport of ozone produced from U.S. emissions. Instead, air quality model simulations are used to estimate USB ozone. The 2006 Air Quality Criteria Document

(AQCD) for ozone (U.S. EPA, 2006a) followed this approach after concluding that background ozone concentrations could not be determined exclusively from ozone measurements because of long-range transport of ozone originating from U.S. anthropogenic precursors even at the most remote monitoring locations. At the time that the 2006 Ozone AQCD (U.S. EPA, 2006a) was published, GEOS-Chem (v4.3.3) (Fiore et al., 2003) was the only model documented in the literature for calculating background ozone concentrations, and it was used for the 2006 AQCD estimates of background ozone. Global-scale simulations like those obtained from GEOS-Chem for the 2006 AQCD had coarse spatial resolution, on the order of 100 km, and may not have adequately resolved topographic features in complex terrain or concentration gradients of ozone and its precursors in areas with large emissions, including urban areas and large point sources. A common approach to achieve finer scale spatial resolution is to use nested modeling systems, in which a coarse resolution global scale CTM is used to provide the boundary condition data for a higher resolution regional-scale model. This approach was described in the 2013 Ozone ISA (U.S. EPA, 2013) using the regional CTMs CMAQ and CAMx with boundary conditions taken from the global-scale CTM GEOS-Chem. The 2013 Ozone ISA also reported background ozone estimates using just coarse resolution global-scale models.

- CTMs still remain the preferred approach for estimating USB or other measures of background ozone, but since publication of the 2013 Ozone ISA (U.S. EPA, 2013), coupled global/regional models rather than global CTMs have become more widely applied.
- A major advance in methodology since the 2013 Ozone ISA is the capability for estimating USB ozone using global CTMs coupled with higher resolution regional models (Jaffe et al., 2018), and regional models such as CMAQ (Byun and Schere, 2006) and CAMx (Emery et al., 2012) are typically used to estimate USB or other measures of background ozone for air quality management applications. Boundary conditions are set using output from a global CTM (Lefohn et al., 2014; Emery et al., 2012) and U.S. anthropogenic emissions in the global CTM can also be set to zero (Emery et al., 2012).
- Background ozone is estimated using either zero-out simulations (USB) or source apportionment simulations (USB_{AB}). The most widely used approach for measuring USB or other measures of background ozone is the "zero-out" method, in which anthropogenic U.S. emissions are set to zero in a model simulation to estimate USB ozone in the absence of U.S. anthropogenic emissions (see Section 1.2.2.1). In the source apportionment approach, all emissions sources are included in the model, and reactive tracer species are used to track the mass contributions of USB sources and U.S. anthropogenic emissions to ozone (see Section 1.2.2.2). Both zero-out and source apportionment modeling approaches require the use of global-scale CTMs to provide boundary condition data for the finer resolution, regional-scale models.

1.8.1.1 Zero-Out and Other Source Sensitivity Approaches

In the model zero-out sensitivity approach to estimate USB, the model simulations include all international emissions and U.S. natural emissions, but U.S. anthropogenic emissions are set to zero. This approach provides a model estimate of the lowest ozone levels that would occur in the absence of U.S.

anthropogenic emissions. This is the most widely used approach for estimating USB. Other source sensitivity approaches are described below.

- The zero-out method is part of a larger set of methods called model sensitivity analyses which can be used to assess how ozone responds to changes in emissions (Huang et al., 2013a; Reidmiller et al., 2009). There are several categories of sensitivity methods that have been the subject of recent research and evaluation.
- Direct perturbation modeling is the simplest sensitivity method (Galmarini et al., 2017; Wu et al., 2009). The model is run with emissions for each source of interest perturbed, typically with reductions of 20 to 50%, and then the model outputs are compared to the base case run with full emissions. Zero-out is a special case of perturbation modeling in which emissions for a source category or source region are set to zero.
- Adjoint modeling is a variation of perturbation modeling that calculates the sensitivity of a specified model parameter to individual components of the initial model state over the course of a simulation (Zhang et al., 2009; Sandu et al., 2005). Adjoint techniques are well-suited for receptor-oriented applications.
- Decoupled direct methods (DDM) are designed to calculate local linear sensitivities of ozone responses to small emissions perturbations. Similar to adjoint, HDDM uses derivatives of the underlying governing equations within the model to track sensitivity of ozone to emissions for designated sources without actually perturbing the emissions imports. Unlike the direct perturbation and adjoint methods, higher order DDM (HDDM) can be set up to track nonlinear ozone responses to emissions changes (Hakami et al., 2004; Dunker, 1981).
- Path-integral methods are applied to nonlinear ozone responses (Dunker et al., 2017). In path-integral methods, source contributions are determined by integrating sensitivity coefficients over the range of emissions from the background case to the base case. This contrasts with other source apportionment approaches that determine source contributions from the base case chemistry. The disadvantage is that more computational effort is required.
- Results of sensitivity methods are strongly dependent on emission inventories used as input, making evaluation of uncertainties in source estimates critical (Jaffe et al., 2018). This dependence also applies to brute force zero-out and emissions tagging techniques.
- In the model sensitivity approach, contributions to ozone are evaluated by scaling the model response to an emissions change; for example, the contribution of Asian emissions to ozone in the U.S. can be evaluated using a 20% reduction in Asian emissions and multiplying the modeled ozone response by a factor of 5. A key limitation of sensitivity approaches is that ozone can have a nonlinear response depending on the size of the emissions reduction, so scaling the model response may not provide an accurate estimate of the source contribution (Huang et al., 2013a). While the HDDM method can be used to account for nonlinear ozone response, its accuracy decreases when trying to estimate ozone response to very large emissions changes.

1.8.1.2 Source Apportionment Approaches

As an alternative to model sensitivity approaches, source apportionment techniques track source contributions to ozone formation without perturbing emissions. Tracking techniques use reactive tracer species to tag specific emissions source categories or source regions and then track the ozone produced by

emissions from those source groups (Cohan and Napelenok, 2011; Grewe et al., 2010). A challenge in the use of source apportionment techniques is that both VOC and NO_X precursors contribute to the production of ozone, so rules must be developed to assign ozone production to either the VOC or NO_X source groups.

- Tagging approaches include CAMx Ozone Source Apportionment Technology (OSAT) (http://www.camx.com/) and CMAQ Integrated Source Apportionment Method (ISAM) (Kwok et al., 2015). These approaches assign ozone production to either the tagged VOC or NO_X precursors depending on whether the ozone is produced in a VOC sensitive or NO_X sensitive chemical regime.
- Tagging can be applied to track contributions to ozone production based on source regions or source types (Fiore et al., 2002; Wang et al., 1998) or to ozone transportation from the stratosphere (Zhang et al., 2014; Lin et al., 2012a).
- Other tagging approaches that have been developed attribute source contributions to a single precursor, either NO_X or VOC. For rural or remote areas in which ozone is mostly produced in NO_X-sensitive chemical regimes, tracers can be used to track the source contributions from NO_X emissions (Pfister et al., 2013; Emmons et al., 2012). For urban areas where ozone is mostly produced in VOC-sensitive conditions, tracers can be used to track the source contributions from VOC emissions (Butler et al., 2011; Ying and Krishnan, 2010).
- Tagging of ozone source contributions is more complex when natural and anthropogenic precursors react to produce ozone. The CAMx model source apportionment technique includes an option for preferentially attributing ozone production to anthropogenic precursors (Jaffe et al., 2018) when anthropogenic precursors react with natural precursors.
- Tracking techniques have been used to define an emissions-influenced background (EIB) ozone concentration (see Section 1.2.2.4) that addresses the reduced lifetime of ozone that is transported from the stratosphere or produced from natural and international precursors due to reaction with and is chemically destroyed by anthropogenic emissions (Lefohn et al., 2014).

1.8.1.3 Differences between Zero-Out and Source Apportionment Approaches

Due to the nonlinear character of ozone chemistry, removing emissions in model sensitivity or model zero-out simulations will give a slightly different answer than tracking emissions contributions to ozone production in a source apportionment approach. For this reason, USB estimated with a source apportionment approach is identified in this document as apportionment-based USB (USB_{AB}) following (Dolwick et al., 2015), while USB without qualification (and without a subscript) generally refers to USB based on zero-out or other source sensitivity-based modeling approaches (see Section 1.2.2.1). The zero-out approach is more suited for answering the question "what ozone levels would exist in the absence of all U.S. emissions?" while the source apportionment approach is more suited for answering the question "what amount of current ozone comes from background sources?" The difference between USB and USB_{AB} is small in remote areas most strongly affected by USB sources, but can be substantial in urban areas strongly affected by anthropogenic sources that influence both production and destruction of ozone (Dolwick et al., 2015).

- Comparison of U.S. background estimates between the zero-out approach using CMAQ and a tagged source apportionment method using CAMx gave similar April to October mean estimates in rural areas, but the CAMx source apportionment approach produced lower estimates in urban areas (Dolwick et al., 2015).
- Differences in seasonal mean MDA8 U.S. background estimates from the zero-out and source apportionment approaches were less than 2.5 ppb at 75% of locations after base case model bias correction (Dolwick et al., 2015).
- Differences between USB and USB_{AB} in urban areas indicate that ozone reductions resulting from a reduction of U.S. anthropogenic ozone precursor emissions could be partially offset by the absence of interactions with U.S. anthropogenic emissions that destroy USB ozone. However, this offset may not apply to other photochemical oxidants that are produced along with U.S. anthropogenic ozone (see Section 1.2.1).

1.8.1.4 Other Approaches for Estimating Background Ozone

One additional recently developed approach to estimating background ozone involves fitting a running average of ozone concentrations over a long period to an exponential decay function (Parrish et al., 2017b).

- This approach is difficult to compare with modeling studies that rely on a more rigorous definition of background. The regression approach also requires numerous assumptions, including that U.S. emissions asymptotically approach zero and that background estimates remain constant over time.
- In addition, results reported in Parrish et al. (2017b) suggest that estimates of background ozone are sensitive to assumptions of the exponential decay rate and the years of data included in the analysis.
- It has been suggested that estimates using this approach are more representative of baseline ozone concentrations plus some additional unquantified amount of ozone produced from local U.S. anthropogenic emissions, rather than background concentration as defined by various modeling approaches (Jaffe et al., 2018).

1.8.1.5 Uncertainties and Model Disagreement

Jaffe et al. (2018) reviewed recent modeling results and reported that USB ozone estimates contain uncertainties of about 10 ppb for seasonal average concentrations, with higher uncertainty for MDA8 average concentrations. Because of uncertainty in model predictions, simple bias correction approaches are useful to adjust model results for bias and error. However, these approaches might not be reliable if the model has large errors in USB ozone and locally produced ozone. Accordingly, days with poor model performance are typically excluded when using model results to estimate USB or other measures of background ozone (Fiore et al., 2014). There have been continued efforts to improve model performance and better understand biases and uncertainties involved in the application of CTMs to estimating USB or other measures of background ozone:

- While determining an overall uncertainty for USB ozone is challenging, confidence in estimates of USB ozone or other measures of background ozone can be evaluated by comparing results from multiple models and approaches. Several direct comparisons of results between models have recently been reported. A complete table of model comparisons was recently published in Jaffe et al. (2018).
- In many cases, discrepancies have been attributed to differences in model representations of various processes. For example, higher seasonal mean values were estimated in both spring and summer with the AM3 model compared with other models, most likely due to different model representations of stratosphere-troposphere exchange, wildfires, lightning source and chemistry, and isoprene oxidation chemistry (Fiore et al., 2014). Differences in Asian transport have also been observed (Huang et al., 2013a), and differences in how convection is modeled have been shown to have a large influence on transport (Orbe et al., 2017).
- Differences in seasonal mean ozone estimated with a regional model using four sets of boundary conditions from different global models (AM3, MOZART, Hemispheric CMAQ, and GEOS-Chem) exceeded 10 ppb and on individual days, differences as high as 15 ppb were observed (Hogrefe et al., 2018).
- Multimodel approaches have been carried out to investigate the influence of intercontinental transport on ground-level ozone concentrations throughout North America and Europe (Galmarini et al., 2017). This approach could help to estimate USB ozone in areas where large differences between model results are observed (Jaffe et al., 2018).

1.8.2 Concentrations and Trends of U.S. Background (USB) and Baseline Ozone

The 2013 Ozone ISA (U.S. EPA, 2013) summarized estimates of USB, NAB, and natural background ozone from the published literature using the CTMs GEOS-Chem, CAMx, and CMAQ. Higher USB and NAB concentrations were estimated in the western U.S. than in the eastern U.S., especially in the intermountain West and Southwest. NAB was also found to constitute a larger fraction of modeled ozone at the upper end of the concentration distribution in the intermountain West than in other regions of the country. Higher USB and NAB concentrations were also estimated at elevations greater than 1,500 m than at lower elevations. The east versus west and the high versus low elevation differences were both similar in magnitude to the estimated uncertainty for CTM seasonal mean USB concentrations of 10 ppb (Jaffe et al., 2018) described in Section 1.8.1. As detailed in this section, more recent research has confirmed these broad features of higher USB in the West than in the East and at higher elevations, and has provided new evidence for both an inverse relationship between relative USB contribution and total ozone concentration and a leveling off of baseline ozone concentrations that have been increasing since monitoring was begun.

1.8.2.1 New U.S. Background (USB) and North American Background (NAB) Estimates

A greater variety of approaches has led to a wider range of USB and NAB estimates than reported in the 2013 Ozone ISA (U.S. EPA, 2013). Jaffe et al. (2018) summarized model results from 14 publications in a supplementary table that reported seasonal mean NAB concentrations or seasonal mean concentrations based on alternative background metrics that ranged widely from 20−50 ppb. Geographic trends were generally similar to those described in the 2013 Ozone ISA. Additional modeling supports the prediction of higher NAB and USB estimates at high-elevation sites in the western U.S. than in the eastern U.S. or along the Pacific Coast.

- Fiore et al. (2014) estimated summer NAB ozone concentrations ranging from 25 to 40 ppb at high-elevation sites in the western U.S. compared with 20 to 30 ppb in the eastern U.S.
- Dolwick et al. (2015) estimated April to October mean USB concentrations of 40 to 45 ppb at intermountain west monitors, compared with 25 to 35 ppb along the Pacific Coast.
- Guo et al. (2018b) estimated seasonal means for spring of 41 ppb for U.S. EPA Region 8, the region most closely corresponding to the intermountain West, but seasonal means for all other U.S. EPA regions were narrowly distributed from 34 to 37 ppb.

1.8.2.2 Seasonal Trends in U.S. Background (USB) and Baseline Ozone

The 2013 Ozone ISA (U.S. EPA, 2013) reported higher seasonal mean USB and NAB concentration estimates in spring than in summer for most regions of the U.S, and these results are consistent with earlier modeling estimates (Fiore et al., 2003). However, while some new results consistent with this pattern have been reported, other results suggest that summer USB and baseline ozone concentrations can be comparable to or greater than spring concentrations. This is significant because numerous studies of USB and other measures of background ozone have focused on spring as the season with the greatest USB concentrations, in part because major sources of USB have been reported to make greater contributions to ozone concentrations in the spring (see Section 1.3.2.1 and Section 1.5.2).

- Recent publications have come up with conflicting conclusions about seasonal trends in USB. Higher seasonal mean USB concentrations in spring than in winter were reported for intermountain western sites (Fiore et al., 2014).
- Fiore et al. (2014) reported higher seasonal mean NAB concentrations in spring than in summer at high-elevation western U.S. sites, consistent with the 2013 Ozone ISA (U.S. EPA, 2013).
- Region-wide seasonal mean USB concentrations greater in summer than spring were reported for most U.S. regions (Guo et al., 2018b). Improvement of isoprene-NO_X chemistry was proposed as the reason for the difference in results compared with earlier modeling results like those of (Fiore et al., 2014).
- Jaffe et al. (2018) reported comparable median spring and summer baseline ozone concentrations at elevations >1 km in the western U.S., while below 1-km baseline ozone concentrations were higher in spring.

- These patterns in seasonal mean USB concentrations are important for identifying atmospheric processes leading to high USB concentrations and for understanding total ozone exposures over long periods but are less relevant for estimating USB concentrations on days with high MDA8 concentrations.

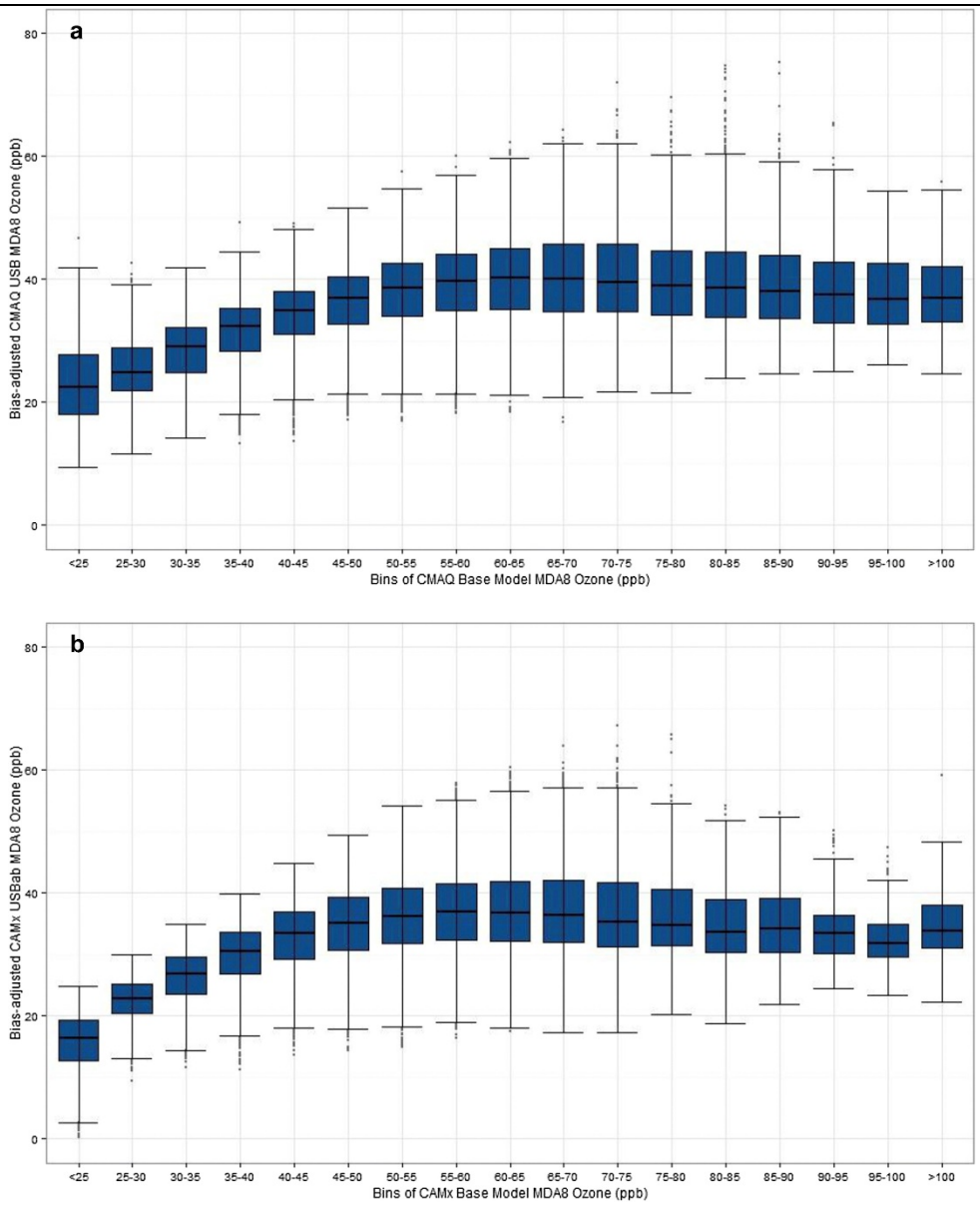
1.8.2.3 U.S. Background (USB) Contribution to Ambient Air Ozone as a Function of Ozone Concentration

USB estimates generally make up a decreasing fraction of total ozone concentration, with increasing total ozone concentrations in the eastern U.S. and at urban locations in the western U.S. Fiore et al. (2002) first described model results of lower background concentrations under conditions favorable for accumulation of high ozone concentrations. Before definitions of USB, NAB, or natural background had been established (see Section 1.2.2.1), they defined background ozone as ozone produced outside of the U.S. boundary layer, and they estimated average afternoon background ozone concentrations ranging from 15−30 ppb in the eastern U.S. and 25−35 ppb in the western U.S., but only 15 ppb during stagnant meteorological conditions.

- Figure 1-11 and Figure 1-12 based on CMAQ and CAMx model results for USB from 2007 do not show an inverse relationship between USB and total ozone concentrations across the U.S., but the results do show that USB concentrations do not increase with total ozone concentration above 60 ppb total ozone concentration, resulting in decreasing predicted relative contributions of USB to total ozone at higher total ozone concentrations (Dolwick et al., 2015).
- Fiore et al. (2014) also described NAB and observed ozone concentrations as largely uncorrelated in the eastern U.S., and Guo et al. (2018b) reported little difference between average USB concentration and USB concentrations on the 10 highest ozone days the eastern U.S. Lefohn et al. (2014) described a decreasing trend of relative EIB contribution with increasing total ozone concentration.
- At low-elevation and urban sites in the western U.S., ozone concentrations estimated as USB, NAB, or EIB (see Section 1.2.1.1) contributions were also reported to be independent of overall ozone concentration, resulting in a decreasing relative background contribution with increasing total ozone concentration (Guo et al., 2018a; Guo et al., 2018b; Dolwick et al., 2015; Lefohn et al., 2014).
- In contrast, model results have shown increasing USB and NAB concentrations with increasing ozone concentration at high-elevation western U.S. sites (Fiore et al., 2014; Lefohn et al., 2014).
- The absence of an inverse relationship between absolute USB concentration and total ozone concentration like that described by Fiore et al. (2002) in the modeling results of Dolwick et al. (2015), Guo et al. (2018b), and others is consistent with observed meteorological influences on ozone concentration (see Section 1.5). As the highest ozone concentrations have decreased, they might now occur under a wider variety of meteorological conditions and might not be limited to the stagnation conditions that suppress USB concentrations (see Section 1.5). However, it is still the case that the relative USB contribution on days with the highest ozone concentrations is usually predicted to be smaller than the seasonal mean USB contribution.
- While the average USB fraction has been shown to decrease on high ozone days compared with low ozone days, there are some instances of high USB fraction on high ozone days as shown by

outliers above 0.75 in Figure 1-13a and Figure 1-14a between 70 and 90 ppb. These are most often associated with model-predicted ozone events from wildfires.

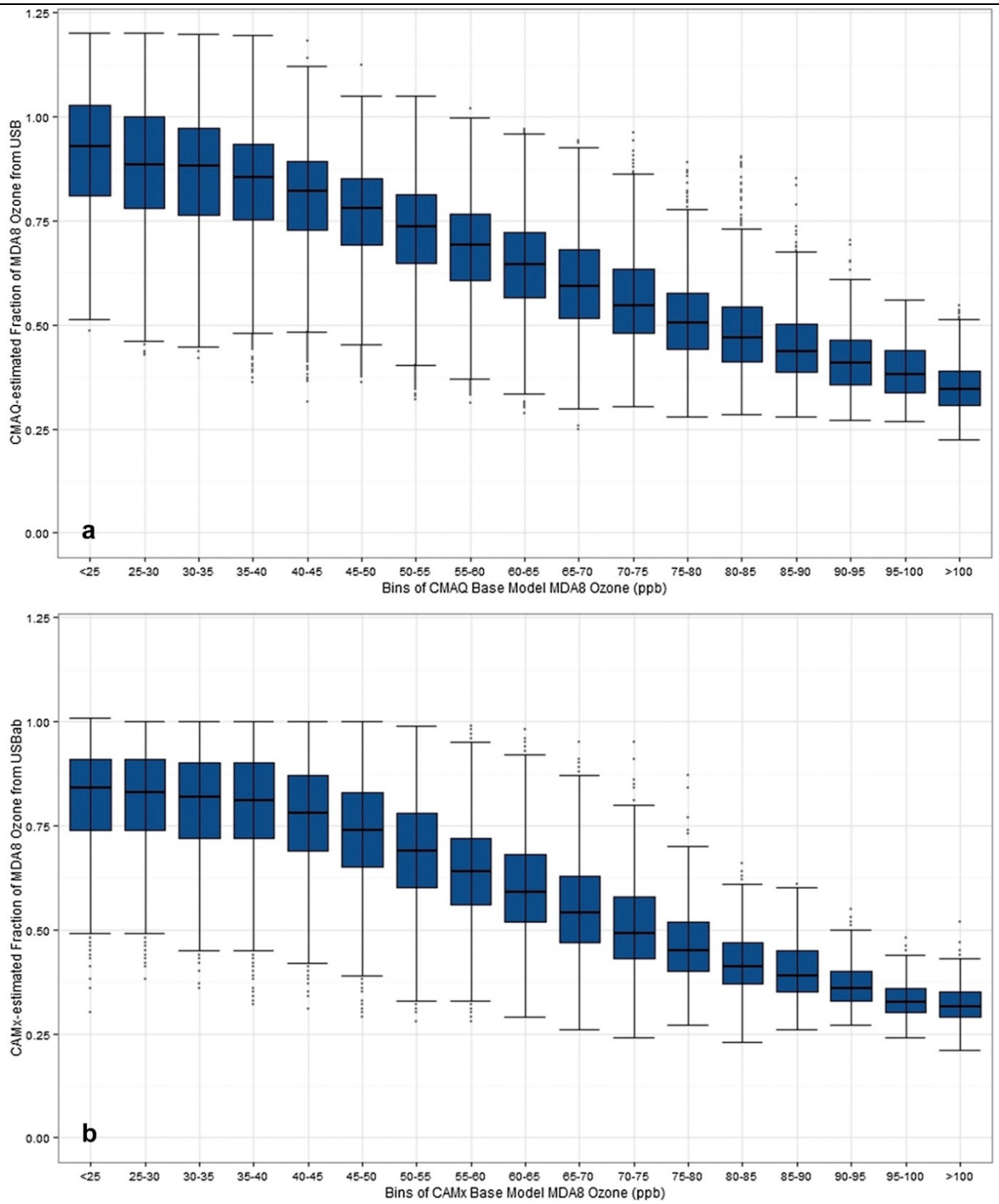
- There is consistent evidence across several studies using different background measurement approaches that USB or other background concentration estimates on most days with high ozone concentrations have been generally predicted to be similar to or smaller than seasonal mean USB ozone estimates in the eastern U.S. and in urban and low-elevation areas of the western U.S., and an inverse relationship between relative USB contribution and total ozone concentration in these areas has been consistently predicted. This contrasts with high-elevation locations in the western U.S., where USB and NAB have been consistently predicted to increase with total ozone concentration.



ppb = parts per billion.

Source: Dolwick et al. (2015).

Figure 1-13 CMAQ (a) and CAMx (b) estimates of daily distributions of bias-adjusted USB MDA8 ozone concentration (ppb) for the period April−October 2007, binned by base model MDA8 ozone concentration ranges.



ppb = parts per billion.

Source: Dolwick et al. (2015).

Figure 1-14 CMAQ (a) and CAMx (b) estimates of daily distributions of bias-adjusted USB ozone fraction at monitoring locations across the western U.S. for the period April−October 2007, binned by base model MDA8 ozone concentration ranges.

1.8.2.4 Long-Term Trends in U.S. Background (USB) and Baseline Ozone

Characterization of long-term trends in USB and baseline ozone presents numerous challenges because of inter-annual variability in and complex interactions between precursor emissions, meteorological events and synoptic patterns, surface deposition, atmospheric circulation, and stratosphere-troposphere exchange (Young et al., 2018; Lin et al., 2015). Further complications arise from unknown errors in emission inventories, limitations of coarse-resolution models in resolving baseline conditions, and known and unknown weaknesses in model representation of chemical and physical processes (Young et al., 2018). Other challenges are introduced by the short observation period and sparse geographic coverage of surface ozone monitoring efforts (Parrish et al., 2017a; Lin et al., 2015). Satellite retrievals provide greater spatial coverage at mid tropospheric levels. However, the period of satellite data collection of 10 years is too short for robust trend analysis (Lin et al., 2015), and satellites are poorly suited for detecting ground-level ozone. In spite of these limitations, there have been some studies on long-term trends in USB and baseline ozone. However, it is largely limited to high-elevation sites in the western U.S. or measurements made aloft, where increasing USB trends were reported until recently. The most recent analyses suggest that this trend has now slowed or reversed.

- Simulated USB ozone trends exhibit poor agreement with monitoring measurements, as well as between different global models (Young et al., 2018; Parrish et al., 2017a). The relative importance between weaknesses in model processes, inaccuracies in model inputs, and inadequate representativeness of measurements as contributors to model disagreement are poorly understood (Young et al., 2018). Based on a series of modeling studies to investigate USB trends, Lin et al. (2015) concluded that accurate quantification of USB requires greater spatial density and temporal frequency in the observational data used in evaluating and improving models than currently exist in the western U.S.
- For context, on average, both annual mean and annual 4th-highest MDA8 ozone values exhibit a lack of trend or a decreasing trend at most rural U.S. monitoring sites (Jaffe et al., 2018; Simon et al., 2015). High-elevation western U.S. sites are the exceptions. Until recently, model results and baseline measurements suggested a long-term increasing trend in both USB and baseline ozone at high-elevation western U.S. sites in the troposphere in the spring (Lin et al., 2017; Zhang and Jaffe, 2017; Gratz et al., 2015; Parrish et al., 2014; Cooper et al., 2012; Parrish et al., 2012).
- An estimated increase of 0.3 to 0.5 ppb/year of USB in spring over the western U.S. in the two decades after 1990 was largely attributed to a tripling of Asian NO_X emissions (Section 1.3.1), with a smaller contribution for increasing global methane concentrations [Section 1.3.1; Lin et al. (2017)].
- Although inter-annual variability makes it difficult to evaluate, there is evidence from baseline monitoring, satellite retrievals, and chemical transport modeling that the ozone resulting from transport from Asia (Section 1.3.1) reached a maximum before 2012 and has been decreasing since then (Parrish et al., 2017a; Oetjen et al., 2016), probably as a result of well-documented decreasing Asian precursor emissions (Liu et al., 2017a; Duncan et al., 2016; Krotkov et al., 2016).
- The existing literature on USB trends has focused mainly on monthly or seasonal means. Model uncertainties are higher, but have not been quantitatively estimated for metrics based on shorter

averaging times like MDA8 (Jaffe et al., 2018), and modeling capabilities for reproducing ozone trends might be different between mean values and other percentiles (Young et al., 2018).

- There is little evidence to suggest that USB is still increasing even in the western U.S. Analyses have been largely limited to the western U.S. high elevations, and these appear to show signs of slowing or even reversing, although this should be considered in the context of high inter-annual variability, poor model agreement, and sparse monitoring coverage that present serious challenges for USB trends analysis.

1.9 Summary

This Appendix reviews scientific advances in atmospheric ozone research relevant to this review of the NAAQS for ozone and other photochemical oxidants and the related air quality criteria. The primary focus is on new evidence concerning the contributions of ozone from natural and non-U.S. sources.

- For this assessment, U.S. background (USB) ozone is defined as ambient ozone that would be present at ground level within the U.S. in the absence of all U.S. anthropogenic ozone precursor emissions. Major contributors to ground-level USB ozone concentrations are stratospheric exchange, international transport, wildfires, lightning, global methane emissions, and natural biogenic and geogenic precursor emissions (Section 1.2).
- Ozone formed in the troposphere is, primarily, the product of photochemical reactions between NO_X and carbon-containing compounds including VOCs, CO, and methane. Major source sectors that emit these ozone precursors include: motor vehicles, EGUs, other industrial processes involving fuel combustion, agricultural processes, wildfires, and vegetation. These emissions can be emitted within or outside the U.S. Emissions trends vary by pollutant, source sector, and source location. Domestic anthropogenic emissions of ozone precursors have largely declined over the past 15−20 years (Section 1.3).
- While ozone is ordinarily a warm-season pollutant, unusually high concentration ozone events have occurred in the winter in two western mountain basins, the Uinta and Upper Green River Basins. Local winter meteorology and high emissions from oil and gas extraction operations appear to be the principal drivers of winter ozone formation, in these locations.

  Continuing research on the role of halogen chemistry in boundary-layer ozone concentrations indicates that the process may serve as an ozone sink in coastal urban environments. When added to model chemical mechanisms, halogen chemistry appears to correct previous overprediction of ozone concentrations (Section 1.4).
- The effects of local precursor emissions controls can be masked by meteorological variability. Inter-annual variability in climate has been shown to play a role in influencing total ozone concentrations across the U.S., as well as USB levels. The El Niño-Southern Oscillation cycle directly affects the frequency of springtime stratosphere-troposphere exchange events, as well as the efficiency of international air pollutant transfer processes impacting the western U.S. (Section 1.5).
- Ozone measurement capabilities have improved since the previous ozone assessment, including establishment of a new FRM and enhanced use of satellite-based remote sensing methods. At the same time, there have been notable advances in regional CTM methods, including improvement in characterizing halogen chemistry, land cover, near surface meteorology, dry deposition,

stratosphere-troposphere exchange, biogenic emissions, and integration with meteorological models (Section 1.6).

- For the 2015−2017 time period, the 98th percentile MDA1, MDA8, and DA24 concentrations are 78, 69, and 53 ppb, respectively. Over the same time period, median 2015−2017 MDA1, MDA8, and DA24 ozone concentrations are 44, 40, and 30 ppb, respectively. Nationally, the ambient ozone concentration distribution is compressing as 95th percentile concentrations are decreasing at the same time 5th percentile concentrations are increasing. This change is consistent with expected reductions in NO_X, which destroys ozone at low ozone concentrations and produces ozone at higher ozone concentrations (Section 1.7).
- Models consistently predict higher USB ozone concentrations at higher elevations in the western U.S. than in the eastern U.S. or along the Pacific coast. Across the ensemble of available modeling studies in the literature, seasonal mean USB concentrations are estimated to range from 20−50 ppb. These model estimates of seasonal mean USB ozone contain uncertainties of about 10 ppb for seasonal average concentrations with higher uncertainty for max daily 8-hour avg concentrations. Uncertainties in emissions, transport processes, and chemistry contribute to model result uncertainties. There have been continued efforts to improve model performance and better understand biases and uncertainties involved in the application of CTMs to estimating USB. With the exception of high-elevation locations in the western U.S., model simulations suggest that domestic anthropogenic sources have a greater proportional contribution on the highest ozone days. Trends in baseline ozone levels suggested a rising contribution from natural and international sources through approximately 2010. Recently, however, this trend has shown signs of slowing or even reversing, possibly due to decreasing East Asian precursor emissions (Section 1.8).

1.10 References

Abatzoglou, JT; Kolden, CA; Balch, JK; Bradley, BA. (2016). Controls on interannual variability in lightning-caused fire activity in the western US [Letter]. Environ Res Lett 11. http://dx.doi.org/10.1088/1748-9326/11/4/045005.

Ahmadov, R; Mckeen, S; Trainer, M; Banta, R; Brewer, A; Brown, S; Edwards, PM; de Gouw, JA; Frost, GJ; Gilman, J; Helmig, D; Johnson, B; Karion, A; Koss, A; Langford, A; Lerner, B; Olson, J; Oltmans, S; Peischl, J; Petron, G; Pichugina, Y; Roberts, JM; Ryerson, T; Schnell, R; Senff, C; Sweeney, C; Thompson, C; Veres, PR; Warneke, C; Wild, R; Williams, EJ; Yuan, B; Zamora, R. (2015). Understanding high wintertime ozone pollution events in an oil- and natural gas-producing region of the western US. Atmos Chem Phys 15: 411-429. http://dx.doi.org/10.5194/acp-15-411-2015.

Albers, JR; Perlwitz, J; Butler, A, myH; Birner, T; Kiladis, GN; Lawrence, ZD; Manney, GL; Langford, AO; Dias, J. (2018). Mechanisms governing interannual variability of stratosphere-to-troposphere ozone transport. J Geophys Res Atmos 123: 234-260. http://dx.doi.org/10.1002/2017JD026890.

Altshuller, AP; Lefohn, AS. (1996). Background ozone in the planetary boundary layer over the United States. J Air Waste Manag Assoc 46: 134-141. http://dx.doi.org/10.1080/10473289.1996.10467445.

Appel, KW; Napelenok, SL; Foley, KM; Pye, HOT; Hogrefe, C; Luecken, DJ; Bash, JO; Roselle, SJ; Pleim, JE; Foroutan, H; Hutzell, WT; Pouliot, GA; Sarwar, G; Fahey, KM; Gantt, B; Gilliam, RC; Heath, NK; Kang, D; Mathur, R; Schwede, DB; Spero, TL; Wong, DC; Young, JO. (2017). Description and evaluation of the Community Multiscale Air Quality (CMAQ) modeling system version 5.1. GMD 10: 1703-1732. http://dx.doi.org/10.5194/gmd-10-1703-2017.

Astitha, M; Luo, H; Rao, ST; Hogrefe, C; Mathur, R; Kumar, N. (2017). Dynamic evaluation of two decades of WRF-CMAQ ozone simulations over the contiguous United States. Atmos Environ 164: 102-116. http://dx.doi.org/10.1016/j.atmosenv.2017.05.020.

Bader, W; Bovy, B; Conway, S; Strong, K; Smale, D, an; Turner, AJ; Blumenstock, T; Boone, C; Coen, MC; Coulon, A; Garcia, O; Griffith, DT; Hase, F; Hausmann, P; Jones, N; Krummel, P; Murata, I; Morino, I; Nakajima, H; O'Doherty, S; Paton-Walsh, C; Robinson, J; Sandrin, R; Schneider, M; Servais, C; Sussmann, R; Mahieu, E. (2017). The recent increase of atmospheric methane from 10 years of ground-based NDACC FTIR observations since 2005. Atmos Chem Phys 17: 2255-2277. http://dx.doi.org/10.5194/acp-17-2255-2017.

Barth, MC; Cantrell, CA; Brune, WH; Rutledge, SA; Crawford, JH; Huntrieser, H; Carey, LD; Macgorman, D; Weisman, M; Pickering, KE; Bruning, E; Anderson, B; Apel, E; Biggerstaff, M; Campos, T; Campuzano-Jost, P; Cohen, R; Crounse, J; Day, DA; Diskin, G; Flocke, F; Fried, A; Garland, C; Heikes, B; Honomichl, S; Hornbrook, R; Huey, LG; Jimenez, JL; Lang, T; Lichtenstern, M; Mikoviny, T; Nault, B; O'Sullivan, D; Pan, LL; Peischl, J; Pollack, I; Richter, D; Riemer, D; Ryerson, T; Schlager, H; St Clair, J; Walega, J; Weibring, P; Weinheimer, A; Wennberg, P; Wisthaler, A; Wooldridge, PJ; Ziegler, C. (2015). The deep convective clouds and chemistry (DC3) field campaign. Bull Am Meteorol Soc 96: 1281-1309. http://dx.doi.org/10.1175/BAMS-D-13-00290.1.

Bash, JO; Baker, KR; Beaver, MR. (2016). Evaluation of improved land use and canopy representation in BEIS v3.61 with biogenic VOC measurements in California. GMD 9: 2191-2207. http://dx.doi.org/10.5194/gmd-9-2191-2016.

Brey, SJ; Fischer, EV. (2016). Smoke in the city: How often and where does smoke impact summertime ozone in the United States? Environ Sci Technol 50: 1288-1294. http://dx.doi.org/10.1021/acs.est.5b05218.

Busilacchio, M; Di Carlo, P; Aruffo, E; Biancofiore, F; Salisburgo, CD; Giammaria, F; Bauguitte, S; Lee, J; Moller, S; Hopkins, J; Punjabi, S; Andrews, S; Lewis, AC; Parrington, M; Palmer, PI; Hyer, E; Wolfe, GM. (2016). Production of peroxy nitrates in boreal biomass burning plumes over Canada during the BORTAS campaign. Atmos Chem Phys 16: 3485-3497. http://dx.doi.org/10.5194/acp-16-3485-2016.

Butler, TJ; Vermeylen, FM; Rury, M; Likens, GE; Lee, B; Bowker, GE; Mccluney, L. (2011). Response of ozone and nitrate to stationary source NO(x) emission reductions in the eastern USA. Atmos Environ 45: 1084-1094. http://dx.doi.org/10.1016/j.atmosenv.2010.11.040.

Byun, D; Schere, KL. (2006). Review of the governing equations, computational algorithms, and other components of the models-3 community multiscale air quality (CMAQ) modeling system [Review]. Appl Mech Rev 59: 51-77. http://dx.doi.org/10.1115/1.2128636.

Carlton, AG; Baker, KR. (2011). Photochemical modeling of the Ozark isoprene volcano: MEGAN, BEIS, and their impacts on air quality predictions. Environ Sci Technol 45: 4438-4445. http://dx.doi.org/10.1021/es200050x.

CCSP (U.S. Climate Change Science Program). (2003). Strategic plan for the U.S. climate change science program: A report by the climate change science program and the subcommittee on global change research. http://www.climatescience.gov/Library/stratplan2003/final/ccspstratplan2003-all.pdf.

Cheadle, LC; Oltmans, SJ; Petron, G; Schnell, RC; Mattson, EJ; Herndon, SC; Thompson, AM; Blake, DR; Mcclure-Begley, A. (2017). Surface ozone in the Colorado northern Front Range and the influence of oil and gas development during FRAPPE/DISCOVER-AQ in summer 2014. 5. http://dx.doi.org/10.1525/elementa.254.

Clifton, OE; Fiore, AM; Correa, G; Horowitz, LW; Naik, V. (2014). Twenty-first century reversal of the surface ozone seasonal cycle over the northeastern United States. Geophys Res Lett 41: 7343-7350. http://dx.doi.org/10.1002/2014GL061378.

Cohan, DS; Napelenok, SL. (2011). Air quality response modeling for decision support. Atmosphere (Basel) 2: 407-425. http://dx.doi.org/10.3390/atmos2030407.

Cooper, OR; Gao, RS; Tarasick, D; Leblanc, T; Sweeney, C. (2012). Long-term ozone trends at rural ozone monitoring sites across the United States, 1990-2010. J Geophys Res Atmos 117. http://dx.doi.org/10.1029/2012JD018261.

Cuchiara, GC; Li, X; Carvalho, J; Rappenglueck, B. (2014). Intercomparison of planetary boundary layer parameterization and its impacts on surface ozone concentration in the WRF/Chem model for a case study in Houston/Texas. Atmos Environ 96: 175-185. http://dx.doi.org/10.1016/j.atmosenv.2014.07.013.

Cuesta, J; Eremenko, M; Liu, X; Dufour, G; Cai, Z; Hoepfner, M; von Clarmann, T; Sellitto, P; Foret, G; Gaubert, B; Beekmann, M; Orphal, J; Chance, K; Spurr, R; Flaud, JM. (2013). Satellite observation of lowermost tropospheric ozone by multispectral synergism of IASI thermal infrared and GOME-2 ultraviolet measurements over Europe. Atmos Chem Phys 13: 9675-9693. http://dx.doi.org/10.5194/acp-13-9675-2013.

Dennis, R; Fox, T; Fuentes, M; Gilliland, A; Hanna, S; Hogrefe, C; Irwin, J; Rao, ST; Scheffe, R; Schere, K; Steyn, D; Venkatram, A. (2010). A framework for evaluating regional-scale numerical photochemical modeling systems. Environ Fluid Mech 10: 471-489. http://dx.doi.org/10.1007/s10652-009-9163-2.

Ding, J; Miyazaki, K; Johannes van der A, R; Mijling, B; Kurokawa, J; Cho, S; Janssens-Maenhout, G; Zhang, Q; Liu, F; Levelt, PF. (2017). Intercomparison of NOx emission inventories over East Asia. Atmos Chem Phys 17: 10125-10141. http://dx.doi.org/10.5194/acp-17-10125-2017.

Dolwick, P; Akhtar, F; Baker, KR; Possiel, N; Simon, H; Tonnesen, G. (2015). Comparison of background ozone estimates over the western United States based on two separate model methodologies. Atmos Environ 109: 282-296. http://dx.doi.org/10.1016/j.atmosenv.2015.01.005.

Duncan, BN; Lamsal, LN; Thompson, AM; Yoshida, Y; Lu, Z; Streets, DG; Hurwitz, MM; Pickering, KE. (2016). A space-based, high-resolution view of notable changes in urban NOx pollution around the world (2005-2014). J Geophys Res Atmos 121: 976-996. http://dx.doi.org/10.1002/2015JD024121.

Duncan, BN; Prados, AI; Lamsal, LN; Liu, Y; Streets, DG; Gupta, P; Hilsenrath, E; Kahn, RA; Nielsen, JE; Beyersdorf, AJ; Burton, SP; Fiore, AM; Fishman, J; Henze, DK; Hostetler, CA; Krotkov, NA; Lee, P; Lin, M; Pawson, S; Pfister, G; Pickering, KE; Pierce, RB; Yoshida, Y; Ziemba, LD. (2014). Satellite data of atmospheric pollution for US air quality applications: Examples of applications, summary of data end-user resources, answers to FAQs, and common mistakes to avoid. Atmos Environ 94: 647-662. http://dx.doi.org/10.1016/j.atmosenv.2014.05.061.

Dunker, AM. (1981). Efficient calculation of sensitivity coefficients for complex atmospheric models. Atmos Environ 15: 1155-1161. http://dx.doi.org/10.1016/0004-6981(81)90305-X.

Dunker, AM; Koo, B; Yarwood, G. (2017). Contributions of foreign, domestic and natural emissions to US ozone estimated using the path-integral method in CAMx nested within GEOS-Chem. Atmos Chem Phys 17: 12553-12571. http://dx.doi.org/10.5194/acp-17-12553-2017.

Edwards, PM; Brown, SS; Roberts, JM; Ahmadov, R; Banta, RM; Degouw, JA; Dubé, WP; Field, RA; Flynn, JH; Gilman, JB; Graus, M; Helmig, D; Koss, A; Langford, AO; Lefer, BL; Lerner, BM; Li, R; Li, SM; Mckeen, SA; Murphy, SM; Parrish, DD; Senff, CJ; Soltis, J; Stutz, J; Sweeney, C; Thompson, CR; Trainer, MK; Tsai, C; Veres, PR; Washenfelder, RA; Warneke, C; Wild, RJ; Young, CJ; Yuan, B; Zamora, R. (2014). High winter ozone pollution from carbonyl photolysis in an oil and gas basin. Nature 514: 351-354. http://dx.doi.org/10.1038/nature13767.

Emery, C; Jung, J; Downey, N; Johnson, J; Jimenez, M; Yarvvood, G; Morris, R. (2012). Regional and global modeling estimates of policy relevant background ozone over the United States. Atmos Environ 47: 206-217. http://dx.doi.org/10.1016/j.atmosenv.2011.11.012.

Emili, E; Barret, B; Massart, S; Le Flochmoen, E; Piacentini, A; El Amraoui, L; Pannekoucke, O; Cariolle, D. (2014). Combined assimilation of IASI and MLS observations to constrain tropospheric and stratospheric ozone in a global chemical transport model. Atmos Chem Phys 14: 177-198. http://dx.doi.org/10.5194/acp-14-177-2014.

Emmons, LK; Hess, PG; Lamarque, JF; Pfister, GG. (2012). Tagged ozone mechanism for MOZART-4, CAM-chem and other chemical transport models. GMD 5: 1531-1542. http://dx.doi.org/10.5194/gmd-5-1531-2012.

Fang, Y; Fiore, AM; Horowitz, LW; Levy II, H; Hu, Y; Russell, AG. (2010). Sensitivity of the NOy budget over the United States to anthropogenic and lightning NOx in summer. J Geophys Res 115. http://dx.doi.org/10.1029/2010JD014079.

Field, RA; Soltis, J; Mccarthy, MC; Murphy, S; Montague, DC. (2015). Influence of oil and gas field operations on spatial and temporal distributions of atmospheric non-methane hydrocarbons and their effect on ozone formation in winter. Atmos Chem Phys 15: 3527-3542. http://dx.doi.org/10.5194/acp-15-3527-2015.

Finlayson-Pitts, BJ; Pitts, JN, Jr. (2000). Chemistry of the upper and lower atmosphere: Theory, experiments and applications. In Chemistry of the upper and lower atmosphere: Theory, experiments and applications. San Diego, CA: Academic Press. http://dx.doi.org/10.1016/B978-0-12-257060-5.X5000-X.

Fiore, A; Jacob, DJ; Liu, H; Yantosca, RM; Fairlie, TD; Li, Q. (2003). Variability in surface ozone background over the United States: Implications for air quality policy. J Geophys Res 108: 4787. http://dx.doi.org/10.1029/2003JD003855.

Fiore, AM; Jacob, DJ; Bey, I; Yantosca, RM; Field, BD; Fusco, AC; Wilkinson, JG. (2002). Background ozone over the United States in summer: origin, trend, and contribution to pollution episodes. J Geophys Res 107: 4275. http://dx.doi.org/10.1029/2001JD000982.

Fiore, AM; Oberman, JT; Lin, MY; Zhang, L; Clifton, OE; Jacob, DJ; Naik, V; Horowitz, LW; Pinto, JP; Milly, GP. (2014). Estimating North American background ozone in U.S. surface air with two independent global models: Variability, uncertainties, and recommendations. Atmos Environ 96: 284-300. http://dx.doi.org/10.1016/j.atmosenv.2014.07.045.

Fiore, AM; West, JJ; Horowitz, LW; Naik, V; Schwartzkopf, MD. (2008). Characterizing the tropospheric ozone response to methane emission controls and the benefits to climate and air quality. J Geophys Res 113: D08307. http://dx.doi.org/10.1029/2007JD009162.

Fischer, EV; Zhu, L; Payne, VH; Worden, JR; Jiang, Z, he; Kulawik, SS; Brey, S; Hecobian, A; Gombos, D; Cady-Pereira, K; Flocke, F. (2018). Using TES retrievals to investigate PAN in North American biomass burning plumes. Atmos Chem Phys 18: 5639-5653. http://dx.doi.org/10.5194/acp-18-5639-2018.

Foley, KM; Hogrefe, C; Pouliot, G; Possiel, N; Roselle, SJ; Simon, H; Timin, B. (2015). Dynamic evaluation of CMAQ part I: Separating the effects of changing emissions and changing meteorology on ozone levels between 2002 and 2005 in the eastern US. Atmos Environ 103: 247-255. http://dx.doi.org/10.1016/j.atmosenv.2014.12.038.

Galmarini, S; Koffi, B; Solazzo, E; Keating, T; Hogrefe, C; Schulz, M; Benedictow, A; Griesfeller, J; Janssens-Maenhout, G; Carmichael, G; Fu, J; Dentener, F. (2017). Technical note: Coordination and harmonization of the multi-scale, multi-model activities HTAP2, AQMEII3, and MICS-Asia3: simulations, emission inventories, boundary conditions, and model output formats. Atmos Chem Phys 17: 1543-1555. http://dx.doi.org/10.5194/acp-17-1543-2017.

Gantt, B; Sarwar, G; Xing, J; Simon, H; Schwede, D; Hutzell, WT; Mathur, R; Saiz-Lopez, A. (2017). The impact of iodide-mediated ozone deposition and halogen chemistry on surface ozone concentrations across the continental United States. Environ Sci Technol 51: 1458-1466. http://dx.doi.org/10.1021/acs.est.6b03556.

Gaudel, A; Cooper, OR; Ancellet, G; Barret, B; Boynard, A; Burrows, JP; Clerbaux, C; Coheur, PF; Cuesta, J; Cuevas, E; Doniki, S; Dufour, G; Ebojie, F; Foret, G; Garcia, O; Granados Muños, MJ; Hannigan, J; Hase, F; Huang, G; Hassler, B; Hurtmans, D; Jaffe, D; Jones, N; Kalabokas, P; Kerridge, B; Kulawik, SS; Latter, B; Leblanc, T; Le Flochmoën, E; Lin, W; Liu, J; Liu, X; Mahieu, E; McClure-Begley, A; Neu, JL; Osman, M; Palm, M; Petetin, H; Petropavlovskikh, I; Querel, R; Rahpoe, N; Rozanov, A; Schultz, MG; Schwab, J; Siddans, R; Smale, D; Steinbacher, M; Tanimoto, H; Tarasick, D; Thouret, V; Thompson, AM; Trickl, T; Weatherhead, E; Wespes, C; Worden, H; Vigouroux, C; Xu, X; Zeng, G; Ziemke, J. (2018). Tropospheric ozone assessment report: Present-day distribution and trends of tropospheric ozone relevant to climate and global atmospheric chemistry model evaluation. Elementa: Science of the Anthropocene 6: 39. http://dx.doi.org/10.1525/elementa.291.

Gong, X; Kaulfus, A; Nair, U; Jaffe, DA. (2017). Quantifying O3 impacts in urban areas due to wildfires using a generalized additive model. Environ Sci Technol 51: 13216-13223. http://dx.doi.org/10.1021/acs.est.7b03130.

Gratz, LE; Jaffe, DA; Hee, JR. (2015). Causes of increasing ozone and decreasing carbon monoxide in springtime at the Mt. Bachelor Observatory from 2004 to 2013. Atmos Environ 109: 323-330. http://dx.doi.org/10.1016/j.atmosenv.2014.05.076.

Grewe, V; Tsati, E; Hoor, P. (2010). On the attribution of contributions of atmospheric trace gases to emissions in atmospheric model applications. GMD 3: 487-499. http://dx.doi.org/10.5194/gmd-3-487-2010.

Guenther, A; Geron, C; Pierce, T; Lamb, B; Harley, P; Fall, R. (2000). Natural emissions of non-methane volatile organic compounds, carbon monoxide, and oxides of nitrogen from North America. Atmos Environ 34: 2205-2230. http://dx.doi.org/10.1016/S1352-2310(99)00465-3.

Guo, JJ; Fiore, AM; Murray, LT; Jaffe, DA; Schnell, JL; Moore, CT; Milly, GP. (2018). Average versus high surface ozone levels over the continental USA: Model bias, background influences, and interannual variability. Atmos Chem Phys 18: 12123-12140. http://dx.doi.org/10.5194/acp-18-12123-2018.

Hakami, A; Odman, MT; Russell, AG. (2004). Nonlinearity in atmospheric response: A direct sensitivity analysis approach. J Geophys Res Atmos 109: 1-12. http://dx.doi.org/10.1029/2003JD004502.

Hall, SJ; Reyes, L; Huang, W; Homyak, PM. (2018). Wet spots as hotspots: Moisture responses of nitric and nitrous oxide emissions from poorly drained agricultural soils. Jour Geo Res: Biog 123: 3589-3602. http://dx.doi.org/10.1029/2018JG004629.

Helmig, D; Thompson, CR; Evans, J; Boylan, P; Hueber, J; Park, JH. (2014). Highly elevated atmospheric levels of volatile organic compounds in the Uintah Basin, Utah. Environ Sci Technol 48: 4707-4715. http://dx.doi.org/10.1021/es405046r.

Henderson, BH; Akhtar, F; Pye, HOT; Napelenok, SL; Hutzell, WT. (2014). A database and tool for boundary conditions for regional air quality modeling: description and evaluation. GMD 7: 339-360. http://dx.doi.org/10.5194/gmd-7-339-2014.

Henneman, LRF; Holmes, HA; Mulholland, JA; Russell, AG. (2015). Meteorological detrending of primary and secondary pollutant concentrations: Method application and evaluation using long-term (2000-2012) data in Atlanta. Atmos Environ 119: 201-210. http://dx.doi.org/10.1016/j.atmosenv.2015.08.007.

Hickman, JE; Huang, Y; Wu, S; Diru, W; Groffman, PM; Tully, KL; Palm, CA. (2017). Non-linear response of nitric oxide fluxes to fertilizer inputs and the impacts of agricultural intensification on tropospheric ozone pollution in Kenya. Global Change Biol 23: 3193-3204. http://dx.doi.org/10.1111/gcb.13644.

Hoesly, RM; Smith, SJ; Feng, L; Klimont, Z; Janssens-Maenhout, G; Pitkanen, T; Seibert, JJ; Vu, L; Andres, RJ; Bolt, RM; Bond, TC; Dawidowski, L; Kholod, N; Kurokawa, JI; Li, M; Liu, L; Lu, Z; Moura, MCP; O'Rourke, PR; Zhang, Q. (2018). Historical (1750-2014) anthropogenic emissions of reactive gases and aerosols from the Community Emissions Data System (CEDS). GMD 11: 369-408. http://dx.doi.org/10.5194/gmd-11-369-2018.

Hogrefe, C; Liu, P; Pouliot, G; Mathur, R; Roselle, S; Flemming, J; Lin, M; Park, RJ. (2018). Impacts of different characterizations of large-scale background on simulated regional-scale ozone over the continental United States. Atmos Chem Phys 18: 3839-3864. http://dx.doi.org/10.5194/acp-18-3839-2018.

Hu, L; Millet, DB; Baasandorj, M; Griffis, TJ; Turner, P; Helmig, D; Curtis, AJ; Hueber, J. (2015). Isoprene emissions and impacts over an ecological transition region in the US Upper Midwest inferred from tall tower measurements. J Geophys Res Atmos 120: 3553-3571. http://dx.doi.org/10.1002/2014JD022732.

Huang, LL; Pan, C; Yu, TT; Guo, K, un; Wang, X, hui; Zhang, J, unYan; Wang, H, zhi; Gao, S. (2014). BENEFICAL THERAPEUTIC EFFECT OF CHINESE HERBAL XINJI'ERKANG FORMULA ON HYPERTENSION-INDUCED RENAL INJURY IN THE 2-KIDNEY-1-CLIP HYPERTENSIVE RATS. African Journal of Traditional, Complementary and Alternative Medicines 11: 16-27. http://dx.doi.org/10.4314/ajtcam.v11i5.3.

Huang, M; Bowman, KW; Carmichael, GR; Pierce, RB; Worden, HM; Luo, M; Cooper, OR; Pollack, IB; Ryerson, TB; Brown, SS. (2013a). Impact of Southern California anthropogenic emissions on ozone pollution in the mountain states: Model analysis and observational evidence from space. J Geophys Res Atmos 118: 12784-12803. http://dx.doi.org/10.1002/2013JD020205.

Huang, M; Carmichael, GR; Chai, T; Pierce, RB; Oltmans, SJ; Jaffe, DA; Bowman, KW; Kaduwela, A; Cai, C; Spak, SN; Weinheimer, AJ; Huey, LG; Diskin, GS. (2013b). Impacts of transported background pollutants on summertime western US air quality: model evaluation, sensitivity analysis and data assimilation. Atmos Chem Phys 13: 359-391. http://dx.doi.org/10.5194/acp-13-359-2013.

Huntrieser, H; Lichtenstern, M; Scheibe, M; Aufmhoff, H; Schlager, H; Pucik, T; Minikin, A; Weinzierl, B; Heimerl, K; Fuetterer, D; Rappengluck, B; Ackermann, L; Pickering, KE; Cummings, KA; Biggerstaff, MI; Betten, DP; Honomichl, S; Barth, MC. (2016). On the origin of pronounced O-3 gradients in the thunderstorm outflow region during DC3. J Geophys Res Atmos 121: 6600-6637. http://dx.doi.org/10.1002/2015JD024279.

Im, U; Bianconi, R; Solazzo, E; Kioutsioukis, I; Badia, A; Balzarini, A; Baro, R; Bellasio, R; Brunner, D; Chemel, C; Curci, G; Flemming, J; Forkel, R; Giordano, L; Jimenez-Guerrero, P; Hirtl, M; Hodzic, A; Honzak, L; Jorba, O; Knote, C; Kuenen, JJP; Makar, PA; Manders-Groot, A; Neal, L; Perez, JL; Pirovano, G; Pouliot, G; San Jose, R; Savage, N; Schroder, W; Sokhi, RS; Syrakov, D; Torian, A; Tuccella, P; Werhahn, J; Wolke, R; Yahya, K; Zabkar, R; Zhang, Y; Zhang, J; Hogrefe, C; Galmarini, S. (2015). Evaluation of operational on-line-coupled regional air quality models over Europe and North America in the context of AQMEII phase 2. Part I: Ozone. Atmos Environ 115: 404-420. http://dx.doi.org/10.1016/j.atmosenv.2014.09.042.

Jaffe, DA; Cooper, OR; Fiore, AM; Henderson, BH; Tonnesen, GS; Russell, AG; Henze, DK; Langford, AO; Lin, M; Moore, T. (2018). Scientific assessment of background ozone over the US: Implications for air quality management. 6. http://dx.doi.org/10.1525/elementa.309.

Jaffe, DA; Wigder, NL. (2012). Ozone production from wildfires: A critical review. Atmos Environ 51: 1-10. http://dx.doi.org/10.1016/j.atmosenv.2011.11.063.

Karamchandani, P; Morris, R; Wentland, A; Shah, T; Reid, S; Lester, J. (2017). Dynamic evaluation of photochemical grid model response to emission changes in the south coast air basin in California. Atmosphere (Basel) 8. http://dx.doi.org/10.3390/atmos8080145.

Kim, PS; Jacob, DJ; Liu, X; Warner, JX; Yang, K; Chance, K; Thouret, V; Nedelec, P. (2013). Global ozone-CO correlations from OMI and AIRS: constraints on tropospheric ozone sources. Atmos Chem Phys 13: 9321-9335. http://dx.doi.org/10.5194/acp-13-9321-2013.

Kirschke, S; Bousquet, P; Ciais, P; Saunois, M; Canadell, JG; Dlugokencky, EJ; Bergamaschi, P; Bergmann, D; Blake, DR; Bruhwiler, L; Cameron-Smith, P; Castaldi, S; Chevallier, F; Feng, L; Fraser, A; Heimann, M; Hodson, EL; Houweling, S; Josse, B; Fraser, PJ; Krummel, PB; Lamarque, JF; Langenfelds, R, ayL; Le Quere, C; Naik, V; O'Doherty, S; Palmer, PI; Pison, I; Plummer, D; Poulter, B; Prinn, RG; Rigby, M; Ringeval, B; Santini, M; Schmidt, M; Shindell, DT; Simpson, IJ; Spahni, R; Steele, LP; Strode, SA; Sudo, K; Szopa, S; van Der Werf, GR; Voulgarakis, A; van Weele, M; Weiss, RF; Williams, JE; Zeng, G. (2013). Three decades of global methane sources and sinks. Nat Geosci 6: 813-823. http://dx.doi.org/10.1038/NGEO1955.

Knote, C; Tuccella, P; Curci, G; Emmons, L; Orlando, JJ; Madronich, S; Baro, R; Jimenez-Guerrero, P; Luecken, D; Hogrefe, C; Forkel, R; Werhahn, J; Hirtl, M; Perez, JL; San Jose, R; Giordano, L; Brunner, D; Yahya, K; Zhang, Y. (2015). Influence of the choice of gas-phase mechanism on predictions of key gaseous pollutants during the AQMEII phase-2 intercomparison. Atmos Environ 115: 553-568. http://dx.doi.org/10.1016/j.atmosenv.2014.11.066.

Knowland, KE; Ott, LE; Duncan, BN; Wargan, K. (2017). Stratospheric intrusion-influenced ozone air quality exceedances investigated in the NASA MERRA-2 reanalysis. Geophys Res Lett 44: 10691-10701. http://dx.doi.org/10.1002/2017GL074532.

Kota, SH; Schade, G; Estes, M; Boyer, D; Ying, Q. (2015). Evaluation of MEGAN predicted biogenic isoprene emissions at urban locations in Southeast Texas. Atmos Environ 110: 54-64. http://dx.doi.org/10.1016/j.atmosenv.2015.03.027.

Krotkov, NA; McLinden, CA; Li, C; Lamsal, LN; Celarier, EA; Marchenko, SV; Swartz, WH; Bucsela, EJ; Joiner, J; Duncan, BN; Boersma, KF; Veefkind, JP; Levelt, PF; Fioletov, VE; Dickerson, RR; He, H; Lu, Z; Streets, DG. (2016). Aura OMI observations of regional SO2 and NO2 pollution changes from 2005 to 2015. Atmos Chem Phys 16: 4605-4629. http://dx.doi.org/10.5194/acp-16-4605-2016.

Kwok, RHF; Baker, KR; Napelenok, SL; Tonnesen, GS. (2015). Photochemical grid model implementation and application of VOC, NOx, and O-3 source apportionment. GMD 8: 99-114. http://dx.doi.org/10.5194/gmd-8-99-2015.

Langford, AO. (1999). Stratosphere-troposphere exchange at the subtropical jet: contribution to the tropospheric ozone budget at midlatitudes. Geophys Res Lett 26: 2449-2452.

Langford, AO; Aikin, KC; Eubank, CS; Williams, EJ. (2009). Stratospheric contribution to high surface ozone in Colorado during springtime. Geophys Res Lett 36: L12801. http://dx.doi.org/10.1029/2009gl038367.

Langford, AO; Alvarez, RJ; Brioude, J; Fine, R; Gustin, MS; Lin, MY; Marchbanks, RD; Pierce, RB; Sandberg, SP; Senff, CJ; Weickmann, AM; Williams, EJ. (2017). Entrainment of stratospheric air and Asian pollution by the convective boundary layer in the southwestern US. J Geophys Res Atmos 122: 1312-1337. http://dx.doi.org/10.1002/2016JD025987.

Langford, AO; Senff, CJ; Alvarez, RJ; Brioude, J; Cooper, OR; Holloway, JS; Lin, MY; Marchbanks, RD; Pierce, RB; Sandberg, SP; Weickmann, AM; Williams, EJ. (2015). An overview of the 2013 Las Vegas Ozone Study (LVOS): Impact of stratospheric intrusions and long-range transport on surface air quality. Atmos Environ 109: 305-322. http://dx.doi.org/10.1016/j.atmosenv.2014.08.040.

Lee, L; Teng, AP; Wennberg, PO; Crounse, JD; Cohen, RC. (2014). On rates and mechanisms of OH and O3 reactions with isoprene-derived hydroxy nitrates. J Phys Chem A 118: 1622-1637. http://dx.doi.org/10.1021/jp4107603.

Lefohn, AS; Emery, C; Shadwick, D; Wernli, H; Jung, J; Oltmans, SJ. (2014). Estimates of background surface ozone concentrations in the United States based on model-derived source apportionment. Atmos Environ 84: 275-288. http://dx.doi.org/10.1016/j.atmosenv.2013.11.033.

Lefohn, AS; Malley, CS; Simon, H; Wells, B; Xu, X; Zhang, L; Wang, T. (2017). Responses of human health and vegetation exposure metrics to changes in ozone concentration distributions in the European Union, United States, and China. Atmos Environ 152: 123-145. http://dx.doi.org/10.1016/j.atmosenv.2016.12.025.

Lefohn, AS; Wernli, H; Shadwick, D; Oltmans, SJ; Shapiro, M. (2012). Quantifying the importance of stratospheric-tropospheric transport on surface ozone concentrations at high- and low-elevation monitoring sites in the United States. Atmos Environ 62: 646-656. http://dx.doi.org/10.1016/j.atmosenv.2012.09.004.

Li, J; Mao, J; Fiore, AM; Cohen, RC; Crounse, JD; Teng, AP; Wennberg, PO; Lee, BH; Lopez-Hilfiker, FD; Thornton, JA; Peischl, J; Pollack, IB; Ryerson, TB; Veres, P; Roberts, JM; Neuman, JA; Nowak, JB; Wolfe, GM; Hanisco, TF; Fried, A; Singh, HB; Dibb, J; Paulot, F; Horowitz, LW. (2018). Decadal changes in summertime reactive oxidized nitrogen and surface ozone over the Southeast United States. Atmos Chem Phys 18: 2341-2361. http://dx.doi.org/10.5194/acp-18-2341-2018.

Lin, M; Fiore, AM; Cooper, OR; Horowitz, LW; Langford, AO; Levy, H; Johnson, BJ; Naik, V; Oltmans, SJ; Senff, CJ. (2012a). Springtime high surface ozone events over the western United States: Quantifying the role of stratospheric intrusions. J Geophys Res 117: D00V22. http://dx.doi.org/10.1029/2012JD018151.

Lin, M; Fiore, AM; Horowitz, LW; Cooper, OR; Naik, V; Holloway, J; Johnson, BJ; Middlebrook, AM; Oltmans, SJ; Pollack, IB; Ryerson, TB; Warner, JX; Wiedinmyer, C; Wilson, J; Wyman, B. (2012b). Transport of Asian ozone pollution into surface air over the western United States in spring. J Geophys Res 117: D00V07. http://dx.doi.org/10.1029/2011JD016961.

Lin, M; Fiore, AM; Horowitz, LW; Langford, AO; Oltmans, SJ; Tarasick, D; Rieder, HE. (2015). Climate variability modulates western US ozone air quality in spring via deep stratospheric intrusions. Nat Commun 6: 7105. http://dx.doi.org/10.1038/ncomms8105.

Lin, M; Horowitz, LW; Oltmans, SJ; Fiore, AM; Fan, S. (2014). Tropospheric ozone trends at Mauna Loa Observatory tied to decadal climate variability. Nat Geosci 7: 136-143. http://dx.doi.org/10.1038/NGEO2066.

Lin, M; Horowitz, LW; Payton, R; Fiore, AM; Tonnesen, G. (2017). US surface ozone trends and extremes from 1980 to 2014: quantifying the roles of rising Asian emissions, domestic controls, wildfires, and climate. Atmos Chem Phys 17: 2943-2970. http://dx.doi.org/10.5194/acp-17-2943-2017.

Lindaas, J; Farmer, DK; Pollack, IB; Abeleira, A; Flocke, F; Roscioli, R; Herndon, S; Fischer, EV. (2017). Changes in ozone and precursors during two aged wildfire smoke events in the Colorado Front Range in summer 2015. Atmos Chem Phys 17: 10691-10707. http://dx.doi.org/10.5194/acp-17-10691-2017.

Liu, F; Beirle, S; Zhang, Q; van der A, RJ; Zheng, B; Tong, D; He, K. (2017a). NOx emission trends over Chinese cities estimated from OMI observations during 2005 to 2015. Atmos Chem Phys 17: 9261-9275. http://dx.doi.org/10.5194/acp-17-9261-2017.

Liu, Z; Liu, Y; Murphy, JP; Maghirang, R. (2017b). Contributions of Kansas rangeland burning to ambient O3: Analysis of data from 2001 to 2016. Sci Total Environ 618: 1024-1031. http://dx.doi.org/10.1016/j.scitotenv.2017.09.075.

Mao, J; Horowitz, LW; Naik, V; Fan, S; Liu, J; Fiore, AM. (2013). Sensitivity of tropospheric oxidants to biomass burning emissions: implications for radiative forcing. Geophys Res Lett 40: 1241-1246. http://dx.doi.org/10.1002/grl.50210.

Martin, RV. (2008). Satellite remote sensing of surface air quality. Atmos Environ 42: 7823-7843. http://dx.doi.org/10.1016/j.atmosenv.2008.07.018.

Mathur, R; Xing, J; Gilliam, R; Sarwar, G; Hogrefe, C; Pleim, J; Pouliot, G; Roselle, S; Spero, TL; Wong, DC; Young, J. (2017). Extending the Community Multiscale Air Quality (CMAQ) modeling system to hemispheric scales: overview of process considerations and initial applications. Atmos Chem Phys 17: 12449-12474. http://dx.doi.org/10.5194/acp-17-12449-2017.

McDonald-Buller, EC; Allen, DT; Brown, N; Jacob, DJ; Jaffe, D; Kolb, CE; Lefohn, AS; Oltmans, S; Parrish, DD; Yarwood, G; Zhang, L. (2011). Establishing policy relevant background (PRB) ozone concentrations in the United States [Review]. Environ Sci Technol 45: 9484-9497. http://dx.doi.org/10.1021/es2022818.

Mebust, AK; Cohen, RC. (2014). Space-based observations of fire NOx emission coefficients: a global biome-scale comparison. Atmos Chem Phys 14: 2509-2524. http://dx.doi.org/10.5194/acp-14-2509-2014.

Mebust, AK; Russell, AR; Hudman, RC; Valin, LC; Cohen, RC. (2011). Characterization of wildfire NOx emissions using MODIS fire radiative power and OMI tropospheric NO2 columns. Atmos Chem Phys 11: 5839-5851. http://dx.doi.org/10.5194/acp-11-5839-2011.

Monks, PS; Archibald, AT; Colette, A; Cooper, O; Coyle, M; Derwent, R; Fowler, D; Granier, C; Law, KS; Mills, GE; Stevenson, DS; Tarasova, O; Thouret, V; von Schneidemesser, E; Sommariva, R; Wild, O; Williams, ML. (2015). Tropospheric ozone and its precursors from the urban to the global scale from air quality to short-lived climate forcer. Atmos Chem Phys 15: 8889-8973. http://dx.doi.org/10.5194/acp-15-8889-2015.

Muñiz-Unamunzaga, M; Borge, R; Sarwar, G; Gantt, B; de la Paz, D; Cuevas, CA; Saiz-Lopez, A. (2018). The influence of ocean halogen and sulfur emissions in the air quality of a coastal megacity: The case of Los Angeles. Sci Total Environ 610-611: 1536-1545. http://dx.doi.org/10.1016/j.scitotenv.2017.06.098.

Murray, LT; Jacob, DJ; Logan, JA; Hudman, RC; Koshak, WJ. (2012). Optimized regional and interannual variability of lightning in a global chemical transport model constrained by LIS/OTD satellite data. J Geophys Res 117: 1-14. http://dx.doi.org/10.1029/2012JD017934.

NAPCA (National Air Pollution Control Administration). (1970). Air quality criteria for photochemical oxidants [EPA Report]. (AP-63). Washington, DC: U.S. Department of Health, Education, and Welfare. http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=9100E2Z7.txt.

NASEM (National Academies of Sciences, Engineering, and Medicine). (2018). Improving characterization of anthropogenic methane emissions in the United States. Washington, D.C.: National Academies Press. http://dx.doi.org/10.17226/24987.

Natraj, V; Liu, X; Kulawik, S; Chance, K; Chatfield, R; Edwards, DP; Eldering, A; Francis, G; Kurosu, T; Pickering, K; Spurr, R; Worden, H. (2011). Multi-spectral sensitivity studies for the retrieval of tropospheric and lowermost tropospheric ozone from simulated clear-sky GEO-CAPE measurements. Atmos Environ 45: 7151-7165. http://dx.doi.org/10.1016/j.atmosenv.2011.09.014.

Nolte, CG; Dolwick, PD; Fann, N; Horowitz, LW; Naik, V; Pinder, RW; Spero, TL; Winner, DA; Ziska, LH. (2018). Air quality. In DR Reidmiller; CW Avery; DR Easterling; KE Kunkel; KLM Lewis; TK Maycock; BC Stewart (Eds.), Impacts, Risks, and Adaptation in the United States: Fourth National Climate Assessment, Volume II (pp. 512538). Washington, DC: U.S. Global Change Research Program. https://nca2018.globalchange.gov/chapter/13/.

Oetjen, H; Payne, VH; Neu, JL; Kulawik, SS; Edwards, DP; Eldering, A; Worden, HM; Worden, JR. (2016). A joint data record of tropospheric ozone from Aura-TES and MetOp-IASI. Atmos Chem Phys 16: 10229-10239. http://dx.doi.org/10.5194/acp-16-10229-2016.

Orbe, C; Waugh, DW; Yang, H; Lamarque, JF; Tilmes, S; Kinnison, DE. (2017). Tropospheric transport differences between models using the same large-scale meteorological fields. Geophys Res Lett 44: 1068-1078. http://dx.doi.org/10.1002/2016GL071339.

Pan, LL; Homeyer, CR; Honomichl, S; Ridley, BA; Weisman, M; Barth, MC; Hair, JW; Fenn, MA; Butler, C; Diskin, GS; Crawford, JH; Ryerson, TB; Pollack, I; Peischl, J; Huntrieser, H. (2014). Thunderstorms enhance tropospheric ozone by wrapping and shedding stratospheric air. Geophys Res Lett 41: 7785-7790. http://dx.doi.org/10.1002/2014GL061921.

Parrish, DD; Lamarque, JF; Naik, V; Horowitz, L; Shindell, DT; Staehelin, J; Derwent, R; Cooper, OR; Tanimoto, H; Volz-Thomas, A; Gilge, S; Scheel, HE; Steinbacher, M; Froehlich, M. (2014). Long-term changes in lower tropospheric baseline ozone concentrations: Comparing chemistry-climate models and observations at northern midlatitudes. J Geophys Res Atmos 119: 5719-5736. http://dx.doi.org/10.1002/2013JD021435.

Parrish, DD; Law, KS; Staehelin, J; Derwent, R; Cooper, OR; Tanimoto, H; Volz-Thomas, A; Gilge, S; Scheel, HE; Steinbacher, M; Chan, E. (2012). Long-term changes in lower tropospheric baseline ozone concentrations at northern mid-latitudes. Atmos Chem Phys 12: 11485-11504. http://dx.doi.org/10.5194/acp-12-11485-2012.

Parrish, DD; Petropavlovskikh, I; Oltmans, SJ. (2017a). Reversal of long-term trend in baseline ozone concentrations at the North American West Coast. Geophys Res Lett 44: 10675-10681. http://dx.doi.org/10.1002/2017GL074960.

Parrish, DD; Young, LM; Newman, MH; Aikin, KC; Ryerson, TB. (2017b). Ozone design values in southern California's air basins: Temporal evolution and US background contribution. J Geophys Res Atmos 122: 11166-11182. http://dx.doi.org/10.1002/2016JD026329.

Pfister, GG; Walters, S; Emmons, LK; Edwards, DP; Avise, J. (2013). Quantifying the contribution of inflow on surface ozone over California during summer 2008. J Geophys Res Atmos 118: 12282-12299. http://dx.doi.org/10.1002/2013JD020336.

Phoenix, DB; Homeyer, CR; Barth, MC. (2017). Sensitivity of simulated convection-driven stratosphere-troposphere exchange in WRF-Chem to the choice of physical and chemical parameterization. 4: 454-471. http://dx.doi.org/10.1002/2017EA000287.

Ran, L; Pleim, J; Gilliam, R; Binkowski, FS; Hogrefe, C; Band, L. (2016). Improved meteorology from an updated WRF/CMAQ modeling system with MODIS vegetation and albedo. J Geophys Res Atmos 121: 2393-2415. http://dx.doi.org/10.1002/2015JD024406.

Rappenglueck, B; Ackermann, L; Alvarez, S; Golovko, J; Buhr, M; Field, RA; Soltis, J; Montague, DC; Hauze, B; Adamson, S; Risch, D; Wilkerson, G; Bush, D; Stoeckenius, T; Keslar, C. (2014). Strong wintertime ozone events in the Upper Green River basin, Wyoming. Atmos Chem Phys 14: 4909-4934. http://dx.doi.org/10.5194/acp-14-4909-2014.

Rasool, QZ; Zhang, R; Lash, B; Cohan, DS; Cooter, EJ; Bash, JO; Lamsal, LN. (2016). Enhanced representation of soil NO emissions in the Community Multiscale Air Quality (CMAQ) model version 5.0.2. GMD 9: 3177-3197. http://dx.doi.org/10.5194/gmd-9-3177-2016.

Rastigejev, Y; Park, R; Brenner, MP; Jacob, DJ. (2010). Resolving intercontinental pollution plumes in global models of atmospheric transport. J Geophys Res 115: D02302. http://dx.doi.org/10.1029/2009JD012568.

Reddy, PJ; Pfister, GG. (2016). Meteorological factors contributing to the interannual variability of midsummer surface ozone in Colorado, Utah, and other western US states. J Geophys Res Atmos 121: 2434-2456. http://dx.doi.org/10.1002/2015JD023840.

Reidmiller, DR; Fiore, AM; Jaffe, DA; Bergmann, D; Cuvelier, C; Dentener, FJ; Duncan; Bryan, N; Folberth, G; Gauss, M; Gong, S; Hess, P; Jonson, JE; Keating, T; Lupu, A; Marmer, E; Park, R; Schultz, MG; Shindell, DT; Szopa, S; Vivanco, MG; Wild, O; Zuber, A. (2009). The influence of foreign vs. North American emissions on surface ozone in the US. Atmos Chem Phys 9: 5027-5042.

Rieder, HE; Fiore, AM; Clifton, OE; Correa, G; Horowitz, LW; Naik, V. (2018). Combining model projections with site-level observations to estimate changes in distributions and seasonality of ozone in surface air over the USA. Atmos Environ 193: 302-315. http://dx.doi.org/10.1016/j.atmosenv.2018.07.042.

Ross, AN; Wooster, MJ; Boesch, H; Parker, R. (2013). First satellite measurements of carbon dioxide and methane emission ratios in wildfire plumes. Geophys Res Lett 40: 4098-4102. http://dx.doi.org/10.1002/grl.50733.

Russell, AR; Perring, AE; Valin, LC; Bucsela, EJ; Browne, EC; Wooldridge, PJ; Cohen, RC. (2011). A high spatial resolution retrieval of NO2 column densities from OMI: Method and evaluation. Atmos Chem Phys 11: 8543-8554. http://dx.doi.org/10.5194/acp-11-8543-2011.

Rydsaa, JH; Stordal, F; Gerosa, G; Finco, A; Hodnebrog, O. (2016). Evaluating stomatal ozone fluxes in WRF-Chern: Comparing ozone uptake in Mediterranean ecosystems. Atmos Environ 143: 237-248. http://dx.doi.org/10.1016/j.atmosenv.2016.08.057.

Ryu, YH; Hodzic, A; Barre, J; Descombes, G; Minnis, P. (2018). Quantifying errors in surface ozone predictions associated with clouds over the CONUS: a WRF-Chem modeling study using satellite cloud retrievals. Atmos Chem Phys 18: 7509-7525. http://dx.doi.org/10.5194/acp-18-7509-2018.

Sandu, A; Daescu, DN; Carmichael, GR; Chai, TF. (2005). Adjoint sensitivity analysis of regional air quality models. Journal of Computational Physics 204: 222-252. http://dx.doi.org/10.1016/j.jcp.2004.10.011.

Sarwar, G; Gantt, B; Schwede, D; Foley, K; Mathur, R; Saiz-Lopez, A. (2015). Impact of Enhanced Ozone Deposition and Halogen Chemistry on Tropospheric Ozone over the Northern Hemisphere. Environ Sci Technol 49: 9203-9211. http://dx.doi.org/10.1021/acs.est.5b01657.

Sarwar, G; Simon, H; Bhave, P; Yarwood, G. (2012). Examining the impact of heterogeneous nitryl chloride production on air quality across the United States. Atmos Chem Phys 12: 6455-6473. http://dx.doi.org/10.5194/acp-12-6455-2012.

Saunois, M; Bousquet, P; Poulter, B, en; Peregon, A; Ciais, P; Canadell, JG; Dlugokencky, EJ; Etiope, G; Bastviken, D; Houweling, S; Janssens-Maenhout, G; Tubiello, FN; Castaldi, S; Jackson, RB; Alexe, M; Arora, VK; Beerling, DJ; Bergamaschi, P; Blake, DR; Brailsford, G; Brovkin, V; Bruhwiler, L; Crevoisier, C; Crill, P; Covey, K; Curry, C; Frankenberg, C; Gedney, N; Hoeglund-Isaksson, L; Ishizawa, M; Ito, A; Joos, F; Kim, HS; Kleinen, T; Krummel, P; Lamarque, JF; Langenfelds, R, ay; Locatelli, R; Machida, T; Maksyutov, S; Mcdonald, KC; Marshall, J; Melton, JR; Morino, I; Naik, V; O'Doherty, S; Parmentier, FJW; Patra, PK; Peng, C; Peng, S; Peters, GP; Pison, I; Prigent, C; Prinn, R; Ramonet, M; Riley, WJ; Saito, M; Santini, M; Schroeder, R; Simpson, IJ; Spahni, R; Steele, P; Takizawa, A; Thornton, BF; Tian, H; Tohjima, Y; Viovy, N; Voulgarakis, A; van Weele, M; van Der Werf, GR; Weiss, R; Wiedinmyer, C; Wilton, DJ; Wiltshire, A; Worthy, D; Wunch, D; Xu, X; Yoshida, Y; Zhang, B; Zhu, Q. (2016). The global methane budget 2000-2012. Earth System Science Data 8: 697-751. http://dx.doi.org/10.5194/essd-8-697-2016.

Schnell, RC; Oltmans, SJ; Neely, RR; Endres, MS; Molenar, JV; White, AB. (2009). Rapid photochemical production of ozone at high concentrations in a rural site during winter. Nat Geosci 2: 120-122. http://dx.doi.org/10.1038/NGEO415.

Schreier, SF; Richter, A; Kaiser, JW; Burrows, JP. (2014). The empirical relationship between satellite-derived tropospheric NO2 and fire radiative power and possible implications for fire emission rates of NOx. Atmos Chem Phys 14: 2447-2466. http://dx.doi.org/10.5194/acp-14-2447-2014.

Schreier, SF; Richter, A; Schepaschenko, D; Shvidenko, A; Hilboll, A; Burrows, JP. (2015). Differences in satellite-derived NOx emission factors between Eurasian and North American boreal forest fires. Atmos Environ 121: 55-65. http://dx.doi.org/10.1016/j.atmosenv.2014.08.071.

Seinfeld, JH; Pandis, SN. (2006). Atmospheric chemistry and physics: From air pollution to climate change. In JH Seinfeld; SN Pandis (Eds.), Atmospheric chemistry and physics: From air pollution to climate change (2nd ed.). Hoboken, NJ: John Wiley & Sons. https://www.wiley.com/en-us/Atmospheric+Chemistry+and+Physics%3A+From+Air+Pollution+to+Climate+Change%2C+2nd+Edition-p-9780471720188.

Shen, L; Mickley, LJ; Tai, APK. (2015). Influence of synoptic patterns on surface ozone variability over the eastern United States from 1980 to 2012. Atmos Chem Phys 15: 10925-10938. http://dx.doi.org/10.5194/acp-15-10925-2015.

Shen, L, u; Mickley, LJ; Leibensperger, EM; Li, M. (2017). Strong dependence of US summertime air quality on the decadal variability of Atlantic Sea surface temperatures. Geophys Res Lett 44: 12527-12535. http://dx.doi.org/10.1002/2017GL075905.

Simon, H; Baker, KR; Phillips, S. (2012). Compilation and interpretation of photochemical model performance statistics published between 2006 and 2012. Atmos Environ 61: 124-139. http://dx.doi.org/10.1016/j.atmosenv.2012.07.012.

Simon, H; Kimura, Y; McGaughey, G; Allen, DT; Brown, SS; Osthoff, HD; Roberts, JM; Byun, D; Lee, D. (2009). Modeling the impact of ClNO2 on ozone formation in the Houston area. J Geophys Res Atmos 114: D00F03. http://dx.doi.org/10.1029/2008jd010732.

Simon, H; Reff, A; Wells, B; Xing, J; Frank, N. (2015). Ozone trends across the United States over a period of decreasing NOx and VOC emissions. Environ Sci Technol 49: 186-195. http://dx.doi.org/10.1021/es504514z.

Skerlak, B; Sprenger, M; Pfahl, S; Tyrlis, E; Wernli, H. (2015). Tropopause folds in ERA-Interim: Global climatology and relation to extreme weather events. J Geophys Res Atmos 120: 4860-4877. http://dx.doi.org/10.1002/2014JD022787.

Stohl, A; Spichtinger-Rakowsky, N; Bonasoni, P; Feldmann, H; Memmesheimer, M; Scheel, HE; Trickly, T; Hubener, S; Ringer, W; Mandl, M. (2000). The influence of stratospheric intrusions on alpine ozone concentrations. Atmos Environ 34: 1323-1354. http://dx.doi.org/10.1016/S1352-2310(99)00320-9.

Stone, D; Sherwen, T; Evans, MJ; Vaughan, S; Ingham, T; Whalley, LK; Edwards, PM; Read, KA; Lee, JD; Moller, SJ; Carpenter, LJ; Lewis, AC; Heard, DE. (2018). Impacts of bromine and iodine chemistry on tropospheric OH and HO2: comparing observations with box and global model perspectives. Atmos Chem Phys 18: 3541-3561. http://dx.doi.org/10.5194/acp-18-3541-2018.

Strode, SA; Rodriguez, JM; Logan, JA; Cooper, OR; Witte, JC; Lamsal, LN; Damon, M; Van Aartsen, B; Steenrod, SD; Strahan, SE. (2015). Trends and variability in surface ozone over the United States. J Geophys Res Atmos 120: 9020-9042. http://dx.doi.org/10.1002/2014JD022784.

Tang, Q; Prather, MJ; Hsu, J. (2011). Stratosphere-troposphere exchange ozone flux related to deep convection. Geophys Res Lett 38: L03806. http://dx.doi.org/10.1029/2010GL046039.

Tanimoto, H; Ikeda, K; Boersma, KF; Ronald, J, vanderA; Garivait, S. (2015). Interannual variability of nitrogen oxides emissions from boreal fires in Siberia and Alaska during 1996-2011 as observed from space. Environ Res Lett 10. http://dx.doi.org/10.1088/1748-9326/10/6/065004.

Tereszchuk, KA; Abad, GG; Clerbaux, C; Hurtmans, D; Coheur, PF; Bernath, PF. (2011). ACE-FTS measurements of trace species in the characterization of biomass burning plumes. Atmos Chem Phys 11: 12169-12179. http://dx.doi.org/10.5194/acp-11-12169-2011.

Trainer, M; Parrish, DD; Buhr, MP; Norton, RB; Fehsenfeld, FC; Anlauf, KG; Bottenheim, JW; Tang, YZ; Wiebe, HA; Roberts, JM; Tanner, RL; Newman, L; Bowersox, VC; Meagher, JF; Olszyna, KJ; Rodgers, MO; Wang, T; Berresheim, H; Demerjian, KL; Roychowdhury, UK. (1993). Correlation of ozone with NOy in photochemically aged air. J Geophys Res 98: 2917-2925.

Travis, KR; Jacob, DJ; Fisher, JA; Kim, PS; Marais, EA; Zhu, L; Yu, K; Miller, CC; Yantosca, RM; Sulprizio, MP; Thompson, AM; Wennberg, PO; Crounse, JD; St Clair, JM; Cohen, RC; Laughner, JL; Dibb, JE; Hall, SR; Ullmann, K; Wolfe, GM; Pollack, IB; Peischl, J; Neuman, JA; Zhou, X. (2016). Why do models overestimate surface ozone in the Southeast United States? Atmos Chem Phys 16: 13561-13577. http://dx.doi.org/10.5194/acp-16-13561-2016.

Tuite, K; Brockway, N; Colosimo, SF; Grossmann, K; Tsai, C; Flynn, J; Alvarez, S; Erickson, M; Yarwood, G; Nopmongcol, U; Stutz, J. (2018). Iodine catalyzed ozone destruction at the Texas coast and Gulf of Mexico. Geophys Res Lett 45: 7800-7807. http://dx.doi.org/10.1029/2018GL078267.

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

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

U.S. EPA (U.S. Environmental Protection Agency). (2006b). Air Quality Criteria for Ozone and Related Photochemical Oxidants. Volume 1 of 3. (EPA/600/R-05/004AF; EPA600R05004AF). Research Triangle Park, NC. National Center for Environmental Assessment.
: Environmental Protection Agency General. http://nepis.epa.gov/exe/ZyPURL.cgi?Dockey=P100CJZN.txt.

U.S. EPA (U.S. Environmental Protection Agency). (2007). Review of the national ambient air quality standards for ozone: Policy assessment of scientific and technical information: OAQPS staff paper [EPA Report]. (EPA/452/R-07/007). Research Triangle Park, NC. https://www3.epa.gov/ttn/naaqs/standards/ozone/data/2007_07_ozone_staff_paper.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2010). Integrated science assessment for carbon monoxide (pp. 1-593). (EPA/600/R-09/019F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=218686.

U.S. EPA (U.S. Environmental Protection Agency). (2011). MOVES (Motor Vehicle Emission Simulator). Available online at http://www.epa.gov/otaq/models/moves/index.htm (accessed January 28, 2011).

U.S. EPA (U.S. Environmental Protection Agency). (2013). Integrated science assessment for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-10/076F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment-RTP Division. http://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=247492.

U.S. EPA (U.S. Environmental Protection Agency). (2015). Implementation of the 2015 Primary Ozone NAAQS: Issues associated with background ozone. White paper for discussion. Research Triangle Park, NC: U.S. Environmental Protection Agency :: U.S. EPA.

U.S. EPA (U.S. Environmental Protection Agency). (2016a). 2014 National Emissions Inventory, version 1 technical support document (Draft). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (2016b). Integrated science assessment for oxides of nitrogen-Health Criteria (final report) [EPA Report]. (EPA/600/R-15/068). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment. http://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=526855.

U.S. EPA (U.S. Environmental Protection Agency). (2016c). Inventory of U.S. greenhouse gas emissions and sinks: 1990-2016. (EPA 430-R-18-003). Washington, D.C. https://www.epa.gov/ghgemissions/inventory-us-greenhouse-gas-emissions-and-sinks.

U.S. EPA (U.S. Environmental Protection Agency). (2017). Profile of version 1 of the 2014 National Emissions Inventory. Washington, DC. https://www.epa.gov/sites/production/files/2017-04/documents/2014neiv1_profile_final_april182017.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2018a). 2014 National Emissions Inventory (NEI) data (Version 2). Washington, DC. Retrieved from https://www.epa.gov/air-emissions-inventories/2014-national-emissions-inventory-nei-data

U.S. EPA (U.S. Environmental Protection Agency). (2018b). Integrated science assessment for oxides of nitrogen, oxides of sulfur and particulate matter -Ecological criteria (2nd external review draft). (EPA/600/R-18/097). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment. http://cfint.rtpnc.epa.gov/ncea/prod/recordisplay.cfm?deid=340671.

U.S. EPA (U.S. Environmental Protection Agency). (2019a). Air markets program data. Available online at https://ampd.epa.gov/ampd/ (accessed

U.S. EPA (U.S. Environmental Protection Agency). (2019b). Air pollutant emissions trends data: Criteria pollutants national tier 1 for 1970-2017. Retrieved from https://www.epa.gov/air-emissions-inventories/air-pollutant-emissions-trends-data

Van Dingenen, R; Crippa, M; Janssens-Maenhout, G; Guizzardi, D; Dentener, F. (2018). Global trends of methane emissions and their impacts on ozone concentrations. (JRC113210; EUR 29394 EN; OP KJ-NA-29394-EN-N). Luxembourg: Office for Official Publications of the European Union. http://dx.doi.org/10.2760/820175.

Vinken, GCM; Boersma, KF; Maasakkers, JD; Adon, M; Martin, RV. (2014). Worldwide biogenic soil NOx emissions inferred from OMI NO2 observations. Atmos Chem Phys 14: 10363-10381. http://dx.doi.org/10.5194/acp-14-10363-2014.

Voulgarakis, A; Telford, PJ; Aghedo, AM; Braesicke, P; Faluvegi, G; Abraham, NL; Bowman, KW; Pyle, JA; Shindell, DT. (2011). Global multi-year O3-CO correlation patterns from models and TES satellite observations. Atmos Chem Phys 11: 5819-5838. http://dx.doi.org/10.5194/acp-11-5819-2011.

Wang, YH; Jacob, DJ; Logan, JA. (1998). Global simulation of tropospheric O-3-NOx-hydrocarbon chemistry 1. Model formulation. J Geophys Res Atmos 103: 10713-10725. http://dx.doi.org/10.1029/98JD00158.

Warneke, C; Geiger, F; Edwards, PM; Dube, W; Petron, G; Kofler, J; Zahn, A; Brown, SS; Graus, M; Gilman, JB; Lerner, BM; Peischl, J; Ryerson, TB; de Gouw, JA; Roberts, JM. (2014). Volatile organic compound emissions from the oil and natural gas industry in the Uintah Basin, Utah: oil and gas well pad emissions compared to ambient air composition. Atmos Chem Phys 14: 10977-10988. http://dx.doi.org/10.5194/acp-14-10977-2014.

Worden, HM; Deeter, MN; Frankenberg, C; George, M; Nichitiu, F; Worden, J; Aben, I; Bowman, KW; Clerbaux, C; Coheur, PF; de Laat, ATJ; Detweiler, R; Drummond, JR, JR; Edwards, DP; Gille, JC; Hurtmans, D; Luo, M; Martinez-Alonso, S; Massie, S; Pfister, G; Warner, JX. (2013). Decadal record of satellite carbon monoxide observations. Atmos Chem Phys 13: 837-850. http://dx.doi.org/10.5194/acp-13-837-2013.

Wu, SL; Duncan, BN; Jacob, DJ; Fiore, AM; Wild, O. (2009). Chemical nonlinearities in relating intercontinental ozone pollution to anthropogenic emissions. Geophys Res Lett 36: L05806. http://dx.doi.org/10.1029/2008gl036607.

Wunch, D; Toon, GC; Blavier, JF; Washenfelder, RA; Notholt, J; Connor, BJ; Griffith, DW; Sherlock, V; Wennberg, PO. (2011). The total carbon column observing network. Philos Transact A Math Phys Eng Sci 369: 2087-2112. http://dx.doi.org/10.1098/rsta.2010.0240.

Xie, Y; Paulot, F; Carter, WPL; Nolte, CG; Luecken, DJ; Hutzell, WT; Wennberg, PO; Cohen, RC; Pinder, RW. (2013). Understanding the impact of recent advances in isoprene photooxidation on simulations of regional air quality. Atmos Chem Phys 13: 8439-8455. http://dx.doi.org/10.5194/acp-13-8439-2013.

Xing, J, ia; Wang, J; Mathur, R; Wang, S; Sarwar, G; Pleim, J; Hogrefe, C; Zhang, Y; Jiang, J; Wong, DC; Hao, J. (2017). Impacts of aerosol direct effects on tropospheric ozone through changes in atmospheric dynamics and photolysis rates. Atmos Chem Phys 17: 9869-9883. http://dx.doi.org/10.5194/acp-17-9869-2017.

Xing, J; Mathur, R; Pleim, J; Hogrefe, C; Gan, CM; Wong, DC; Wei, C; Gilliam, R; Pouliot, G. (2015). Observations and modeling of air quality trends over 1990-2010 across the Northern Hemisphere: China, the United States and Europe. Atmos Chem Phys 15: 2723-2747. http://dx.doi.org/10.5194/acp-15-2723-2015.

Yahya, K; Wang, K; Zhang, Y; Kleindienst, TE. (2015). Application of WRF/Chem over North America under the AQMEII Phase 2-Part 2: Evaluation of 2010 application and responses of air quality and meteorology-chemistry interactions to changes in emissions and meteorology from 2006 to 2010. GMD 8: 2095-2117. http://dx.doi.org/10.5194/gmd-8-2095-2015.

Ying, Q; Krishnan, A. (2010). Source contributions of volatile organic compounds to ozone formation in southeast Texas. J Geophys Res Atmos 115: Article #D17306. http://dx.doi.org/10.1029/2010JD013931.

Young, PJ; Naik, V; Fiore, AM; Gaudel, A; Guo, J; Lin, MY; Neu, JL; Parrish, DD; Rieder, HE; Schnell, JL; Tilmes, S; Wild, O; Zhang, L; Ziemke, J; Brandt, J; Delcloo, A; Doherty, RM; Geels, C; Hegglin, MI; Hu, L; Im, U; Kumar, R; Luhar, A; Murray, L; Plummer, D; Rodriguez, J; Saiz-Lopez, A; Schultz, MG; Woodhouse, MT; Zeng, G. (2018). Tropospheric Ozone Assessment Report: Assessment of global-scale model performance for global and regional ozone distributions, variability, and trends. Elementa: Science of the Anthropocene 6. http://dx.doi.org/10.1525/elementa.265.

Zare, A; Christensen, JH; Gross, A; Irannejad, P; Glasius, M; Brandt, J. (2014). Quantifying the contributions of natural emissions to ozone and total fine PM concentrations in the Northern Hemisphere. Atmos Chem Phys 14: 2735-2756. http://dx.doi.org/10.5194/acp-14-2735-2014.

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

Zhang, L; Jacob, DJ; Kopacz, M; Henze, DK; Singh, K; Jaffe, DA. (2009). Intercontinental source attribution of ozone pollution at western US sites using an adjoint method. Geophys Res Lett 36: L11810. http://dx.doi.org/10.1029/2009gl037950.

Zhang, L; Jacob, DJ; Yue, X; Downey, NV; Wood, DA; Blewitt, D. (2014). Sources contributing to background surface ozone in the US Intermountain West. Atmos Chem Phys 14: 5295-5309. http://dx.doi.org/10.5194/acp-14-5295-2014.

Zhang, L; Jaffe, DA. (2017). Trends and sources of ozone and sub-micron aerosols at the Mt. Bachelor Observatory (MBO) during 2004-2015. Atmos Environ 165: 143-154. http://dx.doi.org/10.1016/j.atmosenv.2017.06.042.

Zhang, R; Cohan, A; Biazar, AP; Cohan, DS. (2017). Source apportionment of biogenic contributions to ozone formation over the United States. Atmos Environ 164: 8-19. http://dx.doi.org/10.1016/j.atmosenv.2017.05.044.

Zhang, Y; Cooper, OR; Gaudel, A; Thompson, AM; Nedelec, P; Ogino, SY; West, JJ. (2016). Tropospheric ozone change from 1980 to 2010 dominated by equatorward redistribution of emissions. Nat Geosci 9: 875-+. http://dx.doi.org/10.1038/NGEO2827.

Zheng, B; Tong, D; Li, M; Liu, F; Hong, C; Geng, G; Li, H; Li, X; Peng, L; Qi, J; Yan, L; Zhang, Y; Zhao, H; Zheng, Y; He, K; Zhang, Q. (2018). Trends in China's anthropogenic emissions since 2010 as the consequence of clean air actions. Atmos Chem Phys 18: 14095-14111. http://dx.doi.org/10.5194/acp-18-14095-2018.

Zhou, Y; Mao, H; Sive, BC. (In Press) Decadal trends and variability in intermountain west surface ozone near oil and gas extraction fields. Atmos Chem Phys. http://dx.doi.org/10.5194/acp-2019-164.

Ziemke, J. R.; Chandra, S; Oman, LD; Bhartia, PK. (2010). A new ENSO index derived from satellite measurements of column ozone. Atmos Chem Phys 10: 3711-3721.

Zoogman, P; Jacob, DJ; Chance, K; Liu, X; Lin, M; Fiore, A; Travis, K. (2014). Monitoring high-ozone events in the US Intermountain West using TEMPO geostationary satellite observations. Atmos Chem Phys 14: 6261-6271. http://dx.doi.org/10.5194/acp-14-6261-2014.

APPENDIX 2 EXPOSURE TO AMBIENT OZONE

Overall Conclusions regarding Estimates of Exposure to Ambient Ozone for Use in Epidemiologic Studies

- Since the 2013 Ozone ISA, advances have been made in several approaches for predicting ambient ozone concentrations as surrogates for exposure. Errors associated with exposure assessment methods are often similar over urban scales because ambient ozone concentrations tend to have low spatial variability.
- For epidemiologic studies of short-term exposure to ambient ozone, the association between exposure estimates and health effects may be underestimated by the measurement or model used to represent exposure, and the effect estimate may have reduced precision. Even when the magnitude of the association is uncertain, the true effect would likely be larger than the estimated association in these cases. The bias and reduction in precision are typically small in magnitude.
- For epidemiologic studies of long-term exposure to ambient ozone, depending on the model and scenario being modeled, the association between exposure estimates and health effects may be underestimated or overestimated. It is much more common for the association to be underestimated because near-road ozone scavenging can result in greater spatial variability due to a reduction in ozone concentration compared with ambient ozone measured at a fixed-site monitor. The bias and reduction in precision are typically small in magnitude.
- Estimating exposure without accounting for time-activity data may result in underestimation of the association and reduced precision. Although the magnitude of the association between exposure estimates and health effects is uncertain, the true effect tends to be larger than the estimated associations in these cases.

2.1 Introduction

This Appendix presents new developments in methodology for developing exposure estimates for epidemiologic studies and interpreting the results, given the strengths and limitations of the exposure assessment data. The Appendix describes concepts and terminology relating to exposure (Section 2.2), methodologies used for exposure assessment (Section 2.3), factors influencing personal exposure to ozone (Section 2.4), copollutant correlations and potential for confounding (Section 2.5), and interpreting exposure measurement error for use in epidemiologic studies (Section 2.6). This Appendix focuses on the ambient air component of personal exposure to ozone, because the NAAQS pertains to ambient ozone. Because there are very few indoor sources of ozone, individuals are typically exposed to ozone from ambient air rather than ozone generated from indoor sources. This Appendix focuses on studies of exposure among the general population. The information provided in this Appendix will be used to help interpret the evidence for the health effects of ozone exposure presented in the health appendices that follow (Appendix 3-Appendix 7).

2.2 Exposure Concepts

A conceptual model of personal exposure to ambient ozone is described in the 2013 Ozone ISA (U.S. EPA, 2013). Ozone in ambient air is generally produced by photochemical oxidation of NO_2, little or no precursor gases occurring in ambient air are transformed to ozone indoors. Indoor ozone therefore either infiltrates from outdoors or is generated by indoor sources, such as air purifiers. Some ozone infiltrating indoors is lost to surface reactions. A variety of metrics and terms are used to characterize air pollution exposure. They are described here to provide clarity for the subsequent discussion.

The *concentration* of ozone is defined as the volume of the pollutant in a given volume of air (e.g., ppb). Concentrations observed in outdoor locations accessible to the public are referred to as ambient concentrations. The term *exposure* refers to contact at the interface of the breathing zone with the concentration of a specific pollutant over a certain period of time (Zartarian et al., 2005), in single or multiple locations. For example, contact with a concentration of 10 ppb ozone for 1-hour would be referred to as a 1-hour exposure to 10 ppb ozone, and 10 ppb is referred to as the *exposure concentration*. As discussed in Appendix 3, dose incorporates the concept of intake into the body (via inhalation).

A location where exposure occurs is referred to as a *microenvironment*, and an individual's daily exposure consists of the time-integrated concentrations in each of the microenvironments visited during the day. Ambient air pollution may penetrate indoors, where it combines with air pollution from indoor sources (*indoor air pollution*) to produce the total measured indoor concentration. *Personal exposure to ambient* ozone is exposure to the ambient fraction of total indoor ozone concentration, together with exposure to ambient ozone concentrations in outdoor microenvironments such as parks, yards, sidewalks, and roads (e.g., while riding on bicycles or motorcycles), is referred to as ambient exposure (Wilson, 2000). This differs from overall total personal exposure, which may also include exposure to indoor air pollution. Personal exposure to ambient ozone is influenced by several factors, including:

- time-activity in different microenvironments (e.g., vehicle, residence, workplace, outdoor);
- climate (e.g., weather, season);
- characteristics of indoor microenvironments (e.g., window openings, draftiness, air conditioning);
- ambient concentrations of NO_X and CO from incomplete combustion (e.g., mobile sources, construction equipment) that are photolyzed to form ozone; and
- scavenging of ozone immediately near roads when NO reacts with ozone to produce NO_2.

Exposure assessment studies are evaluated from the reference point of personal exposure to ambient ozone, with epidemiologic studies of health effects from ambient ozone exposure generally employing concentration as a surrogate for ozone exposure. Because personal exposures are not routinely measured, the term *exposure surrogate* is used in this Appendix to describe a quantity meant to estimate or represent exposure to ambient ozone, such as ozone concentration measured at an ambient monitor (Sarnat et al., 2000). A *fixed-site monitor* (i.e., a monitor with a fixed position) is a type of *ambient air*

monitor used to estimate population average ambient concentrations and their trends over neighborhood and urban scales for epidemiologic studies.

When surrogates are used to estimate exposure in epidemiologic studies, exposure measurement error or exposure misclassification can result. *Exposure measurement error* refers to the bias and uncertainty associated with using concentration metrics to represent the true, but unknown, exposure of an individual or population (Lipfert and Wyzga, 1996). *Exposure misclassification* refers to exposure measurement error that occurs when exposure conditions such as location, timing, or population grouping are assigned incorrectly or with uncertainty. Exposure misclassification and exposure measurement error can result in bias and reduced precision of the *effect estimate* (i.e., the slope of the concentration-response function, in epidemiologic studies). *Bias* refers to the difference between the effect estimate derived from a statistical model and the true effect (Armstrong et al., 1992). Negative bias, or attenuation, of the effect estimate indicates an underestimate of the magnitude of the effect and tends to occur when the exposure surrogate and effect are not well correlated in time or space (temporal correlation is important for short-term studies, while spatial correlation is important for long-term studies) (Armstrong et al., 1992). Low spatial correlation with negative bias of the effect estimate in a long-term study indicates underestimation of the effect and may occur when the exposure measurement is systematically higher than the true population exposure. Positive bias of the effect estimate indicates an overestimate of the magnitude of the effect and may occur when the magnitude of the exposure measurement is systematically lower than the true population exposure (Armstrong et al., 1992). Such an overestimate may occur for an exposed group of people living near a road where O_3 scavenging by NO_X occurs far from a fixed-site monitor measuring a higher concentration (Simon et al., 2016; Cleveland and Graedel, 1979). Exposure measurement error can also lead to incorrect estimation of standard errors around the effect estimate.

Exposure measurement error has two components: (1) exposure measurement error derived from uncertainty in the metric being used to represent exposure and (2) error due to use of a surrogate parameter of interest in the epidemiologic study in lieu of the true exposure, which may be unobservable. *Classical exposure measurement error* is defined as exposure measurement error scattered around the true personal exposure and independent of the level of the measured exposure. Classical exposure measurement error may occur when a fixed-site monitor measuring ambient concentration is imprecise, even if it is accurate, and it is also independent of time and space (Szpiro et al., 2011). Classical exposure measurement error can result in bias of the epidemiologic effect estimate. When variation in the exposure measurements is greater than variation in the true exposures, classical exposure measurement error typically biases the effect estimate negatively (indicating no or lesser effect of the exposure relative to the true effect). This would cause the effect to be underestimated. Classical exposure measurement error can also cause inflation or reduction of the standard error of the effect estimate. *Bergson exposure measurement error* is defined as error scattered around the measured exposure surrogate (in most cases, the measured ambient concentration) and is independent of the true exposure (Goldman et al., 2011; Reeves et al., 1998). Berkson exposure measurement error may occur when the time series of ambient

ozone concentration measured at a monitor differs from the time series of a person's true exposure such that the true variability in the person's ozone exposure goes unmeasured. Berkson exposure measurement error is not expected to bias the effect estimate.

Definitions for *classical-like* and *Berkson-like exposure measurement errors* were developed for exposures estimated using models (Section 2.3.2). These errors can depend on how exposure metrics are averaged across space and time. Szpiro et al. (2011) defined classical-like and Berkson-like exposure measurement errors as errors sharing some characteristics with classical and Berkson exposure measurement errors, respectively, but with some differences. Specifically, classical-like exposure measurement errors can add variability to predicted exposures and can bias effect estimates in a manner similar to pure classical exposure measurement errors, but they differ from pure classical errors in that the variability is around the predicted exposures. Berkson-like exposure measurement errors occur when the modeled exposure does not capture all sources of variability in the true exposure. Berkson-like exposure measurement errors increase the variability around the effect estimate in a manner similar to pure Berkson exposure measurement error, but Berkson-like exposure measurement errors are not independent of predicted exposures, unlike pure Berkson exposure measurement errors. Berkson-like exposure measurement error can lead to bias of the effect estimate in either direction (Szpiro and Paciorek, 2013).

The influence of these types of exposure measurement errors on effect estimates for specific short- and long-term exposure study designs is evaluated in Section 2.6. This review of the influence of error on exposure estimates used in epidemiologic studies informs evaluation of confounding and other biases and uncertainties when considering the health effects evidence in Appendix 3−Appendix 7.

2.3 Exposure Assessment Methods

The 2013 Ozone ISA (U.S. EPA, 2013) reported on fixed-site monitors, passive and active personal samplers, and microenvironmental models. Since that time, many new modeling methods have become available to characterize ozone concentrations at neighborhood, urban, and regional scales for use as exposure surrogates. These methods are described below. Their application in epidemiologic studies of different averaging times is discussed in Section 2.6.1 for short-term exposure studies and in Section 2.6.2 for long-term exposure studies.

2.3.1 Monitoring

This section builds upon discussions from the 2013 Ozone ISA (U.S. EPA, 2013) about fixed-site, area, and personal ozone monitoring. Section 2.3.1.1 describes recent studies of fixed-site monitors, which largely agree with previous studies of fixed-site monitors reported in the 2013 Ozone ISA. Section 2.3.1.2 presents new studies of microenvironmental and personal ozone monitors, the results

of which mostly agree with studies presented in the 2013 Ozone ISA. A new development in using data-processing algorithms to improve the quality of ozone concentration data obtained using low-cost monitors is highlighted.

2.3.1.1 Fixed-Site Monitors

The 2013 Ozone ISA (U.S. EPA, 2013) described the use of two types of fixed-site Federal Reference Method (FRM) ozone monitors to provide a surrogate for ozone exposure: ultraviolet (UV) absorption photometric analyzers and chemiluminescence analyzers. Positive biases were noted for the UV analyzers when concentrations of volatile organic compounds (VOCs), mercury, and humidity were high, although the interference due to humidity was found to be small (Ollison et al., 2013). More than 95% of monitors (including both UV and chemiluminescence analyzers) in the ozone monitoring network, including the Photochemical Assessment Monitoring Stations (PAMS), met the U.S. EPA data quality goal of less than 7% bias and precision for 2005 through 2009. Fixed-site monitors were noted to provide reasonable approximations for ambient concentration when spatial variability of ozone was low.

Recent studies have noted advantages and limitations of using fixed-site monitors to provide an exposure surrogate (Table 2-1). Strengths include high quality assurance of the monitors, ease of assigning exposures to study participants, and availability of multiple years of data to ascertain trends. Several of the studies cited in Table 2-1 cite the lack of data on ozone spatial variability as an uncertainty. However, Dionisio et al. (2014) noted that spatial variability of ozone tends to be lower than that of NO_2, SO_2, or some PM size fractions, so fixed-site monitors may provide an acceptable representation of ozone concentration in many cases. Reported errors from using a fixed-site monitor to estimate long-term exposure tended to be within 6 ppb, ranging from <1 to 7% of reported ozone concentrations. On a short-term basis, considerable gradients in ozone concentrations can occur within urban areas, largely resulting from ozone scavenging by NO emitted by transportation during the daytime (Simon et al., 2016). Modeling analyses suggest that these gradients are sensitive to changes in NO concentrations and thus may not be constant over time (Simon et al., 2016).

2.3.1.2 Personal and Microenvironmental Monitors

The 2013 Ozone ISA (U.S. EPA, 2013) described methods useful for personal or area monitoring. Passive badges are typically used for personal exposure monitoring. Passive badges integrate ozone concentration over a period of 24 hours or longer by measuring reaction of a nitrite filter coating to nitrate. Badges have a method detection limit (MDL) of 5−10 ppb. Portable active monitors may be used for personal exposure or area monitoring. Variations include drawing air past a nitrite coated glass tube for an integrated ozone sample or using UV photometry for continuous ozone monitoring. Studies in the 2013 Ozone ISA reported the MDL for the nitrite coated glass tube to be 10 ppb-hour, while studies

reported in the 2013 Ozone ISA did not report on MDLs for portable UV photometers. High MDLs can lead to uncertainties in exposure when ozone concentrations are low. Other biases and uncertainties in passive badges and portable active monitors were not reported.

Table 2-8 presents recent papers testing continuous or passive personal or microenvironmental ozone monitors. Continuous microenvironmental ozone monitors have demonstrated low bias and correlation >0.8 with Federal Equivalent Method samplers. In the case of the low-cost continuous monitors, reduction of bias was dependent on the way data from the monitors were analyzed. Low-cost monitors tend to experience drift when they are deployed in the field. Use of a random forest method, in which concentrations measured at a specific location are predicted from a combination of temperature, relative humidity, and concentrations measured at other locations in the network, allowed Zimmerman et al. (2018) to correct for drift by analyzing trends at each monitoring site over time, thus decoupling monitor drift from the true ozone concentration signal. Wheeler et al. (2011) measured ozone with a mean concentration of 26 ppb using passive badges with bias and precision well below 1 ppb. With such low bias and precision, uncertainty is reduced because these concentrations are well above the MDL reported in the 2013 Ozone ISA (U.S. EPA, 2013).

2.3.2 Modeling

The 2013 Ozone ISA (U.S. EPA, 2013) reviewed three types of models: models that estimate ambient ozone concentration, microenvironmental models, and air exchange models. Concentration modeling estimates ambient ozone concentrations at locations where monitoring data are not available. Different modeling approaches are described in Section 2.3.2.1 through Section 2.3.2.4. Microenvironmental models use population demographic, time-activity pattern, building characteristic, and air quality data as inputs for stochastic exposure simulations. Air exchange models estimate air exchange rates for buildings based on building characteristics and meteorological variables. Air exchange models describe airflow and are not specific to ozone. These are used as inputs to microenvironmental models and so are not discussed in this ISA.

2.3.2.1 Spatial Interpolation

Spatial interpolation approaches discussed in the 2013 Ozone ISA (U.S. EPA, 2013) include inverse-distance weighting (IDW) models and kriging. These methods use a mathematical function to estimate concentrations of ambient ozone in between outdoor locations where ozone is monitored. They are not resource intensive and can represent concentration with high resolution. However, their limitations include the potential to skew the concentration surface by concentrations measured at one monitor reporting data that are much lower or higher than the majority of monitors, and inaccuracies in capturing true spatial heterogeneity of ozone concentration.

Recent spatial interpolation studies examine the strengths and limitations of three approaches: data averaging, IDW, and kriging (Table 2-9), listed in order of increasing model complexity. Joseph et al. (2013) compared all three interpolation approaches and found that exposure measurement errors were lower for kriging than for IDW and data averaging. An identified strength of the data averaging and IDW approaches is their simplicity. Because ozone typically has lower spatial variability compared with NO_X and SO_X, greater model complexity might not be needed. However, these methods could lead to incorrect model fitting if averaging occurs over an area where variation would be anticipated, such as next to a road (Simon et al., 2016). A model evaluation study conducted for daily data across the city of Montreal, Canada, Buteau et al. (2017) compared IDW with noninterpolation approaches, including land use regression (LUR) and Bayesian Maximum Entropy (BME)-LUR, and found that IDW had the highest interclass correlation coefficient (ICC = 0.89) compared with fixed-site monitors. This finding suggests that IDW can produce well-validated simulations in an urban area, which is consistent with findings of low spatial variability of ozone concentrations across cities.

Kriging applies a distance-based covariance function to estimate the ambient concentration field, which allows for calculation of uncertainties so that the model provides more information about the range of ambient concentrations at a given location (Kethireddy et al., 2014). Like data averaging and IDW, kriging is sensitive to monitor location. Misspecification of the covariance function may not lead to substantial error if the spatial domain has low variability, as is often the case for ozone (U.S. EPA, 2013). All three methods also have the potential for preferential sampling that may lead to overestimation or underestimation of concentrations in some cases (Gelfand et al., 2012). Specifically, Gelfand et al. (2012) developed kriged O_3 concentration surfaces when fitting only to monitors capturing high concentrations (akin to urban areas), only to monitors capturing low concentrations (akin to rural areas), and models that incorporate sites of both type randomly. They found lower out-of-sample prediction error for the random site assignment compared with either the urban-focused or rural-focused preferential sampling model, although the magnitude of the error was not substantially different (high concentration preferential sampling: 22.7 ppb, low concentration preferential sampling: 23.9 ppb, random sampling: 18.0 ppb).

2.3.2.2 Land Use Regression and Spatiotemporal Modeling

The 2013 Ozone ISA (U.S. EPA, 2013) included some discussion of LUR models. LUR models regress observed ozone concentrations on land use (and sometimes additional geographic) covariates and then use the model to predict ambient concentrations where ozone is not measured. The 2013 Ozone ISA cites high concentration resolution as a strength of the LUR approach. However, for ozone, a dearth of monitors near roads prevents accurate characterization of ambient concentration in these locations where scavenging by NO can increase the gradient of ozone concentration. A limited number of studies employed LUR for ozone exposure assessment at the time of the 2013 Ozone ISA. Specifically, validation of LUR model results for annual average ozone in urban areas was low (R^2 = 0.06), but the

model performed better in rural areas (R^2 = 0.62). The 2013 Ozone ISA did not review spatiotemporal modeling.

Recent LUR studies have compared model results to fixed-site monitoring data for ozone concentration and/or alternative models. Comparison with fixed-site monitoring data produced low to moderate R^2 values. In a study of 10 urban areas across the U.S., Clark et al. (2011) reported R^2 = 0.34 for a model of 8-hour daytime ozone (10:00 a.m.−6:00 p.m.). A study of the entire province of Quebec produced an R^2 of 0.47 in a model of avg 8-hour daily ozone (9:00 a.m.−5:00 p.m.) (Adam-Poupart et al., 2014), and the authors discussed larger observed differences between measured and predicted ozone concentrations in Montreal than in the remainder of the province. In contrast to the other urban studies reported in the 2013 Ozone ISA (U.S. EPA, 2013) or more recently, Buteau et al. (2017) found better agreement between LUR estimates and concentrations from fixed-site monitors (interclass correlation coefficient, ICC = 0.67) in a model of avg 8-hour daily ozone (9:00 a.m.−5:00 p.m.) for Montreal.

Like LUR, spatiotemporal models may use land use and geographic covariates to model ozone concentration, but these models may also include a more flexible statistical model formulation and additional modeling inputs, such as kriging or autocorrelation (Wang et al., 2015). Spatiotemporal models were applied in several recent studies to improve predictions of ozone concentrations for exposure assessment studies (Table 2-10). Several studies used BME approaches, although the prior information varied across studies and included kriging, LUR, and an autoregressive function. BME applies known information as a prior geostatistical distribution, maximizes an entropy function of the prior distribution, and then applies a Bayes function to estimate a posterior distribution (i.e., the predicted concentration field) (He and Kolovos, 2018; Christakos, 1990). An important advantage of BME is that it incorporates multiple sources of data into the model, allowing for minimization of errors (Adam-Poupart et al., 2014; Warren et al., 2012). BME can also provide a good representation of variability, with both spatial and temporal variability well represented when autoregressive priors are used (Sahu and Bakar, 2012a, b). However, spatially clustered monitors in the study domain tend to produce more accurate model results. Partial least squares (PLS) have also been used as a framework for spatiotemporal modeling. When a large number of geographic covariates are included in a model, PLS constructs linear combinations of variables, similar to principal component analysis, which are called "scores." The scores are designed to maximize the spatial covariance structure of the concentration field while avoiding model overfitting from inclusion of correlated covariates. PLS was thought to be appropriate for ozone because spatial variability of ozone is low in most locations (except near roads) (Wang et al., 2016; Xu et al., 2016a; Wang et al., 2015). Like BME models, PLS approaches require sufficient input data to produce accurate models.

2.3.2.3 Chemical Transport Modeling

In the 2013 Ozone ISA (U.S. EPA, 2013), chemical transport models (CTMs) were briefly discussed in regard to exposure assessment. CTMs use first principles to characterize the processes that influence ozone formation (EPA, 2018). CTMs require emissions and meteorological data as inputs. The chemistry is specified in the model, and concentrations of air pollutants (e.g., ozone) are output to a discretized grid. However, CTMs are limited by their grid cell resolution, may be resource intensive to run, and contain no time-activity information for possible exposure assignment. The Community Multiscale Air Quality (CMAQ) model (EPA, 2018) may better capture spatial heterogeneity, especially in rural areas, compared to interpolation methods (Bell, 2006). However, a coarse grid size may result in difficulty differentiating ozone concentrations near roadways due to scavenging by NO (Marshall et al., 2008).

The number of studies using CTMs has greatly expanded since the 2013 Ozone ISA (U.S. EPA, 2013) (Table 2-11). A few studies directly compared different CTMs. Bond et al. (2013) compared CMAQ with the Comprehensive Air quality Model with extensions (CAMx) in January and July of 2002 in the southeastern U.S. Results were presented by monitoring network, month, and averaging time (i.e., 1-hour daily max ozone and 8-hour daily max ozone). The configurations between CMAQ and CAMx for this work varied in several ways, including horizontal and vertical advection mechanism and deposition. Both models mostly overpredicted ozone concentrations in January, most likely because neither model accounted for vertical mixing. In July, overprediction occurred for CAMx while underprediction occurred for CMAQ when compared to monitor observations, as noted in Appendix 1 (Section 1.6.2). Underprediction was mostly due to underestimation of emissions precursors and peak temperatures. Wang et al. (2016) modeled surface ozone in Los Angeles, CA over the years 2000−2008 and found that ozone had a greater root mean squared error (RMSE) for the University of California at Davis-California Institute of Technology (UCD-CIT) CTM in rural areas, especially during the warm season. Wang et al. (2016) did not quantify the direction of error. Herwehe et al. (2011) compared CMAQ with Weather Research and Forecasting coupled with Chemistry (WRF/Chem) across the continental U.S. (CONUS) in August 2006. While CMAQ generally performed better than WRF/Chem, both CTMs overpredicted the 8-hour daily max ozone concentration (e.g., mean bias was 3.62 ppb for CMAQ). WRF/Chem likely predicted higher ozone concentrations due to vertical mixing in the boundary layer, dry deposition, and convection schemes. Many studies covered approximately all of the CONUS (Appel et al., 2017; Appel et al., 2013; Appel et al., 2012) or covered localized, urban areas of either the U.S. or Canada (e.g., Houston, TX, Seattle, WA, San Joaquin Valley, CA, Los Angeles, CA, Vancouver, Canada) (Wang et al., 2016; Steyn et al., 2013; Hu et al., 2012; Tsimpidi et al., 2012; Ying and Li, 2011).

The horizontal grid resolution of a CTM can influence the heterogeneity of an ambient concentration surface and the associated exposure estimate, but those effects are generally only seen for areas of peak concentrations. Some studies directly compared how ozone model performance statistics changed with the size of a given grid cell (Yu et al., 2016; Schaap et al., 2015; Thompson and Selin,

2012; Tsimpidi et al., 2012). The majority of these studies found that the cumulative distribution function and summary statistics did not vary greatly among the simulations using different spatial resolutions. However, the upper and lower tails of the distributions had greater error for coarse resolution simulations compared with fine resolution simulations in (Yu et al., 2016). Schaap et al. (2015) compared the Multiscale Chemistry-Transport Model for Atmospheric Composition Analysis and Forecast (CHIMERE), CMAQ, European Monitoring and Evaluation Program (EMEP), Research and Development Center for Global Change (RCGC), and Long Term Ozone Simulation-European Operational Smog (LOTOS-EUROS) models across grid resolutions for rural, suburban, and urban areas. They found that the largest magnitude biases between the model and observations were for the urban simulations, and the models more often overestimated rather than underestimated measurements for all settings. These findings are consistent with older studies not cited in the 2013 Ozone ISA (U.S. EPA, 2013). Cohan et al. (2006) compared CMAQ simulations for 4-, 12-, and 36-km resolutions and found that the 4-km resolution simulation was more sensitive to fluctuations in precursor emissions but observed little difference among the simulations in average ozone concentrations. Similarly, Henderson et al. (2010) found that 1-km grid resolution allowed detection of much larger ozone concentration peaks, but otherwise observed little difference between the 1- and 4-km simulations.

Many short-term CTM studies relevant to short-term exposure assessment either characterized a specific high ozone event (e.g., wildfire), tested a new mechanism of a model (e.g., planetary boundary layer schemes, a new version of the master chemical mechanisms exploring two-way coupling), or both. For example, Baker et al. (2016) explored how the Wallow wildfire and Flint Hills prescribed fire in 2011 influenced ozone concentration in those localized areas. In the case of a wildfire, bias increased by approximately 2 ppb for every 1 ppb increase in estimated ozone contribution from the fire. For prescribed burns, bias increased by approximately 1 ppb for every 1 ppb increase in estimated ozone contribution from the fire. Wong et al. (2012) developed a two-way coupled system for CMAQ in which the WRF and CMAQ components could consistently be executed in and around California for a week in June, 2008 during a wildfire event. For all data, comparison of the model with measurements showed little bias (slope = 0.98) with observable scatter (R = 0.62). When data were limited to AOD >0.5, the model had positive bias (slope = 1.2) but with less scatter (R = 0.75). Given that bias was related to ozone concentration in these studies, the results suggest that bias is greater and emissions more uncertain for wildfires than for prescribed burns.

Inaccurate characterization of cloudiness has been shown to lead to biased or uncertain ozone concentrations due to the influence of photolysis on ozone formation, potentially leading to biased or uncertain exposure estimates. In Ngan et al. (2012), the modeling scheme misrepresented cloud locations, which also affected the modeling of PM due to the deposition and removal processes that occur by precipitation, leading to an overestimation of ozone. Pan et al. (2015) overestimated ozone during part of the modeling time period due to uncertainties in the cloud fraction along with other meteorological variables. Yahya et al. (2016) found that for a 10-year avg of certain cloud variables, ozone concentrations were generally underpredicted for most regions of the U.S.

CTMs have been shown to underestimate high concentrations and overestimate low concentrations, which could impact estimates of peak exposure conditions. In Tsimpidi et al. (2012) the normalized mean bias was slightly negative at a 4-km grid resolution in CMAQ when concentrations less than 40 ppb were excluded from the bias calculations (−7.9%) for the Pacific northwest in July, 2006. However, when all concentrations were included, the statistic became large and positive in magnitude (42.7%). Tsimpidi et al. (2012) attributed overprediction of nighttime ozone concentrations to inaccurate models of vertical diffusivity in CMAQ. Similarly for northern California in July, 2009, Bash et al. (2016) observed overprediction of ozone concentration in CMAQ by a median bias of 29−32% (depending on the biogenic VOC model) when ozone concentrations were less than 60 ppb, while median bias was −8 to −9% when ozone concentration was greater than 60 ppb. Garner et al. (2015) observed a positive mean bias in the early morning hours that decreased through the for Baltimore, MD in July, 2011.

2.3.2.4 Hybrid Approaches

Hybrid models were not reviewed in the 2013 Ozone ISA (U.S. EPA, 2013). Like spatiotemporal models, hybrid models use information from multiple data sources to develop ambient concentration estimates. Several hybrid models combine observed data from fixed-site regulatory monitors with CTMs that are defined over a spatiotemporal grid (Table 2-12). These separate data sources are combined in such a manner that the resulting exposure prediction is a "hybrid" of the input data sources. These approaches are frequently referred to as "data fusion" methods. A CTM predicts ambient ozone concentration at the centroid of the grid. In the "downscaler" method, Berrocal et al. (2012) adjusted CTM data at any point in the domain based on a weighted average of the CTM predictions for surrounding grids such that exposure estimates were predicted at spatial scales finer than the input CTM. This hybrid model had an improved performance (mean squared error, MSE: 45.4 ppb^2, mean absolute error, MAE: 5.0 ppb) compared with either the observed data (MSE: 124 ppb^2, MAE: 8.7 ppb) or the CTM data alone (MSE: 136 ppb^2, MAE: 9.1 ppb) when predicting ozone over the eastern CONUS in summer, 2001 with CMAQ data. Its predictive power was more pronounced in areas far from monitoring locations.

Other studies found similarly improved performance with use of the BME. Xu et al. (2016b) used a BME approach to merge ambient ozone concentration data from the Air Quality System (AQS) database with CAMx simulations modeled at a 36- × 36-km scale and incorporated a regional correction factor to allow for flexible selection of spatial points included in the model. The regionalized model decreased RMSE from 6.7 to 5.5 ppb and increased R^2 from 0.88 to 0.89 for 8-hour daily max ambient ozone concentration. Xu et al. (2017) estimated validation error as the RMSE between the predicted and observed data and found that RMSE was larger when using hourly ambient ozone concentrations as model input compared with 8-hour daily max and 24-hour avg input concentrations to make 8-hour daily max and 24-hour avg predictions, respectively. RMSE was also slightly larger when a 36- × 36-km grid

was used in the CAMx model compared with a 12- × 12-km grid. For the U.S. and Canada, Robichaud and Menard (2014) combined predictions from the CTM Canadian Hemispheric and Regional Ozone and NO_X System (CHRONOS, 2005) and Global Environmental Multi-scale coupled with Model of Air quality and Chemistry (GEM-MACH, 2012) with surface observations from the AQS and Canadian databases through an optimal interpolation scheme in which the model and monitor data were linked through a Kalman filter optimization matrix. This approach is known as Objective Analysis, and it was shown to produce near-zero systematic errors and smaller random errors (of positive magnitude) compared with CTM alone. Reich et al. (2014) employed a more flexible downscaler using spectral methods. When compared with a linear downscaler, the spectral downscaler had a smaller bias and mean squared error in the ambient ozone concentration estimate. Other hybrid methods are more straightforward and use weighting factors to combine data sources (Friberg et al., 2016). This weighting approach reduced error in ambient ozone concentration and increased the spatial correlation compared with using CMAQ alone.

Hybrid models need not be restricted to only CTM and observed data. Di et al. (2017) calibrated satellite-based MODIS ozone column data against GEOS-Chem CTM output. They predicted ground-level 8-hour daily max ambient ozone concentrations as a function of the calibrated satellite data along with surface ambient ozone concentrations in the AQS database and land use variables in a neural network model that can accommodate nonlinearity of the variables. For the years 2000−2012, 10-fold cross-validation bias was reported to be 20% with R^2 of 0.76. Tang et al. (2015b) adjusted CMAQ output with both observed data and MODIS AOD observations from Terra and Aqua satellites. Incorporating observed and satellite data improved the correlation between surface observed data when compared to CMAQ data alone in the southeastern CONUS, with mixed results for mean bias in the prediction of ambient ozone concentration. A recent study of spatial and temporal biases in satellite data informs our understanding of the limitations of hybrid models using satellite data as inputs (Verstraeten et al., 2013). Global column data obtained using Tropospheric Emissions Spectrometer (TES) version 4 were compared with ozonesonde balloon measurements obtained over 2005−2010. Negative biases in ozonesonde concentration measurements up to 8 ppb were noted for June, July, and August in the midlatitudes (coincident with the U.S.). Larger biases were observed for the midlatitudes compared with the subtropics or tropics. These data were obtained at a single time during the early afternoon as the ozonesonde passed each location, so nighttime ambient ozone concentrations were not accounted for.

2.3.2.5 Microenvironmental Modeling

The 2013 Ozone ISA (U.S. EPA, 2013) presented several studies that evaluated integrated microenvironmental exposure (ME) and dose models. ME models apply stochastic sampling of distributions of data for air quality, time-activity patterns, demographic, physiological, and building ventilation variables to predict population exposures in different locations. ME models predict microenvironmental concentrations, exposures, and doses. Advantages identified in the 2013 Ozone ISA

include ability of the user to design analyses for specific populations (assuming that demographic and time-activity data are available) and to include of indoor air sources (which are uncommon for ozone). Limitations include resource intensiveness of the ME models and that indoor exposures cannot be easily validated (Georgopoulos et al., 2005).

Strengths and limitations identified in recent studies (Table 2-13) agree with ME model studies presented in the 2013 Ozone ISA (U.S. EPA, 2013). Dionisio et al. (2014) found that exposure estimates were 72% higher when using the ME model with an incorporated CTM compared with using fixed-site measurements alone as exposure surrogates. The majority of that difference came from inclusion of time-activity data in the ME model. Reich et al. (2012) found that ME models produced lower estimates of exposure than did fixed-site monitors. However, the ME models were not validated by personal monitors, so the extent of this error was unknown.

2.4 Personal Exposure

This section builds upon discussions from the 2013 Ozone ISA (U.S. EPA, 2013) about relationships between indoor and outdoor ambient ozone concentrations and between personal exposure to ambient ozone and ambient ozone concentration. Section 2.4.1 describes recent advances in characterizing time-activity data for exposed people, given advances in global positioning system (GPS) technologies and the continued updating of the Consolidated Human Activity Database (CHAD). Summaries of relevant discussions from the 2013 Ozone ISA are included in Section 2.4.2 and Section 2.4.3, and findings in more recent studies are largely consistent with the findings reported in the 2013 Ozone ISA.

2.4.1 Time-Activity Data

The 2013 Ozone ISA (U.S. EPA, 2013) provided only limited discussion of time-activity patterns. Ozone-averting behavior, or the tendency to stay indoors as much as possible to avoid exposure on days with high ambient ozone concentrations, as reported by the news media, was described as one factor that could change time-activity patterns. Recent technological advances in GPS technologies and expansions to existing time-activity databases have expanded the information base regarding time-activity. Such new tools have enabled an examination of factors that influence time-activity patterns and errors in those relationships.

Data through 2010 are available from the CHAD database to compare time-activity data among different population strata for 25,431 individuals who reported 54,373 days of data (Isaacs, 2014). Percentage and number of person-minutes in different locations based on all individuals with diaries in this version of CHAD during the warm months (April−September) for all day (12:00 a.m.−12:59 p.m.)

and for the afternoon and early evening (12:00 p.m.−8:00 p.m.), assumed to be the period when ozone concentration is at a maximum each day, are presented in Table 2-1 through Table 2-3. Across this population of individuals in CHAD, substantially more time was spent indoors at home for children younger than 6 years and for adults older than 64 years, while teens ages 12−19 years and adults 20−64 years spent the least amount of time indoors at home. Similarly, young children spent the least amount of time in transit, while adults 20−64 years spent the most time in transit. Teens ages 12−19 years spent the largest proportion of the day outdoors, while older adults spent the least amount of time outdoors. Time spent outdoors by young children ages 0−5 years was similar to that for older adults. A separate analysis of CHAD data gauged the percentage of study participants who engaged in outdoor activities (participation rate was defined as the percentage of person-days in spending at least 1 minute outdoors) and the number of minutes spent outdoors per day during the afternoon and early evening (12:00 p.m.−8:00 p.m.), assumed to be the period when ozone concentration is at a maximum each day (Isaacs, 2014). Children and teens ages 4−18 years had the largest participation rate among those spending more than 2 hours outdoors and the largest mean time outdoors per person spending at least 1 minute outdoors, while younger adults (ages 19−35 years) had the highest participation rate among those spending more than 1 minute outdoors, and adults ages 35−50 years had the largest mean outdoors per person among those spending more than 2 hours outdoors. Moreover, Isaacs (2014) calculated that 79% of time spent by children ages 4−18 years and 63% of time spent by adults ages 19−95 years involved at least moderate exertion. When comparing time-activity data by race from the CHAD database (Table 2-2), Hispanic study participants spent slightly more time indoors at home than the total population, while white study participants spent the most time outdoors compared with Asian, black, and Hispanic participants (Isaacs, 2014). However, 11% of participants had missing race/ethnicity data or refused to provide information regarding race/ethnicity, so these results should be interpreted cautiously. Males spent more time outdoors than females (Table 2-3). These studies collectively suggest that older children, males, and those of white race may spend the most time outdoors during warm weather, where they could be exposed to elevated ozone concentrations.

The CHAD database is useful because it provides a detailed picture of time-activity across population groups, and it has a large number of days of data. Several caveats should be noted (Graham and Mccurdy, 2004). CHAD combines data from several different studies conducted over several years. These studies collected data under different circumstances, and in some cases variables could not be combined. Validation techniques for the data may have differed across studies input to CHAD, and it is possible that participants were not precise in providing time increments or that missingness of data could have been handled differently across studies. Moreover, when breaking down the data by age, race, and sex, some studies may have contributed a disproportionate amount of data to that group, because the objective of the individual study may have been to characterize time-activity patterns for a segment of the population rather than for the population as a whole.

Recent studies have focused on the use of GPS technologies, such as in smartphones, to develop detailed time-activity pattern data. This technology has the potential to allow a time-activity study to

overcome limitations of time-activity diaries, such as imprecise estimation of time-location data. For example, Glasgow et al. (2014) analyzed the frequency of Android-based smartphones in recording positional data among a panel of study participants and found that on average 74% of the data was collected over intervals shorter than 5 minutes, which is a marked improvement over many time-activity studies using diaries. Positional errors are also a concern for GIS and GPS-based technologies. Several studies found that median positional errors based on smartphones were less than 26 m (Ganguly et al., 2015; Lane et al., 2013; Wu et al., 2010). Glasgow et al. (2014) observed much larger errors, with an overall median positional accuracy of 342 m and a range from 98 to 1,169 m using an Android-based smartphone, while Wu et al. (2010) observed much smaller errors when comparing two smartphones with three other GPS technologies. The magnitude of positional errors may be important, because positional error has the potential to lead to misclassification of time-activity patterns.

Survey tools to assess time-activity patterns may be subject to recall error among the subjects. Spalt et al. (2015) administered a retrospective survey to all participants in the Multi-Ethnic Study of Atherosclerosis (MESA) Air Study to ascertain time spent indoors and outdoors at home, at work/volunteer/school, in transit, or in other locations. A subset of the study population was asked to complete a detailed time-activity diary in addition to the survey. Correlation between the MESA Air surveys and the time-activity diaries for indoor locations was Spearman $R = 0.63$ for home, Spearman $R = 0.73$ for work/volunteer/school, and Spearman $R = 0.20$ for other indoor locations. Correlation between the MESA Air surveys and the time-activity diaries for outdoor locations was much lower, with Spearman $R = 0.14$ at home, Spearman $R = 0.20$ for work/volunteer/school, and Spearman $R = 0.10$ for other outdoor locations. Correlation between MESA Air surveys and time-activity diaries for individuals in transit was Spearman $R = 0.39$. These results suggest that study participants have better recall of the times spent inside their home or work/volunteer/school compared to other activities, because time spent at home or at work/volunteer/school tends to occur at routine times.

Residential mobility is another source of exposure measurement error in long-term exposure studies. Using a single address to represent exposure concentration over a period of several years may result in either under- or overestimating exposure during the study period. For example, Brokamp et al. (2015) analyzed residential mobility for a cohort of children over the first 7 years of life in Cincinnati, OH and found that 54% of the children changed residential address during that time, resulting in a 4.4% decrease in the cohort's average traffic-related air pollution concentration (defined as black carbon estimates from an LUR model for this study). They also noted that if the birth address is used for exposure estimation during the entire study period, exposure misclassification is increased for those that move earlier (due to more years at the incorrect address) or are more highly exposed (due to a greater likelihood of moving). The Brokamp et al. (2015) study showed that not accounting for residential mobility resulted in bias toward the null. Exposure measurement error due to incorrect home address would be expected to be lower for ozone compared with more spatially variable air pollutants, but it would not necessarily be zero.

Updated time-activity data and tools for assessing time-activity data have improved the general understanding of time-activity data and related uncertainties in recent years. Analysis of CHAD diaries indicated that young children ages 0−5 years were found to spend less time outdoors than older children, teens, and adults, and white respondents spent more time outdoors than their Asian, black, and Hispanic counterparts (Isaacs, 2014). New technologies to assess study participant location, errors related to study participant recall, and residential mobility have been used to determine that location-based errors are within 6% for short- and long-term exposure assessment, while omission of residential mobility can produce bias in the exposure estimate, resulting in negatively biasing the effect estimate for a study of long-term ozone exposure.

Table 2-1 Total and age-stratified percentage of hours spent in different locations from the Consolidated Human Activity Database (Isaacs, 2014), warm season for all hours and for afternoon hours (12:00 p.m.−8:00 p.m.).

Location Type	All	0−5 yr	6−11 yr	12−19 yr	20−64 yr	65+ yr
N (number of individuals)	12,673	2,253	2,010	1,080	5,785	1,403
(%)	(100)	(18)	(16)	(8.5)	(46)	(11)
Warm Season, 12:00 a.m.−11:59 p.m. (person-minutes [%])						
Indoor-residential	31,038,736 (75)	5,932,419 (81)	4,474,880 (74)	1,846,578 (71)	13,593,134 (71)	5,012,405 (83)
Transit	2,359,073 (5.7)	284,770 (3.9)	242,706 (4.0)	134,854 (5.2)	1,352,166 (7.1)	329,628 (5.4)
Indoor-work/school/other	5,956,425 (14)	725,350 (9.9)	906,573 (15)	429,633 (17)	3,342,988 (18)	504,961 (8.3)
Outdoor	1,562,018 (3.8)	214,340 (2.9)	255,882 (4.2)	130,973 (5.1)	793,278 (4.1)	162,906 (2.7)
Uncertain or missing	521,188 (1.3)	163,109 (2.2)	166,890 (2.8)	45,274 (1.7)	74,463 (0.39)	48,180 (0.80)
Warm Season, 12:00 p.m.−8:00 p.m. (person-minutes [%])						
Indoor-residential	9,234,040 (61)	1,867,690 (70)	1,327,384 (60)	529,438 (56)	3,853,625 (55)	1,601,243 (73)
Transit	1,407,828 (9.3)	184,339 (6.9)	157,601 (7.1)	81,709 (8.6)	781,252 (11)	194,099 (8.8)
Indoor-work/school/other	3,240,916 (21)	412,785 (15)	470,148 (21)	225,926 (24)	1,819,323 (26)	288,478 (13)

Table 2-1 (Continued): Total and age-stratified percentage of hours spent in different locations from the Consolidated Human Activity Database (Isaacs, 2014), warm season for all hours and for afternoon hours (12:00 p.m.−8:00 p.m.).

Location Type	All	0−5 yr	6−11 yr	12−19 yr	20−64 yr	65+ yr
Outdoor	1,015,749 (6.7)	154,838 (5.8)	198,851 (9.0)	92,928 (10)	476,679 (6.8)	89,982 (4.1)
Uncertain or missing	190,037 (1.3)	53,793 (2.0)	56,847 (2.6)	14,958 (1.6)	37,363 (0.50)	26,851 (1.2)

Note: Data presented in this table for person-minutes are calculated as the sum of minutes across individuals and percentage is calculated as the percentage of total person-minutes for a given category. Data are filtered by the criteria noted in the column headings. Data were downloaded from: https://www.epa.gov/healthresearch/consolidated-human-activity-database-chad-use-human-exposure-and-health-studies-and.

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Table 2-2 Total and race/ethnicity-stratified percentage of hours spent in different locations from the Consolidated Human Activity Database (Isaacs, 2014), warm season for all hours and for afternoon hours (12:00 p.m.−8:00 p.m.).

Location Type	All	Asian	Black	Hispanic	White	Other
N (number of individuals) (%)	12,673 (100)	248 (2.0)	1,829 (14)	729 (5.8)	8,083 (64)	310 (2.4)
Warm Season, 12:00 a.m.−11:59 p.m. (person-minutes [%])						
Indoor-residential	31,038,736 (75)	693,831 (75)	4,026,861 (75)	2,278,661 (78)	20,590,280 (75)	968,084 (77)
Transit	2,359,073 (5.7)	45,730 (4.9)	309,221 (5.8)	153,744 (5.3)	1,576,269 (5.7)	69,455 (5.5)
Indoor-work/school/other	5,956,425 (14)	153,540 (17)	768,617 (14)	376,251 (13)	3,957,782 (14)	170,660 (14)

Table 2-2 (Continued): Total and race/ethnicity stratified percentage of hours spent in different locations from the Consolidated Human Activity Database (Isaacs, 2014) warm season for all hours and for afternoon hours (12:00 p.m.−8:00 p.m.).

Location Type	All	Asian	Black	Hispanic	White	Other
Outdoor	1,562,018 (3.8)	21,449 (2.3)	160,968 (3.0)	95,143 (3.2)	1,134,048 (4.1)	38,127 (3.0)
Uncertain or missing	521,188 (1.3)	12,810 (1.4)	69,533 (1.3)	23,721 (0.81)	357,941 (1.3)	13,674 (1.1)
Warm Season, 12:00 p.m.−8:00 p.m. (person-minutes [%])						
Indoor-residential	9,234,040 (61)	201,679 (59)	1,166,578 (61)	693,833 (65)	6,157,342 (61)	297,935 (65)
Transit	1,407,828 (9.3)	28,237 (8.3)	188,304 (10)	93,949 (8.8)	935,729 (9.3)	41,811 (9.1)
Indoor-work/school/other	3,240,916 (21)	90,168 (27)	413,189 (22)	208,599 (20)	2,151,117 (21)	90,752 (20)
Outdoor	1,015,749 (6.7)	15,146 (4.5)	113,455 (5.9)	62,517 (5.9)	727,574 (7.2)	25,541 (5.5)
Uncertain or missing	190,037 (1.3)	4,427 (1.3)	38,950 (2.0)	9,393 (0.9)	114,541 (1.1)	5,295 (1.1)

Note: Data presented in this table for person-minutes are calculated as the sum of minutes across individuals and percentage is calculated as the percentage of total person-minutes for a given category. Data are filtered by the criteria noted in the column headings. Data were downloaded from: https://www.epa.gov/healthresearch/consolidated-human-activity-database-chad-use-human-exposure-and-health-studies-and.

Table 2-3 Total and sex-stratified percentage of hours spent in different locations from the Consolidated Human Activity Database (Isaacs, 2014), warm season for all hours and for afternoon hours (12:00 p.m.−8:00 p.m.).

Location Type	All	Female	Male
N (number of individuals) (%)	12,673 (100)	6,821 (54)	5,849 (46)
Warm Season, 12:00 a.m.−11:59 p.m. (person-minutes [%])			
Indoor-residential	31,038,736 (75)	16,945,109 (77)	14,086,470 (73)
Transit	2,359,073 (5.7)	1,235,125 (5.6)	1,123,433 (5.8)
Indoor-work/school/other	5,956,425 (14)	2,996,029 (14)	2,959,731 (15)
Outdoor	1,562,018 (3.8)	657,845 (3.0)	903,889 (4.7)
Uncertain or missing	521,188 (1.3)	236,982 (1.1)	261,327 (1.4)
Warm Season, 12:00 p.m.−8:00 p.m. (person-minutes [%])			
Indoor-residential	9,234,040 (61)	5,069,614 (63)	4,162,147 (59)
Transit	1,407,828 (9.3)	752,611 (9.4)	654,873 (9.3)
Indoor-work/school/other	3,240,916 (21)	1,670,920 (21)	1,569,613 (22)
Outdoor	1,015,749 (6.7)	441,741 (5.5)	573,859 (8.1)
Uncertain or missing	190,037 (1.3)	96,730 (1.2)	93,307 (1.3)

2.4.2 Infiltration

The 2013 Ozone ISA (U.S. EPA, 2013) reviewed literature on indoor-outdoor (I/O) ratios to describe infiltration of ambient ozone into homes and buildings. Ozone generation indoors is uncommon, as described in the 2013 Ozone ISA. Assuming an absence of devices that generate ozone, such as household air purifiers, I/O ratios generally ranged from 0.1−0.4. Higher ratios were observed during the warm season when ambient ozone concentrations are highest.

Table 2-4 summarizes I/O ratios from ozone infiltration studies across the U.S. Several of the studies report I/O ratios below 0.2 when the windows are closed and AER is 0.5/hour or lower. Across studies, I/O tended to increase with higher values of AER from open windows or mechanical ventilation. Studies where air exchange was reported to have been higher, primarily in commercial areas or offices (Ben-David and Waring, 2018; Chan et al., 2014) or where windows were open (Dutton et al., 2013), tended to report higher I/O ratios compared with studies of lower AER, primarily in homes (Singer et al., 2016; Sarnat et al., 2013; Chen et al., 2012). Johnson et al. (2014) also examined ozone infiltration in vehicles, and the mean and range of I/O ratios were between the values for I/O ratio obtained for open versus closed windows or doors.

Sarnat et al. (2013) explored how AER can modify the effect of ozone related to asthma emergency department (ED) visits in Atlanta neighborhoods. Parsing their data by low (<0.227/hour threshold), medium (0.228−0.308/hour) and high AER (>0.309/hour threshold) did not appreciably influence the risk of asthma ED visits, but the ozone level (low: <32 ppb, moderate: 33−53 ppb, high: >54 ppb) was related to an increase in risk ratio from approximately 1 for low ozone to 1.02 for moderate ozone to 1.08 for high ozone. In contrast, the risk ratios of asthma ED visits and $PM_{2.5}$ and NO_X exposures showed some sensitivity to AER. High levels of poverty (8.5% threshold) were associated with high AER. They attributed this observation to old, drafty housing being more prevalent among those in poverty.

Table 2-4 Summary of U.S. studies of ozone infiltration published after 2011.

Reference	Location	Time Period	Population	Microenvironment	Ambient Ozone Concentration	I/O Ratio	Correlation	AER
Sarnat et al. (2013)	Atlanta, GA	January, 1999–December, 2002	Residents above and below poverty	Home	Mean (SD): 41.9 ppb (18.6 ppb)	NR	AER: −0.19	Mean (SD): 0.265/h (0.108/h)
Chen et al. (2012)	NMMAPS cities:	1987–2000	All residents	Home (estimated by model)	NR	Calculated change in indoor ozone per unit change in ambient ozone[a]	NR	Mean
	Atlanta, GA				NR	0.14	NR	0.43/h
	Birmingham, AL				NR	0.14	NR	0.43/h
	Boston, MA				NR	0.20	NR	0.68/h
	Buffalo, NY				NR	0.20	NR	0.70/h
	Chicago, IL				NR	0.18	NR	0.61/h
	Cincinnati, OH				NR	0.16	NR	0.52/h
	Corpus Christi, TX				NR	0.17	NR	0.48/h
	Dallas/Ft. Worth, TX				NR	0.16	NR	0.50/h
	Denver, CO				NR	0.16	NR	0.49/h
	Los Angeles, CA				NR	0.13	NR	0.42/h

Table 2-4 (Continued): Summary of U.S. studies of ozone infiltration published after 2011.

Reference	Location	Time Period	Population	Microenvironment	Ambient Ozone Concentration	I/O Ratio	Correlation	AER
Chen et al. (2012) (cont.)	Miami, FL	1987–2000 (cont.)	All residents (cont)	Home (estimated by model) (cont.)	NR	0.15	NR	0.35/h
	Nashville, TN				NR	0.16	NR	0.51/h
	New York City, NY				NR	0.20	NR	0.62/h
	Phoenix, AZ				NR	0.14	NR	0.42/h
	Seattle, WA				NR	0.17	NR	0.62/h
	St. Louis, MO				NR	0.18	NR	0.58/h
	Washington, DC				NR	0.16	NR	0.54/h
	Worcester, MA				NR	0.18	NR	0.60/h

Table 2-4 (Continued): Summary of U.S. studies of ozone infiltration published after 2011.

Reference	Location	Time Period	Population	Microenvironment	Ambient Ozone Concentration	I/O Ratio	Correlation	AER
Dutton et al. (2013)	Alameda, CA	September 6–December 4, 2011	Office workers	Office 1 windows closed	NR	Mean (SD): 0.18 (0.11) Peak: 0.78	NR	NR
				Office 1 windows open	NR	Mean (SD): 0.37 (0.18) Peak: 0.78	NR	NR
	Oakland, CA	June 15–July 1, 2012		Office 2 windows closed	NR	Mean (SD): NR Peak: 0.52	NR	NR
				Office 2 windows open	NR	Mean (SD): 0.24 (0.10) Peak: 0.52	NR	NR
	El Cerrito, CA	July 2–20, 2012		Office 3 windows closed	NR	Mean (SD): 0.18 (0.07) Peak: 0.54	NR	NR
				Office 3 windows open	NR	Mean (SD): 0.28 (0.14) Peak: 0.54	NR	NR
	Berkeley, CA	NR		Office 4 windows closed	NR	Mean (SD): NR Peak: 0.68	NR	NR
				Office 4 windows open	NR	NR	NR	NR

Table 2-4 (Continued): Summary of U.S. studies of ozone infiltration published after 2011.

Reference	Location	Time Period	Population	Microenvironment	Ambient Ozone Concentration	I/O Ratio	Correlation	AER
Chan et al. (2014)	San Francisco Bay Area, Sacramento Area, Fresno, Los Angeles Area, CA	September 2011–March 2013	Store occupants	Grocery stores	Average, 1-h daily max across stores: 24.1−66.7 ppb, 30.0−79.4 ppb	Mean (range): 0.40 (0.18−0.59)	NR	Across stores: 0.65−1.47/h
				Furniture/hardware stores	Average, 1-h daily max across stores: 30.1–62.1 ppb, 30.1–62.1 ppb	Mean (range): 0.42 (0.29–0.47)	NR	Across stores: 0.39–2.38/h
				Apparel stores	Average, 1-h daily max across stores: 12.1–51.5 ppb, 15.1–59.6 ppb	Mean (range): 0.33 (0.11–0.47)	NR	Across stores: 0.52–2.33/h
Johnson et al. (2014)	Durham, NC	August–September 2012		Various stores	1-h avg: 29–58 ppb (by day)	Mean (range): 0.17 (−0.012–0.78)	NR	NR
				Windows or doors open		Mean (range): 0.44 (0.13–0.780)		
				Windows or doors closed		Mean (range): 0.093 (0.00–0.30)		
				In-vehicle (driving, parked, refueling, roadside)		Mean (range): 0.33 (0.0063–0.70)		
Gall et al. (2011)	Houston, TX	Simulation	Simulated homes	No passive removal materials	NR	Average: 0.16	NR	0.5/h
			Simulated homes	Gypsum, activated carbon cloth, or other removal materials	NR	Average: 0.047–0.12	NR	0.5/h

Table 2-4 (Continued): Summary of U.S. studies of ozone infiltration published after 2011.

Reference	Location	Time Period	Population	Microenvironment	Ambient Ozone Concentration	I/O Ratio	Correlation	AER
Ng et al. (2015)	Atlanta, GA	NR	Simulated box store	ASHRAE prescribed ventilation	Average of daily average (daily peak): 76 ppb (93 ppb)	Calculated as mean indoor/daily average outdoor: daily average (daily peak): 0.13 (0.13)	NR	1.2 L/s-m^2
				Volume-weighted concentrations	Average of daily average (daily peak): 76 ppb (93 ppb)	Calculated as mean indoor/daily average outdoor: daily average (daily peak): 0.079 (0.075)	NR	0.4 L/s-m^2
Lai et al. (2015)	West Lafayette, IN	NR	Test chamber	Infiltration	24.02–53.5 ppb	0.050–0.099	NR	Median (10th–90th percentile) 0.40 (0.15–0.85)
				Simple mechanical ventilation	38.96–39.69 ppb	0.57–0.63	NR	Median (10th–90th percentile) 0.98 (0.22–4.84)
				HVAC	20.11–34.49 ppb	0.15–0.43	NR	Median (10th−90th percentile) 0.98 (0.22–4.84)
				Window open	30.85–51.02 ppb	0.23–0.42	NR	Median (10th−90th percentile) 3.67 (0.74–7.70)
				Façade natural ventilation	22.09–27.68 ppb	0.18–0.33	NR	Median (10th−90th percentile) 3.67 (0.74–7.70)

Table 2-4 (Continued): Summary of U.S. studies of ozone infiltration published after 2011.

Reference	Location	Time Period	Population	Microenvironment	Ambient Ozone Concentration	I/O Ratio	Correlation	AER
Ben-David and Waring (2016)	Miami, FL; Houston, TX; Phoenix, AZ; Atlanta, GA; El Paso, TX; Los Angeles, CA; Philadelphia, PA; Albuquerque, NM; Seattle, WA; Boston, MA; Salt Lake City, UT; Milwaukee, WI; Billings, MT; Fargo, ND	Data from 2013 or earlier	Office buildings	Mechanical ventilation: ASHRAE 62.1	Range of means: 17 ppb (Seattle, WA)–35 ppb (Albuquerque, NM)	Mean (5th–95th percentile): 0.121 (0.116, 0.127)	NR	0.39 (for all locations)
				Mechanical mixed with added outdoor air when thermodynamically favorable	Range of means: 17 ppb (Seattle, WA)–35 ppb (Albuquerque, NM)	NR	NR	0.40 (Miami, FL)–1.4 (Los Angeles, CA)
				Natural ventilation: ASHRAE 62.1	Range of means: 17 ppb (Seattle, WA)–35 ppb (Albuquerque, NM)	Mean (5th–95th percentile): 0.107 (0.0926, 0.128)	NR	0.33 (Miami, FL)–0.39 (Los Angeles, CA and Seattle, WA)
				Natural ventilation with added outdoor air when thermodynamically favorable	Range of means: 17 ppb (Seattle, WA)–35 ppb (Albuquerque, NM)	NR		0.49 (Seattle, WA and Boston, MA)–1.6 (Los Angeles, CA)

Table 2-4 (Continued): Summary of U.S. studies of ozone infiltration published after 2011.

Reference	Location	Time Period	Population	Microenvironment	Ambient Ozone Concentration	I/O Ratio	Correlation	AER
Singer et al. (2016)	Sacramento, CA	January–February 2014	NR	Home	1-h daily max: average per ventilation condition: 44−72 ppb	0.03–0.12	NR	Summer: 0.21−0.31, Fall/Winter: 0.22–0.35
					8-h daily max average per ventilation condition: 37−60 ppb	0.03–0.13	NR	Summer: 0.21−0.31, Fall/Winter: 0.22–0.35
Ben-David and Waring (2018)	15 cities: Miami, FL; Houston, TX; Phoenix, AZ; Memphis, TN; El Paso, TX; San Francisco, CA; Baltimore, MD; Albuquerque, NM; Salem, OR; Chicago, IL; Boise, ID; Burlington, VT; Helena, MT; Duluth, MN; Fairbanks, AK	1999–2015 data from U.S. EPA	Office buildings	Constant air volume ventilation	Hourly average: 17.5 ppb (Miami, FL)–34.0 ppb (Albuquerque, NM)	0.18–0.49	NR	Infiltration: 0.08/h Ventilation: 0.8−3.2/h
				Variable air volume ventilation	Hourly average: 17.5 ppb (Miami, FL)–34.0 ppb (Albuquerque, NM)	0.19–0.51	NR	Infiltration: 0.08/h Ventilation: 0.8−3.2/h

AER = air exchange rate, ASHRAE = American Society of Heating, Refrigeration, and Air-Conditioning Engineers, HVAC = heating, ventilation, and air conditioning, I = indoor ozone air concentration, NR = not reported, O = outdoor ozone air concentration, SD = standard deviation.

[a]I/O was calculated from data provided in Table 1 of Chen et al. (2012) by normalizing to unit ozone rather than the I/O provided in the table per 10 ppb of ozone.

2.4.3 Relationships between Personal Exposure and Ambient Concentration

The 2013 Ozone ISA (U.S. EPA, 2013) reviewed literature on personal exposure-ambient concentration (P/A) ratios where an individual is exposed. P/A ratios generally ranged from 0.1−0.3. Correlations between personal exposure and ambient concentration were reported as 0.05−0.91 over timescales of hours to days. Higher ratios (0.5−0.9) and correlations ($R > 0.64$) were reported in the 2013 Ozone ISA for personal exposure measurements when a greater proportion of time was spent outdoors for studies incorporating timescales up to 14 hours, especially in the vicinity of roadways where ozone titration by NO_X occurs over a small spatial scale.

Results from recent studies of relationships between personal exposure and ambient concentration (Table 2-5) are somewhat consistent with those described in the 2013 Ozone ISA (U.S. EPA, 2013). P/A ratios calculated using data by Chen et al. (2012) for the National Morbidity, Mortality, and Air Pollution Study (NMMAPS) study ranged from 0.25 to 0.30 and accounted for both indoor and outdoor exposure. Jones et al. (2013) noted average P/A of 0.48 with a 95th percentile of 0.83 and correlation of 0.98. During the Moderate and Severe Asthmatics and Their Environment Study (MASAES), Williams et al. (2012) observed no relationship between ozone exposure and personal activities, with a P/A ratio below 0.1 and correlation between ambient ozone concentration and personal ozone exposure of 0.27 for 24-hour integrated sampling periods.

Ozone participates in surface reactions indoors to cause a reduction in concentrations and exposures. For example, ozone has been shown to participate in surface reactions with VOCs such as terpenes, a common ingredient of household cleaners and air fresheners (Waring and Wells, 2015; Springs et al., 2011). Gall et al. (2011) found that activated carbon and gypsum also reacted with ozone to reduce indoor concentrations. Human presence has also been shown to lead to reduced ozone concentrations, because squalene, a natural oil in skin or dust containing skin cells, reacts with ozone (Rim et al., 2018; Fadeyi et al., 2013), potentially reducing inhaled ozone concentrations.

Table 2-5 Studies reporting relationships between personal ozone exposures and ambient ozone concentrations.

Reference	Location	Time Period	Population	Personal Concentration	Ambient Concentration	P/A Ratio	Correlation
Williams et al. (2012)	Detroit, MI	February, 2008–April, 2009	U.S. EPA moderate and severe asthmatics and their environment study panel	Mean (SD): 3.4 ppb (3.6 ppb)	Mean (SD): 29.7 ppb (15.0 ppb)	0.0665 (slope)	P/A: 0.27
Chen et al. (2012)	NMMAPS cities:	1987–2000	All residents	NR	NR	Calculated change in total ozone exposure per unit change in ambient ozone[a]	NR
	Atlanta, GA					0.25	
	Birmingham, AL					0.26	
	Boston, MA					0.30	
	Buffalo, NY					0.30	
	Chicago, IL					0.28	
	Cincinnati, OH					0.27	
	Corpus Christi, TX					0.28	
	Dallas/Ft. Worth, TX					0.27	

Table 2-5 (Continued): Studies reporting relationships between personal ozone exposures and ambient ozone concentrations.

Reference	Location	Time Period	Population	Personal Concentration	Ambient Concentration	P/A Ratio	Correlation
Chen et al. (2012) (cont.)	Denver, CO	1987–2000 (cont.)	All residents (cont.)	NR (cont.)	NR (cont.)	0.27	NR (cont.)
	Los Angeles, CA					0.25	
	Miami, FL					0.26	
	Nashville, TN					0.27	
	New York City, NY					0.30	
	Phoenix, AZ					0.25	
	Seattle, WA					0.30	
	St. Louis, MO					0.29	
	Washington, DC					0.27	
	Worcester, MA					0.27	
Jones et al. (2013)	New York City, NY	June 1–August 31, 2001–2005	Hospital admissions for respiratory diagnoses	Avg 8-h daily max (95th percentile): 12.78 ppb (20.78 ppb)	Avg 8-h daily max: 30.67 ppb	Mean (95th percentile): 0.48 (0.83)	P/A: 0.979

NR = not reported, NMMAPS = National Morbidity, Mortality, and Air Pollution Study; P/A = fo + fipa/(a+k) where P = personal exposure to ambient ozone, A = ambient ozone concentration, fo = fraction of time spent outdoors, fi = fraction of time spent indoors, p = penetration of ozone indoors (assumed 100% prior to losses), a = air exchange rate, k = loss rate.

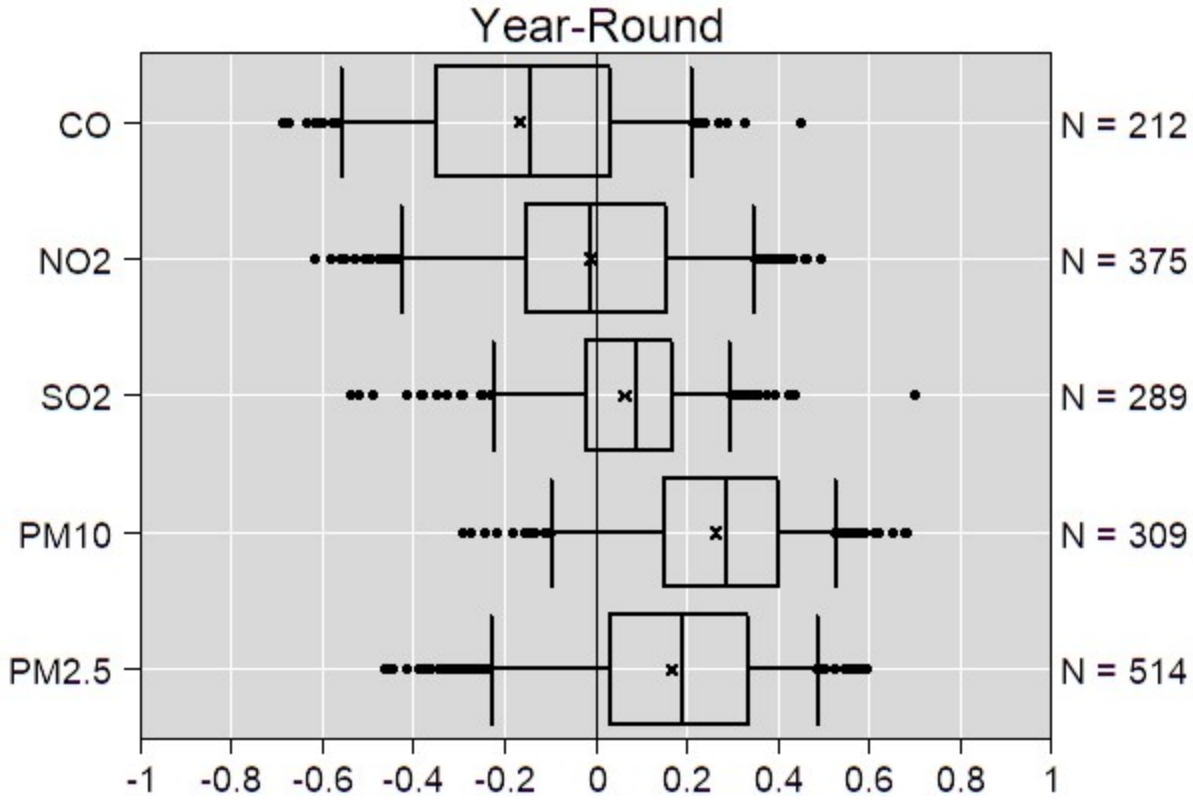
[a]P/A was calculated from Chen et al. (2012) by inputting the data provided in Table 1 into the equation for P/A presented in the 2013 Ozone ISA (U.S. EPA, 2013).

2.5 Copollutant Correlations and Potential for Confounding

Confounding among copollutants can occur when the copollutants are correlated with each other and with the incidence of the health effect being studied (Billionnet et al., 2012). Potential confounding is limited to copollutants in this section, because noise is source-based and would not be expected to correlate with ozone produced by atmospheric chemistry. Other confounders are addressed in the health effects appendices, as detailed in the study quality criteria Annex for Appendix 3.

Correlation of ozone with copollutants can lead to inflation of the effect estimates reported in epidemiologic studies (Goldberg, 2007; Zeger et al., 2000). Winquist et al. (2014) compared joint effects calculated with single-pollutant models with joint effects that account for copollutant correlation. Consistent with older studies, they found that the effect estimates from single pollutant models including ozone were inflated beyond the multipollutant joint effect estimates. Evaluation of copollutant correlation is helpful to understand where there is potential for confounding.

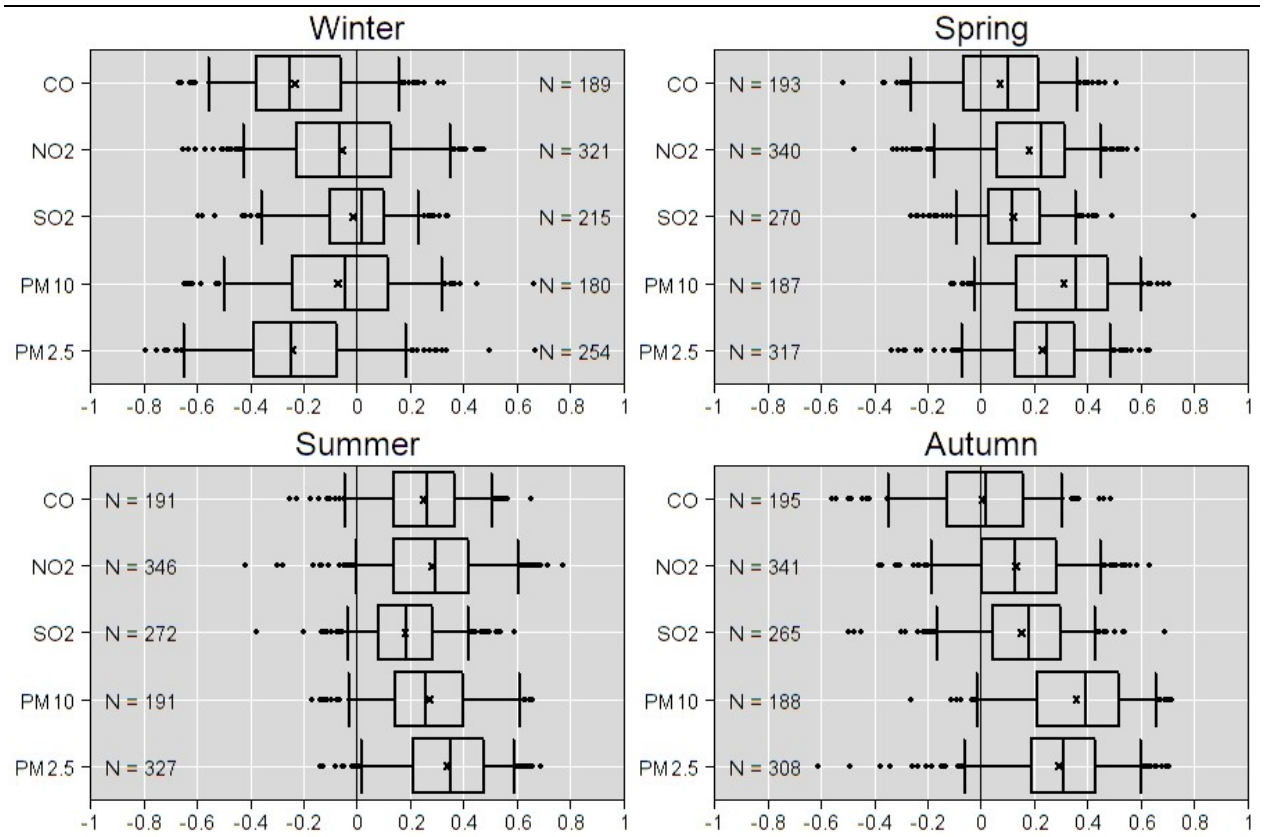
Average copollutant correlations with 8-hour daily max ozone ranged from −0.17 for 8-hour daily max CO to 0.26 for 24-hour avg PM_{10} (Figure 2-1). CO provides a surrogate for traffic-related pollution. While NO_2 also is generally a traffic-related pollutant, it can also be a product of the reaction between NO and O_3 in the near-road environment. Outliers can have correlations as high as 0.7 or −0.7, but the bulk of the data are clustered near zero. During the summer (Figure 2-2), average copollutant correlations are higher and positive for all pollutants, ranging from 0.17 for 1-hour daily max SO_2 to 0.33 for 24-hour avg $PM_{2.5}$. Copollutant correlations over the 25th percentile were generally positive for summer, while the majority of copollutant correlation data were negative during the winter. Given that the majority of the copollutant correlation data are low, confounding of the relationship between ambient ozone exposure and a health effect by exposure to CO, SO_2, NO_2, PM_{10}, or $PM_{2.5}$ is less of a concern for studies of the health effects of ambient ozone exposure compared with studies of the health effects related to exposure of other criteria air pollutants. When copollutant correlations are higher during the warm season, greater risk of copollutant confounding exists.



Note: Daily metrics based on the form of the standards were used for all pollutants (ozone: 8-h daily max, CO: 8-h daily max, NO_2: 1-h daily max, $PM_{2.5}$: 24-h avg, PM_{10}: 24-h avg, SO_2: 1-h daily max). "x" signifies the mean, while the vertical line within each box represents the median. The box covers the interquartile range, and the whiskers cover the 5th to 95th percentiles of the data.

Source: AQS database.

Figure 2-1. Year-round Pearson correlations of 8-hour daily max ozone concentrations with copollutant concentrations measured in AQS 2015−2017.



Note: Daily metrics based on the form of the standards were used for all pollutants (ozone: 8-h daily max, CO: 8-h daily max, NO_2: 1-h daily max, $PM_{2.5}$: 24-h avg, PM_{10}: 24-h avg, SO_2: 1-h daily max). "x" signifies the mean, while the vertical line within each box represents the median. The box covers the interquartile range, and the whiskers cover the 5th to 95th percentiles of the data.

Source: AQS database.

Figure 2-2. Seasonal Pearson correlations of 8-hour daily max ozone concentrations with copollutant concentrations measured in AQS, 2015−2017.

2.6 Interpreting Exposure Measurement Error for Use in Epidemiology Studies

As described in the 2013 Ozone ISA (U.S. EPA, 2013), exposure measurement error, which refers to the biases and uncertainties associated with using concentration metrics as surrogates for the actual exposure of an individual or population (Section 2.2, Exposure Concepts), can be an important contributor to error in epidemiologic study results. Short-term exposure studies include time-series studies, case-crossover studies, and panel studies. Time-series studies generally assess the association of daily health status of a population of thousands or millions of people over the course of multiple years (i.e., thousands of days) across an urban area with estimates of human exposure using a short monitoring

interval (hours to days). In these studies, the community-averaged concentration of an air pollutant measured at ambient monitors is typically used as a surrogate for individual or population ambient exposure. Case-crossover studies use individuals as their own controls and compare exposures during a health event with exposures before and/or after the event occurs. Case-crossover studies can be considered a subset of time-series studies, albeit with different assumptions about baseline risk (Lu and Zeger, 2007), because the conditional logistic regression function used in case-crossover studies is a form of the log-linear model utilized in time-series studies. Therefore, the influence of exposure assessment method on effect estimates for case-crossover studies is not considered separately from time-series studies. Panel studies, which consist of a relatively small sample (typically tens) of study participants followed over a period of days to months, have been used to examine the association of specific health effects with short-term exposure to ambient concentrations of pollutants (e.g., Delfino et al. (1996)). Long-term exposure studies usually are longitudinal cohort studies. A longitudinal cohort epidemiologic study, such as the American Cancer Society (ACS) cohort study, typically involves hundreds or thousands of subjects followed over several years or decades (e.g., Jerrett et al. (2009)). Ambient concentrations are generally aggregated over time and by community as exposure surrogates.

Exposure measurement error can bias epidemiologic associations between ambient pollutant concentrations and health outcomes and tends to widen confidence intervals around those estimates (Sheppard et al., 2005; Zeger et al., 2000). The importance of exposure measurement error depends on the spatial and temporal aspects of the study design. Other factors that could influence exposure estimates include meteorology, instrument errors, unaccounted localized sources of precursor species, use of ambient ozone concentration as a surrogate for exposure to ambient ozone, and the presence of copollutants. This section will summarize information compiled in Section 2.3 about the different methods used for ozone exposure assessment in epidemiologic studies and related strengths, limitations, and errors along with how those errors would influence effect estimates for epidemiologic studies of short-term and long-term ozone exposure (Table 2-6).

2.6.1 Short-Term Exposure

In most short-term exposure time-series epidemiologic studies, changes in the incidence of the health effect are modeled as a function of changes in estimates of ambient exposure, Ea (Davalos et al., 2017). In the absence of indoor ozone sources, E_a can be thought of as a function of the product of ambient concentration, C_a, and a term encompassing time-weighted averaging of microenvironmental exposures and infiltration of ozone. This model is presented in the 2013 Ozone ISA (U.S. EPA, 2013).

2.6.1.1 Time-Series Studies

Time-series epidemiologic studies capturing the exposures and health outcomes of a large cohort frequently use the ambient concentration at a fixed-site monitor or an average of ambient concentrations across monitors as a surrogate for Ea in a statistical model, as detailed in the 2013 Ozone ISA (U.S. EPA, 2013). This is necessary because measuring personal exposures in studies involving thousands of participants is infeasible. Moreover, for time-series epidemiologic studies of short-term exposure, the temporal variability in concentration is of primary importance to relate to variability in the effect estimate (Zeger et al., 2000). Ca can be an acceptable surrogate if the ambient monitor captures the temporal variability of the true air pollutant exposure. Spatial variability in ozone concentrations across the study area could attenuate an epidemiologic study's effect estimate if the exposures are not correlated in time with Ca when ambient monitoring is used to represent exposure in the statistical model. Differences between personal exposure to ambient ozone and C_a due to unaccounted time-activity patterns could bias time-series studies. If exposure assessment methods that more accurately capture spatial variability in the concentration distribution over a study area are employed, then the confidence intervals around the effect estimate may decrease.

A summary of the methods-related studies evaluated in Section 2.3 showed that several methods can be used in time-series studies, because they can provide an estimate for a geographical domain containing a large number of individuals and because the data they provide are of a timescale less than 1 month (Table 2 6). These methods include fixed-site monitors, data averaging, LUR, spatiotemporal modeling, chemical transport models, hybrid models, and microenvironmental models. Among these methods, fixed-site monitors tend to be used more frequently for short-term exposure studies. Short-term exposure studies examine how short-term (hourly, daily, weekly) changes in health effects are related to short-term changes in exposure, so accurate characterization of temporal variability by a fixed-site monitor is more important than accurate characterization of spatial variability. This assumes temporal variability of the exposure is constant over space.

For short-term exposure assessment methods, use of an exposure surrogate may produce inaccuracy when temporal variability in the concentration at the location of measurement or model prediction differs from temporal variability of the true exposure concentration. As a result, the correlation between the exposure surrogate and the incidence of the effect would decrease because the additional scatter in that relationship would flatten the slope of the relationship between the effect and exposure surrogate, causing the true effect of exposure on incidence of the health outcome to be underestimated and imprecise. Darrow et al. (2011) examined spatial variability for ozone concentration measurement timescales (1-hour daily max, 8-hour daily max, commuting hours [7:00 a.m.−10:00 a.m. and 4:00 p.m.−7:00 p.m.], workday hours [8:00 a.m.−7:00 p.m.], and night hours [12:00 a.m. −6:00 a.m.]) and its impact on effect estimates. Over a distance of 60 km, inter-monitor correlation was greater than 0.75 for all but nighttime ozone measurements, indicating low spatial variability during the day. Risk ratios were greater than 1 for each case except for nighttime ozone. This finding implies that most ozone

concentration measurements (excluding nighttime) used as a surrogate for exposure to ambient ozone would produce a small magnitude underestimation of the effect because spatial variability is low over an urban scale. This analysis did not account for microscale ozone scavenging due to a high NO_X gradient near roads. In a recent study, Shmool et al. (2016) used 24-hour avg temporal and spatio-temporal ozone concentrations in models of the risk of inpatient hospitalization or outpatient ED visits for asthma in a case-crossover analysis in New York City. For both outcomes, no difference between models including only a temporal model of ozone concentration or a spatio-temporal model of ozone concentration could be ascertained, implying that spatial variability was not important for this time-series study of ambient ozone exposure. Goldman et al. (2010) simulated the effect of spatial error with and without autocorrelation on risk ratio and found that the risk was slightly underestimated when spatial error was added (with autocorrelation: relative risk, RR = 1.0128 per ppm; without autocorrelation: RR = 1.0126 per ppm compared with a base case RR = 1.0139 with no spatial error added).

Goldman et al. (2012) evaluated the effect of different types of spatial averaging on bias in the risk ratio and the effect of correlation between measured and "true" ambient concentrations of ozone and other air pollutant measures. Concentrations were simulated at alternate monitoring locations using the geostatistical approach described above (Goldman et al., 2010) for the 20-county Atlanta metropolitan area for comparison with measurements obtained directly from monitors at those sites. Geostatistical-simulated concentrations were considered by the authors to be "true" in this study, and other exposure assessment methods were assumed to have some error. Five different exposure assessment approaches were tested: (1) using a single fixed-site ambient monitor, (2) averaging the simulated ambient concentrations across all monitoring sites, (3) performing a population-weighted average across all monitoring sites, (4) performing an area-weighted average across all monitoring sites, and (5) population-weighted averaging of the geostatistical simulation. Goldman et al. (2012) observed that the exposure measurement error was somewhat correlated with both the measured and "true" values, reflecting both Berkson and classical exposure measurement error components. For the single fixed-site ambient monitor, the exposure measurement errors had a moderate positive correlation with the measured value. For the other ambient concentration estimation methods, the exposure measurement errors were moderately negatively correlated with the "true" value, while having positive but lower magnitude correlation with the measured value. Additionally, the exposure bias, given by the ratio of the exposure measurement error to the measured value, was higher in magnitude at the single fixed-site monitor than for the spatial averaging techniques for ozone. Hence, compared with other exposure assessment methods, the effect estimate would likely have greater negative bias (i.e., underestimation of the true effect) with reduced precision when a single fixed-site monitor is used to measure ozone concentration as a surrogate for exposure. However, exposure measurement error is likely to cause some bias and decreased precision for other exposure surrogate methods.

The role of classical and Berkson exposure measurement error on effect estimates has been explored in recent time-series studies. For example, in a time-series study of ED visits for cardiovascular disease, Goldman et al. (2011) simulated the effect of classical and Berkson exposure measurement errors

due to spatiotemporal variability among ambient (fixed-site) or simulated outdoor (i.e., an ambient monitor situated outside the home) air pollutant concentrations over a large urban area, based on the method used in (Goldman et al., 2010). For 8-hour daily max ozone concentrations, the RR per unit mass was negatively biased in the case of classical exposure measurement error (1.0114 compared to the base case of 1.0139) and negligibly positively biased in the case of Berkson exposure measurement error (1.0142). Negative bias means that the true effect was underestimated. The 95% confidence interval range for RR per ppm of ozone was slightly wider for Berkson exposure measurement error (0.0133) compared with classical exposure measurement error (0.0109). In addition to the effect of the correlations and ratios themselves, spatial variation in their values across urban areas also affects time-series epidemiologic results. The Goldman et al. (2010) and Goldman et al. (2012) findings suggest more Berkson exposure measurement error in the spatially resolved ambient concentration metrics compared with the fixed-site ambient monitors, and more classical exposure measurement error for the fixed-site ambient monitor estimate compared with the other exposure assessment techniques. Hence, more bias would be anticipated for the effect estimate calculated from the fixed-site ambient monitor, and more uncertainty would be expected for the effect estimate calculated with the more spatially resolved methods.

A recent study by Strickland et al. (2013) added instrument error to concentrations estimated with a fixed-site monitor, population-weighted average (PWA), unweighted average (UA), and "true" population-weighted average (TPWA) concentration obtained from a grid with 1,054 receptor locations. Berkson exposure measurement error, considered by Strickland et al. (2013) to be the difference in effect estimates from using the TPWA and a 5-km-resolution simulated ambient concentration surface, was 2.21% per ppb ambient ozone. Positive Berkson exposure measurement error suggested that variability in the true exposure concentration was uncharacterized but correlated with the ambient concentration. Median biases for the ambient concentration measurement methods were −16.9% for the fixed-site monitor, −1.6% for PWA, and −2.6% for UA. These biases reflected errors in capturing all components of the ambient concentration (Berkson-like) and the imprecision in the ambient concentration estimate (classical-like). Differences in the magnitude of exposure concentration estimates are not likely to cause substantial bias, but they tend to widen confidence intervals and thus reduce the precision of the effect estimate (Zeger et al., 2000). The more spatially variable air pollutants studied in Goldman et al. (2012) also had more bias in their effect estimates. This occurred across exposure assessment methods but was more pronounced for the fixed-site ambient monitoring data. Note that the Goldman et al. (2010), Goldman et al. (2011), Goldman et al. (2012), and Strickland et al. (2013) studies were performed only in Atlanta, GA. These simulation studies are informative, but similar simulation studies in additional cities would aid generalization of these results.

Introducing errors in the time-series of data rather than across space had a larger impact on effect estimates. For example, Samoli et al. (2014) recently compared effects in four cities (Athens, Greece; London, UK; Milano, Italy; Zurich, Switzerland) estimated using a complete daily time-series with effects where a time-series with only 1 day in 6 was systematically included. For all cities and for results pooled across city, the percentage change in total mortality corresponding to a 10 $\mu g/m^3$ increase in ozone

concentration decreased from positive to negative (of equal or lesser magnitude) with larger confidence intervals when the one-in-six data were used in lieu of the full data set.

Data for time-activity patterns and avoidance behaviors are often omitted from exposure assessment studies. This omission has the potential to add negative bias to and decrease precision of the effect estimate. Bias would result from a reduction in correlation between the exposure surrogate and the incidence of the health effect, while decreased precision could occur when the lack of time-activity data prevents characterization of the true variability in exposure. These errors can potentially occur for fixed-site monitors, data averaging, LUR, spatiotemporal models, CTMs, and hybrid approaches (Table 2-6). Jones et al. (2013) compared respiratory and asthma hospitalization estimates obtained from use of a fixed-site monitor with those obtained from a microenvironmental model to ascertain the impact of time-activity data on the results. Little differences between the mean and confidence interval of the hazard ratios were observed for the entire population for respiratory hospitalizations (fixed-site: 1.013 confidence interval, CI 0.999−1.028; mean error, ME: 1.013, CI 0.998−1.029) and asthma hospitalizations (fixed-site: 1.029, CI 1.010−1.047; ME: 1.029, CI 1.009−1.049). However, differences in hazard ratios were noted for the 5−14 year (fixed-site: 1.056, ME: 1.013), 15−24 year (fixed-site: 1.051, ME: 1.013), 25−64 year (fixed-site: 1.021, ME: 1.013), and ≥65 year (fixed-site: 0.993, ME: 1.015) age groups. The ≥65 year group, which spends the most time indoors (Table 2-1), was the only group for which effect was underestimated by the fixed-site monitor.

2.6.1.2 Panel Studies

The 2013 Ozone ISA (U.S. EPA, 2013) did not comment on potential effects of exposure measurement errors on results of panel studies. Panel studies of the effects of short-term exposure to ozone typically use active or passive microenvironmental monitors to represent exposure (Table 2-6). A strength of the measurement methods is the ability to have a representation of the exposure at the location of the individuals being studied, while a limitation is greater sensitivity to instrument errors. Active monitors are subject to interference from humidity and copollutants, while passive monitors have diffusion-related losses when ozone reacts with the instrument manifold. These instrument errors tend to be small but negative (i.e., the instrument reports a lower concentration than the true concentration). Because panel studies take measurements at the exposed individual sites, correlation between change in effect with change in exposure is less important than estimating the relationship between the ozone exposure and the occurrence of the health effect. As a result, instrument error from use of microenvironmental monitors could add a small amount of positive bias to effect estimates.

2.6.2 Long-Term Exposure

In most epidemiologic studies evaluating long-term exposures, the health effect endpoint is modeled as a function of long-term average ambient ozone exposure, Ea (U.S. EPA, 2013). For cohort epidemiologic studies of long-term exposure to ambient ozone, where the difference in the magnitude of the concentration is of most interest, C_a is used as a surrogate for ambient exposure. Uncertainties in time-activity patterns of exposed individuals and surface losses of ozone can reduce precision in the effect estimates. Spatial variability in ozone concentrations across the study area could lead to bias in the effect estimate if C_a is not representative of E_a. There are limited data regarding whether C_a is a biased exposure surrogate in the near-road environment for epidemiologic studies of long-term exposure. However, ozone is known to be fairly spatially homogeneous at the urban scale (Appendix 1). Spatial variability may be greater in some locations, such as near roads where ozone scavenging occurs due to NO_X chemistry (Kimbrough et al., 2017). Scavenging would result in ozone concentrations that are lower near the road than at a fixed-site monitor located away from the road. It would therefore be anticipated that effects would be underestimated by using fixed-site monitoring data to describe exposures for a population living or working near a road or traveling on a road. Biases in effect estimates would be small but could occur in either direction.

A summary of the methods-related studies evaluated in Section 2.3 showed that several methods have the potential to be used in long-term exposure studies because they can provide an estimate for a geographical domain containing a large number of individuals and because the data they provide are of a timescale greater than 1 month (Table 2-6). These methods include fixed-site monitors, data averaging, IDW, kriging, LUR, spatiotemporal modeling, CTMs, hybrid models, and microenvironmental models. IDW, kriging, LUR, spatiotemporal modeling, CTMs, and hybrid models are spatial concentration prediction models listed in order of increasing sophistication (i.e., producing increasing model fit; Section 2.3.2). In recent studies, hybrid models incorporating CTM output with satellite data have produced simulated ambient ozone concentration surfaces with low spatial model error at a national scale [e.g., (Robichaud and Menard, 2014)]. Higher resolution exposure assessment models are intended to minimize bias and uncertainty in the effect estimate due to spatial variability. Microenvironmental models incorporate time-activity patterns with high spatial resolution ambient ozone concentration predictions to estimate ambient ozone exposures among the population.

Nonspatial sources of exposure measurement error can also influence the effect estimate produced from modeled exposure surrogates. Model misspecification, where an exposure assessment model is not fit with the correct predictive variables, can also lead to bias in the effect estimate in either direction. Omission of time-activity data in the spatiotemporal exposure assessment models can decrease precision in the effect estimate because variability in the exposure is curtailed without time-activity patterns. Likewise, omission of time-activity data can result in negative bias when it causes the spatial correlation between the exposure estimate and the effect to decrease. Dionisio et al. (2014) recently compared effect estimates derived from a microenvironmental model of exposure with effect estimates

derived from a CTM. Using personal exposure as a reference, the effect estimate was considered to be negatively biased when the CTM alone was used. Omission of time-activity data was responsible for 87.6% [RMSE(SD) = −0.85 ppb (0.015 ppb)] of the total bias in the effect estimate.

As described in the 2013 Ozone ISA (U.S. EPA, 2013), spatial variability is typically low at the urban scale, with the exception of near-road areas. Bias related to spatial variability is typically anticipated to be low except where ozone scavenging takes place. Uncharacterized ambient ozone scavenging near a road would mean that a population living or working near roads would have overestimated exposure and a negatively biased effect estimate. When LUR or spatiotemporal models are applied, then bias can occur in either direction if the model is applied in a location different from where it was fit (Table 2-6). Since the 2013 Ozone ISA, Punger and West (2013) estimated exposure using a CTM (CMAQ 4.7.1) and compared the effect estimates using a coarse (36-km) and medium (12-km) grid. Use of a 36-km grid led to a 12% higher effect estimate compared with the medium grid. Dionisio et al. (2014) recently evaluated bias by comparing an effect estimate considering exposure derived from a dispersion model (AERMOD) with an effect estimate considering exposure derived from a fixed-site monitor. Omission of spatial exposure measurement error accounted for 12.4% [RMSE(SD) = −0.12 ppb (0.093 ppb)] of the total bias in the effect estimate, and biases were negative. The standard deviation was a larger proportion of bias in the effect estimate for spatial exposure measurement error compared with bias in the effect estimate related to omission of time-activity data in the exposure assessment. Lopiano et al. (2011) compared effect estimates computed with exposures from three variations each of kriging and parametric bootstrapping. Several cases were evaluated for the set of models. The health effect estimate was slightly overestimated (by ≤2.5%) for the cases where exposure assessment occurred at the location of cases and where some case locations were omitted from the model with prediction of the effect's variability close to the true 95% confidence intervals (within ±2.5%), so negligibly small positive biases in the effect estimates were observed with good coverage. This suggests that the spatial variability for ozone may not be a large source of error.

Table 2-6. Summary of exposure estimation methods, their typical use in ozone epidemiologic studies, and related errors and uncertainties.

Exposure Concentration Assignment Method	Description	Epidemiologic Application	Strengths	Limitations	Exposure Measurement Errors	Influence on Effect Estimates in Epidemiologic Study Results
			Measurement Methods			
Fixed-site monitor [Section 2.3.1.1 Table 2-7; U.S. EPA (2013)]	A FRM or FEM monitor located at a fixed location to measure ambient ozone concentration by chemiluminescence or UV absorption	Short-term exposure studies: surrogate for ambient ozone exposure concentration of a population within a city	Ambient ozone concentration measurements undergo rigorous quality assurance	Measurements of ambient ozone concentration made at a fixed location may differ from an exposed individual's true exposure concentration, and no spatial variation is assumed	Correlation between outdoor ozone concentrations proximal to the receptors and ambient ozone concentration measurements decreases with increasing distance from the monitor, especially in cities with a lot of solar radiation and roadways, where ozone production is high but scavenging occurs near roads (in some cities, correlations >0.80 over distances of 50 km)	Potential for simultaneous decreased precision and negative bias in the effect estimate, because decreased correlation between the exposure surrogate and effect drives the slope towards zero
					Omission of time-activity data	Negative bias and decreased precision in the effect estimate

Table 2-6. (Continued): Summary of exposure estimation methods, their typical use in ozone epidemiologic studies, and related errors and uncertainties.

Exposure Concentration Assignment Method	Description	Epidemiologic Application	Strengths	Limitations	Exposure Measurement Errors	Influence on Effect Estimates in Epidemiologic Study Results
Fixed-site monitor [Section 2.3.1.1 Table 2-7; U.S. EPA (2013)] (cont.)	A FRM or FEM monitor located at a fixed location to measure ambient ozone concentration by chemiluminescence or UV absorption (cont.)	Long-term exposure studies: surrogate for ambient ozone exposure concentration to compare populations within a city or among multiple cities	Ambient ozone concentration measurements undergo rigorous quality assurance (cont.)	Measurements of ambient ozone concentration made at a fixed location may differ from an exposed individual's true exposure concentration, and no spatial variation is assumed (cont.)	Ambient ozone concentration at a receptor location is higher or lower than the ambient ozone concentration measured at the monitor	Potential for bias in the effect estimate in either direction, but likely small in magnitude
					Localized ozone loss processes near roads are not captured	Potential for negative bias in the effect estimate
					Spatial variability of ozone concentration is not characterized	Potential for decreased precision in the effect estimate
					Omission of time-activity data	Negative bias and decreased precision in the effect estimate
Microenvironmental exposure monitor (non-FRM or FEM) (Section 2.3.1.2 Table 2-8)	Typically a miniaturized UV absorption sampler for ozone, where air is pulled through a pump; may be an FEM	Panel studies: ozone exposure (e.g., personal or residential samples) within a geographic area	Ozone concentrations may be obtained at the site of the exposed person and therefore automatically account for time-activity patterns; spatial variability is better captured by deploying monitors with higher spatial density	Non-FEM UV absorption instruments subject to interference from humidity, mercury, and VOCs	Instrument errors more typically lead to positive artifacts from interferences	Instrument errors are typically small but positive and so have the potential to add negative bias to the effect estimate

Table 2-6. (Continued): Summary of exposure estimation methods, their typical use in ozone epidemiologic studies, and related errors and uncertainties.

Exposure Concentration Assignment Method	Description	Epidemiologic Application	Strengths	Limitations	Exposure Measurement Errors	Influence on Effect Estimates in Epidemiologic Study Results
Passive personal exposure monitor (Section 2.3.1.2 Table 2-8)	Ozone is captured on a nitrite-treated substrate via passive exposure for a time period to measure a personal or area sample; oxidation by ozone converts the nitrite to nitrate, which is analyzed by ion chromatography	Panel studies: ambient ozone exposure within a city or among multiple cities	Ozone concentrations are obtained at the site of the exposed person and therefore automatically account for time-activity patterns; low cost	Long duration integrated sampling time (e.g., 7 days) does not allow for time-series analysis; diffusion-related losses to the passive sampler hardware	Diffusion-related losses to the passive sampler hardware have the potential to bias the concentration estimation based both on reduced ozone detection and overestimation of flux to the sampling substrate	Instrument errors are typically small but negative and so have the potential to add positive bias to the effect estimate
Modeling Methods						
Data averaging (Section 2.3.2.1 Table 2-9)	Averaging across multiple monitors during the same time window and within a geographical area, such as a city or county, typically using fixed-site monitoring data	Short-term exposure studies: surrogate for ambient ozone exposure concentration of a population within a city	Ambient ozone concentration measurements undergo rigorous quality assurance; averaging scheme designed for population or trend of interest; simple to implement		Correlation between outdoor ozone concentrations proximal to the receptors and ambient ozone concentration measurement at a centrally located fixed-site monitor decreases with increasing distance from the monitor, especially in cities with a lot of solar radiation and roadways, where ozone production is high but scavenging occurs near roads (in some cities, correlations >0.80 over distances of 50 km)	Low correlation potentially leads simultaneously to decreased precision and to negative bias in the effect estimate due to decreased correlation between the exposure surrogate and effect
					Omission of time-activity data	Negative bias and decreased precision in the effect estimate

Table 2-6. (Continued): Summary of exposure estimation methods, their typical use in ozone epidemiologic studies, and related errors and uncertainties.

Exposure Concentration Assignment Method	Description	Epidemiologic Application	Strengths	Limitations	Exposure Measurement Errors	Influence on Effect Estimates in Epidemiologic Study Results
Data averaging (Section 2.3.2.1 Table 2-9) (cont.)	Spatial averaging (area averaging, population-weighted averaging), typically using fixed-site monitoring data	Long-term exposure studies: surrogate for ambient ozone exposure concentration, usually within a city or geographic region	Ambient ozone concentration measurements undergo rigorous quality assurance; averaging scheme designed for population or trend of interest; simple to implement (cont.)	Measurements of ambient ozone concentration made at a fixed location may differ from an exposed individual's true exposure concentration, and either no spatial variation is assumed or spatial variation is assumed to be well represented by the averaging scheme; errors in average concentration can be caused by one errant monitor; in areas where different monitors peak on different days, this method will mute overall temporal variation	Localized ozone loss processes near roads are not captured	Potential for negative bias in the effect estimate
					Assumption of constant ozone concentration within some geographic area	Potential for decreased precision in the effect estimate
					Omission of time-activity data	Negative bias and decreased precision in the effect estimate

Table 2-6. (Continued): Summary of exposure estimation methods, their typical use in ozone epidemiologic studies, and related errors and uncertainties.

Exposure Concentration Assignment Method	Description	Epidemiologic Application	Strengths	Limitations	Exposure Measurement Errors	Influence on Effect Estimates in Epidemiologic Study Results
Inverse-distance weighting (Section 2.3.2.1 Table 2-9)	Measured ambient ozone concentrations are interpolated to estimate ambient ozone concentration surfaces across regions; IDW uses an inverse function of distance to monitors	Long-term exposure studies: surrogate for ambient ozone exposure concentration, usually within a city or geographic region	High spatial resolution	Does not account for atmospheric chemistry or meteorology; over-smoothing is possible based on smoothing function between monitors	Ozone concentration is overly smoothed	Potential for negative bias with decreased precision in the effect estimate
					Omission of time-activity data	Negative bias and decreased precision in the effect estimate
					Localized ozone loss processes near roads are not captured	Potential for negative bias in the effect estimate
Kriging (Section 2.3.2.1 Table 2-9)	Measured ambient ozone concentrations are interpolated to estimate ambient ozone concentration surfaces across regions	Long-term exposure studies: surrogate for ambient ozone exposure concentration, usually within a city or geographic region	High spatial resolution	Does not account for atmospheric chemistry or meteorology; over-smoothing is possible based on smoothing function between monitors	Ozone concentration is overly smoothed	Potential for negative bias with decreased precision in the effect estimate
					Omission of time-activity data	Negative bias and decreased precision in the effect estimate
					Localized ozone loss processes near roads are not captured	Potential for negative bias in the effect estimate

Table 2-6. (Continued): Summary of exposure estimation methods, their typical use in ozone epidemiologic studies, and related errors and uncertainties.

Exposure Concentration Assignment Method	Description	Epidemiologic Application	Strengths	Limitations	Exposure Measurement Errors	Influence on Effect Estimates in Epidemiologic Study Results
Land use regression (Section 2.3.2.2 Table 2-10)	Measured ambient ozone concentrations are regressed on local variables (e.g., land use factors); the resulting model is used to estimate ambient ozone concentrations at specific locations	Short-term and long-term exposure studies: surrogate for ambient ozone exposure concentration, usually across a city but sometimes among multiple cities	High spatial resolution	Does not account for precursor emission rates, dispersion, or atmospheric chemistry and may account for meteorology only in terms of wind speed and wind direction, depending on model formulation; has limited generalizability to other locations; uncertainties are highest where training monitors are sparse	Potential for model misspecification	Short-term exposure studies: potential for negative bias with decreased precision in the effect estimate Long-term exposure studies: potential bias in the effect estimate in either direction
					Model is applied to a location different from where it was fit	Short-term exposure studies: potential for negative bias with decreased precision in the effect estimate Long-term exposure studies: potential bias in the effect estimate in either direction
					Omission of time-activity data	Negative bias and decreased precision in the effect estimate

Table 2-6. (Continued): Summary of exposure estimation methods, their typical use in ozone epidemiologic studies, and related errors and uncertainties.

Exposure Concentration Assignment Method	Description	Epidemiologic Application	Strengths	Limitations	Exposure Measurement Errors	Influence on Effect Estimates in Epidemiologic Study Results
Spatiotemporal model (Section 2.3.2.2 Table 2-10)	Measured ambient ozone concentrations are modeled by a spatial average, spatially varying covariates, and a spatiotemporal residual; the resulting model is used to estimate ambient ozone concentrations at specific locations	Short-term and long-term exposure studies: surrogate for ambient ozone exposure concentration, usually across a city but sometimes among multiple cities	High spatial resolution; flexible modeling framework allows for minimization of errors	Does not account for precursor emission rates, dispersion, or atmospheric chemistry and may account for meteorology only in terms of wind speed and wind direction, depending on model formulation; has limited generalizability to other locations; uncertainties are highest where training monitors are sparse	Potential for model misspecification	Short-term exposure studies: potential for negative bias with decreased precision in the effect estimate Long-term exposure studies: potential bias in the effect estimate in either direction
					Model is applied to a location different from where it was fit	Short-term exposure studies: potential for negative bias with decreased precision in the effect estimate Long-term exposure studies: potential bias in the effect estimate in either direction
					Omission of time-activity data	Negative bias and decreased precision in the effect estimate

Table 2-6. (Continued): Summary of exposure estimation methods, their typical use in ozone epidemiologic studies, and related errors and uncertainties.

Exposure Concentration Assignment Method	Description	Epidemiologic Application	Strengths	Limitations	Exposure Measurement Errors	Influence on Effect Estimates in Epidemiologic Study Results
Chemical transport model (Section 2.3.2.3 Table 2-11)	Grid-based ambient ozone concentrations are estimated from precursor emissions, meteorology, and atmospheric chemistry and physics	Short-term and long-term exposure studies: surrogate for ambient ozone exposure concentration, sometimes within a city but more typically across a larger region	Accounting for precursor emission rates, mixing height, atmospheric stability, meteorology, atmospheric chemistry, and complex terrain	Limited grid cell resolution (i.e., grid cell length scale is typically 4−36 km); spatial smoothing of local ozone precursor emissions	Localized ozone loss processes near roads are not captured because grid cell scale is too large	Short-term exposure studies: potential for negative bias with decreased precision in the effect estimate Long-term exposure studies: potential for negative bias in the effect estimate
					Omission of time-activity data	Negative bias and decreased precision in the effect estimate

Table 2-6. (Continued): Summary of exposure estimation methods, their typical use in ozone epidemiologic studies, and related errors and uncertainties.

Exposure Concentration Assignment Method	Description	Epidemiologic Application	Strengths	Limitations	Exposure Measurement Errors	Influence on Effect Estimates in Epidemiologic Study Results
Hybrid approaches (Section 2.3.2.4 Table 2-12)	Grid-based ambient ozone concentrations are estimated from precursor emissions, meteorology, and atmospheric chemistry and physics and bias corrected based on monitoring data	Short-term and long-term exposure studies: surrogate for ambient ozone exposure concentration, sometimes within a city but more typically across a larger region	Accounting for ozone precursor emission rates, mixing height, atmospheric stability, meteorology, atmospheric chemistry, and complex terrain; bias correction improves model results, particularly where biases are large; fusing model results with monitoring, satellite, and chemical transport model data helps to minimize exposure measurement errors	The modeling process can be resource intensive; spatial smoothing of local precursor emissions sources; has limited generalizability to other locations; uncertainties are highest where training monitors are sparse	Potential for model misspecification	Short-term exposure studies: potential for negative bias with decreased precision in the effect estimate Long-term exposure studies: potential bias in the effect estimate in either direction
					Model is applied to a location different from where it was fit	Short-term exposure studies: potential for negative bias with decreased precision in the effect estimate Long-term exposure studies: potential bias in the effect estimate in either direction
					Omission of time-activity data	Negative bias and decreased precision in the effect estimate

Table 2-6. (Continued): Summary of exposure estimation methods, their typical use in ozone epidemiologic studies, and related errors and uncertainties.

Exposure Concentration Assignment Method	Description	Epidemiologic Application	Strengths	Limitations	Exposure Measurement Errors	Influence on Effect Estimates in Epidemiologic Study Results
Microenvironmental modeling (e.g., APEX, SHEDS [Section 2.3.2.5 Table 2-13])	Estimates distributions of microenvironmental ozone concentrations, exposures, and doses for populations (e.g., census tracts) based on air quality data, demographic variables, and activity patterns	Short-term and long-term exposure studies	Accounts for variability of ozone exposures across large populations; accounts for different concentrations in different microenvironments; accounts for location-activity information	Models simulate individuals and their exposures; they do not model actual individuals but simulated representative individuals based on the population being modeled	The modeled distributions of ambient ozone concentration, indoor:outdoor pollutant ratios, and time-activity patterns may differ from the true distributions, depending on model inputs	Potential for decreased precision in the effect estimate

APEX = air pollutants exposure model; BC = black carbon; CPC = condensation particle counter; FEM = Federal Equivalent Method; FRM = Federal Reference Method; IDW = inverse-distance weighting; SHEDS = stochastic human exposure and dose simulation.

2.7 Conclusions

The 2013 Ozone ISA (U.S. EPA, 2013) focused on personal exposure to ozone. Recently published data on I/O ratios (Section 2.4.2), P/A ratios (Section 2.4.3), and associated correlations (Section 2.5) are similar to those presented in the 2013 Ozone ISA. Likewise, ambient ozone characteristics, including its spatial distribution over urban scales, high variability near roads, and seasonal and diurnal variation, have not changed substantially since the 2013 Ozone ISA. More information is now available on losses of ozone at surfaces, and those studies support the published ratios. Personal measurements of ozone exposure (Section 2.3.1.2) from active or passive monitors are subject to interference from humidity, mercury, and VOCs (active monitors) or from deposition to the sampling manifold (passive monitors). This can lead to positively biased concentrations and negatively biased effect estimates.

Fixed-site monitors are still widely in use as ozone exposure surrogates [(U.S. EPA, 2013), Section 2.3.1.1], given the low spatial variability typical of ozone in many places. Biases tend to be small in magnitude for this reason. Localized atmospheric chemistry near NO_X sources (i.e., ozone sinks) such as highways may result in overestimation of exposure when ozone concentration monitored away from the highway is used in an epidemiologic analysis. Data averaging techniques (Section 2.3.1.2), including IDW and kriging, provide spatial interpolation where monitors are sparse, but they can produce a less precise exposure estimate compared with hybrid or spatiotemporal models.

The largest development since the 2013 Ozone ISA (U.S. EPA, 2013) is the expanded availability of models to predict ozone concentrations as surrogates for exposure assessment, thereby addressing a key uncertainty for modeling ozone exposure where measurements are not available, such as in rural areas. Both LUR and spatiotemporal modeling (Section 2.3.2.2) can be subject to model misspecification, where the model is not fit with the optimal set of variables. Larger exposure measurement errors can occur if the models are fit to one location and then applied in a different location. Use of CTMs has greatly expanded in usage and in number of available models (Section 2.3.2.3). Misspecification can occur through inadequate characterization of emissions, meteorology, and chemistry. High magnitude errors most typically happen around low and high ozone modeled outputs. Spatiotemporal models sometimes become the framework for incorporating CTMs, satellite data (Section 2.3.2.4), and fixed-site monitoring data (Section 2.3.2.1) into a single hybrid model. By combining so many sources of data, overfitting may be a larger concern for the hybrid model than for other exposure estimation models.

For epidemiologic studies of short-term exposure to ozone, effect estimates potentially have decreased precision and negative bias if the correlation between the exposure surrogate and the health effect is lower than the correlation between the true exposure and the health effect (Table 2-6). Negative bias with decreased precision may occur for fixed-site monitors or any of the spatial interpolation methods. Attenuation of the effect estimate may also occur for LUR, spatiotemporal, and hybrid models

when they are misspecified or fit to a different geographical area than where they are applied. Fixed-site monitors and CTMs may also produce negatively biased effect estimates if individuals are exposed to localized areas of low ozone, such as near roads where ambient ozone is scavenged by NO_X so that the monitor or modeled estimate of ozone exposure is higher than the true exposure. ***In these cases, use of the exposure surrogate generally leads to underestimation of the association between short-term exposure to ambient ozone and the health effect with reduced precision. Although the magnitude of the association between ambient ozone and the health effect is uncertain, the evidence indicates that the true effect is typically larger than the effect estimate in these cases.***

Panel studies tend to use microenvironmental or personal monitors to measure exposure at the locations of individuals in a study. ***The small instrument errors observed for active and passive monitors can lead to small but positive biases in the effect estimates for short-term exposures to ozone.***

For epidemiologic studies of long-term exposure to ozone, when concentrations measured at fixed-site monitors are used as exposure surrogates, effect estimates have the potential to be biased in either direction. However, it is more common that these methods contribute to underestimation of the effect, and the magnitude of bias is likely small given that ozone concentration does not vary over space as much as other criteria pollutants, such as NO_X or SO_2 (Table 2-6). Localized ozone scavenging by NO_X creates potential for negative bias in the effect estimate, if people are exposed on or near a roadway with traffic but have their exposures estimated by concentrations measured at a monitor positioned away from that location. The assumption of a constant ozone concentration within some radius of the monitor or model receptor location also may reduce precision for fixed-site monitors and CTMs. Coarse horizontal grid resolution in CTMs can reduce the spatial heterogeneity of the true exposure. Smoothing may also lead to reduced precision for the data averaging schemes presented. Model misspecification and model fit in a location apart from the field study in LUR, spatiotemporal models, and hybrid models may cause bias in either direction. ***Depending on the model and scenario being modeled, the true effect of long-term exposure to ambient ozone may be underestimated or overestimated by the model. It is much more common for the effect estimate to be underestimated, and the bias is typically small in magnitude.***

For most exposure estimation methods, omission of time-activity data may lead to negative bias and decreased precision of the effect estimates, because exposure variability is largely uncharacterized (Section 2.4.1). That was demonstrated by the comparison of exposure and effect estimates based on monitored concentrations with exposure and health estimates based on microenvironmental models (Section 2.3.2.5) that do use time-activity data from sample populations. ***Estimating exposure without accounting for time-activity data may result in underestimation of the true effect and reduced precision. Although the magnitude of the association between ozone and the health effect is uncertain, the evidence suggests that the true effect of ambient ozone exposure is larger than the effect estimate when time-activity data are not considered in the analysis.***

2.8 Evidence Inventories—Data Tables to Provide Supporting Information

Validation measures are used to evaluate concentrations measured or modeled, as described in Section 2.3. Method performance measures are listed below and are included in Table 2-7 through Table 2-13:

Unpaired predicted-to-observed peak ozone ratio (AUP)	$\frac{P_{i,peak} - O_{i,peak}}{P_{i,peak}}$
Mean bias (MB)	$\frac{1}{N}\sum_{i=1}^{N}(P_i - O_i)$
Mean error (ME)	$\frac{1}{N}\sum_{i=1}^{N}\lvert P_i - O_i\rvert$
Mean-squared error (MSE)	$\frac{1}{N}\sum_{i=1}^{N}(P_i - O_i)^2$
Root-mean-squared error (RMSE)	$\sqrt{\frac{1}{N}\sum_{i=1}^{N}(P_i - O_i)^2}$
Fractional bias (FB)	$\frac{P_i - O_i}{P_i + O_i}$
Fractional error (FE)	$\left\lvert\frac{P_i - O_i}{P_i + O_i}\right\rvert$
Gross error (GE)	$\frac{1}{N}\sum_{i=1}^{N}\lvert P_i - O_i\rvert$
Mean normalized bias (MNB) (−100% to +∞)	$\frac{1}{N}\sum_{i=1}^{N}\left(\frac{P_i - O_i}{O_i}\right)$
Mean normalized error (MNE) (0% to +∞)	$\frac{1}{N}\sum_{i=1}^{N}\left\lvert\frac{P_i - O_i}{O_i}\right\rvert$
Normalized mean bias (NMB) (−100% to +∞)	$\frac{\sum_{i=1}^{N}(P_i - O_i)}{\sum_{i=1}^{N}O_i}$
Normalized mean error (NME) (0% to +∞)	$\frac{\sum_{i=1}^{N}\lvert P_i - O_i\rvert}{\sum_{i=1}^{N}O_i}$
Mean fractional bias (MFB) (−200 to +200%)	$\frac{2}{N}\sum_{i=1}^{N}\left(\frac{P_i - O_i}{P_i + O_i}\right)$

Mean fractional error (MFE)
(0 to +200%)

$$\frac{2}{N}\sum_{i=1}^{N}\left|\frac{P_i - O_i}{P_i + O_i}\right|$$

Coefficient of determination (R^2)

$$\frac{\left\{\sum_{i=1}^{N}\left(O_i - \overline{O}\right)\left(P_i - \overline{P}\right)\right\}^2}{\sum_{i=1}^{N}\left(O_i - \overline{O}\right)^2 \sum_{i=1}^{N}\left(P_i - \overline{P}\right)^2}$$

Mean absolute error (MAE)

$$\frac{\sum_{i=1}^{N}|P_i - O_i|}{N}$$

Index of agreement (IOA)

$$\frac{\sum_{i=1}^{n}(P_i - O_i)^2}{\sum_{i=1}^{n}(|P_i - \bar{O}| + |O_i - \bar{O}|)^2}$$

NB, FB, FE, U R, NB, NE, NGE,
AUP, CSI, FAR, UPA, MNGE,

P_i and O_i are prediction and observation at the ith monitoring site, respectively; N is the number of monitoring sites.

Table 2-7 Studies informing assessment of exposure measurement error when concentrations measured by fixed-site monitors are used for exposure surrogates.

Reference	Monitor	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Blanchard et al. (2011)	Observed, fixed-site ambient monitors only from SEARCH, U.S. EPA PAMS, U.S. EPA STN, IMPROVE monitoring networks	L: in and near the Atlanta metropolitan area; T: between the years 1999 and 2007; P: entire population considered	Observed fixed-site ambient monitors only, protocols of observed data were referenced in previous publications	Long-term exposure	Mean summer quartiles of peak 8-h ozone in ppb, 44.85, 55.73, 64.93, 80.23	Observed data collected directly; speciation collected; several years of data collected	Spatial variability is limited to monitoring locations	NR
Hackbarth et al. (2011)	Daily 8-h max ozone within 20 miles of the zip code centroid weighted by inverse distance from U.S. EPA's fixed-site monitors	L: California; T: 2005–2007; P: those with ER visits for cardio, resp, asthma	Observed data used in inverse-distance weighting validated from U.S. EPA	Short-term exposure	Ozone daily average in CA between 2005 and 2007 because 37.1−73.8 ppb	Observed data used directed with ER data	Sensitivity analysis of exposure method not explored (e.g., buffer size of nearest neighbor)	Ozone daily average across California = 39.9 ppb, standard error = 0.225 ppb

Table 2-7 (Continued): Studies informing assessment of exposure measurement error when concentrations measured by fixed-site monitors are used for exposure surrogates.

Reference	Monitor	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Joseph et al. (2013)	Nearest monitor	L: Houston and Los Angeles; T: select days in 2009–2011	Comparison with 10–20 monitors in each metropolitan area	Short-term exposure	Houston = 31.0−112.5 ppb; Los Angeles = 10.8–117.1 ppb	Simple to implement	Overfitting may be caused by just one incorrect parameter; does not capture the underlying phenomena	N = 10 RMSE =. 54−21.01 ppb, n = 20 RMSE = 8.41–19.06 ppb
Dionisio et al. (2014)	Ambient monitor	L: Atlanta, GA; T: 1999–2002; P: Entire population	Comparison with dispersion model	Long-term exposure	NR	Less spatial variability in ozone, so fixed-site monitors do a better job than for spatially variable pollutants	Uncertainty in areas where there are known sinks (e.g., near roads)	Mean (SD) exposure measurement error for omission of spatial variability: −0.055 (0.037); bias on effect estimate for omission of spatial variability: −0.12 (0.093)

Table 2-7 (Continued): Studies informing assessment of exposure measurement error when concentrations measured by fixed-site monitors are used for exposure surrogates.

Reference	Monitor	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Ollison et al. (2013)	Comparison between FRM (Thermo-scientific 49C) and FEM ambient monitors (two models: Teledyne Model 211 and Teledyne Model 265E). The Teledyne Model 265E has since been certified as an FRM	L: Houston, TX; T: August 26–November 19, 2010; P: entire population	Comparison between ambient monitor types using air spiked with known quantity of ozone	Short-term exposure	8-h daily max = average 22 ppb, maximum 94 ppb, 19 values and 6 days with 8-h daily max above 75 ppb	Average and maximum data compare well; frequent calibrations and zero/span improved data quality	Positive bias of FRM due to water vapor, gas-phase mercury, and VOCs	Differences between monitors presented graphically but not reported
Johnson et al. (2014)	FEM ambient monitor	L: Durham, NC; T: September, 2012; P: Entire population	Comparison with an FRM and a microenviron-mental model	Short-term exposure	10-min avg value = 33.0−55.2 ppb	Average values are only slightly higher than FRM	Potentially high positive errors in vicinity of VOC sources	In the vicinity of VOC sources, ozone concentration from the FEM was several hundred percentage higher (depending on the source) than a microenviron-mental monitor that compared well with the FRM

Table 2-7 (Continued): Studies informing assessment of exposure measurement error when concentrations measured by fixed-site monitors are used for exposure surrogates.

Reference	Monitor	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Buteau et al. (2017)	Fixed-site monitors reporting data for 8-h daily max	L: Montreal, Quebec, Canada; T: January 1, 1991–December 31, 2002; P: Entire population	Comparison with BME, IDW, and back-extrapolation LUR	Short-term exposure	8-h daily max; mean (SD) = 27.9 ppb (15.2 ppb) median = 26.3 ppb	Most accurate measure of ozone	Lacks spatial resolution	ICC mean (95% CI) vs. IDW 0.89 (0.89, 0.89), vs. LUR w/back-extrapolation 0.67 (0.47, 0.78), vs. BME 0.64 (0.41, 0.77)

Table 2-7 (Continued): Studies informing assessment of exposure measurement error when concentrations measured by fixed-site monitors are used for exposure surrogates.

Reference	Monitor	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Yu et al. (2018)	Centralized monitor reporting data for 8-h daily max	L: Atlanta, GA; T: 2011; P: Entire population	Comparison with fixed-site monitors	Short-term exposure	NR	Accurate capture of temporal variation	No spatial resolution, autocorrelation introduces bias	Urban site: MB = −2.54 ppb, ME = 4.15 ppb, RMSE = 5.71 ppb, MNB = −5%, MNE = 10%, NMB = −6%, NME = 9%, MFB = −6%, MFE =10%, R^2 = 0.91, Slope = 1.02; Rural site: MB = −2.43 ppb, ME = 7.30 ppb, RMSE = 9.29 ppb, MNB = −7%, MNE = 18%, NMB = −5%, NME = 16%, MFB = −9%, MFE = 19%, R^2 = 0.70, Slope = 0.67

AQS = Air Quality System; BME = Bayesian maximum entropy; ER = emergency room; ICC = interclass correlation coefficient; U.S. EPA = Environmental Protection Agency; IDW = inverse-distance weighting; IMPROVE = Interagency Monitoring of Protected Visual Environments; L = location; LUR = land use regression; MB = mean bias; ME = mean error; MFB = mean fractional bias; MFE = mean fractional error; MNB = mean normalized bias; MNE = mean normalized error; NMB = normalized mean bias; NME = normalized mean error; NR = not reported; OK = ordinary kriging; P = population; PAMS = Photochemical Assessment Monitoring System; RMSE = root mean squared error; SD = standard deviation; SEARCH = Southeastern Aerosol Research and Characterization; STN = Speciation Trends Network; T = time; UK = universal kriging.

Table 2-8 Studies informing assessment of exposure measurement error when concentrations measured by personal and microenvironmental monitors are used for exposure surrogates.

Reference	Monitor	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Wheeler et al. (2011)	Ogawa passive badge (diffusion-based)	L: Windsor, Ontario, CAN; T: Winter and summer, 2005–2006; P: Adults and children with asthma	Inter-sampler comparison; comparison to fixed-site monitors	Panel study	Mean (SD) = 26 ppb (9 ppb); median = 25 ppb	Can be used for personal, indoor, and outdoor sampling	Integrated measurements, negative bias and decreased precision	Median bias = −0.24 ppb precision = 0.09 ppb
Bart et al. (2014)	Gas sensitive semiconductor monitors	L: Lower Fraser Valley, British Columbia, Canada; T: May–September, 2012; P: Entire population	Collocate 10 GSS microsensors around each of 10 fixed-site monitors	Panel study	NR (shown on figure)	Low cost, easy to deploy over many locations; provides real-time measurements	Interferents: humidity, NO	MB = −1 ppb SE = 6 ppb
Zimmerman et al. (2018)	Low cost sensor (Real-Time Affordable Multipollutant sensors) data filtered through model to improve data quality based on data across geographical area	L: Pittsburgh, PA; T: August 3, 2016–February 7, 2017; P: Carnegie Mellon University campus population	Deployed a dense network of low cost samplers then applied one of two models (random forest or multiple linear regression) to smooth data for ozone	Short-term exposure	15-min avg time; NR	Allows for better spatial coverage	Sensitivity to model quality and input data quality	Multiple linear regression MAE avg (SD) = 5.1 ppb (0.6 ppb) R = 0.81 Random forest MAE average (SD) = 0.7 ppb (0.1 ppb) R = 0.99

Table 2-8 (Continued): Studies informing assessment of exposure measurement error when concentrations measured by personal and microenvironmental monitors are used for exposure surrogates.

Reference	Monitor	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Sagona et al. (2018)	Personal ozone monitor operates by UV light absorption at 254 nm wavelength (2B Technologies)	L: Piscataway, NJ; T: July, 2014; P: Panel of volunteers	Comparison of personal monitors with FEM; intercomparison of personal monitors	Panel study	Outdoor test range (5-min avg time) = 0–65 ppb; indoor test range (1-min avg time) = 30–55 ppb; chamber test range (1-min avg time) = 85−125 ppb	Good accuracy when compared with FEM	Measurable bias observed during personal monitor intercomparison; correlations between personal monitors dropped when VOCs were introduced to the test chamber	Intercomparison chamber: R = 0.947, slope = 0.82 outdoor: R = 0.991, slope = 1.08 indoor; comparison with FEM: outdoor R = 0.982, slope = 0.92 indoor R = 0.867, slope = 0.88

FEM = Federal Equivalent Method; L = location; MAE = mean absolute error; MB = mean bias; P = population; Pearson R = correlation coefficient; SD = standard deviation; SE = standard error; T = time; VOC = volatile organic compound.

Table 2-9 Studies informing assessment of exposure measurement error when concentrations modeled by spatial interpolations methods are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
				Data Averaging				
Joseph et al. (2013)	Simple averaging	L: Houston and Los Angeles; T: Select days in 2009–2011	Comparison with 10–20 monitors in each metropolitan area	Short-term exposure	Houston = 31.0−112.5 ppb; Los Angeles = 10.8–117.1 ppb	Simple to implement	Overfitting may be caused by just one incorrect parameter; does not capture the underlying phenomena	Houston: n = 10 RMSE = 11.30−15.35 ppb, n = 20 RMSE = 10.77−15.07 ppb; Los Angeles: n = 10 RMSE = 15.16−25.13 ppb, n = 20 RMSE = 12.96−24.35

Table 2-9 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by spatial interpolations methods are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Yu et al. (2018)	Average of 10 monitors located across the city reporting data for 8-h daily max	L: Atlanta, GA; T: 2011; P: Entire population	Comparison with fixed-site monitors	Short-term exposure	NR	No bias due to autocorrelation	Low spatial resolution	Urban site: MB = −0.75 ppb, ME = 4.00 ppb, RMSE = 5.73 ppb, MNB = −1%, MNE = 10%, NMB = −2%, NME = 9%, MFB = 0%, MFE = 9%, R^2 = 0.91, Slope = 1.14 Rural site: MB = −0.53 ppb, ME = 5.00 ppb, RMSE = 6.52 ppb, MNB = −1%, MNE = 12%, NMB = −1%, NME = 11%, MFB = −3%, MFE = 12%, R^2 = 0.80, Slope = 0.80
Inverse-Distance Weighting								
Buteau et al. (2017)	IDW of data from fixed-site monitors	L: Montreal, Quebec, Canada; T: January 1, 1991–December 31, 2002; P: Entire population	Comparison with BME, back-extrapolation LUR, and fixed-site monitors	Short-term exposure	8-h daily max; mean (SD) = 28.1 ppb (13.0 ppb) median = 26.5 ppb	Low spatial variability of ozone may negate limitation	Quality of model depends on spatial density of monitors	ICC mean (95% CI) vs. fixed-site monitor = 0.89 (0.89, 0.89), vs. LUR w/back-extrapolation = 0.62 (0.59, 0.64), vs. BME = 0.76 (0.72, 0.78)

Table 2-9 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by spatial interpolations methods are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Yu et al. (2018)	IDW of data from 10 fixed-site monitors	L: Atlanta, GA; T: 2011; P: Entire population	Comparison with fixed-site monitors	Short-term exposure	NR	Better spatial resolution than monitor-based approaches	Quality of model depends on spatial density of the monitors	Urban site: MB = −1.27 ppb, ME = 2.94 ppb, RMSE = 4.31 ppb, MNB = −2%, MNE = 7%, NMB = −3%, NME = 7%, MFB = −3%, MFE = 7%, R^2 = 0.95, Slope = 1.05 Rural site: MB = −2.43 ppb, ME = 4.31 ppb, RMSE = 5.44 ppb, MNB = −6%, MNE = 11%, NMB = −5%, NME = 10%, MFB = −7%, MFE = 11%, R^2 = 0.88, Slope = 0.89

Table 2-9 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by spatial interpolations methods are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
					Kriging			
Joseph et al. (2013)	Ordinary and universal kriging	L: Houston and Los Angeles; T: Select days in 2009–2011	Comparison with 10–20 monitors in each metropolitan area	Short-term exposure	Houston = 31.0−112.5 ppb; Los Angeles = 10.8–117.1 ppb	Simple to implement	Overfitting may be caused by just one incorrect parameter, does not capture the underlying phenomena	Houston: n = 10 valid pts RMSE = 8.53−13.12 ppb, n = 20 RMSE = 7.56−12.72 ppb; Los Angeles: n = 10 valid pts RMSE = 12.55−19.30 ppb, n = 20 RMSE = 11.04−17.84 ppb
Liu et al. (2011)	Universal kriging	L: Eastern and midwestern U.S.; T: May 15–September 11, 1995 10:00–17:00; P: Entire population	Comparison of model points with concentrations from 375 monitors reporting to AQS	Long-term exposure	NR	As a traditional method, this is better established	Assumes linearity or some simplified function between sampling points	Kriging Model 1 Daily RMSE = 8.58–22.67 ppb; Kriging Model 2 Daily RMSE = 8.65–21.11 ppb

Table 2-9 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by spatial interpolations methods are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Joseph et al. (2013)	Ordinary and universal kriging	L: Houston and Los Angeles; T: Select days in 2009–2011	Comparison with 10–20 monitors in each metropolitan area	Short-term exposure	Houston = 31.0−112.5 ppb Los Angeles = 10.8–117.1 ppb	Yields superior validation compared to other methods	Overfitting may be caused by just one incorrect parameter	Houston: OK n = 10 valid pts RMSE = 7.01−10.39 ppb, n = 20 RMSE = 5.84−9.59 ppb UK n = 10 RMSE = 8.15−13.29 ppb, n = 20 RMSE = 6.36−10.42 ppb Los Angeles: OK n = 10 valid pts RMSE = 12.21−16.79 ppb, n = 20 RMSE = 10.20−19.22 ppb UK n = 10 RMSE = 12.43−18.96 ppb, n = 20 RMSE = 10.85−19.70 ppb

Table 2-9 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by spatial interpolations methods are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Kethireddy et al. (2014)	Ordinary kriging	L: Texas cities; T: 2012; P: Entire population	Comparison with monitors reporting to the Texas Air Monitoring Information System	Short-term exposure	Mean (SD) for select hours 3/25/2012 2:00 p.m. = 68.2 ppb (6.15 ppb) 4/24/2012 2:00 p.m. = 65 ppb (6.32 ppb) 5/17/2012 2:00 p.m. = 72.7 ppb (10 ppb) 6/28/2012 3:00 p.m. = 70 ppb (15.7 ppb) 7/21/2012 2:00 p.m. = 47 ppb (17 ppb) 8/20/2012 3:00 p.m. = 71 ppb (12 ppb)	Prediction uncertainty can be calculated	Accuracy depends on input data, proximity between points used to fit the model	For the same select hours ME = −0.000166 to 0.000407 RMSE = 0.004823 to 0.00956 standardized mean = −0.02046 to 0.0270 RMSE standardized = 0.714 to 1.099 avg std error = 0.00527 to 0.0105

Table 2-9 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by spatial interpolations methods are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Gelfand et al. (2012)	Kriging (approach is unspecified)	L: California; T: 2008; P: Entire population	Compare kriged ozone surface to monitors reporting to AQS; kriged surface is fit to high concentration monitors, low concentration monitors, randomly selected monitors, or all monitors	Long-term exposure	NR	Comparison of monitor selection allows for evaluation of best practices (i.e., use of randomly selected monitors); otherwise, selection of high monitors causes overestimation of concentrations and vice versa	Preferential sampling causes overestimation or underestimation	RMSE high monitors = 22.7 ppb, low monitors = 23.9 ppb, randomly selected monitors = 18.0 ppb, all monitors: 18.0 ppb

Table 2-9 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by spatial interpolations methods are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Yu et al. (2018)	Kriging of data from 10 fixed-site monitors	L: Atlanta, GA; T: 2011; P: Entire population	Comparison with fixed-site monitors	Short-term exposure	NR	Better spatial resolution than monitor-based approaches	Quality of model depends on spatial density of the monitors	Urban site: MB = −2.32 ppb, ME = 4.85 ppb, RMSE = 9.53 ppb, MNB = −4%, MNE = 11%, NMB = −5%, NME = 11%, MFB = −6%, MFE = 13%, R^2 = 0.74, Slope = 0.87 Rural site: MB = −4.34 ppb, ME = 5.19 ppb, RMSE = 8.35 ppb, MNB = −10%, MNE = 12%, NMB = −10%, NME = 12%, MFB = −12%, MFE = 14%, R^2 = 0.74, Slope = 0.82

AQS = Air Quality System; BME = Bayesian maximum entropy; ICC = interclass correlation coefficient; IDW = inverse-distance weighting; L = location; LUR = land use regression; MB = mean bias; ME = mean error; MFB = mean fractional bias; MFE = mean fractional error; MNB = mean normalized bias; MNE = mean normalized error; NMB = normalized mean bias; NME = normalized mean error; NR = not reported; OK = ordinary kriging; P = population; RMSE = root mean squared error; SD = standard deviation; T = time; UK = universal kriging.

Table 2-10 Studies informing assessment of exposure measurement error when concentrations modeled by land use regression or spatiotemporal models are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
				Land Use Regression				
Clark et al. (2011)	Land use regression with variables related to the built environment, climate, transportation, and income	L: 100 U.S. urban areas across the U.S.; T: May–September 1990; P: Entire population	Observed data used in model was validated	Short-term exposure	8-h daytime avg during ozone season arithmetic mean is 45 ppb	Observed data used	Observed data were sparse	Model LUR R^2 = 0.34
Adam-Poupart et al. (2014)	Land use regression mixed-effects model incorporating temperature, precipitation, day of year, road density, and latitude	L: Montreal, Quebec, Canada; T: May–September 1990–2009; P: Entire population	Cross-validation against NAPS monitoring data from 2005	Short- and long-term exposure	8-h daily max; NR	More accurate than BME variation in some cases	Output quality depends on quality of input data	R^2 = 0.466, RMSE = 8.747 ppb, percentage change in MSE = −19.9% (compared with a BME-LUR hybrid model)

Table 2-10 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by land use regression or spatiotemporal models are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Buteau et al. (2017)	Land use regression with back-extrapolation, LUR model built from 76 monitors and included variables for land use and the built environment	L: Montreal, Quebec, Canada; T: January 1, 1991–December 31, 2002; P: Entire population	Comparison with BME, IDW, and fixed-site monitors	Short-term exposure	8-h daily max; mean (SD) = 21.5 ppb (15.8 ppb) median = 17.5 ppb	Easy to implement	Depends on quality of independent variables used to fit model	ICC mean (95% CI) vs. fixed-site monitor = 0.67 (0.47, 0.78), vs. IDW = 0.62 (0.59, 0.64), vs. BME = 0.37 (0.16, 0.52)
Spatiotemporal Models								
Warren et al. (2012)	Two-stage model with Stage 1 with Bayesian kriging of weather patterns and Stage 2 as an underlying process specific to the pollutant	L: 13 counties in eastern Texas; T: 2002–2004; P: Preterm births	See prior references by Fuentes and Raftery (2005) and Fuentes (2009)	Long-term exposure	NR	Bayesian framework pulls information from different sources to minimize error	It is difficult to distinguish how ozone exposure is dealt with in the model, based on the data provided	NR

Table 2-10 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by land use regression or spatiotemporal models are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Chai et al. (2013)	U.S. National Air Quality Forecast Capability (NAQFC) 12-km horizontal resolution	L: CONUS; T: 2010; P: Entire population	Model compared with observed data from AQS	Short- and long-term exposure	Figure 4: between 15 and 50 ppb daily avg across domain Figure 5: daily average across urban, suburban, rural between 15 and 50 ppb Figure 6: across six region daily avg between 10 and 60 ppb; Figure 8: average at monitors for August 2010 between 8 and 50 ppb Figure 10: by average hour by region between 0 and 80 ppb	Validation method compared to observed data; multiple timescales explored; regional differences explored	Future predictions are not of interest	MB = 5.6 ppb RMSE = 15.4 ppb; weekly MB = 9.2 ppb
Adam-Poupart et al. (2014)	Bayesian maximum entropy model with priors from land use regression	L: Montreal, Quebec, Canada; T: May–September 1990–2009; P: Entire population	Cross-validation against NAPS monitoring data from 2005	Long-term exposure	NR	Accurate when monitoring stations clustered in study area	When monitors are not clustered where people live, model quality is reduced	R^2 = 0.414, RMSE = 9.164 ppb, percentage change in MSE = −23.0%

Table 2-10 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by land use regression or spatiotemporal models are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Wang et al. (2016)	Spatiotemporal-LUR incorporating the UCD-CIT chemical transport model with meteorology modeled by WRF v.3.1.1 on a 4-km grid, ST-LUR alone	L: Los Angeles and Riverside Counties; T: 2000–2008; P: Entire population	10-fold cross-validation against 37 monitors for each variation of model	Short- and long-term exposure	8-h daily max; range = 10−50 ppb (long-term avg)	Improved integration of different models, takes advantage of spatial residuals	Modeling approach used 2-week data, did not look at 8-h daily max	ST-LUR: RMSE = 5.64 ppb R^2 = 0.86; ST-LUR + CTM: RMSE = 4.65 ppb, R^2 = 0.87
Wang et al. (2015)	Spatiotemporal model drawing from universal kriging, spatiotemporal trend, and spatiotemporal residuals	L: Baltimore, Chicago, Los Angeles, New York, St. Paul, Winston-Salem; T: 1993–2013; P: MESA Air study participants	10-fold cross-validation against home and AQS monitors in each city	Short- and long-term exposure	2-week avg; NR	Low spatial variability of ozone concentration across space allows this method to be more applicable	Missing ozone data during cold seasons may limit the applicability of the model	Overall cross-validation MSE R^2: Baltimore = 0.90, Chicago = 0.71, Los Angeles = 0.67, New York = 0.60, St. Paul = 0.91, Winston-Salem = 0.66 Overall cross-validation R^2: Baltimore = 0.89, Chicago = 0.72, Los Angeles = 0.78, New York = 0.61, St. Paul = 0.90, Winston-Salem = 0.76

Table 2-10 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by land use regression or spatiotemporal models are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Xu et al. (2016a)	Partial least squares model with or without universal kriging	L: Baltimore, MD; T: February 11–22, 2012 and June 18–27, 2012; P: Participants in the MESA-Air study	LOOCV, using comparison with measurements obtained on a mobile monitoring platform	Short-term exposure	2-week avg; range Summer = 50.5−93.6 ppb, Winter = 20.6−37.4 ppb	PLS + UK model accounts well for spatial residuals	There would be more confidence in the cross-validation if the monitoring periods were run for more days; averaging times for cross-validation monitors varied by season, meteorology was not accounted for	LOOCV R^2 PLS summer = 0.55 winter = 0.40, PLS + UK summer = 0.71 winter = 0.58
Sahu and Bakar (2012b)	Bayesian autoregressive models	L: Eastern U.S.; T: 1997–2006; P: Entire population	Comparison of model variations with concentrations from 69 fixed-site monitors (622 sites used to fit the model)	Long-term exposure	Range: annual 4th highest = 48.5−109 ppb, 3-yr avg = 50.6–100.2 ppb	Accurate, good representation of both spatial and temporal variability, can be used for downscaling CTMs	Because the model takes meteorological inputs, it cannot be validated with meteorological data	Annual fourth highest: RMSE = 5.24 ppb MAE = 4.17 ppb; 3-year avg RMSE = 4.21 ppb, MAE = 3.36 ppb

Table 2-10 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by land use regression or spatiotemporal models are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Sahu and Bakar (2012a)	Dynamic linear and Bayesian autoregressive models	L: New York State; T: July–August 2006; P: Entire population	Comparison of model variations with concentrations from 4 fixed-site monitors (25 sites used to fit the model)	Short-term exposure	Range: median of 8-h daily max = 25–70 ppb	Autoregressive model performs well	Model performance depends on parameter selection; need to run the model for the same domain for which it is fit	MSE dynamic linear model = 58.42 ppb^2 Autoregressive model = 46.16 ppb^2 (RMSE dynamic linear model = 7.64 ppb, autoregressive model = 6.79 ppb)
Buteau et al. (2017)	Bayesian maximum entropy model drawing output from a land use regression as its prior	L: Montreal, Quebec, Canada; T: January 1, 1991–December 31, 2002; P: Entire population	Comparison with IDW, back-extrapolation LUR, and fixed-site monitors	Short-term exposure	8-h daily max; mean (SD) = 30.0 ppb (9.1 ppb) median = 29.8 ppb	Captures spatial variability more completely	Higher complexity compared with other models	ICC mean (95% CI) vs. fixed-site monitor = 0.64 (0.41, 0.77), vs. IDW = 0.76 (0.72, 0.78), vs. LUR w/back-extrapolation = 0. 37 (0.16, 0.52)

Table 2-10 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by land use regression or spatiotemporal models are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Gong et al. (2017)	HYSPLIT v4.9 with GDAS 1° × 1° data with a GAM, satellite data from HMS	L: Eight western U.S. cities (i.e., Houston, Boise, Denver, Fort Collins, Provo, Salt Lake City, Spokane); T: May to September for 2008 to 2015; P: Entire population	Model compared (and built) with surface, fixed-site monitoring data	Short- and long-term exposure	8-h daily max ozone for Houston between 0 and 120 ppb; Table 3: obs 8-h daily max ozone mean = 39.29 ppb, no smoke n = 1,082, smoke n = 41, no smoke residuals = −0.33, smoke residuals = 8.10; 8-h daily max ozone for Provo site between 20 and 80 ppb	Multiple models used together (e.g., HYSPLIT and HMS and obs data)	Limited cities explored in western U.S.	Model-obs comparison R^2 = 0.816 for Houston

Table 2-10 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by land use regression or spatiotemporal models are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Chang et al. (2015)	Stochastic partial differential equation	L: Worldwide; T: 2000–2005; P: Entire population	Cross-validated model against measurements across the world	Long-term exposure	NR (graphed for entire world but not reported)	Model performs better over longer time periods	Large domain means local problems with model fitting	Cross validation SE December–February = 6.55−11.95 ppb March–May = 5.86–9.96 ppb June–August = 6.13−8.65 ppb September–November = 5.82−11.23 ppb

AQS = air quality system; BME = Bayesian maximum entropy; CI = confidence interval; CTM = chemical transport model; GAM = generalized additive model; GDAS = Global Data Assimilation System; HMS = Hazard Mapping System; ICC = interclass correlation coefficient; IDW = inverse-distance weighting; L = location; LOOCV = leave one out cross validation; LUR = land use regression; MAE = mean absolute error; MB = mean bias; MESA = Multiethnic Study of Atherosclerosis; MSE = mean squared error; NAPS = National Air Pollution Surveillance; NAQFC = National Air Quality Forecast Capability; NR = not reported; P = population; PLS = partial least squares; RMSE = root mean squared error; SD = standard deviation; SE = standard error; ST = spatiotemporal; T = time; UCD-CIT = University of California at Davis-California Institute of Technology model; UK = universal kriging; WRF = Weather Research and Forecasting model.

Table 2-11 Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Hutzell et al. (2012)	CMAQ modeled output version 4.7.1 with a 36-km grid and nested 12-km grid	L: Eastern CONUS; T: January and July in 2002; P: Entire population	CMAQ with SAPRC07T mechanism compared with SAPRC−99 mechanism; both mechanisms compared with observed fixed-site monitoring data	Short-term exposure	NR (shown graphically)	Specific mechanism in CMAQ investigated during and not during the ozone season	Modeling time is relatively short, so an epi application may be limited	Mechanisms and observed data compared January NMB = −16 to 16 ppb, July NMB = −20 to 20 ppb; R^2 = 0.7–0.8, January RMSE = 7.46–7.59 ppb, July RMSE = 12.4–13.6 ppb, January MB = −1.11 to −1.37 ppb, July MB = 5.2–7.1 ppb, January NMB = −4.1 to −5.1 %, July NMB = 9.2–12.6%, January ME = 5.77–5.86 ppb, July ME = 9.73−10.6 ppb, January NME = 1.5–21.8%, July NME = 17.2−18.8 % between the two mechanism

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Li et al. (2012)	CMAQ 4.6 with nested grids (36 and 12 km) going down to 4-km grid size	L: Southeast Texas; T: 3 weeks of hourly ozone between August 16 and September 6, 2000; P: Entire population	CMAQ with SAPRC07 mechanism compared with SAPRC99 mechanism; both mechanisms compared with observed fixed-site monitoring data	Short-term exposure	8-h avg during ozone episode; hourly ozone NR (shown graphically)	The scale of the CMAQ data was very fine, and the specific mechanisms were directly compared	Only 3 weeks of data investigated	MFE by site for S99 = 0.14–0.33 ppb and 0.25 ppb overall, MFE by site for S07 = 0.17–32 ppb and 0.25 ppb overall, MFB by site for S99 = −0.21 to−0.04 ppb and −0.12 overall, MFB by site for S07 = −0.24 to −0.07 ppb and −0.16 overall, accuracy of paired peak by site for S99 = −0.29–0.00 ppb and −0.16 overall, accuracy of paired peak by site for S07 = −0.29 to −0.05 ppb and −0.20 overall, n = 27–138 and 1,887 overall comparing mechanisms to observed data

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Emery et al. (2012)	GEOS-Chem 8-03-01 on a 2- × 2.5-degree grid, CAMx 5.30 run on a 36-km and then 12-km grid size	L: CONUS; T: Hourly ozone data for all of 2006; P: Entire population	Comparison done between GEOS-Chem and collocated observed data	Short- and long-term exposure	Summer average for 2006; NR (shown graphically)	Two different modeling methods are compared and are both are compared to observed data	The coarse grid of GEOS-Chem may be a weakness	R^2 for CAMx = 0.34–0.61, R^2 for GEOS-Chem = 0.21–0.66 between observed and modeled; R^2 = 0.50 of fraction of days >60, >65, >70 ppb for CAMx and R^2 = 0.42, 0.47, 0.47 for GEOS-Chem and R^2 = 0.42, 0.30, 0.25 for GEOS-Chem, number of days with certain ozone ranges 0–240

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
McDonald-Buller et al. (2011)	GEOS-Chem (it is not clear the version of GEOS-Chem used with resolution 0.5 × 0.67 degree), on a 4- × 5-degree global grid	L: CONUS, with global comparisons of TES, OMI, GEOS-Chem (TES AK), and GEOS-Chem (OMI AK); T: 8-h daily max from March–May 2006 compared with June–August 2006, with results displayed between 2006 and 2008; P: Entire population	Comparison done with fixed-site CASTNet monitors, global scales compared with OMI, TES, GEOS-Chem (TES AK), and GEOS-Chem (OMI AK)	Short- and long-term exposure	NR (shown graphically)	Modeled output compared to observed data and other modeled data	Assumption that observed data is representative	No exposure measurement error presented in tables

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Ying and Li (2011)	CMAQ-MCM (Master Chemical Mechanism) with three nested domains (36 km, 12 km, 4 km), CMAQ 4.6 with MCM 3.1	L: Houston-Galveston Bay area; T: 3-week ozone episode period between August 16 and September, 2000; P: Entire population	CMAQ-MCM compared with CMAQ with SAPRC07 (version not stated) with fixed-site U.S. EPA monitors from the AIRS database	Short term exposure	Hourly ozone differences between the two models ranged from 4–12 ppb for averaged across the ozone episode	Very fine scale spatial resolution, two modeled compared, both modeled compared with observed data	Only measured during an ozone episode, so longer ozone values may be less represented	Bias between the two models between 4 and 12 ppb

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Schere et al. (2011)	CTM of CMAQ using the boundary conditions of GEOS-Chem 8-03-01 (not clear the CMAQ version or grid cell resolution), CHIMERE at a 0.25-degree horizontal resolution (note clear on the CHIMERE version)	L: CONUS, Western Europe; T: Hourly ozone data for the entirety of 2006; P: Entire population	CMAQ over CONUS compared with observed ozonesonde, CHIMERE with AQMEII boundary conditions compared with typical CHIMERE boundary conditions over CONUS, CMAQ with AQMEII boundary conditions compared with GEOS-Chem boundary conditions over Western Europe, CHIMERE with 3-h boundary conditions compared with monthly climatology over Western Europe	Short-term exposure	NR (shown graphically)	Varying boundary conditions of multiple models compared over different parts of the world also compared with observed data, vertical profiles also explored	Not clear the version of each model	Seasonal differences in surface ozone MB = −20 to 20 ppb, MB for four different 3-month periods between = −3 to 3 ppb, difference in standard deviation of modeled ozone = 0–2.5 ppb
Brauer et al. (2012)	TM5 CTM model with 0.1 degree resolution	L: global model; T: 1990, 2005; P: Entire population	TM5 data evaluated elsewhere	Long term exposure	NR (shown graphically)	Multiple data sources merged together including observed data	Only one data source available for ozone	No exposure measurement errors found in tables or figures

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Simon et al. (2013)	CMAQ 4.7.1 with 12-km horizontal resolution	L: Eastern U.S. from CONUS; T: Hourly ozone in July and August 2005; P: Entire population	Modeled output compared with observed ozone data from AQS coming from fixed-site monitors; CMAQ 4.7.1 using brute force emissions changes	Short-term exposure	NR (shown graphically)	A NAAQS application was applied to the method, method allows for specification with chemical processes with HDDM, method clearly explained; method compared to observed data	Greater uncertainties associated with introducing a new process to the CMAQ model, results only shown for selected monitors; model only run for 2 mo	RMSE between HDDM and brute-force method for selected stations by ozone concentration, RMSE = 6 ppb at Charlotte site, RMSE = 4–7 at Detroit site

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Tsimpidi et al. (2012)	CMAQ 4.7 with 12-km horizontal resolution with nested 4-km grid in Seattle with a DDM-3D	L: Seattle, WA; T: Hourly data for July 12–24, 2006; P: Entire population	CMAQ 4-km resolution compared with 12 and 36 km, compared with observed data from fixed-site monitors from AQS	Short-term exposure	NR (shown graphically)	Method was compared to observed data; the paper explored the effect of grid resolutions	This study only looks at a 12 days study period, so this study is not appropriate for long-term exposure, the short time period does not capture low values well, the short time period would not be representative of typical long-term exposures	Comparing modeled to observed to hourly, maximum hourly, max 8-h ozone to different grid resolutions MB, GE, RMSE, NMB, NME, mean obs = 30.2–57.0 ppb for 4 km, 30.3–57.0 ppb for 12 km, 38.3–65.1 ppb for 36km, mean mod = 39.1–43.1 ppb for 4 km, 39.1–58.2 ppb for 12 km, 48.6–68.9 ppb for 36 km, n 68–1,610 for 4 km, 138–3,283 for 12 km, 9,375–226,597 for 36 km, MB = −4.5 to 12.9 ppb for 4 km,

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Tsimpidi et al. (2012) (cont.)	CMAQ 4.7 with 12-km horizontal resolution with nested 4-km grid in Seattle with a DDM-3D (cont)	L: Seattle, WA; T: Hourly data for July 12–24, 2006; P: Entire population (cont.)	CMAQ 4-km resolution compared with 12 and 36 km, compared with observed data from fixed-site monitors from AQS (cont.)	Short-term exposure (cont.)	NR (shown graphically) (cont.)	Method was compared to observed data; the paper explored the effect of grid resolutions (cont.)	This study only looks at a 12 days study period, so this study is not appropriate for long-term exposure, the short time period does not capture low values well, the short time period would not be representative of typical long-term exposures (cont.)	−6.2 to 8.8 ppb for 12 km, 1.5–10.9 ppb for 36 km, MAGE = 14.3–18.5 ppb for 4 km, 14.3–15.8 ppb for 12 km, 12.4–16.1 ppb for 36 km, RMSE = 19.4–22.2 ppb for 4 km, 17.7−19.6 ppb for 12 km, 16.5–20.9 ppb for 36 km, NMB = −7.9 to 42.7% for 4 km, −11.0–28.9% for 12 km, 2.6−26.8% for 36 km, NME = 25.1–61.3% for 4 km, 25.1–52.2% for 12 km, 21.5–42.0% for 36 km; sensitivity of ozone from NO_X and VOC is percentage change in ppb = 0.000–0.050%

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Ferreira et al. (2012)	CAMx (version unclear, but its citation is from 2010) on a 24-km grid, MM5 3.7 (the met data) on a 1-degree grid, emissions in a 12-km grid based on NEI, Canadian emissions inventory, 1999 Mexican BRAVO inventory, biogenic emissions from BEIS 3.14, fire emissions from HMS and SMARTFIRE (2006), point sources from Continuous Emissions Monitoring data	L: North America; T: Hourly ozone data from all of 2006; P: Entire population	Comparison to observed fixed-site monitors	Short- and long-term exposure	Summer 2006 daily ozone = between 20 and 45 ppb	Modeled output compared to observed data, the method focuses on three particular ozone periods of concern	Although an MM5-CAMx combination is part of the AQMEII initiative, the method was not compared to any other modeling method, inputs of emissions inventory and met data has poorer performance	Correlation between modeled and observed as a function of RMSE and normalized SD, R = 0.5–0.6

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Hu et al. (2012)	UCD-CIT model	L: San Joaquin Valley, CA and entire state of California; T: July 27–August 2, 2000; P: Entire population	Compare model results from two photochemistry modules with concentrations from CARB observation sites	Short-term exposure	Mapped concentrations from different emissions sources but did not tabulate	Study designed to interpret different emissions sources' impacts on concentrations and to evaluate different model variations	4-km resolution not fine enough to detect local gradients and small-scale variation in sources	SAPRC-07: 1-h daily max SJV bias = −15.3 ppb NB = −15.6% gross error 15.6 ppb NGE = 16.0%, domain bias = −12.7 ppb NB = −14.5% gross error = 13.6 ppb NGE = 15.6%, 8 h daily max SJV bias = −12.6 ppb NB = −14.4% gross error = 12.8 ppb NGE = 14.7%, domain bias −10.8 ppb NB = −13.5% gross error = 11.5 ppb NGE = 4.4%; SAPRC-99 = 1-h daily max SJV bias = −0.2 ppb NB = −0.2% gross error = 6.2 ppb NGE = 6.3%, domain bias = −3.6 ppb NB = −4.1%

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Hu et al. (2012) (cont.)	UCD-CIT model (cont.)	L: San Joaquin Valley, CA and entire state of California; T: July 27–August 2, 2000; P: Entire population (cont.)	Compare model results from two photochemistry modules with concentrations from CARB observation sites (cont.)	Short-term exposure (cont.)	Mapped concentrations from different emissions sources but did not tabulate (cont.)	Study designed to interpret different emissions sources' impacts on concentrations and to evaluate different model variations (cont.)	4-km resolution not fine enough to detect local gradients and small-scale variation in sources (cont.)	gross error = 11.5 ppb NGE = 13.1%, 8-h daily max SJV bias = −0.5 ppb NB = −0.5% gross error = 5.7 ppb NGE = 6.6%, domain bias = −2.7 ppb NB = −3.3% gross error = 9.6 ppb NGE = 12.0%
Liu and Zhang (2011)	CMAQ 4.4 over CONUS at 32-km horizontal resolution with MM5 3.4 with NEI 3	L: CONUS; T: hourly ozone from June 12–28 1999; P: Entire population	Comparison of U.S. EPA observed fixed-site monitors	Short-term exposure	1-h mean obs = 53.0–60.3 ppb, 1-h mean mod = 62.0–67.4 ppb, 1 h n = 84–14,659; 8-h daily max mean obs = 46.6–55.0 ppb, 8-h daily max mean mod = 58.3–62.2 ppb, 8-h daily max n = 82–14,619	Many evaluation methods: both the horizontal grids and vertical grids (through flight data) were evaluated with observed data, satellite data used	The horizontal resolution was coarse; modeling period was short and specific so epi application will not be representative of longer term exposure	1-h R = 0.7–0.8, 1-h NMB = 4.9−17.0 %, 1-h NME = 15.6−25.7 %, 8-h daily max R = 0.8–0.8, 8-h daily max NMB = 8.5–25.0%, 8-h daily max NME = 17.0–30.1%

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Wang et al. (2012)	CMAQ 4.7 horizontal grid resolution of 108 km with a dust component called CMAQ-Dust with incorporation of ISORROPIA II, WRF 3.2, 1999 NEI 1	L: Global model; T: April 2001; P: Entire population	CMAQ-Dust compared to observed data from AQS; several versions of the CMAQ model compared with observed data from different U.S. EPA fixed-site monitoring networks	Short-term exposure	In U.S. mean max 1 h modeled from ozone = 43.4−54.1 ppb; AIRS 8-h daily max mean mod = 45.4–51.1 ppb; AIRS max 1 h n = 29,993, mean obs = 52.7 ppb, mean mod = 48.7–54.1 ppb; in Beijing mean 1 max h ozone is 86.8−112.4 ppb, max 1 h, n = 30, mean obs = 95.8 ppb, mean mod = 86.8–109.9 ppb	The module introduced is highly specialized; global model used	The short modeling period may not be appropriate in a long term epi study and may not be representative of exposures outside of this time window; coarse resolution is very coarse, making exposure assignment in an epi study have potential misclassification	In U.S. during dust episode between obs and modeled, R = 0.48–0.54, NMB = −7.3–2.8%, NME = 16.6−18.5%, R = 0.46–0.53, NMB = −4.7 to 6.8%, NME = 17.9–18.8%; R= −0.03 to 0.06, NMB = −9.36 to 17.3%, NME = 25.5–30.6%; ozone difference spatially = −1.5 to 1.5 ppb

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Yu et al. (2012)	CMAQ (version NR) 12 km where WRF-ARW (Advanced Research WRF 3.0) and WRF-NMM are compared	L: Eastern U.S. with flight data over east Texas; T: Hourly data from August 1–October 15, 2006; P: Entire population	Two different modeling methods were compared with observed data from U.S. EPA's fixed-site monitors and observed data from air planes with flight paths and ship data in port	Short-term exposure	1 h for model ARW (model NMM), n = 51,532, mean obs = 48.6 ppb, mean mod = 56.2 ppb (56.7 ppb), 8-h daily max mean obs = 42.7, mean mod = 50.4 ppb (52.0 ppb); mean ± SD for obs = 36.38 ± 24.13	Multiple comparison methods with two different types of modeled and multiple types of observed data (e.g., fixed-site, flight data, ship data)	Version of CMAQ never stated; vertical validation not applicable for an epi setting; because the study was short term, ambient concentrations may not be representative of a longer term exposure	1 h for model ARW (model NMM) MB = 7.5 ppb (8.1 ppb), RMSE = 13.4 ppb (13.9 ppb), NMB = 15.5% (16.7%), NME = 22.3% (22.8%), *R* = 0.76 (0.75), 8-h daily max mean MB = 7.7 ppb (9.3 ppb), RMSE = 12.6 ppb (13.8 ppb), NMB = 18.0% (21.8%), NME = 24.2% (26.4%), *R* = 0.76 (0.74); NMB by ozone concentration = −9.7 to 48.3 ppb; time series between the two WRF models, MB = 2−14 ppb, RMSE = 8–16 ppb,

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Yu et al. (2012) (cont.)	CMAQ (version NR) 12 km where WRF-ARW (Advanced Research WRF 3.0) and WRF-NMM are compared (cont.)	L: Eastern U.S. with flight data over east Texas; T: Hourly data from August 1–October 15, 2006; P: Entire population (cont.)	Two different modeling methods were compared with observed data from U.S. EPA's fixed-site monitors and observed data from air planes with flight paths and ship data in port (cont.)	Short-term exposure (cont.)	1 h for model ARW (model NMM), n = 51,532, mean obs = 48.6 ppb, mean mod = 56.2 ppb (56.7 ppb), 8-h daily max mean obs = 42.7, mean mod = 50.4 ppb (52.0 ppb); mean ± SD for obs = 36.38 ± 24.13 (cont.)	Multiple comparison methods with two different types of modeled and multiple types of observed data (e.g., fixed-site, flight data, ship data) (cont.)	Version of CMAQ never stated; vertical validation not applicable for an epi setting; because the study was short term, ambient concentrations may not be representative of a longer term exposure (cont.)	NMB = 0−0.5%, NME = 0.1–0.5%, *R* = 0.3–0.9; mod NMM = 40.07 ± 22.46, mod ARW = 41.33 ± 20.36, NMB NMM = 10.1%, NMB ARW = 13.6%
Godowitch et al. (2011)	CMAQ 4.7, 12-km horizontal resolution, MM5 3.7.4, SMOKE 2.2	L: Eastern U.S.; T: June–August 2002 hourly ozone data; P: Entire population	Compared with observed data from U.S. EPA's CASTNet, observed data from flights taken in the afternoon of July 2002	Short-term exposure	Daily max 8 h ozone during each day of study period = 40–120 ppb	Methods clearly explained, multiple observed data for evaluation methods	Short term exposure values not representative of longer term exposures	Time series mean of 8-h daily max = 40−80 ppb; 95% time series of 8-h daily max = 55−120 ppb

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Bravo et al. (2012)	CMAQ 4.5.1 12-km horizontal resolution	L: Eastern U.S.; T: 2002; P: Entire population	Comparing CMAQ outputs to observed data	Long-term exposure	County-level seasonal average (April−September) = 25.1–70.0 ppb	Explicit county-level aggregations between two methods were compared	No new method developed, only an evaluation of CMAQ at a county level	Monthly NMB between −2 to 12%; annual average NMB in southeastern U.S. = 10–30% *R* > 0.80 in upper Southeast, Northeast, Ohio River Valley, 0.61−0.80 in Florida, Gulf Coast, Great Lakes
Carlton and Baker (2011)	CMAQ 4.7.1 12-km horizontal resolution, AERO5, CB05, WRF 3.1, 2001 NEI 2 and BEIS 3.14 compared with MEGAN 2.04	L: Oark region of the U.S. covering Missouri, Illinois, Indiana, Kentucky, and northern Arkansas; T: Hourly ozone data from June 15–July 31, 1998; P: Entire population	Biogenic emission from both BEIS 3.14 and MEGAN 2.04 compared with fixed-site monitors (AIRS, CASTNet/IMPROVE network), balloon and aircraft measurements	Short-term exposure	Hourly ozone; NR (shown graphically)	Comparison of biogenic emissions has the specificity to understand the crux of ozone differences in areas with higher isoprene emissions; comparison methods were thorough with comparing two types of modeled data with multiple sources of observed data	Given the short time period, concentrations over a month and half may not be indicative of more long-term exposure; the area is relatively rural so precursor emissions may vary in other parts of the U.S. where more population may be affected	1-1 line with observed data and difference modeled data but not correlation calculated; no clear exposure measures related to ozone found in text, figures, or tables

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Steyn et al. (2013)	WRF 3.1, SMOKE 2.5, CMAQ 4.7.1 with 4-km horizontal resolution, model compared with NRC MM5/CMAQ for 2001, model compared with CALGRID and UAM for 1985	L: Metro Vancouver, Canada; T: July 19–21, 1985, July 17–19, 1995, August 10–12, 2001, June 24–26, 2006; P: Entire population	Models compared with each other, all model compared with historical surface, fixed-site monitors from Canada's NAPS, aloft observed data from aircraft in 1995	Short- and long-term exposure	CMAQ (NRC) for 2001 n = 1,717 (5,948), mean mod = 26.8 ppb (21.8 ppb), mean obs = 21.2 ppb (19.2 ppb); mean mod across years = 21.8–27.6 ppb, mean obs across years = 16.8–27.7 ppb	Very few studies explore long-term concentrations; multiple types of obs data; multiple models compared	Difficulties in validating historical emissions	For CMAQ (NRC) for 2001 MB = 5.7 ppb (2.6 ppb), NMB = 5.7% (13.3%), ME = 11.5 ppb (9.8 ppb), NME = 54.4% (51.2%); for stations T12 in 1985 CMAQ (CALGRID, UAM) MAE = -20.0 ppb (25.0 ppb, 28.2 ppb), RMSE = 23.7 ppb (29.5 ppb, 33.1 ppb), IOA = 0.79 (0.57, 0.44)

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Zhou et al. (2013)	CMAQ 4.7 12-km horizontal resolution, MM5 3.6.3, SMOKE 2.2	L: Eastern U.S.; T: 2002 and 2006; P: Entire population	Observed data from surface fixed-site monitors were compared with CMAQ; 2002 compared with 2006; NO_X SIP Call region compared with data outside of NO_X SIP Call region	Long-term exposure	Only ozone differences are explored and direct ozone concentrations are not explored	There are multiple comparison of this paper: modeled to observed, data from 2002 compared with 2006; ozone by percentage; inside NO_X SIP Call area vs. outside	The paper recognizes the issues of long-range transport of ozone	Relative difference between quantities of obs and mod, in SIP call region (obs is reference): average change (2002−2006) in 8-h daily max = 42.5%, average percentage change in 8-h daily max = 38.9%; outside SIP call region: average change (2002−2006) in 8-h daily max = 66.7%, average percentage change in 8-h daily max = 69.7%

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Appel et al. (2012)	CMAQ 4.7.1 12-km horizontal resolution, met data from both GEOS-Chem and GEMS	L: North America and Europe; T: 2006; P: Entire population	CMAQ-GEMS compared with CMAQ-GEOS-Chem; CMAQ in North American compared with U.S. EPA data and CMAQ in Europe compared with AirBase data	Long-term exposure	NR (shown graphically)	Several different comparison models and observed data used	This may be less amenable to shorter term exposures	MB winter = −3.5 ppb spring −1.8 ppb summer = 4.4 ppb fall = 2.6 ppb; NMB winter = −13.4% spring = −4.1% summer = 9.8% fall = 8.4%; ME winter = 9.0 ppb spring = 9.3 ppb summer = 11.0 ppb fall = 8.8 ppb; NME winter = 34.7% spring = 29.4% summer = 24.2% fall = 28.0%
Cho et al. (2012)	CMAQ (version unknown), MM5 with 4-km horizontal resolution, emissions data from 2006 compared with 2002	L: East Alberta, Canada; T: May–August 2002: P: Entire population	Emissions data compared to each other, all modeled outputs compared to surface, fixed-site observed data (origin of monitors unknown)	Short-term exposure	NR (shown graphically)	Fine scale spatial resolution; complete spatial coverage with CMAQ	Version of some of the model components are unclear; a 4-mo exposure window may not be indicative of a more long term exposure	Hourly ozone at 4 km resolution, May–August across all sites, no threshold: FB = 13%, FE = 39%; 40 ppb threshold: FB = 16%, FE = 20%

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Herwehe et al. (2011)	CMAQ 4.7, WRF-ARW 2.2 compared with WRF/Chem 3.0.1.1 with 12-km horizontal resolution	L: CONUS, observed aloft data from Centreville and Birmingham, AL; T: August 2006; P: Entire population	WRF CMAQ compared with WRF/Chem, modeled data compared with AQS and SEARCH fixed-site surface monitors, observed aloft ozone data	Short-term exposure	NR (shown graphically)	Modeled differences are specific enough to pinpoint differences in modeled ozone; several difference sources of ozone data explored (e.g., fixed site and aloft data)	Given the short time period of the model run, concentrations may not be indicative of typical ozone exposures	RMSE = 11.52 ppb (13.57 ppb), NME = 18.2% (21.5%), MB = 3.62 ppb (6.18 ppb), NMB = 7.4% (12.7%), R = 0.72 (0.66)
Wong et al. (2012)	CMAQ 4.7.1 12-km horizontal resolution with two-way coupled WRF-CMAQ model	L: Portion of California and surrounding states; T: June 20–29, 2008; P: Entire population	Coupled WRF-CMAQ compared with offline WRF with CMAQ, both methods are compared with fixed-site observed monitoring data from AQS	Short-term exposure	NR (shown graphically)	A highly specialized modeled component pin points the differences in modeled ozone; comparisons are made with observed data	The model run is only for a few days during a wildfire; therefore, short-term exposures may be higher than a typical concentration	Comparison between mod and obs: all data (daytime) slope = 0.98, R = 0.62; when AOD > 0.5 slope = 1.2, R = 0.75

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Bond et al. (2013)	CMAQ 4.5.1 with CAMx 4.42 both with MM5 3.7 and a 4-km horizontal resolution	L: South-eastern U.S. in North Carolina, northeastern Georgia, South Carolina, Tennessee, Virginia, and Kentucky; T: Hourly ozone in January and July 2002; P: Entire population	Both CMAQ and CAMx are compared to each other; both modeled compared to observed fixed-site monitors from AQS, CASTNet, SEARCH, and NCDENR	Short-term exposure	January 2002: Mean 1-h max = 31.8–42.0 ppb, 8-h max = 27.5–39.0 ppb across CMAQ and CAMx at locations of AQS, CASTNet, and SEARCH monitors; January 2002: Mean 1-h max = 59.8–74.7 ppb, 8-h max = 55.7−67.3 ppb	Fine-scale horizontal resolution; two different models compared with both model compared to observed data; errors with observed data presented by monitoring network	Short-term exposure during only 2 mo may not be indicative of typical, long term exposures	1-h max, n between 62 and 384, R = 0.5–0.7, NMB = −7.6 to 10.1%, NME = 15.4–24.5%, and 8-h max, n = 61–384, R = 0.6–0.7, NMB = 0.1–15.8%, NME = 19.2–25.4%
Kaynak et al. (2013)	CMAQ 4.5 36-km horizontal resolution	L: CONUS; T: Hourly ozone July 1–August 31, 2004; P: Entire population	Ground-level CMAQ compared with fixed-site U.S. EPA monitors from AIRS, SEARCH, and CASTNet; vertical profiles of CMAQ compared with ICARTT data which includes aircraft, ship, and ozonesonde data	Short-term exposure	NR (shown graphically)	CMAQ data are compared both to surface data and aloft data from multiple sources	Short-term exposure during only 2 mo may not be indicative of typical, long term exposures	CMAQ to observed for the whole U.S., n = 1,267, R = 0.15, MB = 9.191 ppb, RMSE = 12.181 ppb, MNB = 34.77%, MNE = 37.38%; CMAQ vs. ICARTT R^2 = 0.51; obs vs. CMAQ R^2 = 0.15; CMAQ vs. observed, n = 363, R^2 = 0.43, MB = 5.457 ppb, RMSE = 20.856 ppb, MNB = 10.49%, MNE = 29.93%

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Ngan et al. (2012)	CMAQ 4.6 in nested models at 36-, 12-, and 4-km resolution with nudging of the meteorological fields, called "retrospective forecasting" in the paper	L: Greater Houston area, TX; T: August 23–September 9, 2006; P: Entire population	Comparison with monitors reporting to AQS	Short-term exposure	NR (shown graphically)	Nudging of the meteorological variables improves ozone predictions (based on comparison with monitors)	Limitations in prediction of precipitation may cause error because photolysis is influenced by cloudiness	Regular forecasting: U *R* = 0.49, RMSE = 1.60; V *R* = 0.68, RMSE = 1.97 Retrospective forecasting: U *R* = 0.75, RMSE = 1.04; V *R* = 0.82, RMSE = 1.20
Weir et al. (2013)	CMAQ model on a 36-km horizontal resolution (unclear which CMAQ version was used and how CMAQ exposure assignment happened) compared with annual averaged concentrations from observed data from fixed-site monitors from U.S. EPA's AQS using inverse-distance weighting of all monitors within 20 miles	L: U.S.; T: 2005–2006; P: NHANES participants (considered represent-ative of the entire population)	Inverse-distance weightings compared with CMAQ	Long-term exposure	Annual observed ozone (1 yr prior to study participant examination) = 37.5–60.3 ppb mean = 51.5 ppb median = 52.0 ppb; annual CMAQ ozone = 45.6−70.8 ppb mean = 57.2 ppb median = 57.0 ppb	Observed data were compared with CMAQ data; there is an epi application	The coarseness of the exposure assessments may lose spatial heterogeneity; inverse-distance weighting is a crude exposure assignment method	*R* = 0.66 between observed and CMAQ data

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Jaffe et al. (2013)	CMAQ 4.5.1 with 36-km horizontal resolution MM5 3.7 in summer 2012, WRF-Chem 3.2 with 24-km horizontal resolution between June 10 and July 10, 2008, multilinear relationship between 8-h daily max from June–September between 2000 and 2012 for Salt Lake City, Boise, and Reno using observed data only	L: Salt Lake City, Boise, Reno; T: June–September 2000–2012; L: Western U.S.; T: June 10–July 10, 2008; L: CONUS; T: summer 2012; P: Entire population	CMAQ and WRF-Chem have been validated in previous publications; multilinear modeled not validated, but assessed	Short- and long-term exposures	Obs n = 1,449−1,586, obs min = 17.0−24.8 ppb, obs maximum = 82−101.5 ppb, obs mean = 50.9−55.8 ppb, and obs SD = 8.4–11.0	Several different data sources used (e.g., monitoring data, CMAQ, and WRF-Chem), variety of timescales explored	Modeled data never directly compared with obs data; for the short time window, exposure may not be indicative of longer termed exposure	NR

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Chen et al. (2013)	WRF-Chem 3.1.1 4 km horizontal resolution	L: Los Angeles basin, CA; T: May 15–June 15, 2010; P: Entire population	WRF-Chem compared with Caltech supersite, CARB sites, aloft data from NOAA WP-3D from flights on May 4, 14, 19, 20, and June 20	Short-term exposure	Average for May 15–June 8 2010 = 9.1−62.7 ppb	Fine horizontal resolution; multiple observed data set used; both surface and aloft ozone data collected; exploration of NEI emissions	Short-term exposure may not be indicative of typically exposures	(CalNEX supersite) MB = −10.6 ppb, RMSE = 12.2 ppb, R^2 = 0.63; by site MB = −14.6 to −1.1 ppb, RMSE = 10.5–12.6 ppb, R^2 = 0.12–0.74; (CalNEX supersite) RMSE = 12.22 ppb, R^2 = 0.63
Choi (2014)	CMAQ 4.7.1 over CONUS with 12-km horizontal resolution over August 2009 with baseline emissions compared with NO_X satellite-adjusted emissions	L: CONUS; T: August 2009; P: Entire population	Baseline and NO_X satellite-adjusted ozone compared to each other; each method compared with surface, fixed-sited U.S. EPA AQS data	Short-term exposure	NR (shown graphically)	Entire CONUS covered; specific input change of emissions inventory and pin point ozone differences; comparison to observed data	Short-term exposure may not be indicative of typically exposures	Comparison of means across five cities CMAQ-AQS = −31.3 to −13.1%; CMAQ (with satellite-based emissions)-AQS = 9.6−38.1%

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Pongprueksa (2013)	CMAQ 4.7.1, WRF 3.4, CONUS with 36-km horizontal resolution for 2009	L: CONUS; T: 2009; P: Entire population	CMAQ methods were compared with observed data from U.S. EPA's AQS and ozonesonde data collected across CONUS from WOUDC, NOAA, and TOPP	Short- and long-term exposure	NR (shown graphically)	Several different method comparisons used with two types of modeled data and two types of observed data; long-term exposure from CONUS is more indicative of a typical exposure	Horizontal resolution is coarse	Surface observed ozone compared with CMAQ, n = 26,234, MB = 7 ppb, ME = 11 ppb, NMB = 17%, NME = 28%, *R* = 0.53; model performance by region 1–21, CMAQ, MB = 1–12 ppb, ME = 6–13 ppb, NMB = 1–38%, NME = 14–40%, *R* = 0.30–0.62

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Karamchandani et al. (2014)	CMAQ 5.01 with APT with 12-km horizontal resolution	L: Eastern U.S.; T: January 1–15, July 1–15, 2005; P: Entire population	CMAQ APT compared with CMAQ base and both models are compared with fixed-site surface monitors from U.S. EPA's AQS sites	Short-term exposure	Mean in July above 40 ppb was 55.4 ppb, above 60 ppb was 70.2; around point sources in July above 40 ppb was 55.1 ppb, above 60 ppb was 70.7 ppb; around point sources in July above 40 ppb was 56.1 ppb, above 60 ppb was 70.3 ppb	Specific CMAQ component update to see a pointed difference; APT differences explored around point sources	Short-term exposure may not be indicative of a typical exposure in epi studies	In July with 40 ppb cut off CMAQ, n = 49,765, mean obs = 55.4 ppb, mean mod = 53.9 ppb, ratio of means = 0.97, GB = −1.5 ppb, NB = −1.5%, FB = −4.4%, GE = 0.4 ppb, NE = 17.4%, FE = 18.4%, NMB = −2.7%, NME = 16.9%, R^2 = 0.30, CMAQ APT, n = 49,765, mean obs = 55.4 ppb, mean mod = 53.9 ppb, ratio of means = 0.97, GB = −1.5 ppb, NB = −1.4%, FB = −4.4%, GE = 0.4 ppb, NE = 17.4%, FE = 18.4%, NMB = −2.7%, NME = 16.9%,

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Karamchandani et al. (2014) (cont.)	CMAQ 5.01 with APT with 12-km horizontal resolution (cont.)	L: Eastern U.S.; T: January 1–15, July 1–15, 2005; P: Entire population (cont.)	CMAQ APT compared with CMAQ base and both models are compared with fixed-site surface monitors from U.S. EPA's AQS sites (cont.)	Short-term exposure (cont.)	Mean in July above 40 ppb was 55.4 ppb, above 60 ppb was 70.2; around point sources in July above 40 ppb was 55.1 ppb, above 60 ppb was 70.7 ppb; around point sources in July above 40 ppb was 56.1 ppb, above 60 ppb was 70.3 ppb (cont.)	Specific CMAQ component update to see a pointed difference; APT differences explored around point sources (cont.)		R^2 = 0.30; in July with 40 ppb cut off with 5 × 5 grid CMAQ, n = 2,791, mean obs = 55.1 ppb, mean mod = 55.4 ppb, ratio of means = 1.01, GB = 0.3 ppb, NB = −2.1%, FB = −1.3%, GE = 10.0 ppb, NE = 19.0%, FE = 19.6%, NMB = 0.5%, NME = 18.2%, R^2 = 0.22, CMAQ APT, n = 2,791, mean obs = 55.1 ppb, mean mod = 55.2 ppb, ratio of means = 1.00, GB = 0.1 ppb, NB = −1.8%, FB = −1.6%, GE = 9.9 ppb, NE = 18.7%, FE = 19.4%, NMB = 0.1%, NME = 18.0%,

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Karamchandani et al. (2014) (cont.)	CMAQ 5.01 with APT with 12-km horizontal resolution (cont.)	L: Eastern U.S.; T: January 1–15, July 1–15, 2005; P: Entire population (cont.)	CMAQ APT compared with CMAQ base and both models are compared with fixed-site surface monitors from U.S. EPA's AQS sites (cont.)	Short-term exposure (cont.)	Mean in July above 40 ppb was 55.4 ppb, above 60 ppb was 70.2; around point sources in July above 40 ppb was 55.1 ppb, above 60 ppb was 70.7 ppb; around point sources in July above 40 ppb was 56.1 ppb, above 60 ppb was 70.3 ppb (cont.)	Specific CMAQ component update to see a pointed difference; APT differences explored around point sources (cont.)		R^2 = 0.22, in July with 40 ppb cut off with 9 × 9 grid CMAQ n = 7,197, mean obs = 56.1 ppb, mean mod = 55.7 ppb, ratio of means = 0.99, GB = −0.4 ppb, NB = 0.7%, FB = −2.5%, GE = 9.7 ppb, NE = 18.2%, FE = 18.9%, NMB = −0.8%, NME = 17.4%, R^2 = 0.27, CMAQ APT, n = 7,197, mean obs = 56.1 ppb, mean mod = 55.6 ppb, ratio of means = 0.99, GB = −0.5 ppb, NB = 0.5%, FB = −2.6%, GE = 9.7 ppb, NE = 18.1%, FE = 18.8%, NMB = −0.9%, NME = 17.2%, R^2 = 0.27;
								Difference in methods of 8-h daily max ozone for selected days −10 to 10 ppb

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Zhang et al. (2014)	CMAQ 4.7.1 from 2000–2006, 36-km horizontal resolution over CONUS, WRF 3.2.1, 12-km horizontal resolution over the eastern U.S., 4-km horizontal resolution over seven cities (NYC, Pittsburgh, Baltimore, Chicago, Detroit, St. Paul, Winston-Salem)	L: CONUS, eastern U.S., seven U.S. cities in eastern U.S. (NYC, Pittsburgh, Baltimore, Chicago, Detroit, St. Paul, Winston-Salem); T: Hourly ozone between 2000 and 2006; P: Entire population	CMAQ output compared with surface, fixed-site monitoring data from U.S. EPA's AQS	Long-term exposure (through the MESA and WHI-OS studies)	NR (shown graphically)	This paper has fine scale resolution over a sizeable geographical area for a long time period	Limited obs for performed evaluation	Monthly mean ozone for selected cities MNB = −0.4 to 0.4 ppb, NGE = 0.1 to 0.35 ppb, AUP = −0.6 to 0.4 ppb

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Hogrefe et al. (2014)	Model comparison including CHIMERE (36 km), DEHM (50 km), CAMx (12, 15, and 24 km), CMAQ (12, 18, 24 km), AURAMS (45 km), Polair3D (24 km), MUSCAT (24 km), SILAM (24 km), EMEP (50 km), LOTOS/ EUROS (25 km); versions not reported	L: North America and Europe; T: May 1–September 30, 2006; P: Entire population	Comparison with measurements by: calculating MBE and RMSE in comparison with monitors in eight synoptic regions (Northeast, Midwest, Southeast, Northwest, California, Southwest, Northern Europe, Southern Europe)	Short-term exposure	NR	This study compares several different models and breaks down the comparison by meteorological zone	Not all data are easily discernible, as presented in the paper	(Across synoptic patterns) RMSE NE = 8.2−12.8 ppb MW = 9.3–14.5 ppb SE = 10.5−13.1 ppb NW = 8.7–11.9 ppb CA = 10.8−15.2 ppb SW = 10.2–10.9 ppb NEu = 8.0−15.1 ppb SEu = 9.7–12.4 ppb; MB NE = −5.8 to −0.8 ppb MW = −6.3 to 2.0 ppb SE = −9.1 to −4.4 ppb NW = −5.1 to −2.1 ppb CA = −3.9 to −1.9 ppb SW = 1.8 to 3.0 ppb NEu = 0.7 to 7.2 ppb SEu = 0.2 to 4.0 ppb

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Yahya et al. (2014)	WRF/Chem-MADRID using WRF/Chem 3.0, CB05, 12-km horizontal resolution over the southeastern U.S. comparing biogenic emission from MEGAN2, satellite-derived fire emissions (SD-Fire), and MEGAN2 + SD-Fire	L: Eastern U.S. states; T: May to September 2009–2011, December to February 2009–2012, sensitivity analysis performed July 2011; P: Entire population	WRF/Chem-MADRID compared with surface, fixed-site monitors from AQS, CASTNet, IMPROVE, and SEARCH	Long-term exposure	Avg 8-h daily max during ozone season = 41.4–48.6 ppb; avg 8-h daily max during winter = 31.1–36.0 ppb	Long term exposure is more indicative of typical exposes; sensitivity analysis was extensive	Forecasting daily ozone only 1 day forward is not an ideal exposure methodology in an epi setting	Between model and obs during ozone season 1-h max R = 0.3 to 0.7, NMB = −6.0 to 15.5%, NME = 17.6–27.1%, 8-h max R = 0.4 to 0.7, NMB = −4.5 to 14.6% NME = 17.8–26.1%; sensitivity analysis for July 2011 for 1-h max R = 0.5, NMB −0.9 to 10.1%, NME = 20.6–24.2%, and 8-h max R = 0.5–0.6, NMB = 1.6–12.8%, NME = 20.7−25.4%; sensitivity analysis for July 2011 for 1-h max accuracy = 85.9–91.5%, bias = 0.9–2.2, CSI = 15.6–19.1%, FAR = 67.7–78.3%, POD = 25.0–46.6%,

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Yahya et al. (2014) (cont.)	WRF/Chem-MADRID using WRF/Chem 3.0, CB05, 12-km horizontal resolution over the southeastern U.S. comparing biogenic emission from MEGAN2, satellite-derived fire emissions (SD-Fire), and MEGAN2 + SD-Fire (cont.)	L: Eastern U.S. states; T: May to September 2009–2011, December to February 2009–2012, sensitivity analysis performed July 2011; P: Entire population (cont.)	WRF/Chem-MADRID compared with surface, fixed-site monitors from AQS, CASTNet, IMPROVE, and SEARCH (cont.)	Long-term exposure (cont.)	Avg 8-h daily max during ozone season = 41.4–48.6 ppb; avg 8-h daily max during winter = 31.1–36.0 ppb (cont.)	Long term exposure is more indicative of typical exposes; sensitivity analysis was extensive (cont.)	Forecasting daily ozone only 1 day forward is not an ideal exposure methodology in an epi setting (cont)	8-h daily max accuracy = 66.5–74.0%, bias = 1.1–1.7, CSI = 27.4–30.6%, FAR = 58.8–63.3%, POD = 44.2–62.1%
Li et al. (2014a)	WRF-CHEM using CMAQ 4.6 and ISORROPIA 1.7 horizontal resolution of 2 km	L: California-Mexico border region; T: May 15–16, May 90–30, June 4–5, June 13–14, 2010; P: Entire population	WRF-Chem compared with surface, fixed-site monitors	Short-term exposure	NR (shown graphically)	High spatial resolution	Only having a few days of modeled concentration during an ozone episode is not indicative of more typical long-term exposures	Obs and mod by station MB = −7.6 to 13.5 ppb, R^2 = 0.20–0.79, RMSE = 17.1−22.0 ppb

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Pan et al. (2014)	NAQFC-beta (coupled NAM-CMAQ 4.7.1 with mobile sources defined from 2005 MOBILE6 + 05 to 12 projections, point sources from 2010 CEM + DOE Annual Energy Outlook, nonroad from CSAPR, Canadian emissions from 2006 EI, with 12-km horizontal resolution over CONUS	L: CONUS; T: July 2011; P: Entire population	Modeled output compared with surface, fixed-site monitors from U.S. EPA's AQS and CTM with base-case emissions	Short-term exposure	Average hourly ozone by hour NR (shown graphically)	Complete cover of CONUS; incremental change of model inputs pinpoints differences between two different models	Ozone concentrations in 1 summer month is not indicative of more long-term exposures	Bias U.S. emissions urban = 7.08 ppb, suburban = 7.48 ppb, rural = 7.80 ppb; U.S. + Canadian emissions urban = 6.16 ppb, suburban = 6.22 ppb, rural = 5.93 ppb

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Cuchiara et al. (2014)	ARW-WRF, WRF/Chem 3.5 with 4-km horizontal resolution, with four planetary boundary layer schemes from YSU, MJY, ACM2, QNSE. YSU, and ACM2 are computed based on the bulk Richardson number, which is the ratio of buoyancy to turbulence caused by shear stresses. MYJ and QNSE are computed based on eddy-diffusivity, or atmospheric mixing	L: Houston, TX; T: October 5, 2006; P: Entire population	All four PBL schemes compared to each other and to observed, fixed-site monitors from U.S. EPA's CAMS and aloft observed data from ozonesonde and aircrafts	Short-term exposure	NR (shown graphically)	Very fine scale resolution; small, incremental changes in model pin points model differences and assumptions	Highly localized space/time modeling scenario is not indicative of more long-term exposures	Statistics across sites for four boundary layer schemes: YSU *R* = 0.79–0.92, bias = 0.59–0.99, RMSE = 13.20–21.02 ppbv; MYJ *R* = 0.70–0.90, bias 0.64–1.05, RMSE = 12.17–20.76 ppbv; ACM2 *R* = 0.37–0.77, bias = 0.75–1.26, RMSE = 18.53–25.77 ppbv; QNSE *R* = 0.54–0.71, bias = 0.72–1.09, RMSE = 15.59–24.85 ppbv

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Thompson and Selin (2012)	CAMx 4.5.3 with 36-, 12-, 4-, and 2-km horizontal resolution	L: Houston, Galveston, Brazoria area, TX; T: August 13–September 15, 2006; P: Entire population	All modeled output compared to each other and to surface, fixed-site monitors for air quality monitors in the region	Short-term exposure	Population-weighted maximum ozone across days; NR (shown graphically)	Very fine-scale horizontal resolution of the model	Limited temporal run and spatial coverage of the model may not be indicative of long-term exposures	Average MNGE across sites 36-km resolution = 74%, 12-km resolution = 63%, 4-km resolution = 26%, 2-km resolution = 25%
Lu et al. (2014)	CMAQ 5.0, SMOKE 3.0, WRF 3.3, CB05 with 4-km horizontal resolution	L: Mid-southern U.S. (Mississippi, Arkansas, Tennessee, Alabama); T: July 2011, February 2012; P: Entire population	CMAQ compared against surface, observed fixed-site monitors from U.S. EPA's AQS	Short-term exposure	Hourly ozone NR (shown graphically)	Fine scale resolution; CMAQ has complete coverage in spatial domain	2 mo of short-term exposure is not necessarily indicative of typical exposures	Hourly ozone in July NMB = 48.2%, RMSE = 20.9 ppb, UPA = 28%, *R* = 0.67
Wang and Zhang (2014)	CMAQ 4.7 with 12-km horizontal resolution with offline dry deposition, inline dry deposition, four difference sensitivity analysis with the inline dry deposition	L: Eastern U.S.; T: January and July 2002; P: entire population	All modeled runs compared against each other and modeled data compared against surface, observed fixed-site monitors from CASTNet, IMPROVE, AQS, SEARCH, NADP, and NC DENR	Short-term exposure	Mean in January 2002 for 8-h daily max ozone = between 26.8 and 29.2 ppb	There are several different comparison modeled methods	2 mo of short-term exposure is not necessarily indicative of typical exposures	January, 2002: 8-h daily max ozone NMB = −1.6 to 3.9%, NME = 19.4–21.7%, *R* = 0.70–0.74; July, 2002: 8-h daily max ozone NMB = −2.2–4.3%, NME = 15.4−16.8%, *R* = 0.75–0.77

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Xing et al. (2015)	CMAQ 5.0 with 108-km horizontal resolution, EDGAR 4.2, EDGAR HTAP 1	L: Northern Hemisphere; T: 1990–2010; P: Entire population	CMAQ compared to surface, observed, fixed-site monitors from AQS, CASTNet, IMPROVE (U.S.), EMEP, AIRBASE (Europe), API (China), and WDCGG (global)	Short- and long-term exposure	No figures or tables showing concentration of ozone	Excellent spatial coverage and temporal coverage	Coarse resolution across the U.S.	Comparison of model with CASTNet network spring: *R* = 0.52 MB = −22.8 μg/m^3 NMB = −13.6% RMSE = 29.7 μg/m^3 NME = 16.1%; summer: *R* = 0.59 MB = −14.3 μg/m^3 NMB = −8.1% RMSE = 30.5 μg/m^3 NME = 14.5%; fall: *R* = 0.60 MB = −3.9 μg/m^3 NMB = −2.5% RMSE = 23.5 μg/m^3 NME = 12.4%; winter *R* = 0.51 MB = −3.6 μg/m^3 NMB = −3.2% RMSE = 10.1 μg/m^3 NME = 7.6%

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Herron-Thorpe et al. (2014)	CMAQ 4.6, EGAS, MOBILE 6.2, GVRD, BEIS−3, SMOKE 2.4, BlueSky 3.1 with 12-km horizontal resolution with AIRPACT−3 FEPS Plume Rise compared with AIRPACT−3 SMOKE Plume Rise compared with MOZART−4	L: Pacific Northwest, U.S.; T: July 3–August 2007, June 22–August 27, 2008; P: Entire population	CMAQ compared with surface, fixed-site observed data from U.S. EPA's AQS	Short-term exposure	8-h daily max ozone before (after) smoke event mean obs = 45.8 ppb (42.3 ppb)	Complete coverage with CTM data; multiple data sources used to model ozone	Short time windows may not be indicative of typical exposures	8-h daily max ozone before (after) smoke event R = 0.7 (0.8), MB = −4.7 ppb (−0.7 ppb), ME = 8.9 ppb (7.7 ppb), NMB = −7% (3%), NME = 20 ppb (21 ppb), FB = −10% (−1%), FE = 22% (20%)
Tang et al. (2015a)	CAMx 5.3, MM5 3.7.3, MOZART, emissions for HGB SIP from TCEQ, NLDN, comparison of clouds with GEOS vs. Texas SIP with 12-km horizontal resolution	L: Eastern TX; T: August 13–September 15, 2006; P: Entire population	Photolysis from GEOS is compared with Texas SIP and both modeling methods are compared with surface, fixed-site monitors from U.S. EPA's AQS	Short-term exposure	Monthly avg 8-h daily max ozone NR (shown graphically)	Incremental model changes demonstrate the specific influence of photolysis; multiple comparison methods with multiple model comparison and observed data comparisons	Short time windows may not be indicative of typical exposures	Difference in modeled ozone by day R^2 between = −0.06 to 0.07, NMB = between −0.1 to 0.04%, NME = between −0.1 to 0.02%

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Tessum et al. (2015)	WRF-Chem 3.4 with 12-km horizontal resolution	L: CONUS; T: 2005; P: Entire population	Model compared with surface, fixed-site monitors from U.S. EPA's CASTNet and AQS	Short- and long-term exposure	Annual ozone, annual peak ozone, annual daytime ozone NR (shown graphically)	Comparison to observed data; full spatial coverage of domain; having a year's worth of data make the study applicable to long-term exposures	Short-term exposures were not explored	Annual average MB = 7.92 ppb, ME = 8.58 ppb, MFB = 23%, MFE = 26%, R^2 = 0.37; errors in average daytime ozone per season of WRF-Chem (CMAQ) winter MB = 3.5 ppb (−3.5 ppb), ME = 5.5 ppb (9.0 ppb), NMB = 12% (−13%), NME = 19% (35%), spring MB = 1.5 ppb (−1.8 ppb), ME = 4.6 ppb (9.3 ppb), NMB = 3% (−4%), NME = 10% (29%), summer MB = 9.2 ppb (4.4 ppb), ME = 10.1 ppb (11.0 ppb), NMB = 21% (10%), NME = 23% (24%), fall MB = 5.2 ppb (2.6 ppb), ME = 6.2 ppb (8.8 ppb), NMB = 19% (8%), NME = 23% (28%)

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Hogrefe et al. (2015)	CMAQ 5.0.1, WRF 3.4 with 12-km horizontal resolution	L: CONUS; T: June to August 2006, May to -September 2010; P: Entire population	Model compared with surface, fixed-site monitors from U.S. EPA's AQS	Short- and long-term exposure	Mean during 2006 of 8-h daily max ozone = 32.3–51.1 ppb; mean during 2010 of 8-h daily max ozone = 33.6–47.5 ppb	CMAQ has full coverage of spatial domain	Number of months and different years explored lends itself to both short- and long-term exposures	Avg 8-h daily max ozone June–August 2006: MB = −0.9 to 6.6 ppb, ME = 5.6–10.9 ppb, RMSE = 7.4–14.4 ppb, NMB = −1.9 to 20.5%, NME = 13.8–26.6%, *R* = 0.69–0.78; May–September 2010: MB = −1.9 to 6.6 ppb, ME = 6.6–9.7 ppb, RMSE = 8.7–12.2 ppb, NMB = −5.6 to 14.9%, NME = 13.9–21.8 %, *R* = 0.58–0.78
Tang et al. (2015b)	CMAQ alone (base case)	L: CONUS; T: July 2011; P: Entire population	Modeled outputs compared with AirNow observed data and aircraft measurements from Discover-AQ	Short-term exposure	Hourly ozone in the first half of July 2011 in the northeastern U.S.	Two different observed data sources used; multiple models compared to each other	1 summer month may not be indicative of more long-term exposures	Hourly ozone from July 6–7, 2011 over CONUS *R* = 0.53, MB = 2.54; hourly ozone from July 6–7, 2011 over southeastern U.S. *R* = 0.55, MB = 0.22; *R* between obs and CMAQ alone for aircraft data is 0.604

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Koo et al. (2015)	CAMx 5.4.1 compared with CMAQ 5.0.1-VBS with a 12-km horizontal resolution	L: Eastern U.S.; T: 2005; P: Entire population	Both models compared to each other and models compared to surface, fixed-site monitors from U.S. EPA's AQS, CASTNet, and SEARCH	Long-term exposure	Ozone concentrations not displayed in table or figure for 2005	The shows models sensitivities in two or the most common models; multiple comparison method between models and observed data	CTMs have inherent error	CMAQ: all NMB were within ±15%, all NME were within 25%; CAMx: all NMB were within ±25%, NME were within 30%
Yahya et al. (2015b)	WRF/Chem 3.4.1 with 36-km horizontal resolution for 2006 and 2010	L: CONUS; T: January, February, December 2006 and 2010 with June, July, August 2006 and 2010; P: Entire population	Model compared with surface, observed fixed-site monitors from U.S. EPA's CASTNet and AQS	Short- and long-term exposure	Mean maximum 1 h ozone = between 33.2 and 48.4 ppb, mean 8-h daily max ozone = between 32.7 and 43.8 ppb	Comparison method to obs data; time window lends itself to both short- and long-term exposure	CTMs have inherent errors and horizontal resolution is coarse	*R* 1-h daily max CASTNet = 0.40, AQS = 0.34; *R* = 8-h daily max CASTNet = 0.40, AQS = 0.20; NMB 1-h daily max CASTNet = −30.0%, AQS = −15.8%; NMB = 8-h daily max CASTNet = −25.3%, AQS = −17.0%; NME = 1-h daily max CASTNet = 34.8 and AQS = 28.0%; NME = 8-h daily max CASTNet = 32.0%, AQS = 29.2%

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Pan et al. (2015)	CMAQ 5.0.1, WRF 3.5 base compared with sensitivity analysis of adjusted emissions with 4-km horizontal resolution	L: Southeast TX; T: September 2013; P: Entire population	Models compared to each other and both models compared to TCEQ's CAMS surface, fixed-site monitors	Short-term exposure	Mean obs = 24.4 ppb, mean mod = 32.7 ppb, mean sensitivity analysis = 33.2 ppb	Fine scale resolution; sensitivity analysis of the model	Short-term exposure may not be indicative of typical ozone exposures	Hourly ozone, *R* = 0.73, IOA = 0.80, MB = 8.3 ppb, *R* of model difference = 0, IOA model different = 0, MB model difference = 0.4 ppb
Barrett et al. (2015)	GEOS-Chem model (version not reported) with adjoint	L: U.S., T: 2008–2015, P: Entire population	Comparison with observations from fixed-site monitors reporting to the AQS	Long-term exposure study (contribution from VW emissions)	Average addition of 2.6 ppbv across U.S. due to excess NO_X emissions	Nationwide model, 1,200 monitoring sites used for validation	Low spatial resolution (50 km), model based on 2005 emissions inventory (emissions have dropped over time)	Mean NMB = 25.3%, SD of NMB = 17.9% (NMB calculated from 1-h daily max)

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Friberg et al. (2016)	CMAQ 4.5 alone and annual adjustment	L: Georgia; T: 2002–2008, P: Entire population	Cross-validation by fixed-site monitors	Long-term exposure study	Mean (IQR) = 47.6 ppb (22.0 ppb) for 8-h daily max	Low mean bias, low RMSE, and relatively high R^2 (compared to application of the model for other pollutants), and errors are minimized through the model-weighting approach	Errors in measurements used as input are propagated into the model, limited spatial coverage of monitors increases errors (although this is less of a limitation for ozone and other secondary pollutants)	CMAQ MFE = 0.18, MFB = 0.11, NME = 16.5%, NMB = 8.58%, MB = 0.004, RMSE = 0.01, R^2 (cross-validation) = 67.1%; CMAQ with annual adjustment MFE = 0.17, MFB = 0.03, NME = 15.0%, NMB = 0.14%, MB = 6.9×10^{-5}, RMSE = 0.01, R^2 (cross-validation) = 67.2%
Tao et al. (2016)	NU-WRF model, focused on impact of trans-Pacific aerosol transport	L: Contiguous U.S.; T: March 21−June 30, 2010; P: Entire population	Comparison with observations from fixed-site monitors reporting to the AQS	Short- and long-term exposure	Mean = 30–50 ppbv for 3-mo avg; impact of transpacific PM on ozone concentrations = −0.33 ppbv to 0.50 ppbv	Enabled analysis of the influence of meteorology and Asian air pollution on U.S. ozone concentrations, low mean bias	27-km resolution may lead to bias because not all cloud chemistry can be represented, study did not examine the model's internal variability	(Mean, range) NB = −2.8% (−28.2, 28.0%), NGE = 18.8% (11.9,29.7%)

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Li et al. (2016a)	WRF-Chem 3.6.1 with nested 36-, 12-, and 4-km domains	L: Central Valley, CA; T: June 23–August 1, 2005 (first few days considered spin-up); P: Entire population (6.5 million residents)		Short- and long-term exposure	NR	Inclusion of irrigation and cloud cover on model output; the nested model design includes 4-km resolution, which is sufficiently fine to capture dynamics in rural settings	Planetary boundary layer designation in the model may be uncertain (different approaches have been used in different studies).	Irrigation not included: MB = −6.1 ppb, NMB = −24.6%, NME = 28.9%, MNB = −23.9%, MNGE = 28.3%, *R* = 0.63, IOA: 0.81, RMSE = 18.0 ppb; irrigation inclusion: MB = −5.4 ppb, NMB = −21.1%, NME = 26.0%, MNB = −21.5%, MNGE = 26.1%, *R* = 0.70, IOA: 0.83, RMSE = 17.6 ppb

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Yahya et al. (2015a)	WRF-Chem 3.4.1 at 36-km resolution with initial and boundary conditions downscaled from global models	L: Contiguous U.S.; T: 2001, 2006, 2010; P: Entire population	Comparison with observations from fixed-site monitors reporting to the AQS and CASTNet	Long-term exposure	(Model values at sites for networks mentioned) 2001 CASTNet 8-h max = 39.0 ppb, CASTNet 1-h max = 38.3 ppb, AQS 8-h max = 44.6 ppb, AQS 1-h max = 49.4 ppb; 2006 CASTNet 8-h max = 38.4 ppb, CASTNet 1-h max = 39.3 ppb, AQS 8-h max = 43.2 ppb, AQS 1-h max = 48.1 ppb; 2010 CASTNet 8-h max = 38.2 ppb, CASTNet 1-h max = 38.6 ppb, AQS 8-h max = 41.8 ppb, AQS 1-h max = 47.3 ppb	Extensive comparisons made at different time averages and validation data sets, validation on meteorological variables as well	Lower resolution (36 km)	2001 CASTNet 8-h max: MB = −4.8 ppb, NMB = −11.0%, NME = 28.2%, CASTNet 1-h max = MB: −7.9 ppb, NMB = −17.2%, NME = 30.1%, AQS 8-h max = MB: −0.3 ppb, NMB = −0.7%, NME = 29.9%, AQS 1-h max MB = −1.7 ppb, NMB = −3.3%, NME = 28.5%; 2006 CASTNet 8-h max: MB = −5.2 ppb, NMB = −11.8%, NME = 27.1%, CASTNet 1-h max: MB = −8.3 ppb, NMB = −17.4%, NME = 28.7%, AQS 8-h max:

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Yahya et al. (2015a) (cont.)	WRF-Chem 3.4.1 at 36-km resolution with initial and boundary conditions downscaled from global models (cont.)	L: Contiguous U.S.; T: 2001, 2006, 2010; P: Entire population (cont.)	Comparison with observations from fixed-site monitors reporting to the AQS and CASTNet (cont.)	Long-term exposure (cont.)	Model values at sites for networks mentioned) 2001 CASTNet 8-h max = 39.0 ppb, CASTNet 1-h max = 38.3 ppb, AQS 8-h max = 44.6 ppb, AQS 1-h max = 49.4 ppb; 2006 CASTNet 8-h max = 38.4 ppb, CASTNet 1-h max = 39.3 ppb, AQS 8-h max = 43.2 ppb, AQS 1-h max = 48.1 ppb; 2010 CASTNet 8-h max = 38.2 ppb, CASTNet 1-h max = 38.6 ppb, AQS 8-h max = 41.8 ppb, AQS 1-h max = 47.3 ppb (cont.)	Extensive comparisons made at different time averages and validation data sets, validation on meteorological variables as well (cont.)	Lower resolution (36 km) (cont.)	MB = −1.2 ppb, NMB = −2.8%, NME = 27.5%, AQS 1-h max MB = −2.2 ppb, NMB: = −4.5%, NME = 26.3%; 2010 CASTNet 8-h max: MB = −5.7 ppb, NMB = −13.0%, NME = 26.9%, CASTNet 1-h max: MB = −8.8 ppb, NMB = −18.6%, NME = 28.7%, AQS 8-h max: MB = −0.4 ppb, NMB = −1.1%, NME = 26.1%, AQS 1-h max MB = −1.1 ppb, NMB = −2.3%, NME = 25.3%

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Li et al. (2014b)	WRF-Chem 3.5.1 with nested 36-, 12-, 4-, and 1-km domains	L: Phoenix, AZ; T: June 9, 2011 and May 14, 2012; P: Entire population	Comparison with 24 fixed-site monitors	Short-term exposure	Hourly ozone NR (shown graphically)	Late afternoon heat island captured well	Simulations overestimated wind speed	June 9, 2011: MB = −1.69 ppb, RMSE = 14.70 ppb, NMB = −6.32%, NME = 15.32%, MNB = −5.59%, MNGE = 15.70%, IOA = 0.80, *R* = 0.75; May 14, 2012: MB = −1.50 ppb, RMSE = 14.75 ppb, NMB = −6.50%, NME = 14.43%, MNB = −5.60%, MNGE = 15.76%, IOA = 0.81, *R* = 0.74
Ran et al. (2016)	CMAQ 5.0.2/WRF 3.4 with MODIS leaf area index model included in some runs	L: Contiguous U.S., southern Canada, northern Mexico; T: April, August, October 2006; P: Entire population	Comparison with observations from fixed-site monitors reporting to the AQS	Long-term exposure	CMAQ: April 2006 = 52.70 ppb, August 2006 = 55.00 ppb, October 2006 = 42.2.0 ppb; CMAQ + MODIS: April 2006 = 55.40 ppb, August 2006 = 57.10 ppb, October 2006 = 44.60 ppb	Addition of leaf area index allows for consideration of role of vegetation	12-km resolution	CMAQ: April 2006: RMSE = 9.51 ppb, MAE = 7.33 ppb, MB = 3.94 ppb; August 2006: RMSE = 12.80 ppb, MAE = 9.70 ppb, MB = 4.84 ppb; October 2006: RMSE = 10.10 ppb, MAE = 8.20 ppb, MB = 5.34 ppb

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Godowitch et al. (2015)	CMAQ 5.0.2 with 12-km resolution with WRF/FDDA meteorology and boundary conditions from a global GEOS-Chem simulation	L: Eastern U.S.; T: June 1–August 31, 2002; P: Entire population	Comparison with observations from fixed-site monitors reporting to the AQS, tower sensors at one location (Raleigh, NC), and DISCOVER-AQ flight sensors	Short- and long-term exposure	Eastern U.S.: meteorology Model 1 = 50.1 ppbv, Model 2 = 48.2 ppbv; northeastern U.S.: Model 1 = 49.6 ppbv, Model 2 = 47.5 ppbv; CASTNet: Model 1 = 52.9 ppbv, Model 2 = 51.1 ppbv	Use of continually updated data improves accuracy of model	12-km resolution still inhibits urban studies	Eastern U.S.: meteorology Model 1 MB = 9.8 ppbv, MAE 13.5 = ppb, Fp 42%, Model 2 MB = 7.9 ppbv, MAE = 12.6 ppb, Fp 58%; northeastern U.S.: Model 1: MB = 8.2 ppbv, MAE = 12.4 ppb, Fp = 41%, Model 2: MB = 6.1 ppbv, MAE = 11.5 ppb, Fp = 59%; CASTNet: Model 1: MB = 10.8 ppbv, MAE = 13.3 ppb, Fp = 42%, Model 2: MB = 9 ppbv, MAE = 12.5 ppb, Fp = 58%
Li et al. (2016b)	CMAQ 5.0.2 with WRF/FDDA meteorological model and assimilation of meteorological data	L: Southeast TX, southwest LA; T: September 2013; P: Entire population	Comparison with observations from fixed-site monitors reporting to the AQS	Short-term exposure	Without assimilation mean = 33.7 ppb, SD = 14.1 ppb; with assimilation mean = 30.6 ppb, SD = 17.4 ppb	Data assimilation improves representation of short-term variability in concentration field, better captures hot spots	4-km resolution misses spatial variation	Without assimilation: IOA = 0.78, RMSE = 14.9 ppb, MAE = 12.3 ppb, MB = 9.3 ppb; with assimilation: IOA = 0.83, RMSE = 13.8 ppb, MAE = 11.0 ppb, MB = 6.1 ppb

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Garner et al. (2015)	National Air Quality Forecast Capability combines CMAQ 4.5 with WRF-NMM, also tested beta version with full gas and aerosol mechanism	L: Baltimore, MD; T: July 2011; P: Entire population	Comparison with observations from fixed-site monitors reporting to the AQS	Short-term exposure	NR	NAQFC-beta provides more mechanistic information, despite having higher error	Both versions of the model overpredict at low concentrations and underpredict at high concentrations	NAQFC: Corr = 0.69–0.82, RMSE = 13.59−18.75 ppb, MB = −1.15 to 8.96 ppb, NMB = −2.28 to 22.34%; NAQFC-beta: Corr = 0.67–0.81, RMSE = 15.81–20.92 ppb, MB = 3.40–13.84 ppb, NMB = 6.75–34.49%
Wang et al. (2016)	UCD-CIT chemical transport model with meteorology modeled by WRF 3.1.1 on a 4-km grid	L: Los Angeles and Riverside counties, CA; T: 2000–2008; P: Entire population	10-fold cross-validation against 37 monitors for each variation of model	Short- and long-term exposure	8-h daily max, annual averages (annual average used for summary stats)	CTM accounts for atmospheric chemistry, long-range transport of ozone and its precursors, and biogenic VOCs	Positive bias in the concentration, more variability compared with spatiotemporal models	RMSE = 8.83 ppb, R^2 = 0.56

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Seltzer et al. (2016)	CMAQ 5.0.2/WRF 3.4.1 with 36-km domain with met fields downscaled	L: CONUS; T: January 1, 2000–December 31, 2010; P: Entire population	Comparison of model predictions with observations from monitors reporting to the AQS	Short- and long-term exposure	Median (range estimated from graph) March–May = 45−50 ppb, June–August = 55–65 ppb, September–November = 45−55 ppb, December–February = 35–40 ppb	Variability is accurately captured	Model is positively biased in the summer and fall and negatively biased in the winter	Mean (SD) of median bias across years March–May = 0.9 ppb (0.7 ppb), June–August = 9.7 ppb (1.16 ppb), September–November = 10.9 ppb (0.96 ppb), December–February = 6.7 ppb (1.01 ppb)

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Yahya et al. (2016)	WRF/Chem 3.6.1 with 36-km horizontal resolution	L: CONUS; T: 2001–2010; P: Entire population	Model compared with surface, fixed-site monitors from U.S. EPA's AQS and CASTNet	Long-term exposure	AQS Hourly ozone mean obs = 29.3 ppb, mean sim = 32.1 ppb, 8-h daily max mean obs = 43.7 ppb, mean sim = 45.9 ppb; CASTNet hourly mean obs = 35.0 ppb, mean sim = 31.9 ppb, 1-h daily max mean obs = 47.4 ppb, mean sim = 38.5 ppb, 8-h daily max, mean obs = 43.3 ppb, mean sim = 37.9 ppb	Long-term modeling well suited for long-term exposures	Temperature typically overpredicted during the winter, overpredictions of biogenic emissions in rural areas	Vs AQS hourly ozone *R* = 0.6, MB = 2.8 ppb, NMB = 9.7%, NME = 22.4%; vs. AQS maximum 1-h ozone mean obs = 48.9 ppb, mean sim = 49.7 ppb, *R* = 0.6, MB = 0.8 ppb, NMB = 1.7%, NME = 7.9%; vs. AQS 8-h daily max *R* = 0.6, MB = 2.2 ppb, NMB = 5.0%, NME = 9.3%; vs. CASTNet hourly ozone *R* = 0.7, MB = −3.1 ppb, NMB = −8.8%, NME = 19.8%; CASTNet maximum 1-h ozone *R* = 0.4, MB = −8.9 ppb, NMB = −18.8 ppb, NME = 31.4%; vs. CASTNet maximum 8 h ozone *R* = 0.5, MB = −5.4 ppb, NMB = −12.5%, NME = 29.6%

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Baker et al. (2016)	CMAQ 5.0.2, 2011 NEI 2, SMOKE 3.6.5, WRF 3.4.1 with 12 km horizontal resolution	L: Wallow fire (eastern Arizona and western New Mexico, U.S.) and Flint Hills fire (eastern Kansas, U.S.); T: June 1–6, 2011 (Wallow), April 1–5, 2011 (Flint Hills); P: Entire population	Model compared with surface, fixed-site monitors from U.S. EPA's CASTNet	Short term exposure	Hourly ozone NR (shown graphically)	Localized spatiotemporal region allows for measuring ozone from a specific event; comparison to observed data appropriate	Short time period may not be indicative of typical ozone exposures	Bias presented as a function of ozone concentration for wildfire and prescribed burn. Wildfire increase in bias of approximately 2 ppb for every 1 ppb increase in estimated ozone contribution from fire; prescribed burn increase in bias of approximately 1 ppb for every 1 ppb increase in estimated ozone contribution from fire

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Bash et al. (2016)	CMAQ 5.0.2, WRF 3.3, biogenic emission from BEIS 3.61 with 4-km horizontal resolution; sensitivity analysis includes BEIS 3.14, BEIS 3.61 WRF par, MEGAN 2.1 WRF par	L: Central and northern California; T: June 3–July 31, 2009; P: Entire population	Models compared to each other and compared to observed, fixed-site monitors from U.S. EPA's AQS	Short-term exposure	Average hourly obs ozone greater than 60 ppb = 70.9 ppb, less than 60 = 32.0 ppb, average mod hourly ozone greater than 60 ppb = between 62.1 and 64.8, less than 60 ppb = between 40.7 and 41.7 ppb	This incremental improvement in modeled inputs allow for seeing pointed concentration changes; fine spatial resolution	Localized spatiotemporal modeling domain may not be typical of average ozone exposures	Biases and errors when using satellite parameterization of weather model: ozone greater than 60 ppb: median bias = −8 to −9 ppb, median error = 13–14 ppb, MB = −6.2 to −5.5 ppb, ME = 11–12 ppb, FB = −10.1 to −9.5%, FE = 16.7–17.8%; less than 60 ppb: median bias = 29–32 ppb, median error = 32–34 ppb, MB = 8.8−9.7 ppb, ME = 11.1–11.8 ppb, FB = 29.8−31.9%, FE = 36.4−37.9%
Appel et al. (2017)	WRF 3.7 and CMAQ 5.1	L: CONUS; T: 2011 annual simulation; P: Entire population	Annual, monthly, seasonal and diurnal evaluations provided against AQS data	Long-term exposure	NR	Benchmark study of state-of-the-art CTM science and evaluation	12-km resolution	NR

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Pleim et al. (2016)	WRF 3.7 and CMAQ 5.1	L: CONUS; T: 3-week simulation August 10–30, 2006; P: Entire population	Daily evaluation against 1,144 sites	Short-term exposure	NR	Improvements made in Land-Surface Model and PBL model provides more accurate ozone simulations	12-km resolution, 3-week simulation	NR
Pan et al. (2017a)	WRF 3.4 and CMAQ 5.0.1	L: Houston, TX; T: 1-day simulation September 25, 2013; P: Entire population	Hourly evaluation with TCEQ monitors	Short-term exposure	NR	4-km resolution	1-day study	With improvements in both meteorological and emissions input, model under-prediction of peak ozone concentrations (>100 ppb), improves with mean biases decreasing from 50 to 9 ppb
Muñiz-Unamunzaga et al. (2018)	WRF 3.71, CMAQ 5.1	L: Greater Los Angeles area, CA; T: September 2006; P: Entire population	Hourly and month-long aggregated evaluation against eight AQS sites	Short- and long-term exposure	NR	4-km resolution	1-mo analysis	Inclusion of marine halogen and sulfur concentrations reduced model overprediction as mean bias is reduced from 13.5 to 4.9% across the domain and month

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
U.S. EPA (2011)	MM5 3.7.4, CMAQ 4.7.1	L: CONUS; T: 2005 annual simulation; P: Entire population	Hourly and 8-h daily max	Short-term exposure	NR	CONUS annual simulation	12-km resolution	For 8-h daily max: bias ranged from −2 to −9 ppb, error ranged from 9 to 9 ppb, fractional bias ranged from −3 to −14% and fractional error 12 to 15%. For maximum daily hourly, bias ranged from −4 to −9 ppb. Error 10 to 11 ppb and FB −6 to −13 % and FE 14 to 15%
Henneman et al. (2017b)	WRF 3.6.1 and CMAQ 5.0.2	L: Eastern U.S.; T: Two, 2-year periods, 2001–2002, 2011–2012; P: Entire population	Hourly and 8-h daily max evaluation against more than 500 AQS sites for each of the 4 yr	Short-term exposure	NR	4 full yr of simulation/ evaluation	12-km resolution and only 13 vertical layers.	Correlations ranged from 0.63 −0.67 for hourly to 0.67−0.72 for 8-h daily max; NMB ranged from −7.5 to −13% (hourly) 1.6– 4.3% (8-h daily max); NME ranged from 11–23% (hourly) and 16–21% (8-h daily max) depending on year

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Henneman et al. (2017a)	WRF 3.6.1, CMAQ 5.0.2	L: Atlanta GA; T: 2001; P: Entire population	8-h daily max	Short-term exposure	NR	NR	12-km resolution, only one location in downtown Atlanta was used in the evaluation	For all 8-h daily max: NMB = −0.8%, NME = 27.3%, MB = −0.4%, *r* = 0.70. for 8-h daily max > 60 ppb: NMB = −16.8%, NME = 19.7%, MB = −12.2%, *r* = 0.43.
Pan et al. (2017b)	WRF 3.7 with CMAQ 5.0.2	L: Houston TX; T: September 2013; P: Entire population	Hourly concentrations	Short-term exposure	NR	1 and 4 km simulations	Only two observations sites from the TCEQ	Evaluation statistics were provided in supplementary material. *R* ranged from 0.75–0.77; MB from 10−13 ppb
Nopmongcol et al. (2017)	GEOS-Chem 9.1.3, CAMx 6.1	L: CONUS; T: 2005; P: Entire population	8-h daily max from AQS and CASTNet monitors	Short-term exposure	NR	NR	36-km resolution	AQS sites: Annual NMB = 7.7%, NME = 15%, *r* = 0.76, RMSE = 11.20; CASTNet sites: Annual NMB = 0.4%, NME = 14%, *r* = 0.52, RMSE = 19.40
Matichuk et al. (2017)	WRF 3.4, CMAQ 5.0.2	L: Utah (Uinta Basin); T: 10 days in 2013; P: Entire population	Hourly ozone at a dozen “field study” locations.	Short-term exposure	NR	4-km resolution	10-day period in February	Model bias ranged from 15 to 60 ppb

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Seltzer et al. (2017)	GEOS-Chem	L: CONUS; T: Two, 2-year annual simulations (2004–2006, 2009–2011); P: Entire population	R6MA1 (running 6-mo avg of the 1-h daily max) and 8-h daily max of over 1,000 AQS sites	Short-term exposure	NR	Annual simulations	Grid resolutions were 2.0 × 2.5° and 0.5 × 0.666°	CONUS values of the NMB of 8-h daily max ranged from 2.0 to 6.6 depending on simulation year
Solazzo et al. (2017)	WRF, CMAQ	L: CONUS; T: 2010 annual simulation; P: Entire population	Hourly ozone concentrations	Short-term exposure	NR	CONUS	12-km resolution	Annual MSE ranged from 28.6 to 79.3 ppb[2]
Hall et al. (2012)	WRF 3.1, CMAQ 4.7.1	L: CONUS; T: 2008 annual simulation; P: Entire population	Hourly and 8-h daily max ozone at 1,176 AQS sites	Short-term exposure	NR	Annual simulation	12-km resolution	Evaluation was segregated into seasons and eight CONUS subregions. NMB ranged from −10.4 to 19.5%, FB ranged from −10.1 to 20.0%; NME ranged from 11.2−25.3% and FE ranged from 11.9−25.3%

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Zhang and Ying (2011)	MM5, CMAQ (version NR)	L: Houston TX MSA; T: 12 days only in August 2000; P: Entire population	60 AQS sites , hourly data	Short-term exposure	NR	4-km resolution	Only a 12 day simulation	MNB ranged from −0.3 to +0.2%
Castellanos et al. (2011)	MM5 3.6, CMAQ 4.5.1	L: Eastern U.S.; T: May 15–September 15, 2000; P: Entire population	612 AQS sites and 85 CASTNet sites, hourly ozone	Short- and long-term exposure	NR	NR	12-km resolution, short-term study	R^2 for urban sites 0.55, for rural sites 0.49, hourly biases were 6–12 ppb during rural nighttime and −1 to 3 ppb during urban afternoons
Napelenok et al. (2011)	MM5 3.6.3, CMAQ 4.7.1	L: Eastern U.S.; T: June 1–August 31, 2002 and 2005; P: Entire population	684 AQS sites, DM8H ozone	Short-term exposure	NR	NR	12-km simulation, paper focused on a dynamical evaluation using two emission scenarios and less on actual evaluation with observations	NMB ranged from 0.8% in 2002 to 2.6%in 2005; NME ranged from 16.6% in 2002 to 17.6% in 2005
Tang et al. (2011)	MM5 3.6.1 and CMAQ 4.5	L: Houston, TX MSA; T: 7-day period September 2006; P: Entire population	58 AQS sites in southeastern Texas	Short-term exposure	NR	4-km resolution	7-day simulation/ validation	MNE = 15.4%, MNB = −4.9%, R^2 = 0.49

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Yu et al. (2018)	CMAQ 5.0.2 36- × 36-km resolution	L: Atlanta, GA; T: 2011; P: Entire population	Comparison with fixed-site monitors	Short-term exposure	NR	Better spatial resolution than monitor-based approaches	Relatively low spatial resolution (36 km)	Urban site: MB = −4.5 ppb, ME = 8 ppb, RMSE = 12 ppb, MNB = −9%, MNE = 21%, NMB = −10%, NME = 18%, MFB = −13%, MFE = 23%, R^2 = 0.65, Slope = 0.81; Rural site: MB = −0.73 ppb, ME = 5.92 ppb, RMSE = 7.64 ppb, MNB = 4%, MNE = 15%, NMB = 2%, NME = 13%, MFB = 2%, MFE = 14%, R^2 = 0.69, Slope = 0.85

Table 2-11 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by chemical transport modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors

ACM2 = Asymmetric Convective Model Version 2; AIRBASE = European air quality database; AIRPACT-3 = Air Indicator Report for Public Awareness and Community Tracking Version 3 ; AIRS = Aerometric Information Retrieval System; AOD = aerosol optical density; APT = Advanced Plume Treatment; AQMEII = Air Quality Model Evaluation International Initiative; AQS = Air Quality System; ARW = Advanced Research Weather; AUP = unpaired predicted-to-observed peak ozone ratio; AURAMS = Unified Regional Air Quality Modeling System; BEIS = Biogenic Emissions Inventory System; BME = Bayesian maximum entropy; BRAVO = Mexican Emissions Inventory System; CA = California; CALGRID = California Grid Simulations; CAMS = Continuous Monitoring Station; CAMx = Comprehensive Air Quality Model; CARB = California Air Resources Board; CASTNet = Clean Air Status and Trends Network; CB_{05} = carbon bond mechanism of CMAQ; CEM = continuous emissions modeling; CMAQ = Community Multiscale Air Quality model; CONUS = continental U.S.; CSAPR = Cross-State Air Pollution Rule; CSI = critical success index; CTM = chemical transport model; DDM-3D = decoupled direct method in three dimensions; DEHM = Danish Eulerian Hemispheric Model; DOE = Department of Energy; EDGAR = Emissions Database for Global Atmospheric Research; EMEP = European Monitoring and Evaluation Program; FAR = false alarm ratio; FB = fractional bias; FDDA = four-dimensional data assimilation; FE = fractional error; FEPS = Fire Emissions Production Simulator; Fp = percentage of cases where simulation results were close to observations; GB = gross bias; GE = gross error; HDDM = Hierarchical Bayesian Diffusion Drift Model; HTAP = Hemispheric Transport of Air Pollutants; ICARTT = International Consortium for Atmospheric Research on Transport and Transformation; ICC = interclass correlation coefficient; IDW = inverse-distance weighting; IMPROVE = Interagency Monitoring of Protected Visual Environments; IOA = index of agreement; IQR = inter-quartile range; L = location; LUR = land use regression; MADRID = Model of Aerosol Dynamics, Reaction, Ionization, and Dissolution; MAE = mean absolute error; MB = mean bias; MCM = master chemical mechanism; ME = mean error; MEGAN2 = Model for Gases and Aerosols from Nature Version 2; MESA = Multiethnic Study of Atherosclerosis; MFB = mean fractional bias; MFE = mean fractional error; MM5 = Mesoscale Model Version 5; MNB = mean normalized bias; MNE = mean normalized error; MNGE = mean normalized gross error; MOBILE6 = mobile emission model; MODIS = Moderate Resolution Imaging Spectroradiometer; MOZART = Model for Ozone and Related Chemical Tracers; MYJ = Mellor-Yamada-Janjic; NADP = National Atmospheric Deposition Program; NAM = North American mesoscale; NAPS = National Air Pollution Surveillance; NB = normalized bias; NC DENR = North Carolina Department of Environment and Natural Resources; NEI = National Emissions Inventory; NEu = Northern Europe; NGE = normalized gross error; NAQFC = National Air Quality Forecasting Capability; NMB = normalized mean bias; NME = normalized mean error; NMM = nonhydrostatic mesoscale model; NOAA = National Oceanographic and Atmospheric Administration; NR = not reported; NRC = National Research Council; NU = NASA-Unified; NW = northwest; NYC = New York City; OK = ordinary kriging; OMI = Ozone Monitoring Instrument; P = population; PBL = planetary boundary layer; POD = probability of detection; QNSE = Quasi Normal-Scale Elimination; *R* = Pearson correlation; RMSE = root mean squared error; SD = standard deviation; SE = southeast; SEARCH = Southeastern Aerosol Research and Characterization; SEu = Southern Europe; SIP = State Implementation Plan; SJV = San Joaquin Valley; SMOKE = Sparse Matrix Operator Kernel Emissions model; SW = southwest; T = time; TCEQ = Texas Commission on Air Quality; TES = Tropospheric Emissions System; TOPP = Tropospheric Ozone Pollution Project; UAM = Urban Airshed Model; UCD-CIT = UC Davis-California Institute of Technology model; UK = universal kriging; UPA = unpaired normalized bias; VOC = volatile organic compound; VW = Volkswagen; WDCGG = World Data Centre for Greenhouse Gases; WHI-OS = Women's Health Initiative Observational Study; WOUDC = World Ozone and Ultraviolet Data Centre; WRF = Weather Research Forecasting model; YSU = Yonsei University.

Table 2-12 Studies informing assessment of exposure measurement error when concentrations modeled by hybrid approaches are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Di et al. (2017)	Neural network incorporating OMI column data calibrated to ground level ozone concentration predicted by GEOS-Chem, land use variables, and monitoring network	L: CONUS; T: 8-h daily max ozone for 2000–2012; P: Medicare population	10-fold cross validation against monitor data reporting to AQS for annual fourth-highest 8-h daily max	Long-term exposure	Annual 4th highest ozone concentration by region: Northeast = 0.05–0.085 ppm, Southeast = 0.055–0.075 ppm, West = 0.055–0.07 ppm, National = 0.055–0.06 ppb	Good spatial coverage, high spatial resolution; high R^2 and low RMSE	Potential for model overfitting	Mean R^2 = 0.76, RMSE = 7.36 ppb; spatial R^2 = 0.80, RMSE = 2.91 ppb; temporal R^2 = 0.75, RMSE = 6.79 ppb; bias = 1.20 ppb; slope = 0.99

Table 2-12 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by hybrid approaches are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Hao et al. (2012)	IDW of CMAQ data at the block group level (version of CMAQ unclear) for two different grid resolutions: 36 and 12 km	L: CONUS; T: 8-h daily max ozone for 2006; P: Entire population	CMAQ grid resolutions of 36 and 12 km compared	Short-term exposure	98th percentile of ozone for 2006 = between 39.8 and 100.7 ppb (unclear which data source produced these concentrations), 90th percentile of ozone for 2006 = between 36.7 and 84.4 ppb (again, unclear which data source produced these concentrations)	CMAQ data have been well validated	Methods are unclear for many of the figures in the paper	Number of monitoring sites between 790 and 897, n between 195,035 and 232,081, mean absolute deviation (12 km) between 3.51 and 4.53 ppb, mean absolute deviation (36 km) between 2.98 and 3.23 ppb, *R* (12 km) between 0.94 and 0.96, *R* (36 km) between 0.96 and 0.97

Table 2-12 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by hybrid approaches are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Hystad et al. (2012)	Historically calibrated hybrid model, with a separate model for each historical year, using a regression by at a 21-km horizontal resolution by combining Canadian Hemispheric and Regional Ozone NO_X System (CHRONOS) with ozone obs from Canada and the U.S. from 2004–2006 with historical calibration through NAPS monitors, interpolation to specific locations were done with: (1) IDW and (2) regression with ozone calibration and population density at 10-km buffers around NAP stations	L: Canada; T: Annual ozone from 1975–1994; P: Entire population	Historical model compared with surface, fixed-site monitors from NAPS	Long-term exposure	IDW exposure estimates from NAPS monitors N = 6,919, mean = 23.2 ppb, SD = 3.7 ppb, min = 12.9 ppb, IQR = 4.6 ppb, maximum = 35.4 ppb, linear model N = 6,919, mean = 26.4 ppb, SD = 3.4 ppb, min = 18.1 ppb, IQR = 4.7 ppb, maximum = 37.2	Very few studies examine long-term exposures to ozone	Some aspects of methodology were not clear	Cross-validation with 10% of monitoring data CHRONOS-IDW R^2 = 0.39, RMSE = 5.29 ppb; CHRONOS-linear R^2 = 0.56, RMSE = 4.48 ppb

Table 2-12 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by hybrid approaches are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Davis et al. (2011)	GLM method developed between CMAQ output and modeled met and observed data and observed met	L: 8-h max ozone data from 74 cities across the eastern U.S.; T: May through September from 2002 to 2005; P: Entire population	CMAQ output is compared to observed data; modeled met data are compared to observed met data; both GLM models are compared to each other	Long-term exposure	8-h max ozone = between 0 and 150 ppb	GLM method based on CMAQ data were directly compared with observed data; all models developed were highly localized	Because the GLM models were developed for each location, the GLM model may not have predictive power spatially	R^2 between ozone and fitted ozone, obs R^2 = 0.74 and CMAQ R^2 = 0.70; monitoring station-specific GLM model by R^2 between 50.0 and 80.0

Table 2-12 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by hybrid approaches are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Berrocal et al. (2012)	Downscaled CMAQ (version not stated) at 12-km resolution downscaled with either a Gaussian Markov random field or a univariate model	L: East coast U.S.; T: July 4, July 20, August 9, 2001; P: Entire population	Comparison with monitors reporting to AQS	Short-term exposure	NR (shown graphically)	Data assimilation improves model fit for different variations of downscaling, and fine-tune model enhancements improve fit further	GMRFs cause some oversmoothing to blur predictive maps	CMAQ: PMSE = 135.9, PMAE = 9.1, 95% PI NR CP NR; regressor PMSE = 124.2, PMAE = 8.7, 95% PI NR CP NR; ordinary kriging: PMSE = 60.9, PMAE = 5.8, 95% PI = 30.6 CP = 94.8%; downscaler: PMSE = 53.1, PMAE = 5.3, 95% PI = 30.4 CP = 94.9%; GMRF downscaler: PMSE = 50.3, PMAE = 5.2, 95% PI = 29.4 CP = 94.9%; smoothed downscaler: PMSE = 45.4, PMAE = 5.0, 95% PI = 27.7 CP =95.0%

Table 2-12 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by hybrid approaches are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Liu et al. (2011)	Multiscale Air Quality Simulation Platform (MAQSIP) model, 6-km resolution with Bayesian downscaling to monitoring data	L: Eastern and midwestern U.S.; T: May 15–September 11, 1995 10:00–17:00; P: Entire population	Comparison of model points with concentrations from 375 monitors reporting to AQS	Long-term exposure	NR	Method connects measurements with model results through latent processes; the model has flexibility	Computationally intensive; this version did not include a space-time framework; model assumed measurements and model outputs are Gaussian processes	Daily RMSPE = 6.98–18.55 ppb

Table 2-12 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by hybrid approaches are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Pongprueksa (2013)	CMAQ 4.7.1, WRF 3.4, CONUS with 36-km horizontal resolution for 2009 merged with Tropospheric Emission Spectrometer (TES) L3 (using TES data as is and shifted by 10 ppb to better account for boundary conditions)	L: CONUS; T: 2009; P: Entire population	CMAQ using satellite data were compared to the typical conditions for CMAQ, both CMAQ methods were compared with observed data from U.S. EPA's AQS and ozonesonde data collected across CONUS from WOUDC, NOAA, and TOPP	Short- and long-term exposure	Ozonesonde; annual 8-h daily max ozone in southern states for 2009; 8-h daily max from Texas; annual 8-h daily max ozone across CONUS	Addition of satellite data reduces error and uncertainty both in the upper atmosphere and in the troposphere	Satellite data overestimates tropospheric ozone	Surface observed ozone compared CMAQ-TES: n = 26,234, MB = 9 ppb, ME = 12 ppb, NMB = 23%, NME = 31%, *R* = 0.56, compared with CMAQ-TESadj: n = 26,234, MB = 4 ppb, ME = 10 ppb, NMB = 10%, NME = 24%, *R* = 0.58; model performance by region n = 1–21 sites, CMAQ-TES, MB = 3–15 ppb, ME = 7–15 ppb, NMB = 6–45%, NME = 14–46%, *R* = 0.46–0.64, and CMAQ-TESadj, MB = −3 to 9 ppb, ME = 6–11 ppb, NMB = −6 to 28%, NME = 14–30%, *R* = 0.48–0.66

Table 2-12 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by hybrid approaches are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Reich et al. (2014)	Spectral downscaling using surface, fixed-site monitors from U.S. EPA's AQS and CASTNet and CMAQ 5.0.1 with a 12-km horizontal resolution	L: CONOUS; T: July 2005; P: Entire population	Spectral downscaling compared with no CMAQ, linear downscaler, and kernel smoothed downscaler, all comparison methods are compared against observed data	Short-term exposure	1-day avg ozone concentration	Method is explicitly stated; hybrid models allows for strength of both CTMs and obs data	Short-term exposure is not indicative of longer ozone exposures; collocation needed for validation preferentially selects higher ozone areas	Spatial prediction: monitors only MSE = 62.8 ppb^2, bias = −0.14 ppb, variance = 66.3 ppb^2 CP = 0.91, linear downscaler MSE = 57.5 ppb^2, bias = −0.26 ppb, variance = 56.2 ppb^2 CP = 0.91, spectral downscaler MSE = 53.7 ppb^2, bias = −0.23 ppb, variance = 53.3 ppb^2 CP = 0.91, kernel downscaler 12-km resolution MSE = 54.9 ppb^2, bias = −0.23 ppb, variance = 54.8 ppb^2 CP = 0.91, kernel downscaler 60-km resolution MSE = 58.7 ppb^2, bias = −0.17 ppb, variance = 59.2 ppb^2 CP

Table 2-12 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by hybrid approaches are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Reich et al. (2014 (cont.)	Spectral downscaling using surface, fixed-site monitors from U.S. EPA's AQS and CASTNet and CMAQ 5.0.1 with a 12-km horizontal resolution (cont.)	L: CONOUS; T: July 2005; P: Entire population (cont.)	Spectral downscaling compared with no CMAQ, linear downscaler, and kernel smoothed downscaler, all comparison methods are compared against observed data (cont.)	Short-term exposure (cont.)	1-day avg ozone concentration (cont.)	Method is explicitly stated; hybrid models allows for strength of both CTMs and obs data (cont.)	Short-term exposure is not indicative of longer ozone exposures; collocation needed for validation preferentially selects higher ozone areas (cont.)	= 0.91, kernel downscaler 120-km resolution MSE = 60.9 ppb^2, bias = −0.14 ppb, variance = 62.9 ppb^2 CP = 0.91; nonspatial prediction: monitors only MSE = 339.7 ppb^2, bias = −6.17 ppb, variance = 302.0 ppb^2 CP = 0.89, linear downscaler MSE = 202.1 ppb^2, bias = −2.80 ppb, variance = 177.7 ppb^2 CP = 0.89, spectral downscaler MSE = 145.7 ppb^2 bias = 0.57 ppb, variance = 129.1 ppb^2 CP = 0.89, kernel downscaler 12-km resolution MSE = 151.2 ppb^2,

Table 2-12 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by hybrid approaches are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Reich et al. (2014 (cont.)	Spectral downscaling using surface, fixed-site monitors from U.S. EPA's AQS and CASTNet and CMAQ 5.0.1 with a 12-km horizontal resolution (cont.)	L: CONOUS; T: July 2005; P: Entire population (cont.)	Spectral downscaling compared with no CMAQ, linear downscaler, and kernel smoothed downscaler, all comparison methods are compared against observed data (cont.)	Short-term exposure (cont.)	1-day avg ozone concentration (cont.)	Method is explicitly stated; hybrid models allows for strength of both CTMs and obs data (cont.)	Short-term exposure is not indicative of longer ozone exposures; collocation needed for validation preferentially selects higher ozone areas (cont.)	bias = −0.06 ppb, variance = 134.8 ppb^2 CP = 0.89, kernel downscaler 60-km resolution MSE = 157.6 ppb^2, bias = 1.06 ppb, variance = 142.9 ppb^2 CP = 0.89, kernel downscaler 120 km resolution MSE = 169.1 ppb^2, bias = 0.93 ppb, variance = 151.7 ppb^2 CP = 0.89 Full model MSE 24.97, ppb MAE 3.80 ppb, CP 85.7%, avg length of PI 15.70 ppb; reduced model MSE 24.66 ppb, MAE 3.79 ppm, CP 85.5%, avg length of PI 13.67 ppb

Table 2-12 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by hybrid approaches are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Paci et al. (2013)	Eta-CMAQ at 12-km scale (CMAQ version not specified), downscaled to 717 ozone monitors	L: Eastern and midwestern U.S.; T: August 1–14, 2011; P: Entire population	Compare predicted ozone concentration to ozone measured at monitors set aside from the analysis for validation	Short-term exposure	NR	Works well for forecasting because the downscaler is based on temporal gradients	Method is linear, so it may not handle more complex temporal models well	Full model MSE 24.97 ppb^2, MAE 3.80 ppb, CP 85.7%, avg length of PI 15.70 ppb; reduced model MSE 24.66 ppb^2, MAE 3.79 ppb, coverage probability 85.5%, avg length of PI 13.67 ppb
Tang et al. (2015b)	Optimal interpolation (OI) hybrid method with AirNow data, MODIS AOD from Terra and Aqua satellites incorporated into CMAQ 5.0.2, WRF-ARW 3.4.1 with relative uncertainties of 0.4, up to 0.6, up to 1.0, respectively, with 12-km horizontal resolution	L: CONUS; T: July 2011; P: Entire population	Modeled outputs compared with AirNow observed data and aircraft measurements from Discover-AQ	Short-term exposure	Hourly ozone in the first half of July 2011 in northeastern U.S. NR (shown graphically)	Two different observed data sources used; multiple models compared to each other	1 summer mo may not be indicative of more long-term exposures	Hourly ozone from July 6–7, 2011 over CONUS for optimal interpolation (OI) 1–4, R = 0.52−0.58, MB = 1.06–2.36; hourly ozone from July 6–7, 2011 over southeastern U.S. for OI 1–4, R = 0.58–0.61, MB = −1.40 to 0.43; R between obs and OI 4 for aircraft data is 0.753

Table 2-12 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by hybrid approaches are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Huang et al. (2015)	CAMx 6.10 with 4-km horizontal resolution with land cover compared generated from MODIS and TCEQ; models compared with each other and with ambient monitoring sites (unclear who is responsible for these sites)	L: Eastern Texas; T: June 2006; P: Entire population	Models compared with each other and with ambient monitoring sites (unclear who is responsible for these sites)	Short-term exposure	NR	Fine scale resolution; this speaks to the importance of the effect of model inputs to measured concentration	Limited spatial and temporal coverage may not be indicative of a typical exposure concentration	Difference between obs and simulation: CAMx with MODIS mean = 2–6 ppb, max = >20 ppb; CAMx with TCEQ data mean = 2 ppb, max = 30 ppb

Table 2-12 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by hybrid approaches are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Friberg et al. (2016)	CMAQ 4.5 fused with observational data by three methods: fusing CMAQ with interpolated observations, scaling CMAQ fields to observations that are corrected for seasonal bias, and combining these methods through a weighted model	L: Georgia; T: 2002–2008; P: Entire population	Cross-validation by fixed-site monitors	Long-term exposure	Mean (IQR) = 47.6 ppb (22.0 ppb) for 8-h daily max	Low mean bias, low RMSE, and relatively high R^2 (compared to application of the model for other pollutants), and errors are minimized through the model-weighting approach	Errors in measurements used as input are propagated into the model, limited spatial coverage of monitors increases errors (although this is less of a limitation for ozone and other secondary pollutants)	Interpolated observations MFE = 0.16, MFB = 0.02, NME = 14.7%, NMB = −0.57%, MB = −2.7e–4, RMSE = 0.01, R^2 (cross-validation): 68.7%; for optimized method MFE = 0.05, MFB = 0.01, NME = 4.49%, NMB = 0.03%, MB = 1.5e–5, RMSE = 0.003, R^2 (cross-validation) = 97.0%; weighted combination of methods MFE = 0.10, MFB = 0.02, NME = 8.57%, NMB = 0.03%, MB = 1.3e–5, RMSE = 0.006, R^2 (cross-validation) = 87.1%

Table 2-12 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by hybrid approaches are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Xu et al. (2016b)	CAMx 5.30 at nested 36-km domain over the CONUS and 12-km domain over the eastern U.S., with BME implemented by a model with parameters held constant across the CONUS [CAMP] and with regional parameters [RAMP]	L: Contiguous U.S.; T: 2005; P: Entire population	Comparison with observations from fixed-site monitors reporting to the AQS, tower sensors at one location (Raleigh, NC), and DISCOVER-AQ flight sensors	Long-term exposure	NR	BME enables estimation of concentration below scale of CTM simulation, better spatial and temporal validation with RAMP model, computationally efficient and straightforward approach	Uncertainty in concentration estimates increases with distance from the monitors	CAMP: RMSE 0 km = 5.675 ppb, 36 km = 6.442 ppb, 72 km = 6.966 ppb, 108 km = 7.250 ppb R^2 0 km = 0.884, 36 km = 0.853, 72 km = 0.831, 108 km = 0.819; RAMP: RMSE 0 km = 5.445 ppb, 36 km = 6.109 ppb, 72 km = 6.531 ppb, 108 km = 6.732 ppb R^2 0 km = 0.893, 36 km = 0.866, 72 km = 0.849, 108 km = 0.841

Table 2-12 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by hybrid approaches are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Bash et al. (2016)	CMAQ 5.0.2, WRF 3.3, biogenic emission from BEIS 3.61 with 4-km horizontal resolution; sensitivity analysis include BEIS 3.14, BEIS 3.61 SAT (from MODIS) par, MEGAN 2.1 SAT (from MODIS) par	L: Central and northern CA, U.S.; T: June 3–July 31, 2009; P: Entire population	Models compared to each other and compared to observed, fixed-site monitors from U.S. EPA's AQS	Short-term exposure	Average hourly obs ozone greater than 60 ppb = 70.9 ppb, less than 60 = 32.0 ppb, average mod hourly ozone greater than 60 ppb = between 62.1 and 64.8, less than 60 ppb = between 40.7 and 41.7 ppb	This incremental improvement in modeled inputs allow for seeing pointed concentration changes; fine spatial resolution	Localized spatiotemporal modeling domain may not be typical of average ozone exposures	Biases and errors when using satellite parameterization of weather model: ozone greater than 60 ppb = median bias −9 to −12 ppb, median error = 13–14 ppb, MB = −6.6 to −8.8 ppb, ME = 11−11.9 ppb, FB = −10.8 to −14.1%, FE = 16.8–18.3%; less than 60 ppb: median bias = 29 ppb, median error = 32 ppb, MB = 8.7 ppb, ME = 11 ppb, FB = 29.4−30%, FE = 36.2–36.4%
Xu et al. (2017)	CAMx with observations integrated using BME	L: CONUS; T: 2005 annual simulation; P: Entire population	Hourly, 8-h daily max and 24-h avg	Short-term exposure	NR	CONUS simulations using observations to improve ozone simulation	36- and 12-km simulations	*r* range from 0.78–0.82; RMSE = 5.2−6.3 ppb

Table 2-12 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by hybrid approaches are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Robichaud and Menard (2014)	An objective analysis (OA) scheme is developed to integrate predictions from CHRONOS and GEM-MACH CTMs (with 21-km horizontal resolution for CHRONOS and 15-km resolution for GEM-MACH) with surface observations	L: Canada and U.S.; T: 2001–2012; P: Canadian Census Health and Environment Cohort	Models compared to each other and to observed, fixed-site monitors from the Canadian Meteorological Centre and the AQS	Long-term exposure	NR	Very small or no biases were observed; automated process	Impact of NO_X on spatial variability of ozone would not be captured over coarse grid	Frequency of model predictions within a factor of two of the observations: Canada = 0.654−0.927; U.S. = 0.641−0.969

Table 2-12 (Continued): Studies informing assessment of exposure measurement error when concentrations modeled by hybrid approaches are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Yu et al. (2018)	CMAQ-kriging hybrid model where CMAQ 5.0.2, 36- × 36-km resolution was run, ratios calculated between CMAQ and observations across the surface, CMAQ output was adjusted by those ratios, and then the surface was kriged to interpolate between grid centroids (Friberg et al., 2016)	L: Atlanta, GA; T: 2011; P: Entire population	Comparison with fixed-site monitors	Short-term exposure	NR	Improves spatial resolution over CMAQ alone and monitor-based approaches	Complex model design is more difficult to implement compared with CMAQ alone or the CMAQ-kriging hybrid model	Urban site: MB = −5.8 ppb, ME = 6 ppb, RMSE = 7 ppb, MNB = −12%, MNE = 14%, NMB = −13%, NME = 14%, MFB = −13%, MFE = 15%, R^2 = 0.95, slope = 1.20; Rural site: MB = −1.79 ppb, ME = 3.98 ppb, RMSE = 5.16 ppb, MNB = −4%, MNE = 10%, NMB = −4%, NME = 9%, MFB = −5%, MFE = 10%, R^2 = 0.88, slope = 0.88

Table 2-13 Studies informing assessment of exposure measurement error when concentrations modeled by microenvironmental modeling are used for exposure surrogates.

Reference	Model	Location, Time Period, and Population	Measurement Evaluation Technique	Epidemiology Applications	Concentrations Measured	Strengths	Limitations	Exposure Measurement Errors
Dionisio et al. (2014)[a]	Stochastic Human Exposure and Dose Simulation (SHEDS) model	L: Atlanta, GA; T: 1999–2002; P: Entire population	Comparison with dispersion model	Long-term exposure	8-h daily max ozone, NR	More precise model	Computationally intensive, but might not be needed	Mean (SD) exposure measurement error: population = −0.66 (0.029) spatial = −0.055 (0.037), total = −0.72 (0.010)
Reich et al. (2012)	Air Pollutant Exposure (APEX) model	L: Philadelphia; T: June–August, 2001; P: Entire population	Fivefold cross-validation against monitors reporting to AQS	Short-term exposure	Daily average ozone NR	Predicts exposure rather than providing a surrogate for exposure	The model includes many assumptions; accuracy is limited to quality of input data	Comparison is shown graphically−linear relationship between predictions and observations, but there are many instances where the model is positively biased

APEX = Air Pollution Exposure model; AQS = Air Quality System; NR = not reported; SHEDS = Stochastic Human Exposure and Dose Simulation

[a]Data were obtained from the study author.

2.9 References

Adam-Poupart, A; Brand, A; Fournier, M; Jerrett, M; Smargiassi, A. (2014). Spatiotemporal modeling of ozone levels in Quebec (Canada): a comparison of kriging, land-use regression (LUR), and combined Bayesian maximum entropy-LUR approaches. Environ Health Perspect 122: 970-976. http://dx.doi.org/10.1289/ehp.1306566.

Appel, KW; Chemel, C; Roselle, SJ; Francis, XV; Hu, RM; Sokhi, RS; Rao, ST; Galmarini, S. (2012). Examination of the Community Multiscale Air Quality (CMAQ) model performance over the North American and European domains. Atmos Environ 53: 142-155. http://dx.doi.org/10.1016/j.atmosenv.2011.11.016.

Appel, KW; Napelenok, SL; Foley, KM; Pye, HOT; Hogrefe, C; Luecken, DJ; Bash, JO; Roselle, SJ; Pleim, JE; Foroutan, H; Hutzell, WT; Pouliot, GA; Sarwar, G; Fahey, KM; Gantt, B; Gilliam, RC; Heath, NK; Kang, D; Mathur, R; Schwede, DB; Spero, TL; Wong, DC; Young, JO. (2017). Description and evaluation of the Community Multiscale Air Quality (CMAQ) modeling system version 5.1. GMD 10: 1703-1732. http://dx.doi.org/10.5194/gmd-10-1703-2017.

Appel, KW; Pouliot, GA; Simon, H; Sarwar, G; Pye, HOT; Napelenok, SL; Akhtar, F; Roselle, SJ. (2013). Evaluation of dust and trace metal estimates from the Community Multiscale Air Quality (CMAQ) model version 5.0. GMD 6: 883-899. http://dx.doi.org/10.5194/gmd-6-883-2013.

Armstrong, BK; White, E; Saracci, R. (1992). Principles of exposure measurement in epidemiology. New York, NY: Oxford University Press.

Baker, KR; Woody, MC; Tonnesen, GS; Hutzell, W; Pye, HOT; Beaver, MR; Pouliot, G; Pierce, T. (2016). Contribution of regional-scale fire events to ozone and PM2.5 air quality estimated by photochemical modeling approaches. Atmos Environ 140: 539-554. http://dx.doi.org/10.1016/j.atmosenv.2016.06.032.

Barrett, SRH; Speth, RL; Eastham, SD; Dedoussi, IC; Ashok, A; Malina, R; Keith, DW. (2015). Impact of the Volkswagen emissions control defeat device on US public health. Environ Res Lett 10. http://dx.doi.org/10.1088/1748-9326/10/11/114005.

Bart, M; Williams, DE; Ainslie, B; Mckendry, I, an; Salmond, J; Grange, SK; Alavi-Shoshtari, M; Steyn, D; Henshaw, GS. (2014). High density ozone monitoring using gas sensitive semi-conductor sensors in the lower fraser valley, british columbia. Environ Sci Technol 48: 3970-3977. http://dx.doi.org/10.1021/es404610t.

Bash, JO; Baker, KR; Beaver, MR. (2016). Evaluation of improved land use and canopy representation in BEIS v3.61 with biogenic VOC measurements in California. GMD 9: 2191-2207. http://dx.doi.org/10.5194/gmd-9-2191-2016.

Bell, ML. (2006). The use of ambient air quality modeling to estimate individual and population exposure for human health research: A case study of ozone in the Northern Georgia region of the United States. Environ Int 32: 586-593. http://dx.doi.org/10.1016/j.envint.2006.01.005.

Ben-David, T, om; Waring, MS. (2016). Impact of natural versus mechanical ventilation on simulated indoor air quality and energy consumption in offices in fourteen US cities. Build Environ 104: 320-336. http://dx.doi.org/10.1016/j.buildenv.2016.05.007.

Ben-David, T; Waring, MS. (2018). Interplay of ventilation and filtration: Differential analysis of cost function combining energy use and indoor exposure to PM2.5 and ozone. Build Environ 128: 320-335. http://dx.doi.org/10.1016/j.buildenv.2017.10.025.

Berrocal, VJ; Gelfand, AE; Holland, DM. (2012). Space-time data fusion under error in computer model output: An application to modeling air quality. Biometrics 68: 837-848. http://dx.doi.org/10.1111/j.1541-0420.2011.01725.x.

Billionnet, C; Sherrill, D; Annesi-Maesano, I. (2012). Estimating the health effects of exposure to multi-pollutant mixture [Review]. Ann Epidemiol 22: 126-141. http://dx.doi.org/10.1016/j.annepidem.2011.11.004.

Blanchard, CL; Hidy, GM; Tanenbaum, S; Edgerton, ES. (2011). NMOC, ozone, and organic aerosol in the southeastern United States, 19992007: 3. Origins of organic aerosol in Atlanta, Georgia, and surrounding areas. Atmos Environ 45: 1291-1302. http://dx.doi.org/10.1016/j.atmosenv.2010.12.004.

Bond, TC; Doherty, SJ; Fahey, DW; Forster, PM; Berntsen, T; Deangelo, BJ; Flanner, MG; Ghan, S; Kärcher, B; Koch, D; Kinne, S; Kondo, Y; Quinn, PK; Sarofim, MC; Schultz, MG; Schulz, M; Venkataraman, C; Zhang, H; Zhang, S; Bellouin, N; Guttikunda, SK; Hopke, PK; Jacobson, MZ; Kaiser, JW; Klimont, Z; Lohmann, U; Schwarz, JP; Shindell, D; Storelvmo, T; Warren, SG; Zender, CS. (2013). Bounding the role of black carbon in the climate system: A scientific assessment. J Geophys Res Atmos 118: 5380-5552. http://dx.doi.org/10.1002/jgrd.50171.

Brauer, M; Amann, M; Burnett, RT; Cohen, A; Dentener, F; Ezzati, M; Henderson, SB; Krzyzanowski, M; Martin, RV; Van Dingenen, R; van Donkelaar, A; Thurston, GD. (2012). Exposure assessment for estimation of the global burden of disease attributable to outdoor air pollution. Environ Sci Technol 46: 652-660. http://dx.doi.org/10.1021/es2025752.

Bravo, MA; Fuentes, M; Zhang, Y; Burr, MJ; Bell, ML. (2012). Comparison of exposure estimation methods for air pollutants: ambient monitoring data and regional air quality simulation. Environ Res 116: 1-10. http://dx.doi.org/10.1016/j.envres.2012.04.008.

Brokamp, C; Rao, MB; Fan, Z; Ryan, PH. (2015). Does the elemental composition of indoor and outdoor PM2.5 accurately represent the elemental composition of personal PM2.5? Atmos Environ 101: 226-234. http://dx.doi.org/10.1016/j.atmosenv.2014.11.022.

Buteau, S; Hatzopoulou, M; Crouse, DL; Smargiassi, A; Burnett, RT; Logan, T; Cavellin, LD; Goldberg, MS. (2017). Comparison of spatiotemporal prediction models of daily exposure of individuals to ambient nitrogen dioxide and ozone in Montreal, Canada. Environ Res 156: 201-230. http://dx.doi.org/10.1016/j.envres.2017.03.017.

Carlton, AG; Baker, KR. (2011). Photochemical modeling of the Ozark isoprene volcano: MEGAN, BEIS, and their impacts on air quality predictions. Environ Sci Technol 45: 4438-4445. http://dx.doi.org/10.1021/es200050x.

Castellanos, P; Marufu, LT; Doddridge, BG; Taubman, BF; Schwab, JJ; Hains, JC; Ehrman, SH; Dickerson, RR. (2011). Ozone, oxides of nitrogen, and carbon monoxide during pollution events over the eastern United States: An evaluation of emissions and vertical mixing. J Geophys Res 116: D16307. http://dx.doi.org/10.1029/2010JD014540.

Chai, T; Kim, HC; Lee, P; Tong, D; Pan, L; Tang, Y; Huang, J; Mcqueen, J; Tsidulko, M; Stajner, I. (2013). Evaluation of the United States National Air Quality Forecast Capability experimental real-time predictions in 2010 using Air Quality System ozone and NO2 measurements. GMD 6: 1831-1850. http://dx.doi.org/10.5194/gmd-6-1831-2013.

Chan, WR; Cohn, S; Sidheswaran, M; Sullivan, DP; Fisk, WJ. (2014). Contaminant levels, source strengths, and ventilation rates in California retail stores. Indoor Air 25: 381-392. http://dx.doi.org/10.1111/ina.12152.

Chang, KL; Guillas, S; Fioletov, VE. (2015). Spatial mapping of ground-based observations of total ozone. Atmos Meas Tech 8: 4487-4505. http://dx.doi.org/10.5194/amt-8-4487-2015.

Chen, C; Zhao, B; Weschler, CJ. (2012). Assessing the influence of indoor exposure to "outdoor ozone" on the relationship between ozone and short-term mortality in us communities. Environ Health Perspect 120: 235-240. http://dx.doi.org/10.1289/ehp.1103970.

Chen, D; Li, Q; Stutz, J; Mao, Y; Zhang, L, i; Pikelnaya, O; Tsai, J; Haman, C; Lefer, B; Rappenglueck, B; Alvarez, SL; Neuman, JA; Flynn, J; Roberts, JM; Nowak, JB; de Gouw, J; Holloway, J; Wagner, NL; Veres, P; Brown, SS; Ryerson, TB; Warneke, C; Pollack, LB. (2013). WRF-Chem simulation of NOx and O-3 in the LA basin during CalNex-2010. Atmos Environ 81: 421-432. http://dx.doi.org/10.1016/j.atmosenv.2013.08.064.

Cho, S; Mceachern, P; Morris, R; Shah, T; Johnson, J; Nopmongcol, U. (2012). Emission sources sensitivity study for ground-level ozone and PM2.5 due to oil sands development using air quality modeling system: Part I- model evaluation for current year base case simulation. Atmos Environ 55: 533-541. http://dx.doi.org/10.1016/j.atmosenv.2012.02.026.

Choi, Y. (2014). The impact of satellite-adjusted NOx emissions on simulated NOx and O-3 discrepancies in the urban and outflow areas of the Pacific and Lower Middle US. Atmos Chem Phys 14: 675-690. http://dx.doi.org/10.5194/acp-14-675-2014.

Christakos, G. (1990). A bayesian maximum-entropy view to the spatial estimation problem. Math Geol 22: 763-777. http://dx.doi.org/10.1007/BF00890661.

Clark, LP; Millet, DB; Marshall, JD. (2011). Air quality and urban form in U.S. urban areas: Evidence from regulatory monitors. Environ Sci Technol 45: 7028-7035. http://dx.doi.org/10.1021/es2006786.

Cleveland, WS; Graedel, TE. (1979). Photochemical air pollution in the northeast United States. Science 204: 1273-1278. http://dx.doi.org/10.1126/science.204.4399.1273.

Cohan, DS; Hu, Y; Russell, AG. (2006). Dependence of ozone sensitivity analysis on grid resolution. Atmos Environ 40: 126-135. http://dx.doi.org/10.1016/j.atmosenv.2005.09.031.

Cuchiara, GC; Li, X; Carvalho, J; Rappenglueck, B. (2014). Intercomparison of planetary boundary layer parameterization and its impacts on surface ozone concentration in the WRF/Chem model for a case study in Houston/Texas. Atmos Environ 96: 175-185. http://dx.doi.org/10.1016/j.atmosenv.2014.07.013.

Darrow, LA; Klein, M; Sarnat, JA; Mulholland, JA; Strickland, MJ; Sarnat, SE; Russell, AG; Tolbert, PE. (2011). The use of alternative pollutant metrics in time-series studies of ambient air pollution and respiratory emergency department visits. J Expo Sci Environ Epidemiol 21: 10-19. http://dx.doi.org/10.1038/jes.2009.49.

Davalos, AD; Luben, TJ; Herring, AH; Sacks, JD. (2017). Current approaches used in epidemiologic studies to examine short-term multipollutant air pollution exposures [Review]. Ann Epidemiol 27: 145-153.e141. http://dx.doi.org/10.1016/j.annepidem.2016.11.016.

Davis, J; Cox, W; Reff, A; Dolwick, P, at. (2011). A comparison of CMAQ-based and observation-based statistical models relating ozone to meteorological parameters. Atmos Environ 45: 3481-3487. http://dx.doi.org/10.1016/j.atmosenv.2010.12.060.

Delfino, RJ; Coate, BD; Zeiger, RS; Seltzer, JM; Street, DH; Koutrakis, P. (1996). Daily asthma severity in relation to personal ozone exposure and outdoor fungal spores. Am J Respir Crit Care Med 154: 633-641. http://dx.doi.org/10.1164/ajrccm.154.3.8810598.

Di, Q; Rowland, S; Koutrakis, P; Schwartz, J. (2017). A hybrid model for spatially and temporally resolved ozone exposures in the continental United States. J Air Waste Manag Assoc 67: 39-52. http://dx.doi.org/10.1080/10962247.2016.1200159.

Dionisio, KL; Baxter, LK; Chang, HH. (2014). An empirical assessment of exposure measurement error and effect attenuation in bipollutant epidemiologic models. Environ Health Perspect 122: 1216-1224. http://dx.doi.org/10.1289/ehp.1307772.

Dutton, SM; Banks, D; Brunswick, SL; Fisk, WJ. (2013). Health and economic implications of natural ventilation in California offices. Build Environ 67: 34-45. http://dx.doi.org/10.1016/j.buildenv.2013.05.002.

Emery, C; Jung, J; Downey, N; Johnson, J; Jimenez, M; Yarvvood, G; Morris, R. (2012). Regional and global modeling estimates of policy relevant background ozone over the United States. Atmos Environ 47: 206-217. http://dx.doi.org/10.1016/j.atmosenv.2011.11.012.

EPA, US. (2018). CMAQ (Version 5.2.1) [Computer Program]: Zenodo. Retrieved from https://zenodo.org/record/1212601#.XGFtoVxKjIV

Fadeyi, MO; Weschler, CJ; Tham, KW; Wu, WY; Sultan, ZM. (2013). Impact of human presence on secondary organic aerosols derived from ozone-initiated chemistry in a simulated office environment. Environ Sci Technol 47: 3933-3941. http://dx.doi.org/10.1021/es3050828.

Ferreira, J; Rodriguez, A; Monteiro, A; Miranda, AI; Dios, M; Souto, JA; Yarwood, G; Nopmongcol, U; Borrego, C. (2012). Air quality simulations for North America-MM5-CAMx modelling performance for main gaseous pollutants. Atmos Environ 53: 212-224. http://dx.doi.org/10.1016/j.atmosenv.2011.10.020.

Friberg, MD; Zhai, X; Holmes, HA; Chang, HH; Strickland, MJ; Sarnat, SE; Tolbert, PE; Russell, AG; Mulholland, JA. (2016). Method for fusing observational data and chemical transport model simulations to estimate spatiotemporally resolved ambient air pollution. Environ Sci Technol 50: 3695-3705. http://dx.doi.org/10.1021/acs.est.5b05134.

Fuentes, M. (2009). Statistical issues in health impact assessment at the state and local levels. Air Qual Atmos Health 2: 47-55. http://dx.doi.org/10.1007/s11869-009-0033-3.

Fuentes, M; Raftery, AE. (2005). Model evaluation and spatial interpolation by Bayesian combination of observations with outputs from numerical models. Biometrics 61: 36-45. http://dx.doi.org/10.1111/j.0006-341X.2005.030821.x.

Gall, ET; Corsi, RL; Siegel, JA. (2011). Barriers and opportunities for passive removal of indoor ozone. Atmos Environ 45: 3338-3341. http://dx.doi.org/10.1016/j.atmosenv.2011.03.032.

Ganguly, R; Batterman, S; Isakov, V; Snyder, M; Breen, M; Brakefield-Caldwell, W. (2015). Effect of geocoding errors on traffic-related air pollutant exposure and concentration estimates : Supplemental materials [Supplemental Data]. J Expo Sci Environ Epidemiol 25.

Garner, GG; Thompson, AM; Lee, P; Martins, DK. (2015). Evaluation of NAQFC model performance in forecasting surface ozone during the 2011 DISCOVER-AQ campaign. J Atmos Chem 72: 483-501. http://dx.doi.org/10.1007/s10874-013-9251-z.

Gelfand, AE; Sahu, SK; Holland, DM. (2012). On the Effect of Preferential Sampling in Spatial Prediction. Environmetrics 23: 565-578. http://dx.doi.org/10.1002/env.2169.

Georgopoulos, PG; Wang, SW; Vyas, VM; Sun, Q; Burke, J; Vedantham, R; Mccurdy, T; Ozkaynak, H. (2005). A source-to-dose assessment of population exposures to fine PM and ozone in Philadelphia, PA, during a summer 1999 episode. J Expo Anal Environ Epidemiol 15: 439-457. http://dx.doi.org/10.1038/sj.jea.7500422.

Glasgow, ML; Rudra, CB; Yoo, EH; Demirbas, M; Merriman, J; Nayak, P; Crabtree-Ide, C; Szpiro, AA; Rudra, A; Wactawski-Wende, J; Mu, L. (2014). Using smartphones to collect time-activity data for long-term personal-level air pollution exposure assessment. J Expo Sci Environ Epidemiol 26: 356-364. http://dx.doi.org/10.1038/jes.2014.78.

Godowitch, JM; Gilliam, RC; Rao, ST. (2011). Diagnostic evaluation of ozone production and horizontal transport in a regional photochemical air quality modeling system. Atmos Environ 45: 3977-3987. http://dx.doi.org/10.1016/j.atmosenv.2011.04.062.

Godowitch, JM; Gilliam, RC; Roselle, SJ. (2015). Investigating the impact on modeled ozone concentrations using meteorological fields from WRF with an updated four-dimensional data assimilation approach. Atmos Pollut Res 6: 305-311. http://dx.doi.org/10.5094/APR.2015.034.

Goldberg, MS. (2007). On the interpretation of epidemiological studies of ambient air pollution. J Expo Sci Environ Epidemiol 17: S66-S70. http://dx.doi.org/10.1038/sj.jes.7500629.

Goldman, GT; Mulholland, JA; Russell, AG; Gass, K; Strickland, MJ; Tolbert, PE. (2012). Characterization of ambient air pollution measurement error in a time-series health study using a geostatistical simulation approach. Atmos Environ 57: 101-108. http://dx.doi.org/10.1016/j.atmosenv.2012.04.045.

Goldman, GT; Mulholland, JA; Russell, AG; Srivastava, A; Strickland, MJ; Klein, M; Waller, LA; Tolbert, PE; Edgerton, ES. (2010). Ambient air pollutant measurement error: characterization and impacts in a time-series epidemiologic study in Atlanta. Environ Sci Technol 44: 7692-7698. http://dx.doi.org/10.1021/es101386r.

Goldman, GT; Mulholland, JA; Russell, AG; Strickland, MJ; Klein, M; Waller, LA; Tolbert, PE. (2011). Impact of exposure measurement error in air pollution epidemiology: Effect of error type in time-series studies. Environ Health 10: 61. http://dx.doi.org/10.1186/1476-069X-10-61.

Gong, X; Kaulfus, A; Nair, U; Jaffe, DA. (2017). Quantifying O3 impacts in urban areas due to wildfires using a generalized additive model. Environ Sci Technol 51: 13216-13223. http://dx.doi.org/10.1021/acs.est.7b03130.

Graham, SE; Mccurdy, T. (2004). Developing meaningful cohorts for human exposure models. J Expo Anal Environ Epidemiol 14: 23-43. http://dx.doi.org/10.1038/sj.jea.7500293.

Hackbarth, AD; Romley, JA; Goldman, DP. (2011). Racial and ethnic disparities in hospital care resulting from air pollution in excess of federal standards. Soc Sci Med 73: 1163-1168. http://dx.doi.org/10.1016/j.socscimed.2011.08.008.

Hall, ES; Eyth, AM; Phillips, SB; Mason, R. (2012). Hierarchical Bayesian model (HBM): Derived estimates of air quality for 2008: Annual report. (EPA/600/R-12/048). U.S. Environmental Protection Agency.

Hao, Y; Flowers, H; Monti, MM; Qualters, JR. (2012). U.S. census unit population exposures to ambient air pollutants. Int J Health Geogr 11: 3. http://dx.doi.org/10.1186/1476-072X-11-3.

He, J; Kolovos, A. (2018). Bayesian maximum entropy approach and its applications: a review. Stoch Environ Res Risk Assess 32: 859-877. http://dx.doi.org/10.1007/s00477-017-1419-7.

Henderson, BH; Jeffries, HE; Kim, BU; Vizuete, WG. (2010). The influence of model resolution on ozone in industrial volatile organic compound plumes. J Air Waste Manag Assoc 60: 1105-1117.

Henneman, LRF; Chang, HH; Liao, K, uoJen; Lavoue, D; Mulholland, JA; Russell, AG. (2017a). Accountability assessment of regulatory impacts on ozone and PM2.5 concentrations using statistical and deterministic pollutant sensitivities. Air Qual Atmos Health 10: 695-711. http://dx.doi.org/10.1007/s11869-017-0463-2.

Henneman, LRF; Liu, C; Hu, Y; Mulholland, JA; Russell, AG. (2017b). Air quality modeling for accountability research: Operational, dynamic, and diagnostic evaluation. Atmos Environ 166: 551-565. http://dx.doi.org/10.1016/j.atmosenv.2017.07.049.

Herron-Thorpe, FL; Mount, GH; Emmons, LK; Lamb, BK; Jaffe, DA; Wigder, NL; Chung, SH; Zhang, R; Woelfle, MD; Vaughan, JK. (2014). Air quality simulations of wildfires in the Pacific Northwest evaluated with surface and satellite observations during the summers of 2007 and 2008. Atmos Chem Phys 14: 12533-12551. http://dx.doi.org/10.5194/acp-14-12533-2014.

Herwehe, JA; Otte, TL; Mathur, R; Rao, ST. (2011). Diagnostic analysis of ozone concentrations simulated by two regional-scale air quality models. Atmos Environ 45: 5957-5969. http://dx.doi.org/10.1016/j.atmosenv.2011.08.011.

Hogrefe, C; Pouliot, G; Wong, D; Torian, A; Roselle, S; Pleim, J; Mathur, R. (2015). Annual application and evaluation of the online coupled WRF-CMAQ system over North America under AQMEII phase 2. Atmos Environ 115: 683-694. http://dx.doi.org/10.1016/j.atmosenv.2014.12.034.

Hogrefe, C; Roselle, S; Mathur, R; Rao, ST; Galmarini, S. (2014). Space-time analysis of the Air Quality Model Evaluation International Initiative (AQMEII) Phase 1 air quality simulations. J Air Waste Manag Assoc 64: 388-405. http://dx.doi.org/10.1080/10962247.2013.811127.

Hu, J; Howard, CJ; Mitloehner, F; Green, PG; Kleeman, MJ. (2012). Mobile source and livestock feed contributions to regional ozone formation in Central California. Environ Sci Technol 46: 2781-2789. http://dx.doi.org/10.1021/es203369p.

Huang, L; Mcdonald-Buller, E; Mcgaughey, G; Kimura, Y; Allen, DT. (2015). Comparison of regional and global land cover products and the implications for biogenic emission modeling. J Air Waste Manag Assoc 65: 1194-1205. http://dx.doi.org/10.1080/10962247.2015.1057302.

Hutzell, WT; Luecken, DJ; Appel, KW; Carter, WPL. (2012). Interpreting predictions from the SAPRC07 mechanism based on regional and continental simulations. Atmos Environ 46: 417-429. http://dx.doi.org/10.1016/j.atmosenv.2011.09.030.

Hystad, P; Demers, PA; Johnson, KC; Brook, J; van Donkelaar, A; Lamsal, L; Martin, R; Brauer, M. (2012). Spatiotemporal air pollution exposure assessment for a Canadian population-based lung cancer case-control study. Environ Health 11: 22. http://dx.doi.org/10.1186/1476-069X-11-22.

Isaacs, K. (2014). The consolidated human activity database - master version (CHAD-Master) technical memorandum. Washington, DC: U.S. Environmental Protection Agency, National Exposure Research Laboratory. https://www.epa.gov/sites/production/files/2015-02/documents/chadmaster_091814_1.pdf.

Jaffe, DA; Wigder, N; Downey, N; Pfister, G; Boynard, A; Reid, SB. (2013). Impact of wildfires on ozone exceptional events in the Western U.S. Environ Sci Technol 47: 11065-11072. http://dx.doi.org/10.1021/es402164f.

Jerrett, M; Burnett, RT; Pope, CA, III; Ito, K; Thurston, G; Krewski, D; Shi, Y; Calle, E; Thun, M. (2009). Long-term ozone exposure and mortality. N Engl J Med 360: 1085-1095. http://dx.doi.org/10.1056/NEJMoa0803894.

Johnson, T; Capel, J; Ollison, W. (2014). Measurement of microenvironmental ozone concentrations in Durham, North Carolina, using a 2B Technologies 205 Federal Equivalent Method monitor and an interference-free 2B Technologies 211 monitor. J Air Waste Manag Assoc 64: 360-371. http://dx.doi.org/10.1080/10962247.2013.839968.

Jones, RR; Oezkaynak, H; Nayak, SG; Garcia, V; Hwang, SA; Lin, S. (2013). Associations between summertime ambient pollutants and respiratory morbidity in New York City: Comparison of results using ambient concentrations versus predicted exposures. J Expo Sci Environ Epidemiol 23: 616-626. http://dx.doi.org/10.1038/jes.2013.44.

Joseph, J; Sharif, HO; Sunil, T; Alamgir, H. (2013). Application of validation data for assessing spatial interpolation methods for 8-h ozone or other sparsely monitored constituents. Environ Pollut 178: 411-418. http://dx.doi.org/10.1016/j.envpol.2013.03.035.

Karamchandani, P; Johnson, J; Yarwood, G; Knipping, E. (2014). Implementation and application of sub-grid-scale plume treatment in the latest version of EPA's third-generation air quality model, CMAQ 5.01. J Air Waste Manag Assoc 64: 453-467. http://dx.doi.org/10.1080/10962247.2013.855152.

Kaynak, B; Hu, Y; Russell, AG. (2013). Analysis of NO, NO2, and O3 between model simulations and ground-based, aircraft, and satellite observations. Water Air Soil Pollut 224: 1674. http://dx.doi.org/10.1007/s11270-013-1674-2.

Kethireddy, SR; Tchounwou, PB; Ahmad, HA; Yerramilli, A; Young, JH. (2014). Geospatial interpolation and mapping of tropospheric ozone pollution using geostatistics. Int J Environ Res Public Health 11: 983-1000. http://dx.doi.org/10.3390/ijerph110100983.

Kimbrough, S; Owen, RC; Snyder, M; Richmond-Bryant, J. (2017). NO to NO 2 conversion rate analysis and implications for dispersion model chemistry methods using Las Vegas, Nevada near-road field measurements. Atmos Environ 165: 23-34. http://dx.doi.org/10.1016/j.atmosenv.2017.06.027.

Koo, B; Kumar, N; Knipping, E; Nopmongcol, U; Sakulyanontvittaya, T; Odman, MT; Russell, AG; Yarwood, G. (2015). Chemical transport model consistency in simulating regulatory outcomes and the relationship to model performance. Atmos Environ 116: 159-171. http://dx.doi.org/10.1016/j.atmosenv.2015.06.036.

Lai, D; Karava, P; Chen, Q. (2015). Study of outdoor ozone penetration into buildings through ventilation and infiltration. Build Environ 93: 112-118. http://dx.doi.org/10.1016/j.buildenv.2015.06.015.

Lane, KJ; Scammell, MK; Levy, JI; Fuller, CH; Parambi, R; Zamore, W; Mwamburi, M; Brugge, D. (2013). Positional error and time-activity patterns in near-highway proximity studies: an exposure misclassification analysis. Environ Health 12: 75. http://dx.doi.org/10.1186/1476-069X-12-75.

Li, G; Bei, N; Zavala, M; Molina, LT. (2014a). Ozone formation along the California Mexican border region during Cal-Mex 2010 field campaign. Atmos Environ 88: 370-389. http://dx.doi.org/10.1016/j.atmosenv.2013.11.067.

Li, J; Georgescu, M; Hyde, P; Mahalov, A; Moustaoui, M. (2014b). Achieving accurate simulations of urban impacts on ozone at high resolution. Environ Res Lett 9. http://dx.doi.org/10.1088/1748-9326/9/11/114019.

Li, J; Mahalov, A; Hyde, P. (2016a). Impacts of agricultural irrigation on ozone concentrations in the Central Valley of California and in the contiguous United States based on WRF-Chem simulations. Agr Forest Meteorol 221: 34-49. http://dx.doi.org/10.1016/j.agrformet.2016.02.004.

Li, J; Zhang, H; Ying, Q. (2012). Comparison of the SAPRC07 and SAPRC99 photochemical mechanisms during a high ozone episode in Texas: Differences in concentrations, OH budget and relative response factors. Atmos Environ 54: 25-35. http://dx.doi.org/10.1016/j.atmosenv.2012.02.034.

Li, X; Choi, Y; Czader, B; Roy, A; Kim, H; Lefer, B; Pan, S. (2016b). The impact of observation nudging on simulated meteorology and ozone concentrations during DISCOVER-AQ 2013 Texas campaign. Atmos Chem Phys 16: 3127-3144. http://dx.doi.org/10.5194/acp-16-3127-2016.

Lipfert, FW; Wyzga, RE. (1996). The effects of exposure error on environmental epidemiology. In RF Phalen; RC Mannix; MC Tonini (Eds.), The second colloquium on particulate air pollution & human mortality & morbidity (pp. 4-295-294-302). Sacramento, CA: California Air Resources Board.

Liu, P; Zhang, Y. (2011). Use of a process analysis tool for diagnostic study on fine particulate matter predictions in the U.S. - Part I: Model evaluation. Atmos Pollut Res 2: 49-60. http://dx.doi.org/10.5094/APR.2011.007.

Liu, Z; Le, N; Zidek, JV. (2011). An empirical assessment of Bayesian melding for mapping ozone pollution. Environmetrics 22: 340-353. http://dx.doi.org/10.1002/env.1054.

Lopiano, KK; Young, LJ; Gotway, CA. (2011). A comparison of errors in variables methods for use in regression models with spatially misaligned data. Stat Methods Med Res 20: 29-47. http://dx.doi.org/10.1177/0962280210370266.

Lu, D; Cizdziel, JV; Jiang, Y, i; White, L; Reddy, RS. (2014). Numerical simulation of atmospheric mercury in mid-south USA. Air Qual Atmos Health 7: 525-540. http://dx.doi.org/10.1007/s11869-014-0256-9.

Lu, Y, un; Zeger, SL. (2007). On the equivalence of case-crossover and time series methods in environmental epidemiology. Biostatistics 8: 337-344. http://dx.doi.org/10.1093/biostatistics/kxl013.

Marshall, JD; Nethery, E; Brauer, M. (2008). Within-urban variability in ambient air pollution: Comparison of estimation methods. Atmos Environ 42: 1359-1369. http://dx.doi.org/10.1016/j.atmosenv.2007.08.012.

Matichuk, R; Tonnesen, G; Luecken, D; Gilliam, R, ob; Napelenok, SL; Baker, KR; Schwede, D; Murphy, B, en; Helmig, D; Lyman, SN; Roselle, S. (2017). Evaluation of the community multiscale air quality model for simulating winter ozone formation in the uinta basin. J Geophys Res Atmos 122: 13545-13572. http://dx.doi.org/10.1002/2017JD027057.

McDonald-Buller, EC; Allen, DT; Brown, N; Jacob, DJ; Jaffe, D; Kolb, CE; Lefohn, AS; Oltmans, S; Parrish, DD; Yarwood, G; Zhang, L. (2011). Establishing policy relevant background (PRB) ozone concentrations in the United States [Review]. Environ Sci Technol 45: 9484-9497. http://dx.doi.org/10.1021/es2022818.

Muñiz-Unamunzaga, M; Borge, R; Sarwar, G; Gantt, B; de la Paz, D; Cuevas, CA; Saiz-Lopez, A. (2018). The influence of ocean halogen and sulfur emissions in the air quality of a coastal megacity: The case of Los Angeles. Sci Total Environ 610-611: 1536-1545. http://dx.doi.org/10.1016/j.scitotenv.2017.06.098.

Napelenok, SL; Foley, KM; Kang, D; Mathur, R; Pierce, T; Rao, ST. (2011). Dynamic evaluation of regional air quality models response to emission reductions in the presence of uncertain emission inventories. Atmos Environ 45: 4091-4098. http://dx.doi.org/10.1016/j.atmosenv.2011.03.030.

Ng, LC; Persily, AK; Emmerich, SJ. (2015). IAQ and energy impacts of ventilation strategies and building envelope airtightness in a big box retail building. Build Environ 92: 627-634. http://dx.doi.org/10.1016/j.buildenv.2015.05.038.

Ngan, F; Byun, D; Kim, H; Lee, D; Rappenglueck, B; Pour-Biazar, A. (2012). Performance assessment of retrospective meteorological inputs for use in air quality modeling during TexAQS 2006. Atmos Environ 54: 86-96. http://dx.doi.org/10.1016/j.atmosenv.2012.01.035.

Nopmongcol, U; Alvarez, Y; Jung, J; Grant, J; Kumar, N; Yarwood, G. (2017). Source contributions to United States ozone and particulate matter over five decades from 1970 to 2020. Atmos Environ 167: 116-128. http://dx.doi.org/10.1016/j.atmosenv.2017.08.009.

Ollison, WM; Crow, W; Spicer, CW. (2013). Field testing of new-technology ambient air ozone monitors. J Air Waste Manag Assoc 63: 855-863. http://dx.doi.org/10.1080/10962247.2013.796898.

Paci, L; Gelfand, AE; Holland, DM. (2013). Spatio-temporal modeling for real-time ozone forecasting. Spat Stat 4: 79-93. http://dx.doi.org/10.1016/j.spasta.2013.04.003.

Pan, L, i; Tong, D; Lee, P; Kim, HC; Chai, T. (2014). Assessment of NOx and O-3 forecasting performances in the US National Air Quality Forecasting Capability before and after the 2012 major emissions updates. Atmos Environ 95: 610-619. http://dx.doi.org/10.1016/j.atmosenv.2014.06.020.

Pan, S; Choi, Y; Jeon, W; Roy, A; Westenbarger, DA; Kim, HC. (2017a). Impact of high-resolution sea surface temperature, emission spikes and wind on simulated surface ozone in Houston, Texas during a high ozone episode. Atmos Environ 152: 362-376. http://dx.doi.org/10.1016/j.atmosenv.2016.12.030.

Pan, S; Choi, Y; Roy, A; Jeon, W. (2017b). Allocating emissions to 4 km and 1 km horizontal spatial resolutions and its impact on simulated NOx and O-3 in Houston, TX. Atmos Environ 164: 398-415. http://dx.doi.org/10.1016/j.atmosenv.2017.06.026.

Pan, S; Choi, Y; Roy, A; Li, X; Jeon, W; Souri, AH. (2015). Modeling the uncertainty of several VOC and its impact on simulated VOC and ozone in Houston, Texas. Atmos Environ 120: 404-416. http://dx.doi.org/10.1016/j.atmosenv.2015.09.029.

Pleim, J; Gilliam, R; Appel, W; Ran, L. (2016). Recent advances in modeling of the atmospheric boundary layer and land surface in the coupled WRF-CMAQ model. In DG Steyn; N Chaumerliac (Eds.), Air pollution modeling and its application XXIV (pp. 391-396). Cham, Switzerland: Springer. http://dx.doi.org/10.1007/978-3-319-24478-5_64.

Pongprueksa, P. (2013). Application of satellite data in a regional model to improve long-term ozone simulations. J Atmos Chem 70: 317-340. http://dx.doi.org/10.1007/s10874-013-9270-9.

Punger, EM; West, JJ. (2013). The effect of grid resolution on estimates of the burden of ozone and fine particulate matter on premature mortality in the USA. Air Qual Atmos Health 6: 563-573. http://dx.doi.org/10.1007/s11869-013-0197-8.

Ran, L; Pleim, J; Gilliam, R; Binkowski, FS; Hogrefe, C; Band, L. (2016). Improved meteorology from an updated WRF/CMAQ modeling system with MODIS vegetation and albedo. J Geophys Res Atmos 121: 2393-2415. http://dx.doi.org/10.1002/2015JD024406.

Reeves, GK; Cox, DR; Darby, SC; Whitley, E. (1998). Some aspects of measurement error in explanatory variables for continuous and binary regression models. Stat Med 17: 2157-2177. http://dx.doi.org/10.1002/(SICI)1097-0258(19981015)17:19<2157::AID-SIM916>3.0.CO;2-F.

Reich, BJ; Chang, HH; Foley, KM. (2014). A spectral method for spatial downscaling. Biometrics 70: 932-942. http://dx.doi.org/10.1111/biom.12196.

Reich, BJ; Kalendra, E; Storlie, CB; Bondell, HD; Fuentes, M. (2012). Variable selection for high dimensional Bayesian density estimation: Application to human exposure simulation. J R Stat Soc Ser C Appl Stat 61: 47-66. http://dx.doi.org/10.1111/j.1467-9876.2011.00772.x.

Rim, D; Gall, ET; Ananth, S; Won, Y. (2018). Ozone reaction with human surfaces: Influences of surface reaction probability and indoor air flow condition. Build Environ 130: 40-48. http://dx.doi.org/10.1016/j.buildenv.2017.12.012.

Robichaud, A; Menard, R. (2014). Multi-year objective analyses of warm season ground-level ozone and PM2.5 over North America using real-time observations and Canadian operational air quality models. Atmos Chem Phys 14: 1769-1800. http://dx.doi.org/10.5194/acp-14-1769-2014.

Sagona, JA; Weisel, CP; Meng, Q. (2018). Accuracy and practicality of a portable ozone monitor for personal exposure estimates. Atmos Environ 175: 120-126. http://dx.doi.org/10.1016/j.atmosenv.2017.11.036.

Sahu, SK; Bakar, KS. (2012a). A comparison of Bayesian models for daily ozone concentration levels. Stat Methodol 9: 144-157. http://dx.doi.org/10.1016/j.stamet.2011.04.009.

Sahu, SK; Bakar, KS. (2012b). Hierarchical Bayesian autoregressive models for large space-time data with applications to ozone concentration modelling. Appl Stoch Models Bus Ind 28: 395-415. http://dx.doi.org/10.1002/asmb.1951.

Samoli, E; Peng, RD; Ramsay, T, im; Touloumi, G; Dominici, F; Atkinson, RW; Zanobetti, A; Le Tertre, A; Anderson, HR; Schwartz, J; Cohen, A; Krewski, D; Samet, JM; Katsouyanni, K. (2014). What is the impact of systematically missing exposure data on air pollution health effect estimates? Air Qual Atmos Health 7: 415-420. http://dx.doi.org/10.1007/s11869-014-0250-2.

Sarnat, JA; Koutrakis, P; Suh, HH. (2000). Assessing the relationship between personal particulate and gaseous exposures of senior citizens living in Baltimore, MD. J Air Waste Manag Assoc 50: 1184-1198. http://dx.doi.org/10.1080/10473289.2000.10464165.

Sarnat, JA; Sarnat, SE; Flanders, WD; Chang, HH; Mulholland, J; Baxter, L; Isakov, V; Ozkaynak, H. (2013). Spatiotemporally resolved air exchange rate as a modifier of acute air pollution-related morbidity in Atlanta. J Expo Sci Environ Epidemiol 23: 606-615. http://dx.doi.org/10.1038/jes.2013.32.

Schaap, M; Cuvelier, C; Hendriks, C; Bessagnet, B; Baldasano, JM; Colette, A; Thunis, P; Karam, D; Fagerli, H; Graff, A; Kranenburg, R; Nyiri, A; Pay, MT; Rouil, L; Schulz, M; Simpson, D; Stern, R; Terrenoire, E; Wind, P. (2015). Performance of European chemistry transport models as function of horizontal resolution. Atmos Environ 112: 90-105. http://dx.doi.org/10.1016/j.atmosenv.2015.04.003.

Schere, K; Flemming, J; Vautard, R; Chemel, C; Colette, A; Hogrefe, C; Bessagnet, B; Meleux, F; Mathur, R; Roselle, S; Hu, RM; Sokhi, RS; Rao, ST; Galmarini, S. (2011). Trace gas/aerosol boundary concentrations and their impacts on continental-scale AQMEII modeling domains. Atmos Environ 53: 38-50. http://dx.doi.org/10.1016/j.atmosenv.2011.09.043.

Seltzer, KM; Nolte, CG; Spero, TL; Appel, KW; Xing, J, ia. (2016). Evaluation of near surface ozone and particulate matter in air quality simulations driven by dynamically downscaled historical meteorological fields. Atmos Environ 138: 42-54. http://dx.doi.org/10.1016/j.atmosenv.2016.05.010.

Seltzer, KM; Shindell, DT; Faluvegi, G; Murray, LT. (2017). Evaluating Modeled Impact Metrics for Human Health, Agriculture Growth, and Near-Term Climate. J Geophys Res Atmos 122: 13506-13524. http://dx.doi.org/10.1002/2017JD026780.

Sheppard, L; Slaughter, JC; Schildcrout, J; Liu, JS; Lumley, T. (2005). Exposure and measurement contributions to estimates of acute air pollution effects. J Expo Anal Environ Epidemiol 15: 366-376. http://dx.doi.org/10.1038/sj.jea.7500413.

Shmool, JLC; Kinnee, E; Sheffield, PE; Clougherty, JE. (2016). Spatio-temporal ozone variation in a case-crossover analysis of childhood asthma hospital visits in New York City. Environ Res 147: 108-114. http://dx.doi.org/10.1016/j.envres.2016.01.020.

Simon, H; Baker, KR; Akhtar, F; Napelenok, SL; Possiel, N; Wells, B; Timin, B. (2013). A direct sensitivity approach to predict hourly ozone resulting from compliance with the national ambient air quality standard. Environ Sci Technol 47: 2304-2313. http://dx.doi.org/10.1021/es303674e.

Simon, H; Wells, B; Baker, KR; Hubbell, B. (2016). Assessing temporal and spatial patterns of observed and predicted ozone in multiple urban areas. Environ Health Perspect 124: 1443-1452. http://dx.doi.org/10.1289/EHP190.

Singer, BC; Delp, WW; Black, DR; Walker, IS. (2016). Measured performance of filtration and ventilation systems for fine and ultrafine particles and ozone in an unoccupied modern California house. Indoor Air 27: 780-790. http://dx.doi.org/10.1111/ina.12359.

Solazzo, E; Hogrefe, C; Colette, A; Garcia-Vivanco, M; Galmarini, S. (2017). Advanced error diagnostics of the CMAQ and Chimere modelling systems within the AQMEII3 model evaluation framework. Atmos Chem Phys 17: 10435-10465. http://dx.doi.org/10.5194/acp-17-10435-2017.

Spalt, EW; Curl, CL; Allen, RW; Cohen, M; Adar, SD; Stukovsky, KH; Avol, E; Castro-Diehl, C; Nunn, C; Mancera-Cuevas, K; Kaufman, JD. (2015). Time-location patterns of a diverse population of older adults: the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air). J Expo Sci Environ Epidemiol 26: 349-355. http://dx.doi.org/10.1038/jes.2015.29.

Springs, M; Wells, J. R.; Morrison, GC. (2011). Reaction rates of ozone and terpenes adsorbed to model indoor surfaces. Indoor Air 21: 319-327. http://dx.doi.org/10.1111/j.1600-0668.2010.00707.x.

Steyn, DG; Ainslie, B; Reuten, C; Jackson, PL. (2013). A retrospective analysis of ozone formation in the lower Fraser Valley, British Columbia, Canada. Part I: Dynamical model evaluation. Atmos Ocean 51: 153-169. http://dx.doi.org/10.1080/07055900.2013.781940.

Strickland, MJ; Gass, KM; Goldman, GT; Mulholland, JA. (2013). Effects of ambient air pollution measurement error on health effect estimates in time-series studies: a simulation-based analysis. J Expo Sci Environ Epidemiol 25: 160-166. http://dx.doi.org/10.1038/jes.2013.16.

Szpiro, AA; Paciorek, CJ. (2013). Measurement error in two-stage analyses, with application to air pollution epidemiology. Environmetrics 24: 501-517. http://dx.doi.org/10.1002/env.2233.

Szpiro, AA; Paciorek, CJ; Sheppard, L. (2011). Does more accurate exposure prediction necessarily improve health effect estimates? Epidemiology 22: 680-685. http://dx.doi.org/10.1097/EDE.0b013e3182254cc6.

Tang, W; Cohan, DS; Morris, GA; Byun, DW; Luke, WT. (2011). Influence of vertical mixing uncertainties on ozone simulation in CMAQ. Atmos Environ 45: 2898-2909. http://dx.doi.org/10.1016/j.atmosenv.2011.01.057.

Tang, W; Cohan, DS; Pour-Biazar, A; Lamsal, LN; White, AT; Xiao, X; Zhou, W; Henderson, BH; Lash, BF. (2015a). Influence of satellite-derived photolysis rates and NOx emissions on Texas ozone modeling. Atmos Chem Phys 15: 1601-1619. http://dx.doi.org/10.5194/acp-15-1601-2015.

Tang, Y; Chai, T; Pan, L; Lee, P; Tong, D; Kim, HC; Chen, W. (2015b). Using optimal interpolation to assimilate surface measurements and satellite AOD for ozone and PM2.5: A case study for July 2011. J Air Waste Manag Assoc 65: 1206-1216. http://dx.doi.org/10.1080/10962247.2015.1062439.

Tao, Z; Yu, H; Chin, M. (2016). Impact of transpacific aerosol on air quality over the United States: A perspective from aerosol-cloud-radiation interactions. Atmos Environ 125: 48-60. http://dx.doi.org/10.1016/j.atmosenv.2015.10.083.

Tessum, CW; Hill, JD; Marshall, JD. (2015). Twelve-month, 12 km resolution North American WRF-Chem v3.4 air quality simulation: performance evaluation. GMD 8: 957-973. http://dx.doi.org/10.5194/gmd-8-957-2015.

Thompson, TM; Selin, NE. (2012). Influence of air quality model resolution on uncertainty associated with health impacts. Atmos Chem Phys 12: 9753-9762. http://dx.doi.org/10.5194/acp-12-9753-2012.

Tsimpidi, AP; Trail, M; Hu, Y; Nenes, A; Russell, AG. (2012). Modeling an air pollution episode in northwestern United States: identifying the effect of nitrogen oxide and volatile organic compound emission changes on air pollutants formation using direct sensitivity analysis. J Air Waste Manag Assoc 62: 1150-1165. http://dx.doi.org/10.1080/10962247.2012.697093.

U.S. EPA (U.S. Environmental Protection Agency). (2011). Air quality modeling technical support document: Final EGU NESHAP. (EPA-454/R-11-009). U.S. Environmental Protection Agency, Office of Air Quality Planning and Standards, Air Quality Assessment Division.

U.S. EPA (U.S. Environmental Protection Agency). (2013). Integrated science assessment for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-10/076F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment-RTP Division. http://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=247492.

Verstraeten, WW; Boersma, KF; Zorner, J; Allaart, MAF; Bowman, KW; Worden, J. R. (2013). Validation of six years of TES tropospheric ozone retrievals with ozonesonde measurements: implications for spatial patterns and temporal stability in the bias. Atmos Meas Tech 6: 1413-1423. http://dx.doi.org/10.5194/amt-6-1413-2013.

Wang, K, ai; Zhang, Y. (2014). 3-D agricultural air quality modeling: Impacts of NH3/H2S gas-phase reactions and bi-directional exchange of NH3. Atmos Environ 98: 554-570. http://dx.doi.org/10.1016/j.atmosenv.2014.09.010.

Wang, K; Zhang, Y; Nenes, A; Fountoukis, C. (2012). Implementation of dust emission and chemistry into the Community Multiscale Air Quality modeling system and initial application to an Asian dust storm episode. Atmos Chem Phys 12: 10209-10237. http://dx.doi.org/10.5194/acp-12-10209-2012.

Wang, M; Keller, JP; Adar, SD; Kim, S; Larson, TV; Olives, C; Sampson, PD; Sheppard, L; Szpiro, AA; Vedal, S; Kaufman, JD. (2015). Development of long-term spatiotemporal models for ambient ozone in six metropolitan regions of the United States: The MESA Air study. Atmos Environ 123: 79-87. http://dx.doi.org/10.1016/j.atmosenv.2015.10.042.

Wang, M; Sampson, PD; Hu, J; Kleeman, M; Keller, JP; Olives, C; Szpiro, AA; Vedal, S; Kaufman, JD. (2016). Combining land-use regression and chemical transport modeling in a spatiotemporal geostatistical model for ozone and PM2.5. Environ Sci Technol 50: 5111-5118. http://dx.doi.org/10.1021/acs.est.5b06001.

Waring, MS; Wells, JR. (2015). Volatile organic compound conversion by ozone, hydroxyl radicals, and nitrate radicals in residential indoor air: Magnitudes and impacts of oxidant sources. Atmos Environ 106: 382-391. http://dx.doi.org/10.1016/j.atmosenv.2014.06.062.

Warren, J; Fuentes, M; Herring, A; Langlois, P. (2012). Spatial-temporal modeling of the association between air pollution exposure and preterm birth: Identifying critical windows of exposure. Biometrics 68: 1157-1167. http://dx.doi.org/10.1111/j.1541-0420.2012.01774.x.

Weir, CH; Yeatts, KB; Sarnat, JA; Vizuete, W; Salo, PM; Jaramillo, R; Cohn, RD; Chu, H; Zeldin, DC; London, SJ. (2013). Nitrogen dioxide and allergic sensitization in the 2005-2006 National Health and Nutrition Examination Survey. Respir Med 107: 1763-1772. http://dx.doi.org/10.1016/j.rmed.2013.08.010.

Wheeler, AJ; Xu, X; Kulka, R; You, H; Wallace, L; Mallach, G; Van Ryswyk, K; MacNeill, M; Kearney, J; Rasmussen, PE; Dabek-Zlotorzynska, E; Wang, D; Poon, R; Williams, R; Stocco, C; Anastassopoulos, A; Miller, JD; Dales, R; Brook, JR. (2011). Windsor, Ontario Exposure Assessment Study: Design and methods validation of personal, indoor, and outdoor air pollution monitoring. J Air Waste Manag Assoc 61: 324-338. http://dx.doi.org/10.3155/1047-3289.61.3.324.

Williams, R; Rappold, A; Case, M; Schmitt, M; Stone, S; Jones, P; Thornburg, J; Devlin, RB. (2012). Multi-pollutant exposures in an asthmatic cohort. Atmos Environ 61: 244-252. http://dx.doi.org/10.1016/j.atmosenv.2012.07.049.

Wilson, WE. (2000). Size distributions of ambient and indoor particles: does the overlap of fine and coarse particles cause problems in the interpretation of research results?

Winquist, A; Kirrane, E; Klein, M; Strickland, M; Darrow, LA; Sarnat, SE; Gass, K; Mulholland, J; Russell, A; Tolbert, P. (2014). Joint effects of ambient air pollutants on pediatric asthma emergency department visits in Atlanta, 1998-2004. Epidemiology 25: 666-673. http://dx.doi.org/10.1097/EDE.0000000000000146.

Wong, DC; Pleim, J; Mathur, R; Binkowski, F; Otte, T; Gilliam, R; Pouliot, G; Xiu, A; Young, JO; Kang, D. (2012). WRF-CMAQ two-way coupled system with aerosol feedback: Software development and preliminary results. GMD 5: 299-312. http://dx.doi.org/10.5194/gmd-5-299-2012.

Wu, J; Jiang, C; Liu, Z; Houston, D; Jaimes, G; McConnell, R. (2010). Performances of different global positioning system devices for time-location tracking in air pollution epidemiological studies. Environ Health Insights 4: 93-108. http://dx.doi.org/10.4137/EHI.S6246.

Xing, J; Mathur, R; Pleim, J; Hogrefe, C; Gan, CM; Wong, DC; Wei, C; Gilliam, R; Pouliot, G. (2015). Observations and modeling of air quality trends over 1990-2010 across the Northern Hemisphere: China, the United States and Europe. Atmos Chem Phys 15: 2723-2747. http://dx.doi.org/10.5194/acp-15-2723-2015.

Xu, W; Riley, EA; Austin, E; Sasakura, M; Schaal, L; Gould, TR; Hartin, K; Simpson, CD; Sampson, PD; Yost, MG; Larson, TV; Xiu, G; Vedal, S. (2016a). Use of mobile and passive badge air monitoring data for NOX and ozone air pollution spatial exposure prediction models. J Expo Sci Environ Epidemiol 27: 184-192. http://dx.doi.org/10.1038/jes.2016.9.

Xu, Y; Serre, ML; Reyes, J; Vizuete, W. (2016b). Bayesian maximum entropy integration of ozone observations and model predictions: a national application. Environ Sci Technol 50: 4393-4400. http://dx.doi.org/10.1021/acs.est.6b00096.

Xu, Y; Serre, ML; Reyes, JM; Vizuete, W. (2017). Impact of temporal upscaling and chemical transport model horizontal resolution on reducing ozone exposure misclassification. Atmos Environ 166: 374-382. http://dx.doi.org/10.1016/j.atmosenv.2017.07.033.

Yahya, K; He, J; Zhang, Y. (2015a). Multiyear applications of WRF/Chem over continental US: Model evaluation, variation trend, and impacts of boundary conditions. J Geophys Res Atmos 120: 12748-12777. http://dx.doi.org/10.1002/2015JD023819.

Yahya, K; Wang, K, ai; Campbell, P; Glotfelty, T; He, J; Zhang, Y. (2016). Decadal evaluation of regional climate, air quality, and their interactions over the continental US and their interactions using WRF/Chem version 3.6.1. GMD 9: 671-695. http://dx.doi.org/10.5194/gmd-9-671-2016.

Yahya, K; Wang, K; Zhang, Y; Kleindienst, TE. (2015b). Application of WRF/Chem over North America under the AQMEII Phase 2-Part 2: Evaluation of 2010 application and responses of air quality and meteorology-chemistry interactions to changes in emissions and meteorology from 2006 to 2010. GMD 8: 2095-2117. http://dx.doi.org/10.5194/gmd-8-2095-2015.

Yahya, K; Zhang, Y; Vukoyich, JM. (2014). Real-time air quality forecasting over the southeastern United States using WRF/Chem-MADRID: Multiple-year assessment and sensitivity studies. Atmos Environ 92: 318-338. http://dx.doi.org/10.1016/j.atmosenv.2014.04.024.

Ying, Q; Li, J. (2011). Implementation and initial application of the near-explicit Master Chemical Mechanism in the 3D Community Multiscale Air Quality (CMAQ) model. Atmos Environ 45: 3244-3256. http://dx.doi.org/10.1016/j.atmosenv.2011.03.043.

Yu, H; Russell, A; Mulholland, J; Odman, T; Hu, Y; Chang, HH; Kumar, N. (2018). Cross-comparison and evaluation of air pollution field estimation methods. Atmos Environ 179: 49-60. http://dx.doi.org/10.1016/j.atmosenv.2018.01.045.

Yu, K; Jacob, DJ; Fisher, JA; Kim, PS; Marais, EA; Miller, CC; Travis, KR; Zhu, L; Yantosca, RM; Sulprizio, MP; Cohen, R; Dibb, JE; Fried, A; Mikoviny, T; Ryerson, TB; Wennberg, PO; Wisthaler, A. (2016). Sensitivity to grid resolution in the ability of a chemical transport model to simulate observed oxidant chemistry under high-isoprene conditions. Atmos Chem Phys 16: 4369-4378. http://dx.doi.org/10.5194/acp-16-4369-2016.

Yu, S; Mathur, R; Pleim, J; Pouliot, G; Wong, D; Eder, B; Schere, K; Gilliam, R; Rao, ST. (2012). Comparative evaluation of the impact of WRF-NMM and WRF-ARW meteorology on CMAQ simulations for O-3 and related species during the 2006 TexAQS/GoMACCS campaign. Atmos Pollut Res 3: 149-162. http://dx.doi.org/10.5094/APR.2012.015.

Zartarian, VG; Xue, J; Ozkaynak, H; Dang, W; Glen, G; Smith, L; Stallins, C. (2005). A probabilistic exposure assessment for children who contact CCA-treated playsets and decks: Using the stochastic human exposure and dose simulation model for the wood preservative exposure scenario (SHEDS-Wood). U.S. Environmental Protection Agency. http://www.epa.gov/oppad001/reregistration/cca/final_cca_factsheet.htm.

Zeger, SL; Thomas, D; Dominici, F; Samet, JM; Schwartz, J; Dockery, D; Cohen, A. (2000). Exposure measurement error in time-series studies of air pollution: Concepts and consequences. Environ Health Perspect 108: 419-426. http://dx.doi.org/10.1289/ehp.00108419.

Zhang, H; Chen, G; Hu, J; Chen, SH; Wiedinmyer, C; Kleeman, M; Ying, Q. (2014). Evaluation of a seven-year air quality simulation using the Weather Research and Forecasting (WRF)/Community Multiscale Air Quality (CMAQ) models in the eastern United States. Sci Total Environ 473-474: 275-285. http://dx.doi.org/10.1016/j.scitotenv.2013.11.121.

Zhang, HL; Ying, Q. (2011). Contributions of local and regional sources of NO(x) to ozone concentrations in Southeast Texas. Atmos Environ 45: 2877-2887. http://dx.doi.org/10.1016/j.atmosenv.2011.02.047.

Zhou, W; Cohan, DS; Napelenok, SL. (2013). Reconciling NOx emissions reductions and ozone trends in the US, 2002-2006. Atmos Environ 70: 236-244. http://dx.doi.org/10.1016/j.atmosenv.2012.12.038.

Zimmerman, N; Presto, AA; Kumar, SPN; Gu, J; Hauryliuk, A; Robinson, ES; Robinson, AL; Subramanian, R. (2018). A machine learning calibration model using random forests to improve sensor performance for lower-cost air quality monitoring. Atmos Meas Tech 11: 291-313. http://dx.doi.org/10.5194/amt-11-291-2018.

APPENDIX 3 HEALTH EFFECTS—RESPIRATORY

Summary of Causality Determinations for Short- and Long-Term Ozone Exposure and Respiratory Effects

This Appendix characterizes the scientific evidence that supports causality determinations for short- and long-term ozone exposure and respiratory health effects. The types of studies evaluated within this Appendix are consistent with the overall scope of the ISA as detailed in the Preface. In assessing the overall evidence, strengths and limitations of individual studies were evaluated based on scientific considerations detailed in the Annex for Appendix 3. More details on the causal framework used to reach these conclusions are included in the Preamble to the ISA (U.S. EPA, 2015). The evidence presented throughout this Appendix support the following causality conclusions:

Exposure Duration	Causality Determination
Short-term exposure	Causal
Long-term exposure	Likely to be causal

3.1 Short-Term Ozone Exposure

3.1.1 Introduction, Summary from the 2013 Ozone ISA, and Scope for Current Review

The 2013 Ozone ISA concluded that "*short-term ozone exposure is causally associated with respiratory health effects*" [see Chapter 6 of (U.S. EPA, 2013a)]. This conclusion was based largely on controlled human exposure studies demonstrating ozone-related respiratory effects in healthy individuals. Specifically, statistically significant decreases in group mean pulmonary function relative to ozone exposures as low as 60 ppb were observed in young, healthy adults. Additionally, controlled human exposure and toxicological studies demonstrated ozone-induced increases in respiratory symptoms, lung inflammation, airway permeability, and airway responsiveness. The experimental evidence was supported by strong evidence from epidemiologic studies. Specifically, these studies demonstrated associations between ozone concentrations and respiratory hospital admissions and emergency department (ED) visits across the U.S., Europe, and Canada. Most effect estimates ranged from a 1.6 to 5.4% increase in daily

respiratory-related ED visits or hospital admissions in all-year analyses for unit increases[1] in ambient ozone concentrations. This evidence was further supported by a large body of individual-level epidemiologic panel studies demonstrating associations of ambient ozone with respiratory symptoms in children with asthma. Additionally, several multicity studies and a multicontinent study reported associations between short-term increases in ambient ozone concentrations and increases in respiratory mortality. Additional support for a causal relationship was provided by epidemiologic panel studies that observed ozone-associated increases in indicators of airway inflammation and oxidative stress in children with asthma.

Across respiratory endpoints, mechanistic evidence indicated that antioxidant capacity may modify the risk of respiratory morbidity associated with ozone exposure. The potentially elevated risk of populations with diminished antioxidant capacity and the reduced risk of populations with enhanced antioxidant capacity identified in epidemiologic studies was strongly supported by similar findings from controlled human exposure studies and by evidence that characterizes ozone-induced decreases in intra-cellular antioxidant levels as a potential mechanistic pathway for downstream effects.

Along with this mechanistic evidence, animal toxicological and controlled human exposure studies demonstrated ozone-induced increases in airway responsiveness, decreased pulmonary function, allergic responses, lung injury, impaired host defense, and airway inflammation. These findings provided biological plausibility for epidemiologic associations of ambient ozone concentrations with lung function and respiratory symptoms, hospital admissions, ED visits, and mortality. Together, the evidence integrated across controlled human exposure, epidemiologic, and toxicological studies and across the spectrum of respiratory health endpoints support the determination of a causal relationship between short-term ozone exposure and respiratory health effects.

The following section on short-term ozone exposure and respiratory effects begins with an overview of study inclusion criteria (Section 3.1.2) that defines the scope of the literature that was considered for inclusion in the section. The ensuing section presents a discussion of biological plausibility (Section 3.1.3) that provides background for the subsequent sections in which groups of related endpoints are presented in the context of relevant disease pathways. The respiratory effects subsections are organized by outcome group and aim to clearly characterize the extent of coherence among related endpoints (e.g., hospital admissions, symptoms, inflammation). These outcome groups include respiratory effects in healthy populations (Section 3.1.4), respiratory effects in populations with asthma (Section 3.1.5), respiratory effects in other populations with pre-existing conditions (Section 3.1.6), including COPD (Section 3.1.6.1), obese populations or populations with metabolic syndrome (Section 3.1.6.2), and populations with pre-existing cardiovascular disease (Section 3.1.6.3), respiratory infection (Section 3.1.7), combinations of respiratory related disease hospital admissions and ED visits (Section 3.1.8), and respiratory mortality (Section 3.1.9). Finally, Section 3.1.10 comprises a discussion

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

of relevant issues for interpreting the epidemiologic evidence discussed in the preceding sections. Throughout the sections on respiratory health effects, results from recent studies are evaluated in the context of evidence provided by studies that were previously evaluated in the 2013 Ozone ISA (U.S. EPA, 2013a). Study-specific details, including exposure time periods and exposure concentrations in experimental studies, and study design, averaging times, and select results in epidemiologic studies are presented in evidence inventories in Section 3.3.

3.1.2 Population, Exposure, Comparison, Outcome, and Study Design (PECOS) Tool

The scope of this section is defined by a scoping tool that generally describes the relevant Population, Exposure, Comparison, Outcome, and Study Design (PECOS). The PECOS tool defines the parameters and provides a framework to help identify the relevant literature to inform the draft 2019 Ozone ISA. Because the 2013 Ozone ISA concluded there is a causal relationship between short-term ozone exposure and respiratory health effects, the recent epidemiologic studies evaluated in this ISA are limited to study locations in the U.S. and Canada to provide a focus on study populations and air quality characteristics that are most relevant to circumstances in the U.S. The studies evaluated and subsequently discussed within this section were included if they satisfied all of the components of the following PECOS tool:

Experimental studies:

- Population: Study populations of any controlled human exposure or animal toxicological study of mammals at any lifestage
- Exposure: Short-term (on the order of minutes to weeks) inhalation exposure to relevant ozone concentrations (i.e., ≤0.4 ppm for humans, ≤2 ppm for other mammals); while ozone concentrations in animal toxicological studies appear high, it should be noted that deposition of ozone resulting from exposure to 2 ppm ozone in a resting rat is roughly equivalent to deposition of ozone resulting from exposure to 0.4 ppm ozone in an exercising human (Hatch et al., 1994).
- Comparison: Human subjects serve as their own controls with an appropriate washout period or groups may be compared at the same or varied exposure concentrations; or, in toxicological studies of mammals, an appropriate comparison group is exposed to a negative control (i.e., clean air or filtered-air control)
- Outcome: Respiratory effects
- Study Design: Controlled human exposure studies and animal studies meeting the above criteria

Epidemiologic studies:

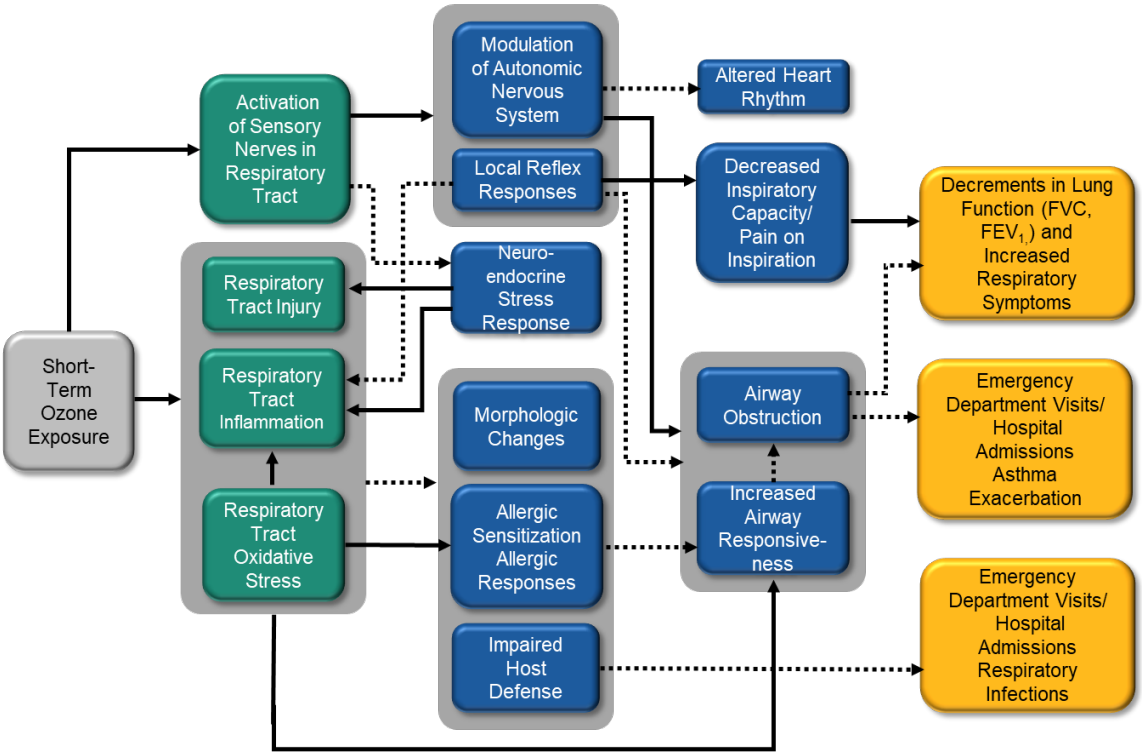
- Population: Any U.S. or Canadian population, including populations or lifestages that might be at increased risk
- Exposure: Short-term exposure (on the order of hours to several days) to ambient concentrations of ozone

- Comparison: Per unit increase (in ppb), or humans exposed to lower levels of ozone compared with humans exposed to higher levels
- Outcome: Change in risk (incidence/prevalence) of respiratory effects
- Study Design: Epidemiologic studies consisting of panel, case-crossover, time-series studies, and case-control studies, as well as cross-sectional studies with appropriate timing of exposure for the health endpoint of interest

3.1.3 Biological Plausibility

This section describes biological pathways that potentially underlie respiratory health effects resulting from short-term exposure to ozone. Figure 3-1 graphically depicts the proposed pathways as a continuum of upstream events, connected by arrows, that may lead to downstream events observed in epidemiologic studies. This discussion of how short-term exposure to ozone may lead to respiratory health effects contributes to an understanding of the biological plausibility of epidemiologic results evaluated later in Section 3.1.4 to Section 3.1.9.

Evidence that short-term exposure to ozone may affect the respiratory tract generally informs two proposed pathways (Figure 3-1). The first pathway begins with the activation of sensory nerves in the respiratory tract that can trigger local reflex responses and transmit signals to regions of the central nervous system that regulate autonomic outflow. The second pathway begins with injury, inflammation, and oxidative stress responses, which are difficult to disentangle. Inflammation generally occurs as a consequence of injury and oxidative stress, but it can also lead to further oxidative stress and injury due to secondary production of reactive oxygen species (ROS) by inflammatory cells.



Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence related to ozone exposure, and the arrows indicate a proposed relationship between those effects. Solid arrows denote evidence of essentiality as provided, for example, by an inhibitor of the pathway or a genetic knockout model used in an experimental study involving ozone exposure. Shading around multiple boxes is used to denote a grouping of these effects. Arrows may connect individual boxes, groupings of boxes, and individual boxes within groupings of boxes. Progression of effects is generally depicted from left to right and color-coded (gray, exposure; green, initial effect; blue, intermediate effect; orange, effect at the population level or a key clinical effect). Here, population level effects generally reflect results of epidemiologic studies. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below.

Figure 3-1 Potential biological pathways for respiratory effects following short-term ozone exposure.

Activation of Sensory Nerves in the Respiratory Tract

Airway sensory nerves in the lower respiratory tract are vagal afferents that carry signals to the nucleus tractus solitarius in the brain. Signals are integrated in the brain and may result in altered autonomic activity that affects the lung (e.g., airway obstruction) or other organs (e.g., altered heart rhythm). In addition, activation of some types of sensory nerves (e.g., C-fibers) leads to local axon reflex responses in the airways that result in altered ventilatory parameters (e.g., altered breathing frequency and inspiratory capacity) and airway obstruction. The release of substance P or other tachykinins from C-fibers and subsequent binding to neurokinin receptors in the airway has been identified as a mechanism

underlying local axon reflex responses. Tachykinins, which mediate bronchoconstriction and neurogenic inflammation, can contribute to increased airway responsiveness. These reflexes at the central-nervous-system or local axon level serve as lung irritant responses—adaptive responses to noxious chemicals that help decrease exposure to that chemical. Activation of vagal afferent pathways in the respiratory tract may also affect stress-responsive regions of the brain and lead to neuroendocrine stress responses that have multiple systemic effects.

Controlled human exposure studies described in the 2013 Ozone ISA (U.S. EPA, 2013a) demonstrated the involvement of sensory nerves and subsequent reflex responses in ozone-induced changes in lung function (Figure 3-1). In studies using pharmacological tools, nociceptive sensory nerves, presumably bronchial and pulmonary C-fibers, were identified as linking ozone exposure to a local axon reflex response that resulted in pain-related respiratory symptoms and inhibition of maximal inspiration. This mechanism underlies the observed decrease in forced vital capacity (FVC) and contributes to the observed decrease in forced expiratory volume in 1 second (FEV_1) in humans exposed to ozone. The essentiality of this mechanism is depicted by the solid lines linking activation of sensory nerves to local reflex responses to decreased inspiratory capacity and increased respiratory symptoms depicted in Figure 3-1. Activation of airway sensory nerves also led to rapid shallow breathing in human subjects exposed to ozone. Similarly, animal toxicological studies described in the 2013 Ozone ISA (U.S. EPA, 2013a) and more recent studies reviewed later in this Appendix demonstrated ozone-induced rapid shallow breathing and other changes in lung ventilatory parameters. Supportive evidence for a role of sensory C-fibers is provided by the association between airway responses to ozone and sensitivity to capsaicin, which is a known activator of sensory C-fibers, found in a recent study in humans (Hoffmeyer et al., 2013). In addition, a recent study in animals demonstrates the involvement of $TRPV_1$ receptors, which are a type of sensory nerve receptor, in ozone-mediated cough (Clay et al., 2016).

Mild airway obstruction, measured as a change in specific airway resistance (sRaw), was observed in humans exposed to ozone (U.S. EPA, 2013a). This response was inhibited by pretreatment with atropine, an inhibitor of muscarinic cholinergic receptors of the parasympathetic nervous system. This pathway is depicted by the solid lines linking activation of sensory nerves to modulation of the autonomic nervous system and to airway obstruction in Figure 3-1. Airway obstruction may contribute to decreases in FEV_1. Studies in humans and animals indicate that airway obstruction resulting from exposure to ozone is also mediated by a local axon reflex through the release of substance P from sensory nerves. Thus, two mechanisms may contribute to ozone-induced airway obstruction—a parasympathetic cholinergic pathway and a substance P-mediated pathway. Furthermore, the autonomic nervous system is implicated in ozone-mediated effects on heart rhythm (Section 4.1.3).

Ozone exposure increased airway responsiveness in humans (U.S. EPA, 2013a). This effect was blocked by atropine pretreatment, implicating a parasympathetic cholinergic process. This mechanism is depicted by the solid lines linking activation of sensory nerves to modulation of the autonomic nervous system and to increased airway responsiveness in Figure 3-1. A correlation was found between airway

responsiveness to methacholine and increases in sRaw in ozone-exposed humans, pointing to an effect of ozone on the parasympathetic nervous system that affects both responses. Animal toxicological studies also provide evidence for ozone-induced increases in airway responsiveness and demonstrate the involvement of the parasympathetic cholinergic pathway, the substance P-mediated pathway, and contributions from arachidonic acid metabolites and cytokines/chemokines (U.S. EPA, 2013a). A recent study showing enhanced bronchoconstriction in a model of vagal nerve electrical stimulation (Verhein et al., 2013) provides additional evidence for ozone-induced increased airway responsiveness. Because airway smooth muscle contraction to electrical field stimulation is a measure of post-ganglionic and parasympathetic-mediated processes, this study provides support for the parasympathetic pathway. Another recent study found that ozone exposure increased release of tachykinins (Barker et al., 2015), further supporting a role for a local axon reflex in mediating the effects of ozone.

Animal models of allergic airway disease share similar phenotypic features with asthma and are used as a surrogate for human asthma. Airway responsiveness was enhanced by ozone exposure to a larger degree in animals with allergic airway disease than in animals without allergic airway disease (U.S. EPA, 2013a; Schelegle and Walby, 2012). This increased airway responsiveness occurred in response to allergens (specific airway responsiveness) and nonallergens (nonspecific airway responsiveness). Moreover, airway resistance in response to ozone exposure was increased to a greater degree in allergic animals than in nonallergic animals (Schelegle and Walby, 2012). This increase in airway resistance was due to bronchoconstriction, and not to other mechanisms that could lead to airway obstruction. The role of vagal afferents in mediating ozone-induced increased airway responsiveness and bronchoconstriction was evaluated by vagotomy and by using pharmacologic tools (Schelegle and Walby, 2012). Vagal lung C-fibers were found to mediate reflex bronchoconstriction and enhance specific airway responsiveness resulting from ozone exposure. Evidence from this study supports an essential role for activation of sensory nerves and the parasympathetic nervous system in enhancing airway responses to ozone in an allergy model. Vagal myelinated fibers mediated an opposing effect (i.e., reflex bronchodilation). A role for neuropeptides such as substance P in mediating the bronchoconstrictive response was also suggested. Other lung irritation effects, besides reflex bronchoconstriction and bronchodilation, were demonstrated in recent studies of allergic airway disease in animals. Ozone exposure was associated with sensory (i.e., upper airway) and pulmonary (i.e., lower airway) irritation in nonallergic animals, but only sensory irritation in allergic animals (Hansen et al., 2016). Ozone-induced rapid, shallow breathing was greater in allergic animals than in nonallergic animals.

Taken together, mechanistic studies may provide biological plausibility for results of epidemiologic panel studies in healthy children and in children with asthma, in which ozone concentrations were associated with decrements in lung function and increased asthma symptoms. Furthermore, they support results of epidemiologic studies showing associations between ozone exposure and asthma-related emergency department visits and hospital admissions.

Injury, Inflammation, and Oxidative Stress

Regarding the second pathway, a large body of evidence from controlled human exposure and animal toxicological studies found injury, inflammation, and oxidative stress responses in healthy individuals and animals exposed to ozone. As described in the 1996 and 2006 Ozone AQCDs (U.S. EPA, 2006, 1996a) and the 2013 Ozone ISA (U.S. EPA, 2013a), some studies in humans found increased numbers of neutrophils, a marker of inflammation, and shed epithelial cells, a marker of injury, in bronchoalveolar lavage fluid (BALF) or sputum. BALF neutrophils correlated in number with sRaw but not with changes in lung volumes. In addition, BALF neutrophils and epithelial cells correlated with the loss of substance P immunoreactivity from neurons in the bronchial mucosa. These findings suggest a common mechanism underlying airway obstruction and inflammation, which possibly involves substance P. In addition, studies from the 2013 Ozone ISA (U.S. EPA, 2013a), and ones published more recently, show that the glutathione S-transferase mu 1(GSTM1) genotype may affect the inflammatory response to ozone in young adults and suggest that greater antioxidant capacity may mitigate the effects of ozone.

Animal toxicological studies described in the 2013 Ozone ISA (U.S. EPA, 2013a) found evidence of oxidative stress resulting from exposure to ozone. This evidence includes decreased levels of ascorbate in the BALF of rodents and decreased levels of glutathione in the respiratory bronchioles of monkeys. In addition, ascorbate deficiency enhanced ozone-induced lung injury. Further support for a role of oxidative stress is provided by a recent study in which Vitamin E supplementation dampened inflammation and airway responsiveness in ozone-exposed animals (Zhu et al., 2016). This relationship is depicted by the solid lines linking respiratory tract oxidative stress to respiratory tract inflammation and to increased airway responsiveness in Figure 3-1. Other studies described in the 2013 Ozone ISA (U.S. EPA, 2013a) found evidence of injury, including increased flux of small solutes from the lung to the plasma and increases in total protein, albumin, and shed epithelial cells in the BALF. In addition, markers of inflammation such as BALF neutrophils and cytokines were observed. Recent studies provide additional evidence for injury and inflammation.in animals exposed to ozone (Section 3.1.4.4.2). Taken together, these studies may provide biological plausibility for results of epidemiologic panel studies in healthy children and children with asthma, in which ozone was associated with markers of pulmonary inflammation and oxidative stress.

Studies described in the 2013 Ozone ISA (U.S. EPA, 2013a) and more recent studies reviewed later in this Appendix provide evidence for numerous cell signaling pathways underlying these oxidative stress, injury, and inflammatory responses. One recent study implicated glucocorticoids in mediating respiratory tract injury and inflammation resulting from ozone exposure (Miller et al., 2016b). In this study, adrenalectomy blocked the effects of ozone on the respiratory tract. This relationship is depicted by the solid lines linking the neuroendocrine stress response to respiratory tract injury and inflammation in Figure 3-1. Evidence for a neuroendocrine stress response initiated by ozone-mediated activation of sensory nerves and propagated systemically has recently been described (see Appendix 7).

Downstream effects of inflammation may result in morphologic changes. The 2013 Ozone ISA (U.S. EPA, 2013a) described mild morphologic changes, such as hyperplasia of the bronchoalveolar duct (rodents) and respiratory bronchioles (monkeys) and mucous cell metaplasia of the nasal epithelium (rodents and monkeys). Recent studies also provide evidence of morphologic changes in the upper and lower respiratory tracts following subacute or repeated exposure to ozone (Harkema et al., 2017; Kumagai et al., 2017; Kumagai et al., 2016; Ong et al., 2016; Cho et al., 2013).

In addition to activating the innate immune system, which is demonstrated by increases in airway neutrophils, ozone exposure affects the adaptive immune system. Alterations in antigen presentation and costimulation by innate immune cells such as macrophages and dendritic cells may lead to T-cell activation, which may enhance host defense or allergic responses. The 2013 Ozone ISA (U.S. EPA, 2013a) describes altered antigen presentation in macrophages and dendritic cells in humans exposed to ozone. Recent studies in animals exposed to ozone demonstrate dendritic cell activation (Brand et al., 2012) and a role for macrophage and T-lymphocyte subpopulations in the resolution of ozone-induced inflammation (Mathews et al., 2015). However, ozone exposure impairs, rather than enhances, host defense. The 2013 Ozone ISA (U.S. EPA, 2013a) describes evidence for altered macrophage function, decreased mucociliary clearance, and increased susceptibility to infectious disease in animals exposed to ozone. Recent studies in animals provide further evidence for increased susceptibility to infection following ozone exposure (Durrani et al., 2012). This demonstration of impaired host defense provides plausibility for epidemiologic findings indicating an association between short-term ozone concentrations and respiratory infection.

However, ozone skews immune responses towards an allergic phenotype. The 2013 Ozone ISA (U.S. EPA, 2013a) describes an animal study in which increased numbers of IgE-containing cells were found in the lungs of mice exposed repeatedly to ozone. Recent studies found increased serum immunoglobulin E (IgE), T helper 2 (Th2) cytokines, thymic stromal lymphopoietin (TSLP), eosinophilic inflammation, and a role for immune lymphoid cells in the development of type 2 immune responses in the upper and lower respiratory tract (Harkema et al., 2017; Kumagai et al., 2017; Kumagai et al., 2016; Ong et al., 2016; Zhu et al., 2016). Vitamin E supplementation was found to dampen allergic responses, implicating ozone-mediated oxidative stress (Zhu et al., 2016). This mechanism is depicted by the solid line connecting respiratory tract oxidative stress and allergic responses in Figure 3-1.

In addition, ozone exposure enhances allergic responses in humans with asthma and in animal models of allergic airway disease. Controlled human exposure studies described in the 2013 Ozone ISA (U.S. EPA, 2013a) provide evidence of increased airway eosinophils and Th2 cytokines, increased expression of IgE receptors on macrophages, and increased expression of CD86 in human subjects with atopy and asthma. Enhanced nasal and airway eosinophilia was seen in human subjects with asthma who were exposed first to allergen and then to ozone. In addition, there is increased uptake of particles by airway macrophages of human subjects with asthma that may also enhance the processing of particulate antigens and lead to greater progression of allergic airway disease. In animal models of allergic airway

disease, ozone exposure leads to enhanced allergic inflammatory responses, goblet cell metaplasia, and upregulated mucin expression, as described in the 2013 Ozone ISA (U.S. EPA, 2013a) and in several recent studies (Harkema et al., 2017; Kumagai et al., 2017; Kumagai et al., 2016; Ong et al., 2016; Bao et al., 2013).

Summary

As described here, there are two proposed pathways by which short-term exposure to ozone may lead to respiratory health effects. One pathway involves the activation of sensory nerves in the respiratory tract leading to lung function decrements, airway obstruction, and increased airway responsiveness. The second pathway involves respiratory tract injury, inflammation, and oxidative stress that may lead to morphologic changes and an allergic phenotype. Respiratory tract inflammation may also lead to altered host defense, which is linked to increased respiratory infections. While experimental studies involving animals or human subjects contribute most of the evidence of upstream effects, epidemiologic studies found associations between exposure to ozone and markers of respiratory tract inflammation, lung function decrements, and ED visits and hospital admissions for asthma and respiratory infection. Together, these proposed pathways provide biological plausibility for epidemiologic evidence of respiratory health effects and will be used to inform a causality determination, which is discussed later in this Appendix (Section 3.1.11).

3.1.4 Respiratory Effects in Healthy Populations

3.1.4.1 Lung Function

3.1.4.1.1 Controlled Human Exposure Studies

Controlled human exposure studies have provided strong and quantifiable exposure response data on the human health effects of ozone for decades (U.S. EPA, 1997). Respiratory responses to acute ozone exposures in the range of ambient concentrations (i.e., ≤80 ppb) include decreased inspiratory capacity; mild bronchoconstriction as demonstrated by increases in sRaw; rapid, shallow breathing patterns 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 (i.e., airway obstruction), contributes to a decrease in the forced expiratory volume in 1 second (FEV_1). Reductions in FVC and increases in sRaw appear to be mediated by different mechanisms.

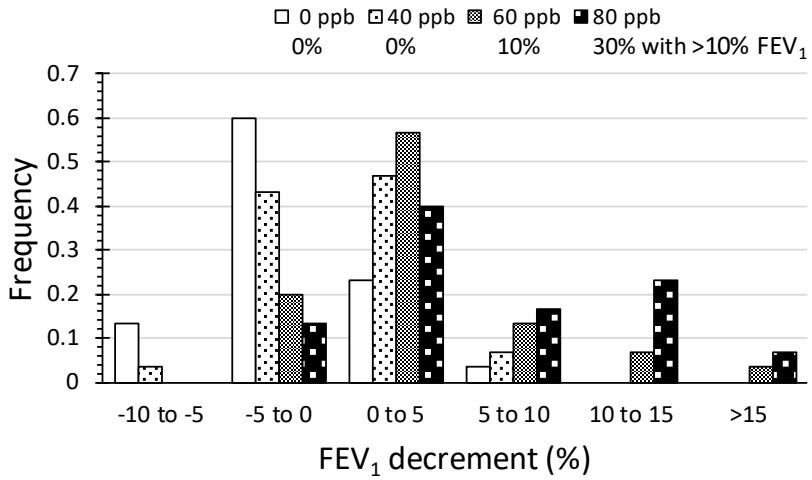
Most of the controlled human exposure studies described in this Appendix have exposed subjects to a constant ozone concentration in a chamber. Cases where subjects were exposed to ozone via a facemask are indicated in the text or figures. However, similar responses between facemask and chamber exposures have been reported for exposures to 80 and 120 ppb O_3 (6.6-hour, moderate quasi-continuous exercise at 40 L/minute) and 300 ppb O_3 (2 hours, heavy intermittent exercise at 70 L/minute) (Adams, 2003a, b, 2002). Some studies [e.g., Adams (2006) and Schelegle et al. (2009)] have increased the ozone concentration in a chamber in a step-wise manner (e.g., rapid change from 70 to 80 ppb each hour over the first 3 to 4 hours of exposure) and then subsequently decreased ozone concentration (e.g., from 80 to 50 ppb on an hourly basis) to achieve a targeted average ozone concentration over a 6.6 hour exposure. Although greater peak responses have been observed in step-wise and triangular (smooth increases and decreases in concentration) exposures versus constant concentration exposure protocols, similar FEV_1 responses have been reported at 6.6 hours regardless of the exposure protocol (i.e., constant versus step-wise) for average ozone exposures to 60, 80, and 120 ppb (Adams, 2006, 2003a; Adams and Ollison, 1997).

The most salient observations from studies reviewed in the 1996 and 2006 ozone AQCDs (U.S. EPA, 2006, 1996a) include: (1) young healthy adults exposed to ≥80 ppb ozone develop significant reversible, transient decrements in pulmonary function and symptoms of breathing discomfort if minute ventilation (Ve) or duration of exposure is increased sufficiently; (2) relative to young adults, children experience similar spirometric responses but lower incidence of symptoms from ozone exposure; (3) relative to young adults, ozone-induced spirometric responses are decreased in older individuals; (4) there is a large degree of inter-subject variability in physiologic and symptomatic responses to ozone, but responses tend to be reproducible within a given individual over a period of several months; and (5) subjects exposed repeatedly to ozone for several days experience an attenuation of spirometric and symptomatic responses on successive exposures, which is lost after about a week without exposure.

Mechanistic studies conducted in humans described in the 2013 Ozone ISA (U.S. EPA, 2013a) contributed to the understanding of neurogenic mechanisms underlying lung function responses in humans exposed to ozone while exercising. Controlled human exposure studies involving exposure to 400−420 ppb ozone provided evidence that nociceptive sensory nerves, presumably bronchial C-fibers, were responsible for pain-related symptoms and inhibition of maximal inspiration that resulted in decreased FVC. Eicosanoids, which are products of arachidonic acid metabolism, may also play a role in this response. Mild airway obstruction, measured as changes in sRaw, in response to ozone exposure, is inhibited by pretreatment with atropine, indicating the involvement of the parasympathetic nervous system. Tachykinins may also contribute to increases in sRaw because ozone exposure (250 ppb) increased substance P in BALF. Moreover, ozone exposure (200 ppb) resulted in a loss of substance P immunoreactivity in the neurons of the bronchial mucosa. Substance P is released by sensory nerves and mediates neurogenic edema and bronchoconstriction. Thus, increased sRaw may be attributed to vagally mediated pathways and to local axon reflexes.

The 2013 Ozone ISA (U.S. EPA, 2013a) included the FEV_1 responses of 150 young healthy adults exposed to 60 ppb [targeted concentration; Kim et al. (2011); Schelegle et al. (2009); Adams (2006)][1] and 31 young healthy adults exposed to 70 ppb (targeted concentration) ozone (Schelegle et al., 2009) for 6.6 hours during quasi-continuous exercise (i.e., 50-minute exercise periods). The moderate exercise level used in these studies is equivalent to walking at a pace of 17 to 18 minutes per mile at a grade of 4 to 5%. Although this is a relatively slow-paced walk, it does account for an average of about 17 miles of walking over six 50-minute exercise periods. On average across studies, the exposures to 60 ppb ozone resulted in a group mean FEV_1 decrement of 2.7%, with 10% of the exposed subjects experiencing greater than a 10% decrement in FEV_1 (see Figure 3-2). Although not consistently statistically significant, these group mean changes in FEV_1 at 60 ppb are consistent across studies, i.e., none observed an average improvement in lung function following a 6.6-hour exposure to 60 ppb ozone. There were no statistically significant effects in respiratory symptoms reported in any of the studies at 60 ppb ozone.

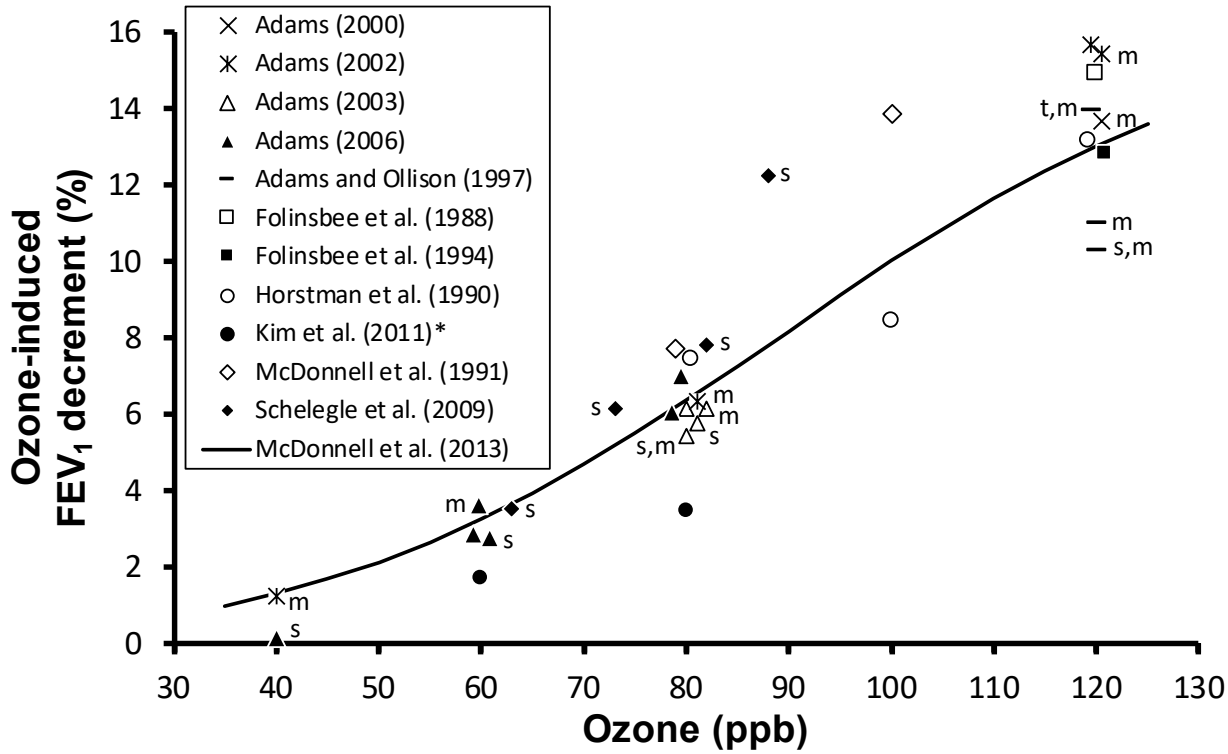
[1] Adams (2006) and Adams (2002) provide data for an additional group (30 of the 150) healthy subjects that were exposed via facemask to 60 ppb (square-wave) ozone for 6.6 hours with moderate exercise (Ve = 23 L/minute per m^2 body surface area [BSA]). These subjects are described on page 133 of Adams (2006) and pages 747 and 761 of Adams (2002). The FEV_1 decrement may be somewhat increased due to a target Ve of 23 L/minute per m^2 BSA relative to other studies that had a target Ve of 20 L/minute per m^2 BSA.



The illustrated data are for 30 subjects in the study conducted by Adams (2006). FEV_1 decrements following each exposure were calculated as pre-exposure FEV_1 minus post-exposure FEV_1 then divided by the pre-exposure FEV_1. The FEV_1 decrements for filtered air (0 ppb ozone) were subtracted from the FEV_1 decrements on ozone exposure days. The data for 60 and 80 ppb are the average of a stepwise exposure day and constant exposure day. During each hour of the exposures, subjects were engaged in moderate quasi-continuous exercise (20 L/minute per m^2 body surface area) for 50 minutes and rest for 10 minutes. Following the 3rd hour, subjects had an additional 35-minute rest period for lunch.

Figure 3-2 Inter-subject variability in forced expiratory volume in one second (FEV_1) decrements in young healthy adults following 6.6 hours of exposure to ozone.

Statistically significant effects on both lung function and respiratory symptoms were observed in young healthy adults following 6.6 hours of exposure to 70 ppb ozone (Schelegle et al., 2009). Illustrated in Figure 3-3 are group mean FEV_1 responses for all 6.6-hour studies of healthy young adults (age 18−35 years), conducted at average exposure concentrations of ≤120 ppb ozone with a target exercise ventilation rate of ~20 L/minute per m^2 body surface area (BSA). During each hour of exposure, subjects were engaged in moderate quasi-continuous exercise for 50 minutes and rest for 10 minutes. During chamber exposure studies, following the third hour, subjects had an additional 35 minute rest period within the chamber for lunch. During facemask exposure studies, following each hour of exposure (50 minutes of exercise followed by 10 minutes of rest), subjects removed their facemask (no ozone or filtered air delivery) for 2−3 minutes for measurement of pulmonary function. Additionally, following measurement of pulmonary function after the third hour of exposure, the facemask remained off (no ozone or filtered air delivery) for a 24 minute lunch period. Thus, for the 6.6-hour facemask studies, there was a total period of ~36 minutes at rest during which there was no delivery of ozone or filtered air exposure, whereas for the chamber studies there was 6.6 hours of continuous ozone or filtered air exposure. Predicted FEV_1 responses are also illustrated by the solid line in Figure 3-3 based on the model described in McDonnell et al. (2013). Predicted FEV_1 responses decrease with age, increase with BMI, and increase with ventilation rates. There are no more recent 6.6-hour ozone exposure studies.



All illustrated studies used a constant target exercise ventilation rate of ~20 L/minute per m^2 body surface area (BSA). For studies using step-wise (s) or triangular (t) increases and decreases in ozone concentration, the FEV_1 response is plotted at the average ozone exposure concentration for the 6.6-hour exposure. Some exposures were conducted using a facemask (m), all other studies were conducted within a chamber. All responses at and above 70 ppb (targeted concentration) were statistically significant relative to filtered air exposure. At 60 ppb, statistically significant FEV_1 responses to square-wave chamber exposures were found by Kim et al. (2011) and in the Adams (2006) study based on the analysis of Brown et al. (2008). With the exception of the Schelegle et al. (2009) data, the data at 60, 80, and 120 ppb have been offset for illustrative purposes. The McDonnell et al. (2013) line illustrates the predicted FEV_1 decrements at 6.6 hours as a function of ozone concentration using Model 3 coefficients for a 23.5-year-old with a BMI of 23.1 kg/m^2 having a ventilation rate during rest and exercise of 6 and 20 L/minute per m^2 BSA. *80 ppb data for 30 health subjects were collected as part of the Kim et al. (2011) study, but only published in Figure 5 of McDonnell et al. (2012).

Adapted from Figure 6-1 of 2013 Ozone ISA (U.S. EPA, 2013a). Studies appearing in the figure legend are: Adams (2006), Adams (2003a), Adams (2002), Adams (2000), Adams and Ollison (1997), Folinsbee et al. (1994), Folinsbee et al. (1988), Horstman et al. (1990), Kim et al. (2011), McDonnell et al. (2013), McDonnell et al. (1991), and Schelegle et al. (2009). *80 ppb data for 30 health subjects were collected as part of the Kim et al. (2011) study, but only published in Figure 5 of McDonnell et al. (2012).

Figure 3-3 Cross-study comparisons of mean ozone-induced forced expiratory volume in one second (FEV_1) decrements in young healthy adults following 6.6 hours of exposure to ozone.

Since the 2013 Ozone ISA, one new study has evaluated the effect of ozone on lung function at concentrations below 80 ppb. The results of this study of older adults (55−70 years) exposed for 3 hours to 0, 70, and 120 ppb ozone appear in both an HEI report (Frampton et al., 2017) and in the scientific literature (Arjomandi et al., 2018) and are discussed in a subsection on lifestage (Section 3.1.4.1.1.1). Several new studies have investigated the effects of 100−300 ppb ozone exposure on lung function [e.g., Biller et al. (2011), Ghio et al. (2014), Hoffmeyer et al. (2013), Madden et al. (2014), Stiegel et al. (2017), Tank et al. (2011)]. Given that lower ambient concentrations are more common currently, any

such studies are most relevant with regard to consideration of mechanistic information or existence of associations between lung function and other indicators of respiratory health. As discussed in Section 6.2.1.1 of the 2013 Ozone ISA, repeated consecutive days of ozone exposure typically show that the FEV_1 response is enhanced on the second day of exposure. Consistent with older studies, Madden et al. (2014) reported that 2 consecutive days of ozone exposure caused a statistically greater decrement in FEV_1 (18.2 ± 4.5%) than the decrement immediately after the first day of ozone exposure (i.e., 9.9 ± 2.5%; $p < 0.05$) an or immediately after ozone exposure (i.e., 10.9 ± 2.6%) preceded by an air exposure on the prior day. Although changes in lung function have generally been found to be unrelated to inflammatory responses of the lung following ozone exposure, significant relationships have been reported between FEV_1 decrements and plasma ferritin [$r = -0.67$, $p = 0.003$; i.e., larger FEV_1 decrements in individuals with lower baseline plasma ferritin, (Ghio et al., 2014) and with the inflammatory cytokine IFN-γ in the blood (Stiegel et al., 2017). Hoffmeyer et al. (2013) used 40 ppb as their control exposure for comparisons against an exposure of 240 ppb. Relatively consistent with the Adams (2002) and Adams (2006) studies of 6.6-hour exposures to 40 ppb, the 4-hour exposure to 40 ppb with two 20-minute periods of light exercise caused no statistically significant changes in lung function in the study by Hoffmeyer et al. (2013). Study-specific details, including exposure concentrations and durations, are summarized in Table 3-4 in Section 3.3.1.

3.1.4.1.1.1 Predicted Lung Function Response to Ozone Exposure in Healthy Adults

The similarities and differences in two models (McDonnell et al., 2012; Schelegle et al., 2012) predicting FEV_1 responses to ozone exposure in healthy adults were described in the 2013 Ozone ISA (U.S. EPA, 2013a). In brief, both are two compartment models that consider a dose of onset in response or a threshold of response. The first compartment in the McDonnell et al. (2012) model considers the level of oxidant stress in response to ozone exposure to increase over time as a function of dose rate (concentration × minute ventilation) and decrease by clearance or metabolization over time according to first order reaction kinetics. In the second compartment of the threshold model, once oxidant stress reaches some threshold level, the decrement in FEV_1 increases as a sigmoid-shaped function of the oxidant stress. In the Schelegle et al. (2012) model, the first compartment acts as a reservoir in which oxidant stress builds up until the dose of onset at which time it spills over into a second compartment. The second compartment is conceptually the same as the first compartment in the McDonnell et al. (2012) model, i.e., oxidant stress increases as a function of dose rate (concentration × minute ventilation) and oxidant stress decreases according to first order clearance kinetics. The oxidant levels in the second compartment of the Schelegle et al. (2012) model are multiplied by a responsiveness coefficient to predict FEV_1 responses. Two new models (Hsieh et al., 2014; McDonnell et al., 2013) have become available since the 2013 Ozone ISA.

The McDonnell et al. (2012) and McDonnell et al. (2013) models were fit to a large dataset consisting of the FEV_1 responses of 741 young healthy adults (104 F, 637 M; mean age 23.8 years) from

23 individual controlled exposure studies conducted in either Chapel Hill, NC or Davis, CA. The models were fit using a SAS procedure specially designed for fitting nonlinear random-effects models. Statistical estimates were obtained for the primary model parameter coefficients, a variance term for inter-subject variability in response, and an error term representing intra-subject variation. McDonnell et al. (2013) provides alternative variance structures relative to the McDonnell et al. (2012) model. McDonnell et al. (2013) partitioned the intra-subject error term to include: (1) random noise in measurement of FEV_1 and (2) increasing variability with increasing FEV_1 response. The addition of random intra-subject noise in the error term allows lower percentiles of the FEV_1 response distribution to have improvements in FEV_1 during ozone exposure.

Hsieh et al. (2014) emulated the mechanistic model developed by Freijer et al. (2002). The Freijer et al. (2002) model predicts changes in FEV_1 to occur as a function of the balance between respiratory cells naïve to ozone exposure and those previously exposed cells having developed some antioxidant protection. An interesting aspect of this model is that it is capable of predicting the effects of consecutive days of ozone exposure on FEV_1 responses. As discussed in Section 6.2.1.1 of the 2013 Ozone ISA, repeated consecutive days of ozone exposure typically show that FEV_1 responses are enhanced on the second day of exposure and become attenuated after 3 to 4 consecutive days of exposure relative to the first ozone exposure day. Hsieh et al. (2014) fitted three parameters of the Freijer et al. (2002) model to best match model-estimated FEV_1 decrements with the Schelegle et al. (2009) data. Overall, across all exposure concentrations (targets of 60, 70, 80, and 87 ppb) and time points (0, 1, 2, 3, 4.6, 5.6, and 6.6 hours), there was an r^2 of 0.73 between the predicted and observed FEV_1 responses. The 70 ppb target exposure in the Schelegle et al. (2009) study is the lowest concentration at which both statistically significant FEV_1 decrements and respiratory symptoms have been observed following 6.6 hours of exposure. Figure 3 of Hsieh et al. (2014) shows that had the Schelegle et al. (2009) study been extended to 8 hours, a 6.14% FEV_1 decrement would be observed at 63 ppb after 8 hours of exposure, the same decrement observed following 6.6 hours of exposure to 70 ppb by Schelegle et al. (2009).

3.1.4.1.1.2 Factors Affecting Lung Function Response to Ozone

Airway Responsiveness

Although ozone exposure has been shown to increase airway responsiveness, fewer studies have assessed whether baseline airway responsiveness is associated with ozone-induced changes in lung function. In the 2006 ozone AQCD (U.S. EPA, 2006), there was limited discussion of Aris et al. (1995) who exposed healthy adults (24 F, 42 M; 27 ± 4.5 years) to 0 and 200 ppb ozone for 4 hours during quasi-continuous exercise (50 minutes at 25 L/minute per m^2 BSA and 10 minutes rest). These authors observed a weak correlation between pre-exposure methacholine responsiveness and ozone-induced increases in sRaw, but not with ozone-induced decreases in FEV_1 and FVC. Recent studies expand upon the previous evidence base, but provide no new evidence per se. Specifically:

- Hoffmeyer et al. (2013) exposed healthy adults (7 F, 8 M; 26 years) to 40 ppb (control exposure) and 240 ppb ozone for 4 hours with two 20-minute exercise (15 L/minute per m^2 BSA) periods. Five subjects having >5% decrement in FEV_1 following the 240 ppb exposure were characterized as responders. There was a tendency towards a greater FEV_1 response to methacholine in the 5 responders as compared to the 10 nonresponders. Responsiveness to capsaicin as a predictor of ozone responses was also examined. Across all subjects, capsaicin responsiveness was correlated with ozone-induced changes in peak expiratory flow ($r = 0.716$, $p = 0.003$) and maximal expiratory flow at 50% of vital capacity ($r = 0.589$, $p = 0.021$), but less so with FEV_1 ($r = 0.417$, $p = 0.122$). The cumulative dose of capsaicin causing two or more coughs was also significantly lower in the ozone responders than nonresponders. The association between ozone and capsaicin responsiveness likely reflected the role of sensory C-fibers.
- Bennett et al. (2016) found statistically greater FVC decrements in obese (19 F; 27.7 ± 5.2 years) than normal weight (19 F; 24 ± 3.7 years) individuals following a 2-hour exposure to 400 ppb ozone. This difference was not associated with methacholine responsiveness on the training day, which was similar between the groups.
- In a large study of individuals with asthma (34 F, 86 M; 32.9 ± 12.9 years), Bartoli et al. (2013) also found the magnitude of ozone-induced FEV_1 response (based on 2-hour exposures to 300 ppb and filtered air) was unrelated to baseline methacholine responsiveness.

Ambient Temperature

Studies reviewed in Section 10.2.9.3 of the 1986 ozone AQCD (U.S. EPA, 1986) suggested an additive effect of increased temperature with ozone exposure on lung function decrements. However, the effect of temperature and humidity on respiratory responses was termed as equivocal in Section 7.2.1.3 of the 1996 ozone AQCD (U.S. EPA, 1996a). In Section 6.5.5 of the 2006 ozone AQCD (U.S. EPA, 2006), a single new study (Foster et al., 2000) was discussed that suggested elevated temperature may partially attenuate spirometric responses but enhance airway reactivity. Discussion of the effect of temperature on responses in controlled human exposure studies was not included in the 2013 Ozone ISA (U.S. EPA, 2013a). Overall, recent studies are consistent with the equivocal findings related to effects of temperature in prior reviews. Specifically:

- Recently, Kahle et al. (2015) exposed healthy volunteers (2 F, 14 M) to filtered air and 300 ppb ozone for 2 hours with intermittent exercise (alternating 15-minute periods rest and exercise at 25 L/minute per m^2 BSA) at both 22°C and 32.5°C. FEV_1 and FVC were significantly reduced by exposure to 300 ppb ozone relative to filtered air, but no significant effect of temperature or ozone-temperature interaction was observed. There was a tendency for smaller ozone-induced FEV_1 and FVC decrements at 32.5°C compared to 22°C.
- In another study, Gomes et al. (2011b) exposed 10 male athletes to filtered air and 100 ppb ozone while completing an 8-km time trial at either 20°C + 50% RH and 31°C + 70% HR. The elevated temperature and humidity with and without ozone significantly decreased running speed. The combination of heat and ozone also significantly increased the athletes perceived exertion level relative to the lower temperature scenario with and without ozone. This study supports a trend for an additive effect of ozone and temperature on decreased exercise performance and perceived exertion level.

- In another high temperature (31°C + 70% HR) ozone study, Gomes et al. (2011a) showed a tendency toward improved exercise performance in nine male athletes during exposure to 100 ppb ozone between vitamin and placebo trials ($p = 0.075$). This is generally consistent with studies reviewed in the 2006 Ozone AQCD (AX6.5.6 Oxidant-Antioxidant Balance).

Cigarette Smoking

Studies reviewed in the 2006 ozone AQCD (U.S. EPA, 2006) and earlier reviews showed active smokers experienced smaller lung function decrements than nonsmokers in response to ozone exposure. A recent study found similar FEV_1 decrements between smokers and nonsmokers:

- In a recent study, Bates et al. (2014) exposed smokers (11 F, 19 M; 24 ± 4 years) and nonsmokers (13 F, 17 M; 25 ± 6 years) to 0 and 300 ppb ozone for 1 hour during light exercise. Statistically significant ozone-induced FEV_1 decrements (about 9−10%) were similar between smokers and nonsmokers. Based on exhaled CO_2 profiles, smokers, but not nonsmokers, showed a reduction in dead space (−6.1 ± 1.2%) and an increase in the alveolar slope (9.1 ± 3.4%). This finding could be caused by nonuniform bronchoconstriction, which would alter the pattern of filling and emptying lung units. An effect on pulmonary ventilation was also reported in the 2006 ozone AQCD; a study by Foster et al. (1997) showed a 24% reduction in the washout rate of the lungs of healthy males following ozone exposure, which remained or developed in 50% of subjects a day after the ozone exposure.

Lifestage

Healthy older subjects (52 F, 35 M; 59.9 ± 4.5 years) were exposed to 0, 70, and 120 ppb ozone for 3 hours during intermittent exercise [15-minute intervals of rest and exercise at 15−17 L/minute per m^2 BSA; Arjomandi et al. (2018)]. Lung function (FEV_1, FVC, FEV_1/FVC, and FEF_{25-75}) was measured 10 minutes before exposure and at 0.25 and 22 hours post-exposure. As has been reported in prior studies [see p. 6-4 of U.S. EPA (2013a)], FEV_1 increased after exercise during exposure to filtered air at both 15 minutes (85 mL; 95% CI: 64−106; paired *t*-test: $p < 0.001$) and 22 hours (45 mL; 95% CI, 26−64; $p < 0.001$) after exposure. The ozone exposures resulted in a smaller exercise-related increase in FEV_1, specifically 15 and 33 mL smaller increase at 70 ppb ($p = 0.12$) and 120 ppb ($p = 0.001$), respectively. The observed FEV_1 and FVC changes following ozone exposure showed no interaction by sex (52 F, 35 M), age (55−70 years), or GSTM1 genotype (57% null, 43% positive). Inflammatory responses measured as part of this study are provided in another section of this document.

While the decrements in lung function observed by Arjomandi et al. (2018) are small, a group mean ozone-induced FEV_1 decrement of only 1.2% (based on group mean changes in lung function provided in Table 2 of the paper) following the 120 ppb exposure, the decrement was not expected in these older subjects at a relatively light activity level and brief 3 hour duration of exposure. For comparison, the McDonnell et al. (2013) model predicts a 2% FEV_1 decrement in 23.8-year-olds (less than the 3% FEV_1 decrement observed and predicted in 6.6 hours studies at 60 ppb) for this exposure protocol and no FEV_1 decrement is predicted in individuals over 48.5 years of age. Results from

Arjomandi et al. (2018) are generally consistent with the prior work of Hazucha et al. (2003), who studied adults up to 60 years of age and showed that lung function decrements decline with age, but may still be present in older adults 50−60 years of age. However, this recent study was conducted at a lower ozone delivery rate than Hazucha et al. (2003), which is more representative of that likely to occur in the ambient environment and shows small lung function decrements may occur in older adults.

3.1.4.1.2 Animal Toxicological Studies

The 2013 Ozone ISA summarized the animal toxicological evidence of changes in lung function resulting from exposure to ozone. Most of the studies involved acute exposures (U.S. EPA, 2013a). Changes in frequency of breathing and tidal volume, reflecting a pattern of rapid, shallow breathing, were commonly observed at ozone concentrations of about 0.2 ppm. Decreased lung volumes were observed in rats exposed to 0.5 ppm, while changes in compliance and resistance were observed at ozone concentrations of 1 ppm and above. Repeated acute exposures over several days led to attenuation of the pulmonary function decrement response. A lung imaging study found that continuous or half-day exposure to 0.5 ppm ozone for several days led to ventilatory abnormalities that suggested narrowing of peripheral small airways and increased airway resistance. While ozone concentrations in animal toxicological studies seem to be high, it should be noted that deposition of ozone resulting from exposure to 2 ppm ozone in a resting rat is roughly equivalent to deposition of ozone resulting from exposure to 0.4 ppm ozone in an exercising human (Hatch et al., 1994).

Studies described in the 2013 Ozone ISA provide evidence that neurogenic mechanisms underlie the changes in lung function observed in animals exposed to ozone (U.S. EPA, 2013a). Activation of sensory nerves in the airway epithelium occurs as a result of ozone exposure. Stimulation of bronchial C-fibers leads to rapid, shallow breathing and other changes in respiratory mechanics in response to ozone. TRPA1 ion channels, which are found on a subset of bronchial C-fibers, may be activated by secondary oxidation products of ozone and components of the extracellular lining fluid in the respiratory tract, such as aldehydes. In addition, arachidonic acid metabolites, such as prostaglandins, may be involved in activation or sensitization of the TRPA1 ion channels. As discussed previously, these airway sensory nerves are vagal afferents that carry signals to the nucleus tractus solitarius neurons in the brain. These pathways can be integrated in the brain, resulting in altered autonomic activity that affects the airways (e.g., bronchoconstriction) or extrapulmonary responses such as changes in heart rhythm. Stress-responsive regions of the brain may also be affected by these vagal afferent pathways from the respiratory tract. In addition, activation of bronchial C-fibers may lead to local axon reflex responses in the airways, such as the release of substance P or other tachykinins, which act through neurokinin receptors to increase airway resistance (i.e., bronchoconstriction).

A large number of recent studies evaluated changes in lung function in response to short-term ozone exposure. Study-specific details are summarized in Table 3-5 in Section 3.3.1. All of these studies were conducted in rodent strains with varying degrees of sensitivity to ozone. Lung function was assessed

by changes in ventilatory parameters such as tidal volume and enhanced pause. Enhanced pause is a measure of respiratory distress that may or may not be related to an increase in airway resistance. Airway resistance can be examined by direct measures of lung mechanics in vivo such as the flexiVent and the pneumotachometer/pressure transducer system, which are both invasive methods. These recent studies, detailed below and grouped by concentration-time profile, demonstrated that exposure to 0.1−2 ppm ozone results in changes in lung function, as measured by altered ventilatory parameters. All of the changes in lung function described below were statistically significant. Changes in enhanced pause and evidence of sensory and pulmonary irritation were observed following acute exposure to 2 ppm ozone. Changes in enhanced pause and tidal volume were observed with acute exposure to 0.5−1 ppm ozone. Repeated exposure to ozone resulted in numerous effects, with decreased respiratory frequency occurring at concentrations of 0.1 ppm ozone.

- Acute exposure of rodents to 2 ppm ozone for 3 hours resulted in increases in enhanced pause (Ghio et al., 2014; Bao et al., 2013; Lee et al., 2013). Evidence for sensory and pulmonary irritation is provided by Hansen et al. (2016). Sensory irritation reflects changes in the upper airways, while pulmonary irritation reflects changes in the lower airways.
- Acute exposure to 0.8−1 ppm ozone for 1−6 hours resulted in alterations in tidal volume and enhanced pause (Gordon et al., 2016b; Dye et al., 2015; Schelegle and Walby, 2012). Alterations were also found following exposure to 0.5 ppm ozone (Dye et al., 2015).
- Repeated ozone exposures with differing concentration-duration profiles also resulted in altered ventilatory parameters.
    - Decreased respiratory frequency—0.1 ppm ozone for 1 hour/day for 10 days (Wolkoff et al., 2012).
    - Increased minute volume and enhanced pause, and decreased relaxation time—1 ppm ozone for 6 hours/day for 2 days (Snow et al., 2016).
    - Increased enhanced pause—1 ppm ozone for 4 hours/day for 1 and 2 days (Miller et al., 2016b) or 1 ppm ozone for 5 hours/day for 2 days (Gordon et al., 2017b) or 0.8 ppm ozone for 4 hours/day for 1 and 2 days (Gordon et al., 2017a).
    - Increased peak expiratory flow and enhanced pause—0.8 ppm ozone for 4 hours/day for 1 and 2 days (Henriquez et al., 2017).
    - No evidence of altered ventilatory parameters was seen in response to 0.25−0.5 ppm ozone for 5−6 hours per day for 2 days (Gordon et al., 2017b; Snow et al., 2016).
- Two studies examined changes in lung function following acute ozone exposure in rodents of varying lifestages.
    - In a study of rodents from adolescence to senescence (Snow et al., 2016), ozone exposure resulted in age-dependent changes in minute volume. Increases in minute volume were observed in 1 month old animals but not in 4, 12, and 24 month old animals exposed to 1 ppm ozone for 6 hours per day for 2 days.
    - In Groves et al. (2013), ozone exposure increased resistance in young adult mice and increased resistance and elastance in older adult mice.

3.1.4.1.3 Epidemiologic Studies

A number of studies evaluated in the 2013 Ozone ISA provided consistent evidence for ozone-related decreases in lung function in healthy children (U.S. EPA, 2013a). Noteworthy evidence of the effect of short-term exposure to ozone on respiratory effects in healthy children came from panel studies with daily assessment of lung function in children attending summer camps (Berry et al., 1991; Spektor and Lippmann, 1991; Avol et al., 1990; Burnett et al., 1990; Higgins et al., 1990; Raizenne et al., 1989; Spektor et al., 1988). Specifically, ozone exposure was consistently associated with decreases in FEV_1 in 7- to 17-year-old children without asthma. Analyses were conducted during summer months in the 1980s and included diverse locations across the U.S. and Canada. Additionally, ozone monitoring generally occurred at the site of the camp, reducing potential exposure measurement error. While associations for peak expiratory flow (PEF) were more variable than those for FEV_1, increases in ambient ozone concentration were generally associated with decreases in PEF. None of the referenced studies examined copollutant models.

In addition to studies of children, the 2013 Ozone ISA evaluated a number of studies that examined lung function in healthy adults. There was consistent evidence of ozone-related lung function decrements in panel studies of adults participating in outdoor recreation, exercise, or work [see Section 6.2.1.2 of the 2013 Ozone ISA U.S. EPA (2013a)]. Like the summer camp studies, these studies had on-site ozone measurements during the time of the outdoor activity, resulting in higher personal exposure and ambient concentration correlations. Cohort and cross-sectional studies that used the average of fixed-site monitors, nearest monitor, or spatial interpolation to assign exposure across a larger study area observed inconsistent evidence of an association between short-term ambient ozone concentrations and lung function in adults and older adults. The inconsistent results relative to panel studies may have been due to differences in study design, geographic location, and/or increased exposure measurement error, among other factors.

A recent study in Canada also reports a positive association between short-term ambient ozone concentrations and lung function effects in a healthy population. Study-specific details, including air quality characteristics and select effect estimates, are highlighted in Table 3-6 in Section 3.3.1. An overview of the evidence is provided below.

- In a randomized crossover study of young adults in Sault Ste. Marie, Canada, Dales et al. (2013) observed decreases in a range of lung function metrics, including FEV_1, FVC, and FEV_1/FVC, associated with ozone concentrations. Participants alternated five consecutive 10-hour days outdoors at two locations and ozone concentrations were measured on-site to reduce potential exposure measurement error. SO_2 concentrations were notably higher at one location near a steel plant, but the lung function associations with ozone were independent of study site.
- Other recent studies of adults examined respiratory effects in the general population (Lepeule et al., 2014; Rice et al., 2013). These studies included both healthy participants and those with

pre-existing respiratory conditions, with asthma or COPD prevalence ranging from 6 to 20%.[1] Because these studies do not directly inform the understanding of the relationship between short-term ozone exposure and lung function in healthy populations or populations with asthma, they are not discussed in either section. However, study specific details can be found in Table 3-7 in Section 3.3.1.

In summary, one recent study, along with studies evaluated in the previous ozone ISA, support the presence of an association between short-term ozone exposure and decreased lung function in healthy populations. Onsite exposure measurement at study site locations has reduced the potential for exposure measurement error in these studies, but the independence of the observed associations relative to other pollutants remains uncertain.

3.1.4.1.4 Integrated Summary for Lung Function

Controlled human exposure studies evaluated in the 1996 and 2006 Ozone AQCDs (U.S. EPA, 2006, 1996a) provided evidence for a number of lung function effects in healthy subjects exposed to ≥80 ppb ozone. Young adults and children experience similar transient decrements in pulmonary function when exposed to ozone, but spirometric responses become less pronounced with increasing age. Further evidence from the 2013 Ozone ISA (U.S. EPA, 2013a) demonstrated decreases in group mean pulmonary function relative to ozone exposures as low as 60 to 70 ppb in young, healthy adults performing moderate exercise. One recent study observed a small, but not statistically significant, group decrease in post-exercise lung function in older adults following 70 ppb exposure.

Like studies in humans, experimental studies in animals also provide evidence of changes in lung function resulting from exposure to ozone. Evidence summarized in the 2013 Ozone ISA indicated changes in the frequency of breathing and tidal volume, decreased lung volume, increased airway resistance, and attenuation of the pulmonary function decrement response following repeated exposures (U.S. EPA, 2013a). Recent evidence further demonstrates changes in ventilatory parameters resulting from ozone exposure. Experimental studies in both humans and animals indicate that changes in lung function, including FEV_1 and sRaw, may be attributed to activation of sensory nerves in the respiratory tract that trigger local and autonomic reflex responses. Specifically, mechanistic studies provide evidence that local reflex responses mediate the observed decreases in inspiratory capacity and pain on inspiration that result in truncated inspiration. In addition, modest increases in airway resistance may occur due to activation of parasympathetic pathways. These changes, along with observed alterations in breathing frequency, are a type of irritant response. Results from recent animal toxicological studies are generally consistent with those described in the 2013 Ozone ISA, reporting changes in ventilatory parameters resulting from acute exposure to 0.5−2 ppm ozone and from repeated exposure to 0.1 ppm ozone.

[1] All epidemiologic results standardized to a 15-ppb increase in 24-hour avg, 20-ppb increase in 8-hour daily max, 25-ppb increase in 1-hour daily max ozone concentrations, or a 10-ppb increase in seasonal/annual ozone concentrations to facilitate comparability across studies.

Epidemiologic studies of lung function in healthy populations are coherent with experimental studies that demonstrate evidence of ozone-related lung function decrements in a controlled environment and provide mechanistic evidence for the plausibility of the observed changes. Most epidemiologic evidence comes from panel studies of healthy children that were previously evaluated in the 2013 Ozone ISA (U.S. EPA, 2013a).

3.1.4.2 Respiratory Symptoms

3.1.4.2.1 Controlled Human Exposure Studies

As described in Section 6.2.1.1 of the 2013 Ozone ISA (U.S. EPA, 2013a), in addition to lung function decrements, controlled human exposure studies clearly indicate ozone-induced increases in respiratory symptoms including pain on deep inspiration, shortness of breath, and cough. In brief, the available evidence during the last review indicated that respiratory symptoms increase with increasing ozone concentration, duration of ozone exposure, and activity level of exposed subjects. For exposures of 1−2 hours to ≥120 ppb, statistically significant respiratory symptoms and effects on FEV_1 were observed when exercise sufficiently increased ventilation rates (McDonnell et al., 1999b). During exposures at rest, 5% of young healthy adults exposed to 400 ppb ozone for 2 hours experienced pain on deep inspiration, but not at 1 hour of exposure. Respiratory symptoms were also not observed following 1 to 2 hours of resting exposure at lower concentrations of 120 to 300 ppb. However, when exposed during light-to-moderate intermittent exercise (22−35 L/minute) to 120 ppb for 2 hours, 9% of individuals experienced pain on deep inspiration, 5% experienced cough, and 4% experienced shortness of breath. For longer duration, 6.6-hour exposures to 80 ppb with moderate exercise, FEV_1 decrements and total respiratory symptoms diverge from filtered air responses after 3 hours and become statistically different by 6.6 hours (Adams, 2006). For the 6.6-hour exposures to ozone, 70 ppb is the lowest concentration where statistically significant ozone-induced lung function decrements and subjective symptoms have been reported (Schelegle et al., 2009). Although several studies have investigated the effects of 6.6-hour exposures during moderate exercise to 60 ppb ozone, none have observed a statistically significant increase in respiratory symptoms following ozone relative to filtered air. There are no new controlled human exposure studies conflicting with the above or contributing a better characterization of ozone-induced respiratory symptoms.

3.1.4.2.2 Animal Toxicological Studies

There were no animal toxicological studies that examined respiratory symptoms in the 2013 Ozone ISA (U.S. EPA, 2013a). In fact, it is difficult or impossible to assess respiratory symptoms such as pain on deep inspiration, shortness of breath, and cough in rodents. Rodents are obligate nasal breathers

and in general do not cough. However, changes in ventilation may be consistent with dyspnea. Further, cough can be elicited in rodents, through the use of an irritant. This reflex is termed a hypertussive response. A recent study in guinea pigs and rabbits found that ozone acts through sensory nerves to enhance coughing that is elicited by citric acid (Clay et al., 2016). Details of this study are summarized in Table 3-5 in Section 3.3.1. Acute exposure to 2 ppm ozone for 0.5−1 hour resulted in statistically significant increases in cough frequency and decreases in time to cough in response to citric acid. Experiments with pharmacological agents implicated TRPV1 receptors, a type of sensory nerve receptor often found on C-fibers, in mediating the hypertussive response to ozone.

3.1.4.2.3 Epidemiologic Studies

The 2013 Ozone ISA did not evaluate any studies that examined respiratory symptoms in study populations consisting solely of healthy populations (i.e., respiratory disease-free) (U.S. EPA, 2013a). Several panel studies of children that were not restricted to healthy individuals, in which asthma prevalence was 50% or less, reported null or negative associations between ambient ozone concentrations and respiratory symptoms, such as cough, wheeze, and phlegm (see Section 3.1.4 of the 2013 Ozone ISA). Notably, the majority of these studies assessed respiratory symptoms through parental reported outcomes, which may be differentially misreported based on asthma status.

3.1.4.2.4 Integrated Summary for Respiratory Symptoms

Controlled human exposure studies evaluated in the 2013 Ozone ISA reported symptoms of cough and pain on deep inspiration corresponding to FEV_1 decrements in healthy young adults exposed to 70 ppb ozone for 6.6 hours (U.S. EPA, 2013a). A recent model can be used to determine the ozone concentration that would lead to the same FEV_1 decrement following an 8-hour exposure (McDonnell et al., 2013). Under the assumption that respiratory symptoms might accompany similar ozone-induced FEV_1 decrements, regardless of exposure duration, the model indicates that an 8-hour exposure to 64 ppb ozone concentration might reasonably be expected to cause an adverse response in young healthy adults.

In coherence with evidence observed in controlled human exposure studies, a recent mechanistic animal toxicological study observed that ozone acts through sensory nerves to induce coughing. In contrast, ozone-induced respiratory symptoms observed in healthy subjects in controlled human exposure and animal toxicological studies were not evident in epidemiologic studies in the general population. However, these epidemiologic studies generally relied on parental reported outcomes that may result in under- or over-reporting of respiratory symptoms, depending on a child's asthma status.

3.1.4.3 Airway Responsiveness

3.1.4.3.1 Controlled Human Exposure Studies

As reviewed in the 2006 Ozone AQCD (U.S. EPA, 2006) and in the 2013 Ozone ISA (U.S. EPA, 2013a), ozone has been shown to cause an increase in airway responsiveness in controlled human exposure studies. In general, airway responsiveness is assessed by increasing inhaled concentrations of a bronchoconstrictive drug and measuring the effect on lung mechanics (FEV_1 or sRaw). A dose-dependent increase in airway responsiveness of young, healthy, nonsmoking males following exposures to 0, 80, 100, and 120 ppb ozone (6.6 hours, quasi-continuous moderate exercise at 39 L/minute) has been demonstrated. Changes in airway responsiveness appear to persist longer than changes in pulmonary function, although this has been studied only on a limited basis. Studies suggest that ozone-induced increases in airway responsiveness usually resolve 18 to 24 hours after exposure, but may persist in some individuals for longer periods. Although FEV_1 decrements and respiratory symptoms become attenuated following several consecutive days of ozone exposure, the ozone-induced increase in airway responsiveness (measured by increase in sRaw upon methacholine challenge) over 5 consecutive days is not attenuated. Increases in airway responsiveness following ozone exposure do not appear to be associated with ozone-induced changes in lung function, respiratory symptoms, or changes in epithelial permeability. First described in the 1986 ozone AQCD (U.S. EPA, 1986), a mechanistic study of subjects exposed to 600 ppb ozone while exercising added to the understanding of mechanisms underlying changes in airway responsiveness caused by ozone exposure. Atropine inhibited an ozone-induced increase in airway responsiveness to histamine, indicating the involvement of the parasympathetic nervous system in this response. A recent study of 38 healthy adult women (average age, 26 years) exposed to 0 and 400 ppb ozone for 2 hours performing light intermittent exercise (15-minute periods of exercise at 25 L/minute and seated rest) showed a tendency (statistical significance not assessed by investigators) for increases in airway responsiveness due to ozone with 4 and 12 subjects being responsive to methacholine after exposure to filtered air and ozone, respectively (Bennett et al., 2016). Study-specific details are summarized in Table 3-8 in Section 3.3.1.

3.1.4.3.2 Animal Toxicological Studies

The 2013 Ozone ISA (U.S. EPA, 2013a) summarized the animal toxicological evidence of increased airway responsiveness resulting from exposure to ozone. In general, airway responsiveness is assessed by measuring the effects of challenge with increasing concentrations of a bronchoconstrictive drug on lung mechanics (FEV_1 or sRaw). Methacholine is the most commonly used nonspecific challenge agent, but histamine and other agents are also used. Most of the studies discussed in the 2013 Ozone ISA (U.S. EPA, 2013a) found increased airway responsiveness in guinea pigs, rats, or mice exposed to 1 ppm and higher concentrations of ozone, although increased airway responsiveness was, in a few cases,

demonstrated after exposure to less than 0.3 ppm ozone. While ozone concentrations in animal toxicological studies seem to be high, it should be noted that deposition of ozone resulting from exposure to 2 ppm ozone in a resting rat is roughly equivalent to deposition of ozone resulting from exposure to 0.4 ppm ozone in an exercising human (Hatch et al., 1994). Studies involving animal models of allergic airway disease are discussed in Section 3.1.5.5.2 because these animal models share phenotypic features with asthma.

Studies described in the 2013 Ozone ISA (U.S. EPA, 2013a) provide evidence that neurogenic mechanisms underlie the increased airway responsiveness observed in animals exposed to ozone (U.S. EPA, 2013a). In one study, eosinophils promoted the activation of airway parasympathetic nerves by releasing major basic protein, which blocked a muscarinic receptor-mediated pathway that attenuates acetylcholine release from the nerves. Acetylcholine, like methacholine, acts on receptors in airway smooth muscle to stimulate bronchoconstriction. In another study, substance P acted through the neurokinin 1 receptor to cause vagally mediated bronchoconstriction. There is also evidence that arachidonic acid metabolites and cytokines/chemokines such as tumor necrosis factor-alpha (TNF-α), C-S-C chemokine receptor type 2 (CXCR2), and macrophage inflammatory protein-1 (MIP-2) play a role in increased airway responsiveness following exposure to ozone. Furthermore, activation of an innate immune pathway involving natural killer cells, interleukin-17 (IL-17), and airway neutrophils was reported to lead to the development of increased nonspecific airway responsiveness following repeated exposure to ozone.

A large number of recent studies evaluated changes in airway responsiveness following acute and repeated ozone exposure in rodent strains with varying degrees of sensitivity to ozone. Study-specific details are summarized in Table 3-5 in Section 3.3.1. A subset of studies investigated the role of specific cell signaling pathways in mediating responses by using genetic knockout models or pharmacologic agents. Airway responsiveness to a challenge agent was often assessed using the flexiVent system to assess respiratory system mechanics. Another invasive method to assess airway resistance—pulmonary inflation pressure following electrical stimulation of the vagal nerve was used in a study by Verhein et al. (2013). Taken together, these recent studies, which are detailed below and grouped by concentration-time profile, demonstrate increases in airway responsiveness following exposure to 0.8−2 ppm ozone. All of the changes in airway responsiveness described below were statistically significant. Ozone exposure enhanced the sensitivity of the airway to vagal nerve stimulation by decreasing muscarinic type 2 receptor function in one study (Verhein et al., 2013) and increased BALF levels of the tachykinin substance P in another study (Barker et al., 2015). Enhanced vagal nerve sensitivity and substance P release due to activation of a local axon reflex in the airways may explain the ability of ozone to act as a nonallergic asthma trigger resulting in bronchoconstriction.

- Acute exposure to 2 ppm ozone for 3 hours resulted in increased airway responsiveness to methacholine or acetylcholine (Cho et al., 2018; Mathews et al., 2018; Malik et al., 2017; Stober et al., 2017; Elkhidir et al., 2016; Kasahara et al., 2015; Razvi et al., 2015; Barreno et al., 2013;

Cho et al., 2013; Sunil et al., 2013); however, no increases were observed in Mathews et al. (2017b) or Cho et al. (2013).

  - This response was persistent over time in Sunil et al. (2013).
  - Several studies provide evidence for cell signaling and other pathways underlying increases in airway responsiveness resulting from acute ozone exposure.
    - TNF-stimulated gene 6 and hyaluronan-heavy chain complexes (Stober et al., 2017)
    - Rho-associated coiled-coil-containing protein kinase [ROCK, (Kasahara et al., 2015)]
    - Dietary short chain fatty acids/gut microbiome (Cho et al., 2018)
    - IL-17 (Mathews et al., 2018)
    - Osteopontin (Barreno et al., 2013)
  - Evidence for ozone exposure-induced release of tachykinins is provided by Barker et al. (2015). Acute ozone exposure (2 ppm for 3 hours) increased levels of the tachykinin substance P levels in the BALF through upstream effects on IL-1β and nerve growth factor.

- Exposure to 2 ppm for 4 hours increased airway responsiveness measured as increased bronchoconstriction in response to electrical stimulation of the vagal nerve (Verhein et al., 2013). Both decreased function of M2 muscarinic receptors and involvement of the p38/JNK pathway were implicated in this response.
- Acute exposure to 0.8−1 ppm ozone for 1−6 hours resulted in increased airway responsiveness (Zychowski et al., 2016; Groves et al., 2012). Rho kinase was implicated in this response (Zychowski et al., 2016).
- Repeated exposure to 1 ppm ozone (3 hours/day for 7 days) resulted in increased airway responsiveness (Zhu et al., 2016). This response was blocked by treatment with Vitamin E, implicating oxidative stress in mediating ozone-induced increased airway responsiveness.
- Acute and repeated exposures to 0.25 and 0.5 ppm ozone did not result in increases in airway responsiveness.

3.1.4.3.3 Integrated Summary for Airway Responsiveness

Controlled human exposure studies and animal toxicological studies evaluated in the 2006 Ozone AQCD (U.S. EPA, 2006) and the 2013 Ozone ISA (U.S. EPA, 2013a) provide consistent evidence of ozone-induced increases in airway responsiveness. In experimental studies in humans, changes in airway responsiveness were less transient than the observed ozone-related lung function changes discussed in Section 3.1.4.1.1. One recent study of healthy adult women showed a tendency for increased airway responsiveness following ozone exposure. In recent experimental animal studies, increases in airway responsiveness resulted from ozone exposures in the range of 0.8 to 2 ppm, but not in response to acute and repeated exposures of 0.25 and 0.5 ppm. Mechanistic studies provide evidence that local reflex responses and activation of parasympathetic pathways mediate increases in airway responsiveness due to

ozone exposure. This may explain the ability of ozone to act as a nonallergic asthma trigger resulting in bronchoconstriction.

3.1.4.4 Respiratory Tract Inflammation, Injury, and Oxidative Stress

3.1.4.4.1 Controlled Human Exposure Studies

As reported in studies reviewed in the 1996 and 2006 ozone AQCDs (U.S. EPA, 2006, 1996a), acute ozone exposure initiates an acute inflammatory response throughout the respiratory tract that has been observed to persist for at least 18−24 hours post-exposure. A single acute exposure (1−4 hours) of humans to moderate concentrations of ozone (200−600 ppb) while exercising at moderate to heavy intensities results in a number of cellular and biochemical changes in the lung, including an inflammatory response characterized by increased numbers of PMNs, increased permeability of the epithelial lining of the respiratory tract, cell damage, and production of proinflammatory cytokines and prostaglandins. These changes also occur in humans exposed to 80 and 200 ppb ozone for 6−8 hours.

The presence of PMNs in the lung has long been accepted as a hallmark of inflammation and is an important indicator that ozone causes pulmonary inflammation. Studies reviewed in the 2006 ozone AQCD showed that inflammatory responses to ozone did not appear to be correlated with changes in lung function in either healthy subjects or those with asthma, but there was some indication of a correlation with changes in sRaw (HEI, 1997; Balmes et al., 1996). The number of PMN and shed epithelial cells (a marker of injury) in the BALF also correlated with loss of substance P immunoreactivity from neurons in the bronchial mucosa in humans following exposure to 200 ppb ozone. Taken together, these findings suggest disparate mechanisms underlying changes in lung volume and inflammation, and a commonality in the mechanisms underlying airway obstruction and inflammation, which possibly involves neurogenic edema.

By the completion of the 2006 ozone AQCD, studies had shown that within-individual inflammatory responses to ozone were reproducible and correlated between repeated exposures. Thus, just as was observed for changes in lung function in response to ozone exposure, some individuals are intrinsically predisposed to having increased PMN responses relative to others. In the 2013 Ozone ISA (U.S. EPA, 2013a), significant ($p = 0.002$) increases in sputum PMN (16−18 hours post-exposure) relative to filtered air responses had been reported for 60 ppb ozone which is the lowest exposure concentration that has been investigated in young healthy adults. There was also some new evidence that GSTM1 genotype may affect inflammatory responses to ozone, with greater PMN levels observed in GSTM1-null subjects 24 hours after ozone exposure [see Genetic Polymorphisms on p. 6-80 of U.S. EPA (2013a)]. Study-specific details, including exposure concentrations and durations, are summarized in Table 3-9 in Section 3.3.1.

- Alexis et al. (2013) conducted a post hoc analysis of sputum PMN collected by Kim et al. (2011) from 24 healthy adults (20−33 years) 18 hours after exposure to 60 ppb ozone or clean air for 6.6 hours with quasi-continuous exercise. Individuals were stratified as PMN responders (10% or greater ozone-induced PMN increase, n = 13) or nonresponders (n = 11). Responders were 13 times more likely to be GSTM1-null than GSTM1-positive. Sputum macrophage phagocytosis was also significantly increased after filtered air in responders compared with nonresponders (51 ± 2% vs. 45 ± 3%, $p < 0.05$). This result is consistent with that of a study in the 2013 Ozone ISA showing macrophage oxidative burst and phagocytic activity was increased in GSTM1-null compared with GSTM1-positive subjects (Alexis et al., 2009). However, a larger study of healthy older adults (52 F, 35 M; 59.9 ± 4.5 years) by Arjomandi et al. (2018) reported a significant increase in PMN following 120 ppb ozone relative to filtered air, which was not dependent on GSTM1 genotype (50 GSTM1-null, 37 GSTM1-positive).

- Bosson et al. (2013) investigated the time course of pulmonary and peripheral PMN following a 2-hour exposure of subjects to 0 and 200 ppb ozone in an exposure chamber with moderate exercise and rest. Following exposures, bronchoscopy was performed at 1.5 hours (5 F, 8 M; 24.6 years), at 6 hours (9F, 6M; 25 years), and at 18 hours (16 F, 13 M; 24.5 years). PMNs were not increased at 1.5 hour post-exposure in either bronchial wash (BW) fluid or BALF. Significant PMN increases were apparent at 6 hours in both the BW (4 times, $p < 0.01$), BAL-fluid sample (1.5 times, $p < 0.05$), and in the bronchial epithelium and submucosa biopsies. At 18 hours, ozone-induced increase in PMN persisted both in BW (2 times, $p = 0.01$) and BALF (1.5 times, $p < 0.05$). However, PMN in biopsies at 18 hours tended to be slightly lower than after air. Based on a metabolomics analysis of BALF samples, Cheng et al. (2018) concluded that the responses at 1 hour reflected oxidative stress, while the responses at 24 hours were consistent with tissue repair. Consistent with prior work, studies using ozone to test anti-inflammatory agents continue to report reproducible inflammatory responses following repeated ozone exposures [e.g., Holz et al. (2015)].

- Emphasizing the need for air control exposures, recent studies show exercise itself increased blood PMNs (Bosson et al., 2013), increased the occurrence of micronuclei in blood PMNs (Holland et al., 2014), and increased the pH of exhaled breath condensate (Hoffmeyer et al., 2015). Studies also show that changes in the blood and lungs should not be viewed as independent of one another. There were significant correlations between PMNs in the lungs with PMNs in the blood, which suggested that peripheral PMNs were reflective of the magnitude of pulmonary inflammation (Bosson et al., 2013). Another study reported that airway inflammation was paralleled by systemic inflammation, with the percentage of PMN increasing in the blood at 5 hours after the start of a 3-hour ozone exposure and returning to baseline by 21 hours post-exposure (Tank et al., 2011).

- There were several analyses of inflammatory responses following ozone exposure in healthy adults (Fry et al., 2014) and groups of individuals with and without asthma (Fry et al., 2012; Hernandez et al., 2012), but included no filtered-air control arm. Without an air control, it is not possible to assess potential effects of exercise and/or the laboratory procedures on results. One of these studies reported that %predicted FEV_1 both before and after ozone exposure did not differ between PMN responders (>10% increase) and nonresponders (Fry et al., 2012). This is consistent with studies reviewed in the 2006 Ozone AQCD showing that spirometric measures lung function and inflammatory responses to ozone are unrelated.

3.1.4.4.1.1 Factors Affecting Pulmonary Inflammation, Injury, and Oxidative Stress

Ambient Temperature

Gomes et al. (2011b) exposed nine male endurance runners (24 ± 6 years) to 0 and 100 ppb ozone at 20°C and 50% RH and at 31°C and 70% RH while they completed an 8 km time trial (i.e., each subject completed four exposures). Nasal lavage was conducted approximately 15 minutes post-exposure. There were no differences in inflammatory markers among the exposures. Although there were no differences between the heat only or ozone only compared to control, levels of nasal Club cells following the high-temperature ozone exposure were significantly increased ($p = 0.03$) relative to the lower temperature air control. Glutathione concentrations were also significantly increased ($p = 0.001$) following the high-temperature ozone exposure relative to the lower temperature air control. The increases in Club cells and glutathione appeared to be additive, but no trend analysis was reported.

Lifestage

As reported in the 1996 and 2006 Ozone AQCDs (U.S. EPA, 2006, 1996a), decrements in lung function and increases in respiratory symptoms in response to ozone exposure decreased with increasing age. However, whether inflammatory responses persisted with increasing age remained unstudied at the time of the 2013 Ozone ISA (U.S. EPA, 2013a). Two recent studies demonstrated inflammatory responses in older adults.

- Arjomandi et al. (2018) investigated changes in sputum markers of inflammation and injury in healthy older adults (52 F, 35 M; 59.9 ± 4.5 years) exposed to 0, 70, and 120 ppb ozone for 3 hours during light to moderate, intermittent exercise. Sputum samples were obtained 22.5 hours post-exposure. A mixed effects model showed marginally significant ($p = 0.012$) concentration-dependent increases in PMNs by 4.1% of total (n.s.; $p = 0.134$) and 8.2% of total (0.003) following 70 and 120 ppb ozone exposures, respectively. Sputum PMN increases following ozone exposure showed no interaction with sex (52 F, 35 M), age (55-70 years), or GSTM1 genotype (57% null, 43% positive). Due to the activity level and duration of exposure, the total delivered ozone dose (120 ppb exposure) was estimated by Arjomandi et al. (2018) to be about 60% of the delivered dose in the Kim et al. (2011) study, which identified a significant increase in sputum PMN in young healthy adults following exposure to 60 ppb ozone. Sputum IL-6, IL-8, TNF-α, and total protein concentrations did not show any significant changes due to ozone exposure.
- Kirsten et al. (2011) studied Bimosiamose effectiveness in mitigating PMN response in healthy older subjects (3 F, 15 M; 43.9 ± 7.4 years) who were found to be responsive (≥20% increase in sputum PMN) following exposure to 250 ppb ozone (no air control) for 3 hours with intermittent exercise (alternating 15 minutes intervals of rest and exercise at 14 L/minute per m^2 BSA). Sputum was collected 3 hour post-exposure. Another nine individuals (age and sex not specified) were also exposed to ozone, but did not experience a sufficient increase in sputum PMN for inclusion in the drug trial. Bimosiamose pretreatment of the 18 PMN responders reduced PMN after ozone exposure to approximately the pre-exposure baseline. This study shows that 2/3 of the

screened subjects, who were older than the 18−35 year old subjects typically examined in studies available in prior reviews, were characterized as PMN responders to ozone.

It is not possible to quantify PMN responses as a function of age due to differences in experimental protocols (i.e., duration of exposure to ozone, ozone concentration, activity level, and post-exposure time of sputum collection). These the studies discussed here and prior studies of younger adults prevent quantification of PMN responses as a function of age; these new studies, nonetheless, show that inflammatory responses following ozone exposure occur in older subjects.

3.1.4.4.2 Animal Toxicological Studies

As discussed in the 2013 Ozone ISA, ozone exposure affects both innate and adaptive immunity. Both tissue damage and foreign pathogens are triggers for activating the innate immune system. This results in the influx of neutrophils, mast cells, basophils, eosinophils, monocytes and dendritic cells and the generation of cytokines such as TNF-α, IL-1, IL-6, keratinocyte-derived chemokine (KC), and IL-17. Innate immunity encompasses the actions of complement and collectins, and the phagocytic functions of macrophages, neutrophils, and dendritic cells. Airway epithelium also contributes to innate immune responses. Innate immunity is highly dependent on cell signaling networks involving the toll like receptor family including toll like receptor 4 (TLR4). Adaptive immunity provides immunologic memory through the actions of B and T cells. Important links between the two systems are provided by dendritic cells and antigen presentation.

The 2013 Ozone ISA summarized the animal toxicological evidence of injury, inflammation, and oxidative stress resulting from exposure to ozone. These responses are hard to disentangle because injury leads to inflammation and inflammation leads to further injury, with oxidative stress mediating both injury and inflammation. A large number of studies have documented injury and inflammation in dogs, rabbits, guinea pigs, rats, mice, and nonhuman primates. Numerous studies evaluated injury by assessing histological lesions. In the lower respiratory tract, airway ciliated epithelial cells and type 1 alveolar cells are the initial targets of ozone exposure and ozone exposure-mediated injury leads to epithelial hyperplasia. In rats, repeated exposure to 0.2 ppm ozone over 7 days resulted in lesions at the junction of the small airways and gas exchange region and included necrotic type 1 cells, hyperplastic type 2 cells, damage to ciliated and nonciliated Club cells, and the accumulation of macrophages. In nonhuman primates (macaques and rhesus monkeys), inflammation and related morphometric changes in necrotic cells, smooth muscle, fibroblasts, and nonciliated bronchiolar cells of the tracheobronchial region of the respiratory tract have been shown after 8 hour exposure to 1 ppm ozone. Repeated exposure of monkeys to 0.2 ppm for 8 hours/day over 7 days also resulted in lesions in the respiratory bronchioles. Repeated exposure to 0.15 ppm ozone over 6 days led to morphometric changes in lung, nose, and vocal cords in monkeys. Mucous cell metaplasia of nasal epithelium has been observed in both rodents and monkeys exposed to ozone over several days.

Impaired epithelial barrier function has also been assessed as an index of injury. Histologic evidence of damage to tight junctions and increased flux of small solutes from lung to plasma have been demonstrated. Other studies have focused on assessing markers in the BALF. For injury, these markers often include total protein, albumin, and shed epithelial cells. For inflammation, they include neutrophils and cytokines/chemokines. The pattern of response varies depending on concentration, duration of exposure, species, and strain. In general, acute (up to 8 hours) exposure to 0.8−2 ppm ozone and subacute exposure (24−72 hours) to 0.3 ppm ozone reproducibly result in increased markers of injury and inflammation in rodents, while acute exposure to 1 ppm ozone produces similar changes in nonhuman primates. Attenuation of inflammatory and injury responses has been observed following repeated exposures for some markers but not others in both rodents and nonhuman primates.

Studies evaluating oxidative stress in animals are fewer in number. However, ozone exposure resulted in decreased levels of ascorbate in BALF of rodents, suggesting that ascorbate reacted with secondary oxidation products produced in the epithelial lining fluid. In addition, ozone exposure decreased glutathione levels in the respiratory bronchioles of rhesus monkeys. Ascorbate deficiency enhanced ozone-induced lung injury, indicating a role for oxidative stress in the response.

Studies described in the 2013 Ozone ISA provide evidence for cell signaling pathways that potentially underlie the injury and inflammation observed in animals exposed to ozone. Key roles have been demonstrated for platelet activating factor, inter-cellular adhesion molecule 1 (ICAM-1), MIP-2, TNF receptor, nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), c-Jun kinase 1 (JNK1), CXCR2, and IL-6 in mediating inflammation. Tachykinins, TLR4, and heat shock protein 70 (HSP70) have been shown to mediate change in barrier function. Pathways that confer protection against injury and inflammation have also been investigated, with protective roles demonstrated for matrix metalloproteinase-9, IL-10, surfactant protein-A (SP-A) (a collectin), club cell secretory protein (CCSP), and metallothionein.

Furthermore, ozone exposure skews immune responses towards an allergic phenotype. For example, increased numbers of IgE-containing cells were found in the lungs of mice exposed to 0.5−0.8 ppm for 4 days. Other studies evaluated the effects of ozone exposure in animal models of allergic airway disease but are discussed in Section 3.1.5.6.2 because these animal models share phenotypic features with asthma.

A large number of recent studies evaluated respiratory tract injury, inflammation, and oxidative stress in response to short-term ozone exposure. Study-specific details are summarized in Table 3-10, Table 3-11, and Table 3-12 in Section 3.3.1. All of these studies were conducted in rodent strains with varying degrees of sensitivity to ozone. Acute exposures generally consisted of a single exposure to 2 ppm ozone for 3 hours. Subacute exposures generally consisted of exposure to 0.3 ppm ozone for 24−72 hours. One study compared responses with acute and subacute exposures over time (Cho et al., 2013). Other exposure concentration−durations, including repeated exposures over several days, have been employed. Some studies investigated the role of specific cell signaling pathways in mediating

responses by using genetic knockout models or pharmacologic agents. Inflammation, injury, and oxidative stress were commonly assessed by measurement of cells and biological markers in the BALF. Some studies also employed histopathology and/or immunocytochemistry of lung tissue. Flow cytometry was used to identify different inflammatory cell subsets and cell surface markers on other cells present in the lavage fluid or lung tissue.

Recent studies, detailed below and grouped according to concentration-time profile, provide additional evidence for ozone-induced inflammation, injury, and oxidative stress. All of these changes, which are described below, were statistically significant. These effects are seen following acute exposure to 0.5−2 ppm ozone, subacute exposure to 0.3−0.7 ppm ozone, and repeated exposure to 0.5−2 ppm ozone. Evidence is provided mainly by BALF markers, but some studies also provide evidence of histological lesions. Responses to 0.1−0.25 ppm ozone are variable, with some studies demonstrating mild histological lesions but not increases in BALF markers. A recent time-course study shows that the earliest measurable response is epithelial barrier injury, as indicated by increased BALF protein levels. Another study shows early activation of NFκB, a transcription factor that upregulates proinflammatory genes, which precedes oxidative stress and a cytokine response. A plausible sequence of events begins with ozone reacting with respiratory tract components to generate an oxidized species that disrupts barrier function and activates innate immunity. A cascade of inflammation, injury, and oxidative stress responses ensues. The influx of airway neutrophils is the hallmark of ozone exposure-induced inflammation, but other inflammatory cell types infiltrate the lung following exposure. Some recent studies focus on macrophage subpopulations that are pro- and anti-inflammatory and that infiltrate to the lung from the spleen. A shift towards anti-inflammatory macrophages is correlated with the resolution of inflammation following an acute exposure. Other studies examine eosinophilic inflammation, an indicator of atopy and T helper 2 immunity. These latter studies, conducted in the nasal and lower airways, demonstrate that repeated exposure to 0.5−0.8 ppm ozone result in airway eosinophilia, increased Th 2 cytokines, and increased mucosubstances, consistent with induced nonatopic asthma and rhinitis. Innate lymphoid cells were found to mediate this response to ozone. Of the many cell-signaling components and other factors tested for inhibitory effects in recent studies, four were found to impact both airway responsiveness and inflammation (ROCK, IL-17, osteopontin, and vitamin E). This finding suggests that there may be common upstream events that trigger both increases in airway responsiveness and inflammatory processes in healthy populations.

- Acute exposure to ozone (2 ppm for 3 hours) resulted in inflammation, injury, or oxidative stress (Cho et al., 2018; Mathews et al., 2018; Tighe et al., 2018; Malik et al., 2017; Mathews et al., 2017a; Stober et al., 2017; Elkhidir et al., 2016; Mishra et al., 2016; Barker et al., 2015; Cabello et al., 2015; Kasahara et al., 2015; Razvi et al., 2015; Williams et al., 2015; Ghio et al., 2014; Bao et al., 2013; Barreno et al., 2013; Cho et al., 2013; Lee et al., 2013; Sunil et al., 2013; Hulo et al., 2011; Shore et al., 2011).
    - In one case (Mathews et al., 2017b), the evidence for inflammation was minimal given that ozone exposure increased BALF levels of IL-33, but not neutrophils.

- The presence of two different macrophage subpopulations in the lung was reported: classically activated (M1) and alternatively activated (M2), with pro- and anti-inflammatory roles, respectively (Sunil et al., 2013).
- Several studies provide evidence for cell signaling and other pathways underlying inflammation, injury, or oxidative stress effects of acute ozone exposure.
    - TNF receptor 1 (Shore et al., 2011)
    - ROCK (Kasahara et al., 2015)
    - Nuclear factor (erythroid-derived 2)-like 2 [NRF2 (Cho et al., 2013)]
    - Osteopontin (Barreno et al., 2013)
    - Dysregulated iron homeostasis (Ghio et al., 2014)
- Evidence for ozone-induced release of tachykinins is provided by one study (Barker et al., 2015). Tachykinins mediate bronchoconstriction and neurogenic inflammation. Acute ozone exposure increased levels of the tachykinin substance P levels in the BALF through upstream effects on IL-1β and nerve growth factor. Two studies examined inflammation following acute ozone exposure in rodents of varying lifestages or sex. Less inflammation was found in older rodents compared with younger ones (Shore et al., 2011). Females had greater inflammatory responses compared with males (Mishra et al., 2016; Cabello et al., 2015). Acute exposure to ozone (2 ppm, 4−6 hours) also resulted in inflammation, injury, and oxidative stress (Verhein et al., 2013; Yanagisawa et al., 2012).
- Tighe et al. (2018) found that different methods employed for euthanasia, but not for lavage, were a source of variability in the measured indices of inflammation and injury parameters.

- Acute exposure to 0.8−1 ppm ozone for 1−6 hours resulted in inflammation, injury, or oxidative stress (Michaudel et al., 2018; Wong et al., 2018; Francis et al., 2017a; Francis et al., 2017b; Yonchuk et al., 2017; Zychowski et al., 2016; Gabehart et al., 2015; Hatch et al., 2015; Kodavanti et al., 2015; Kumarathasan et al., 2015; Paffett et al., 2015; Ramot et al., 2015; Sunil et al., 2015; Ward et al., 2015; Ward and Kodavanti, 2015; Gonzalez-Guevara et al., 2014; Bhoopalan et al., 2013; Groves et al., 2013; Kummarapurugu et al., 2013; Robertson et al., 2013; Connor et al., 2012; Groves et al., 2012).
    - Some of these were time-course studies.
        - Michaudel et al. (2018) followed changes for up to 48 hours post-exposure to a 1 hour exposure to ozone. The earliest measured response was the injury marker, BALF protein, which was increased 1 hour post-exposure and reflects barrier disruption. This response was followed by increases at 4−6 hours in BALF cytokines and chemokines, lung tissue interstitial macrophages, and another marker of epithelial cell injury . Later responses began at 18 hours and included increases in BALF neutrophils, eosinophils, reactive oxygen-producing cells and cell death markers. The time dependence of effects on tight junction proteins was also reported.
        - Gonzalez-Guevara et al. (2014) examined early responses and found increases in lung tissue TNF-α immediately after 3 and 6 hours of exposure, but not after 1 hour of exposure.
        - NFκB activation, which is an early step in the induction of inflammation, occurred prior to changes in oxidative stress and upregulation of the cytokine TNF-α (Connor et al., 2012).

        - Resolution of inflammation and injury within 72 hours following ozone exposure was demonstrated (Groves et al., 2012). However, airway resistance remained increased, indicating that the lung was functionally compromised.
    - Several studies focused on the accumulation of macrophages in the lung in response to ozone exposure (Francis et al., 2017b; Sunil et al., 2015; Groves et al., 2013).
        - Resident alveolar macrophages were not affected, but infiltrating monocytic and granulocytic cells were increased.
        - Increases in both classically activated macrophages (M1, proinflammatory) and alternatively activated macrophages (M2, anti-inflammatory) were found.
        - A time course study showed that M1 macrophages increased in number rapidly and persisted for 72 hours post-exposure, while M2 macrophages were increased beginning at 72 hours.
        - The spleen was found to be a source for these M1 and M2 cells.
    - Some studies provide evidence for cell signaling and other pathways underlying the inflammatory, injury, or oxidative effects of acute ozone exposure.
        - CD36 (Robertson et al., 2013)
        - IL-33 and ST2 (Michaudel et al., 2018)
        - Glucocorticoids (Thomson et al., 2016)
        - TLR4 (Connor et al., 2012)
        - Galectin (Sunil et al., 2015)
        - C-C chemokine receptor type 2 (CCR2) (Francis et al., 2017a)
    - Other endpoints examined include mucus secretion (Gabehart et al., 2015) and upregulation of glucocorticoid-sensitive genes (Thomson et al., 2016; Thomson et al., 2013).
        - Mucus secretion was not seen in juvenile or adult mice in response to ozone.
        - Transient changes in glucocorticoid-sensitive genes occurred immediately after exposure to ozone.
    - One study provides evidence for respiratory effects of acute ozone exposure in rodents of varying lifestages. In Gabehart et al. (2015), inflammation and injury responses in 2-, 3-, and 6-week-old mice, representing weanling, juvenile, and adult stages, respectively, were examined. Results in 1-week-old mice (neonates) similarly exposed are discussed in the long-term exposure section of this Appendix. In general, responses were smallest in 1-week-old mice and greatest in 6-week-old mice, with responses in the 2- and 3-week-old mice sometimes in between and sometimes as high as responses in the 6-week-old mice. The exception was mucus secretion, which was highest in 1-week-old mice and minimal in 2-week-old mice.
- Acute exposure to 0.25−0.5 ppm ozone resulted in minimal or no changes in inflammation, injury, or oxidative stress markers in BALF (Michaudel et al., 2018; Kodavanti et al., 2015; Kumarathasan et al., 2015; Kurhanewicz et al., 2014; McIntosh-Kastrinsky et al., 2013; Thomson et al., 2013). Histopathological lesions were seen in response to 0.25 and 0.5 ppm ozone (Ramot et al., 2015).

- Subacute exposures to 0.3−0.7 ppm ozone for up to 72 hours resulted in inflammation, injury, and oxidative stress (Che et al., 2016; Mathews et al., 2015; Verhein et al., 2015; Kasahara et al., 2014; Cho et al., 2013; Kasahara et al., 2013; Kasahara et al., 2012).
    - Several studies involving subacute exposure to 0.3 ppm ozone examined the time course of changes in inflammatory cells (Mathews et al., 2015; Kasahara et al., 2014; Kasahara et al., 2013; Kasahara et al., 2012).
        - Increases in BALF neutrophils and protein (a marker of injury) occurred earlier (24 hours) than changes in BALF macrophages (48 hours).
        - These changes persisted for up to 72 hours.
        - Macrophage subpopulations in lung tissue consisted of M1 proinflammatory and M2 anti-inflammatory macrophages, as well as macrophages positive for IL-6 and apoptotic macrophages.
        - Numbers of gamma delta T cells were also increased and contributed to resolution of inflammation that occurred over several days post-exposure.
    - Another study (Che et al., 2016), which involved subacute exposure to 0.7 ppm ozone, found increased IL-17A-producing gamma delta T cells.
    - Dendritic cells were increased by subacute exposure to 0.4 ppm ozone, but there was no impact on neutrophilic inflammation (Brand et al., 2016).
    - Evidence of mucus hypersecretion was found in (Cho et al., 2013).
    - Some studies provide evidence for cell signaling and other pathways underlying inflammation, injury, or oxidative stress or effects on mucus secretion resulting from subacute ozone exposure.
        - Adiponectin (Kasahara et al., 2012)
        - T-cadherin (Kasahara et al., 2013)
        - IL-6 (Kasahara et al., 2014)
        - IL-17A and gamma delta T cells (Mathews et al., 2015)
        - NRF2 (Cho et al., 2013)
        - Notch (Verhein et al., 2015)
        - Mannose binding lectin (Ciencewicki et al., 2016)
        - IL-17A, IL-1R, and caspase (Che et al., 2016)
- Repeated exposure to ozone (0.5−2 ppm), using many different concentration-duration profiles, resulted in inflammation, injury, or oxidative stress in many studies (Snow et al., 2018; Gordon et al., 2017b; Gordon et al., 2017a; Harkema et al., 2017; Henriquez et al., 2017; Kumagai et al., 2017; Zhang et al., 2017; Kumagai et al., 2016; Liu et al., 2016; Miller et al., 2016b; Ong et al., 2016; Zhu et al., 2016; Feng et al., 2015; Gonzalez-Guevara et al., 2014; Tankersley et al., 2013; Wang et al., 2013; Brand et al., 2012; Xiang et al., 2012).
    - Effects were found in the upper (nasal) airways following repeated exposure to 0.5−0.8 ppm ozone for up to 9 days (Harkema et al., 2017; Kumagai et al., 2016; Ong et al., 2016).
    - Some studies of repeated ozone exposures provide evidence for cell signaling and other pathways underlying inflammation, injury, or oxidative stress.

        - Vitamin E (Zhu et al., 2016)
        - Epidermal growth factor (EGF) receptor (Feng et al., 2015)
        - Glucocorticoids and stress hormones (Miller et al., 2016b)
    - Ozone exposure induced inflammation, injury, or oxidative stress in rodents of varying lifestage from adolescence to senescence (Snow et al., 2016).
    - Mucous cell metaplasia indicated by increased mucosubstances, eosinophilic inflammation, Th2 cytokines, and other type 2 immune responses were seen in the upper (nasal) and lower airways (Harkema et al., 2017; Kumagai et al., 2017; Kumagai et al., 2016; Ong et al., 2016).
        - These findings are characteristic of induced nonatopic asthma and rhinitis.
        - A role for immune lymphoid cells in the development of type 2 immunity was demonstrated.
    - Zhu et al. (2016)found effects on Th2 cytokines, mast cell degranulation, serum IgE, and TSLP in the lower respiratory tract that were attenuated by treatment with vitamin E.
    - Brand et al. (2012)found dendritic cell activation and increased T cell number in specific regions of the respiratory tract.
- No evidence of inflammation, injury, or oxidative stress was found in other studies involving repeated exposure to 0.1−0.5 ppm ozone (Gordon et al., 2017b; Snow et al., 2016; Feng et al., 2015; Wolkoff et al., 2012).

3.1.4.4.3 Epidemiologic Studies

A limited number of studies evaluated in the 2013 Ozone ISA (U.S. EPA, 2013a) reported consistent evidence of ozone-related pulmonary inflammation in children without asthma (Berhane et al., 2011; Barraza-Villarreal et al., 2008). There are no recent studies in the U.S. or Canada that examine the relationship between short-term ozone exposure and pulmonary inflammation in healthy populations.

Recent studies have examined pulmonary inflammation in general population studies of children (Patel et al., 2013; Salam et al., 2012), with asthma prevalence ranging from 14 to 47%. Because these studies do not directly inform the understanding of the relationship between short-term ozone exposure and pulmonary inflammation in healthy populations or populations with asthma, they are not discussed in either section. However, study specific details can be found in Table 3-7 in Section 3.3.1.

3.1.4.4.4 Integrated Summary for Respiratory Tract Inflammation, Injury, and Oxidative Stress

Controlled human exposure studies evaluated in the 1996 and 2006 Ozone AQCDs (U.S. EPA, 2006, 1996a) established evidence of respiratory tract inflammation in response to acute ozone exposures. Notably, these inflammatory responses are not correlated with lung function changes, but are at least partially correlated with airway resistance. These results indicate that changes in pulmonary inflammation

and airway obstruction may share similar underlying mechanisms, while inflammation and lung volume (FEV_1) may not. Additionally, the evidence suggested that there is interindividual variability in inflammatory responses to ozone. This was expanded upon in the 2013 Ozone ISA (U.S. EPA, 2013a) in studies that demonstrated GSTM1 genotype interaction with ozone exposure on pulmonary inflammation. Recent studies provide some further evidence that GSTM1-null individuals are more susceptible to ozone-related inflammatory responses, although the evidence is not entirely consistent.

Consistent with experimental studies in humans, a large body of evidence from recent animal toxicological studies and studies previously evaluated in the 2013 Ozone ISA (U.S. EPA, 2013a) demonstrate inflammatory responses to acute, subacute, and repeated ozone exposures in various animal models. Additionally, results from recent experimental animal studies are also consistent with previous findings of ozone-related pulmonary injury (0.3−2 ppm ozone) and oxidative stress (0.15−2 ppm ozone). Mechanistic studies present a plausible pathway by which ozone reacts with respiratory tract components, produces oxidized species that injure barrier function and activates innate immunity, resulting in a cycle of inflammation, injury, and oxidative stress.

A limited number of epidemiologic panel studies evaluated in the 2013 Ozone ISA (U.S. EPA, 2013a) observed evidence of pulmonary inflammation in children without asthma associated with short-term ambient ozone exposure. These results are coherent with results from experimental studies in humans and animals. No recent studies in the U.S. or Canada are available for review.

3.1.4.5 Overall Summary of Respiratory Effects in Healthy Populations

Evidence from recent controlled human exposure studies of respiratory effects in healthy populations is generally consistent with findings from prior assessments (U.S. EPA, 2013a, 2006, 1996a). Notably, there is consistent evidence demonstrating ozone-induced decreases in group mean pulmonary function in young, healthy adults performing moderate exercise. Lung function decrements were observed after ozone exposures as low as 60 to 70 ppb, for young adults, and 120 ppb in older adults. The 2013 Ozone ISA also evaluated studies that indicate symptoms of cough and pain on deep inspiration corresponding to FEV_1 decrements in healthy young adults exposed to 70 ppb ozone for 6.6 hours (U.S. EPA, 2013a).

Controlled human exposure studies evaluated in the 2006 Ozone AQCD (U.S. EPA, 2006) and the 2013 Ozone ISA (U.S. EPA, 2013a) provide consistent evidence of ozone-induced increases in airway responsiveness and inflammation in the respiratory tract and lungs. Recent studies are consistent with previous findings and expand on observed interindividual variability in inflammatory responses, providing additional evidence that GSTM1-null individuals are more susceptible to ozone-related inflammatory responses.

Like studies of humans, experimental studies of animals also provide evidence of respiratory effects resulting from exposure to ozone. Evidence summarized in the 2013 Ozone ISA indicated changes in the frequency of breathing and tidal volume, decreased lung volume, increased airway resistance, and attenuation of the pulmonary function decrement response following repeated exposures to ozone (U.S. EPA, 2013a). Additionally, previously evaluated studies indicate ozone-induced increases in airway responsiveness, inflammation, injury, and oxidative stress. A large body of recent evidence further demonstrates changes in each of the specified endpoints resulting from ozone exposure, providing coherence with results from controlled human exposure studies.

Recent mechanistic studies in humans and animals expand on findings from previously reviewed studies to provide plausible pathways that may underlie the observed respiratory health effects resulting from short-term exposure to ozone. Experimental studies in both humans and animals indicate that changes in lung function may be attributed to activation of sensory nerves in the respiratory tract that trigger local and autonomic reflex responses. Specifically, mechanistic studies provide evidence that local reflex responses mediate the observed decreases in inspiratory capacity and pain on inspiration that result in truncated inspiration. In addition, modest increases in airway resistance may occur due to activation of parasympathetic pathways. Mechanistic studies also present a plausible pathway by which ozone reacts with respiratory tract components, produces oxidized species that injure barrier function, and activates innate immunity, resulting in a cycle of inflammation, injury, and oxidative stress.

Evidence from epidemiologic studies is generally coherent with experimental evidence. Most of the epidemiologic evidence comes from panel studies of healthy children that were previously evaluated in the 2013 Ozone ISA (U.S. EPA, 2013a). Several panel studies of children in summer camps demonstrated decreases in FEV_1 associated with short-term ozone exposure. A smaller body of panel studies in children without asthma also consistently reported associations between ozone and increases in markers of pulmonary inflammation. While there is coherence between epidemiologic and experimental evidence of ozone-induced lung function decrements and pulmonary inflammation, respiratory symptoms were not associated with ozone exposure in a limited number of epidemiologic studies. However, these studies generally relied on parental reported outcomes that may result in under- or over-reporting of respiratory symptoms.

3.1.5 Respiratory Effects in Populations with Asthma

Asthma is a chronic inflammatory lung disease characterized by reversible airway obstruction and increased airway responsiveness. Exacerbation of asthma is associated with symptoms such as wheeze, cough, chest tightness, and shortness of breath. Symptoms may be treated with asthma medication, while uncontrollable symptoms may lead to medical treatment, including ED visits and, in extreme cases, hospital admissions. In characterizing the relationship between ozone and asthma exacerbations, this section sequentially considers the effects of short-term exposure to ozone on hospital admissions and ED

visits for asthma, respiratory symptoms and asthma medication use, lung function, and subclinical effects, such as pulmonary inflammation and oxidative stress, in people with asthma. ED visits for asthma are more common and often less serious than hospital admissions. Generally, only a small fraction of respiratory ED visits result in a hospital admission. Accordingly, the two outcomes may reflect different severities of asthma and are evaluated separately.

3.1.5.1 Hospital Admissions

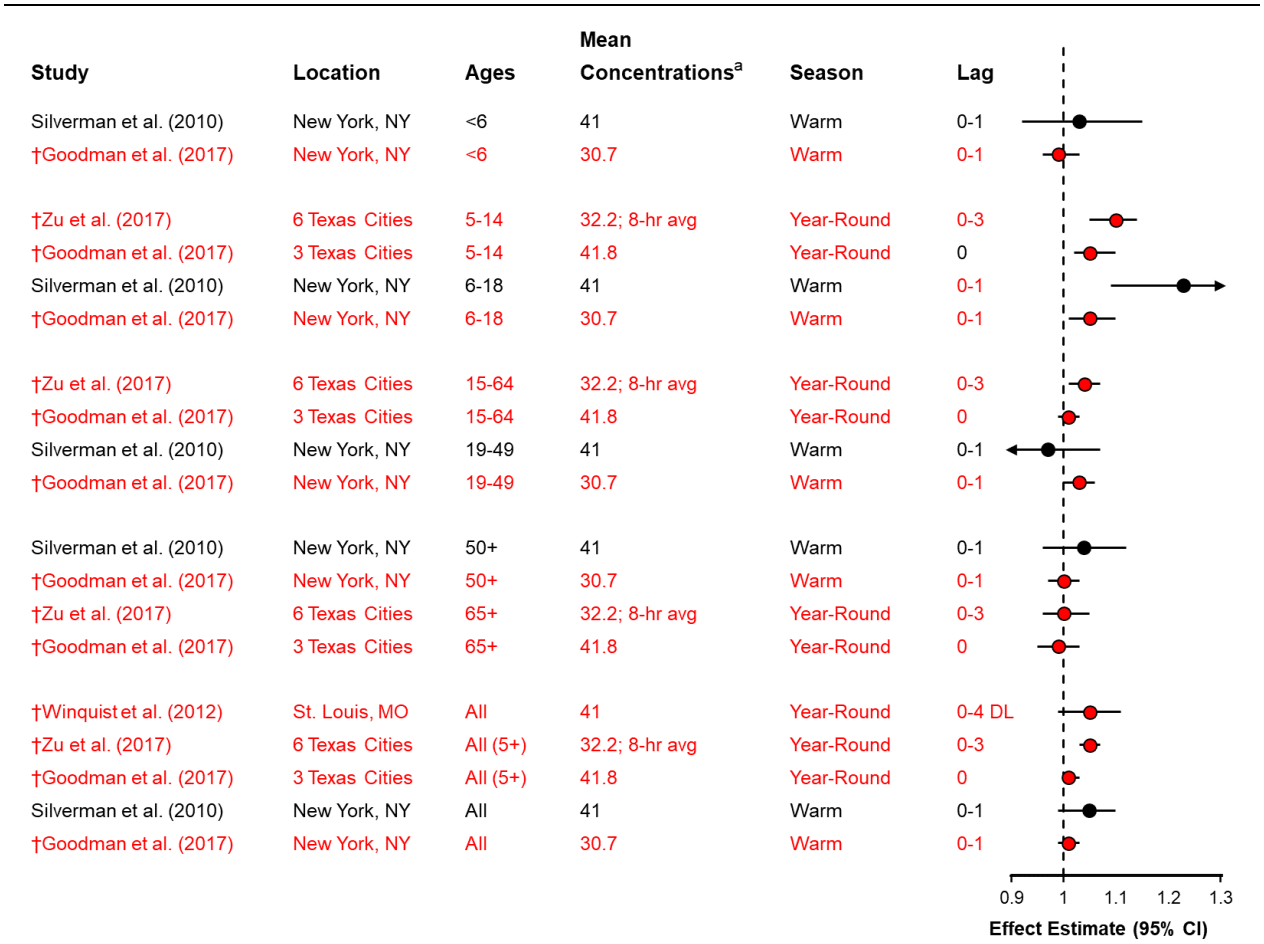
A single study evaluated in the 2013 Ozone ISA (U.S. EPA, 2013a) examined the association between short-term exposure to ozone and hospital admissions for asthma. In New York City, 8-hour max ozone concentrations were associated with severe acute asthma admissions in the warm season (Silverman and Ito, 2010). The authors reported positive associations with non-ICU asthma admissions that were strongest (i.e., of greatest magnitude) for children ages 6 to 18 years, compared to the other age groups examined (ages <6 years, 19−49, 50+, and all ages). The observed effect remained robust to adjustment for $PM_{2.5}$. The authors also performed an analysis examining the shape of the concentration-response (C-R) relationship, which is discussed in more detail in Section 3.1.10.4.

Recent studies expand the existing evidence base and provide consistent evidence of an association between ozone and hospital admissions for asthma (Figure 3-4). Generally, the evaluated studies use 8-hour daily max averaging times, although there are studies that use daily 8-hour avg (Zu et al., 2017) and 24-hour avg (Shmool et al., 2016). The averaging time used in each study, along with other study-specific details, including air quality characteristics and select effect estimates, are highlighted in Table 3-13 in Section 3.3.1. An overview of the evidence is provided below.

- Multicity studies in Texas (Goodman et al., 2017b; Zu et al., 2017) and single-city studies in New York City (Goodman et al., 2017a; Shmool et al., 2016; Sheffield et al., 2015) and St. Louis, MO (Winquist et al., 2012) reported evidence of an association between short-term ozone concentrations and hospital admissions for asthma.
- Shmool et al. (2016) compared monitor-based ozone concentrations to ozone estimated at a 300-m spatial scale using a fusion of monitoring data and land-use regression (LUR). In short, the authors used LUR with local monitoring inputs to estimate seasonal average concentrations within 300 m radial buffers around geocoded participant addresses. The ratio of these spatially-resolved seasonal average concentrations and the citywide averages were multiplied by daily monitor averages to estimate spatially-refined daily exposures. The effect estimates derived from the spatiotemporal model were similar to those estimated using monitored ozone concentrations. These results indicate that the observed association of ozone concentrations with asthma hospital admissions is robust to exposure assignment technique.
- Like previous findings from Silverman and Ito (2010), recent studies that reported age-stratified results (Goodman et al., 2017b; Goodman et al., 2017a; Zu et al., 2017; Sheffield et al., 2015) generally observed ozone-asthma hospital admission associations that were strongest (i.e., greater in magnitude) in younger populations (5 to 18 years of age). Many studies exclude data for children less than 5 years of age due to less reliable asthma diagnosis in young children. Additionally, most studies that examined hospital admissions in adults older than 50 reported null

associations. While most studies observed associations in analyses of all ages combined, stratified analyses suggest that these associations are likely being driven by hospital admissions among children.

- In recent studies, there was some limited evaluation of the shape of the C-R relationship (Zu et al., 2017), potential copollutant confounding (Shmool et al., 2016), and seasonal differences in effect estimates (Goodman et al., 2017a) across the evaluated studies. These topics are discussed in more detail in the Relevant Issues for Interpreting Epidemiologic Evidence section (Section 3.1.10).



DL = distributed lag.

Note: †Studies published since the 2013 Ozone ISA. Black text = studies included in the 2013 Ozone ISA.

[a]Mean concentrations reported in ppb and are for 8-hour daily max averaging times unless otherwise noted.

Results standardized to a 15-ppb increase in 24-hour avg, 20-ppb increase in 8-hour daily max, or 25-ppb increase in 1-hour daily max ozone concentrations. Corresponding quantitative results are reported in Table 3-5.

Figure 3-4 Summary of associations from studies of short-term ozone exposures and hospital admissions for asthma for a standardized increase in ozone concentrations.

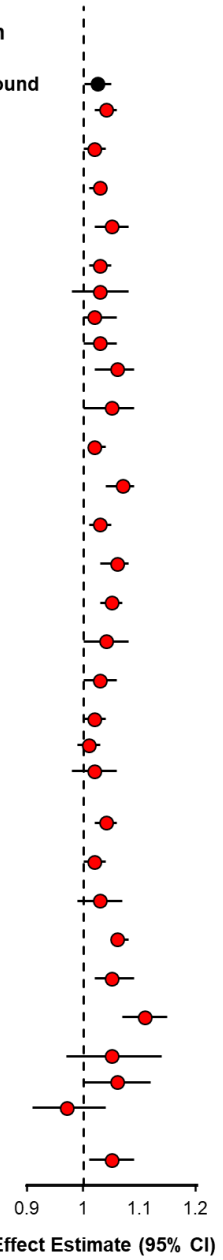
3.1.5.2 Emergency Department (ED) Visits

A number of studies evaluated in the 2013 Ozone ISA (U.S. EPA, 2013a) examined the association between short-term ozone exposure and ED visits for asthma. A multicity study in Canada (Stieb et al., 2009) as well as single-city studies in the U.S. and Canada (Ito et al., 2007; Villeneuve et al., 2007) provided consistent evidence that increased ozone exposure is associated with increases in asthma ED visits. The observed associations were consistently stronger in magnitude in the warm season. Additionally, Ito et al. (2007) reported an association that was robust in copollutant models that adjusted for $PM_{2.5}$, NO_2, SO_2, and CO.

Recent studies continue to present consistent evidence of an association between ozone and ED visits for asthma across a number of study locations, a range of mean ozone concentrations, and a variety of study designs and exposure assignment techniques, including population-weighted monitor averages, CMAQ modeling estimates, and fusions of modeled and monitored data (Figure 3-5). Generally, the evaluated studies use 8-hour daily max averaging times, although there are instances in which the 1-hour daily max (Malig et al., 2016) and 24-hour avg (Szyszkowicz et al., 2018; Sarnat et al., 2013) are used. The averaging time used in each study, along with other study-specific details, including air quality characteristics and select effect estimates, are highlighted in Table 3-14 in Section 3.3.1. Additionally, information on potential copollutant confounding and seasonal differences in effect estimates is presented in the Relevant Issues for Interpreting Epidemiologic Evidence section (Section 3.1.10). An overview of the recent evidence is provided below.

- The strongest evidence of an association between short-term exposure to ozone and ED visits for asthma is presented in multicity studies, including statewide studies conducted in California (Malig et al., 2016), North Carolina (Sacks et al., 2014), and Georgia (Xiao et al., 2016) and in other multicity studies in the U.S. (Barry et al., 2018; Alhanti et al., 2016; Gleason et al., 2014) and Canada (Szyszkowicz et al., 2018).
- Supporting evidence is provided by single-city studies in New York (Shmool et al., 2016; Sheffield et al., 2015), Atlanta (O'Lenick et al., 2017; Strickland et al., 2014; Winquist et al., 2014; Sarnat et al., 2013), and elsewhere (Byers et al., 2015; Sarnat et al., 2015), demonstrating consistent increases in ED visits for asthma corresponding to short-term ozone exposure.
- Most recent studies of ED visits for asthma included all ages and/or focused more specifically on children. The evidence is consistent for both study populations. A limited number of studies examined ED visits for asthma in adults, and reported some evidence that associations exist among these older age groups (Alhanti et al., 2016; Byers et al., 2015).

Study	Location	Ages	Mean Concentration[a]	Lag	Season
Stieb 2009	Multicity, Canada	All	10.3-22.1; 24-hr avg	1	**Year-Round**
†Malig et al. (2016)	California	All	33-55; 1-hr max	0-1	
†Sacks et al. (2014)	North Carolina	All	43.6	0-2	
†Sarnat et al. (2013)	Atlanta, GA	All	41.9; 24-hr avg	0-2	
†Winquist et al. (2012)	St. Louis, MO	All	NR	0-4 DL	
†Barry et al. (2018)	Atlanta, GA	All	37.5-42.2	0-2	
	Birmingham, AL				
	Dallas, TX				
	Pittsburgh, PA				
	St. Louis, MO				
†Sarnat et al. (2015)	St. Louis, MO	All	36.2	0-2 DL	
†Alhanti et al. (2016)	3 U.S. Cities	0-4	37.3-43.7	0-2	
†Strickland et al. (2014)	Atlanta, GA	2-16	42.2	0-2	
†Xiao et al. (2016)	Georgia	2-18	42.1	0-3	
†O'Lenick et al. (2017)	Atlanta, GA	5-17	NR	0-2	
†Alhanti et al. (2016)	3 U.S. Cities	5-18	37.3-43.7	0-2	
†Szyszkowicz et al. (2018)	Multicity, Canada	<20; Females	22.5-29.2; 24-hr avg	1	
		<20; Males			
†Alhanti et al. (2016)	3 U.S. Cities	19-39	37.3-43.7	0-2	
		40-64			
		65+			
†Malig et al. (2016)	California	All	33-55; 1-hr max	0-1	**Warm**
†Sacks et al. (2014)	North Carolina	All	50.1	0-2	
†Byers et al. (2015)	Indianapolis, IN	All (5+)	48.5	0-2	
†Gleason et al. (2014)	New Jersey	3-17	NR	0-2	
†Winquist et al. (2014)	Atlanta, GA	5-17	53.9	0-2	
†Sheffield et al. (2015)	New York, NY	5-17	NR	0-3	
†Byers et al. (2015)	Indianapolis, IN	5-17	48.5	0-2	
		18-44			
		45+			
†Winquist et al. (2014)	Atlanta, GA	5-17	53.9	0-2	**Cold**



Note: †Studies published since the 2013 Ozone ISA.

[a]Mean concentrations reported in ppb and are for 8-hour daily max averaging times unless otherwise noted.

Results standardized to a 15-ppb increase in 24-hour avg, 20-ppb increase in 8-hour daily max, or 25-ppb increase in 1-hour daily max ozone concentrations. Corresponding quantitative results are reported in Table 3-6.

Figure 3-5 Summary of associations from studies of short-term ozone exposures and asthma emergency department (ED) visits for a standardized increase in ozone concentrations.

3.1.5.3 Respiratory Symptoms

Respiratory symptoms in children with asthma, including cough, wheeze, sputum production, shortness of breath, and chest tightness, may indicate an exacerbation of disease. Further, uncontrollable symptoms may lead people with asthma to seek medical care. Thus, studies examining the association between ozone and increases in asthma symptoms and medication use may provide support for the observed increases in asthma hospital admissions and ED visits in children.

3.1.5.3.1 Controlled Human Exposure Studies

As discussed in Section 3.1.4.2.1, controlled human exposure studies of healthy adults clearly demonstrate ozone-induced increases in respiratory symptoms including pain on deep inspiration, shortness of breath, and cough. In Section 7.5.1.2 of the 1996 Ozone AQCDs (U.S. EPA, 1996a), individuals with and without asthma were reported to have similar respiratory symptom responses to ozone exposure; however, the study by Horstman et al. (1995) showed an increased incidence of wheeze in subjects with asthma exposed for 7.6 hours with light quasi-continuous exercise to 160 ppb. These observations are not changed by recently available studies or those in subsequent assessments (U.S. EPA, 2013a, 2006).

3.1.5.3.2 Epidemiologic Studies

A number of epidemiologic panel studies evaluated in the 2013 Ozone ISA (U.S. EPA, 2013a) examined the relationship between short-term ozone exposure and incidence of respiratory symptoms and increased symptom scores in children with asthma. Evidence from a limited number of multicity U.S. studies was inconsistent, but many single-city studies provided evidence of an association (see Section 3.1.4.1 of the 2013 Ozone ISA). Methodological distinctions in the evaluated multicity studies, including lack of power and extended averaging times (e.g., 19-day averages), reduced the consideration given to the observed results. Associations were observed in single-city panel studies across a diversity of locations and ambient ozone concentrations.

One recent panel study of school-aged children in Detroit tracked respiratory symptoms in children with asthma for periods of 14 consecutive days during 11 seasons (Lewis et al., 2013). The authors reported increases in a range of respiratory symptoms, including cough, wheeze, shortness of breath, and chest tightness, associated with increases in 1- and 8-hour daily max ozone concentrations. Associations were generally negative or null at lag 1, but positive at lags 2, 3−5, and 1−5. Consistent with results from studies evaluated in the 2013 Ozone ISA, Lewis et al. (2013) observed associations that were larger in magnitude in children taking corticosteroids. However, these associations were much less precise (i.e., wider 95% CIs) than the associations for children not taking steroids. See Table 3-15 in Section 3.3.1 for complete study details.

3.1.5.3.3 Integrated Summary for Respiratory Symptoms

Controlled human exposure studies provide evidence of ozone-induced increases in respiratory symptoms in individuals with asthma, with respiratory symptom responses that are generally comparable to those from individuals without asthma. A number of epidemiologic panel studies evaluated in the 2013 Ozone ISA also provided evidence that ozone exposure is associated with increased respiratory symptoms in children with asthma. A recent epidemiologic panel study provides additional evidence that ozone concentrations are associated with a range of respiratory symptoms in children with asthma.

3.1.5.4 Lung Function

3.1.5.4.1 Controlled Human Exposure Studies

Based on studies reviewed in the 1996 and 2006 Ozone AQCDs (U.S. EPA, 2006, 1996a) and the 2013 Ozone ISA (U.S. EPA, 2013a), it was concluded that individuals with asthma were at least as sensitive to acute effects of ozone as healthy individuals. In the 2013 Ozone ISA (U.S. EPA, 2013a), the study by Horstman et al. (1995) was recognized as showing clearly larger FEV_1 responses in individuals with asthma relative to those without (19 vs. 10% FEV_1 decrements, respectively, $p = 0.04$) following 7.6 hour exposures to 160 ppb ozone with light quasi-continuous exercise. In asthmatics, ozone-induced FEV_1 decrements were also well correlated with baseline %predicted FEV_1 ($r = 0.53$, $p < 0.05$); that is, responses to ozone increased with severity of disease, and individuals using bronchodilators experienced greater ozone-induced lung function decrements. Based on FEV_1/FVC, this study also showed that the obstructive response to ozone is greater in individuals with asthma than those without. Kreit et al. (1989) also reported a large statistically significant difference in ozone-induced FEV_1 decrements between individuals with asthma and those without (25 vs. 16%, respectively, $p < 0.05$) exposed to 400 ppb ozone with heavy intermittent exercise for 2 hours. Overall, however, the majority of controlled human exposure studies found little to no difference in ozone-induced lung function responses between individuals with and without asthma.

- Since the 2013 Ozone ISA, four controlled human exposure studies examining ozone effects on lung function in individuals with asthma have been published (Arjomandi et al., 2015; Leroy et al., 2015; Bartoli et al., 2013; Fry et al., 2012). Study-specific details, including exposure concentrations and durations, are summarized in Table 3-16 and EI3-13 in Section 3.3.1.
- Neither Arjomandi et al. (2015) nor Fry et al. (2012) reported FEV_1 responses to ozone differentiated by the presence of asthma.
- Consistent with Horstman et al. (1995), in a large study of individuals with asthma (34 F, 86 M; 32.9 ± 12.9 years), Bartoli et al. (2013) found that the magnitude of ozone-induced FEV_1 response increased with decreasing baseline FEV_1 ($p = 0.02$) and a lack of inhaled corticosteroid treatment ($p = 0.04$). This study, however, did not include a healthy nonasthmatic control group, limiting our understanding of differences between asthmatic and nonasthmatic individuals. In a

smaller study of healthy nonasthmatic individuals (5 F, 7 M; 31.8 ± 6.0 years) and subjects with mild asthma (5 F, 3 M; 33.7 ± 10.1 years), although baseline FEV_1 and FEV_1/FVC were significantly lower in asthmatics than nonasthmatics, there was no significant association between the presence of asthma and lung function response to ozone (Leroy et al., 2015). These new studies do not contribute to our understanding of lung function responses to ozone in individuals with asthma relative to those without.

3.1.5.4.2 Animal Toxicological Studies

Several recent studies provide evidence for ozone exposure-induced respiratory effects in animal models of allergic airway disease. These effects include sensory and pulmonary irritation and changes in lung function. Study-specific details are summarized in Table 3-18 and Table 3-19 in Section 3.3.1. All of these changes, which are described below, were statistically significant. Allergic mice exhibited enhanced responses compared with naïve mice. One study provides insight into mechanisms underlying ozone-induced bronchoconstriction. Recent studies, detailed below, are grouped according to concentration-time profile.

- Sensory and pulmonary irritation to acute ozone exposure (2 ppm, 3 hours) were examined in naïve and allergic mice, which were sensitized with ovalbumin. Sensory irritation reflects changes in the upper airways, while pulmonary irritation reflects changes in the lower airways. Bao et al. (2013) found increased baseline enhanced pause, with a greater enhancement seen in allergic mice.Hansen et al. (2016) found sensory irritation in naïve and allergic mice and pulmonary irritation in naïve, but not in allergic, mice.
- Schelegle and Walby (2012) investigated the role of vagal afferents in mediating bronchoconstriction to acute ozone exposure (1 ppm, 8 hours). Direct measurements of airway resistance were made in naïve and allergic rats (sensitized and challenged with nDer f 1). Ozone exposure induced rapid shallow breathing in all the rats, but the response was greatest in the allergic rats. Ozone exposure also increased airway resistance (i.e., bronchoconstriction) in allergic rats, but not in naïve rats. The mechanisms underlying increased airway resistance were explored using vagotomy and pharmacological agents and were found to involve vagal C-fibers, vagal myelinated fibers, and possibly mediators released in the airway. Vagal lung C-fibers mediated the reflex bronchoconstriction to ozone. The vagal myelinated fibers mediate a reflex bronchodilation. Neuropeptides (e.g., substance P) may also be involved in the bronchoconstrictive response. This new study provides evidence that ozone exposure exacerbates bronchoconstriction in allergic animals. Sensory nerve pathways, specifically vagal afferents, played an important role in increased airway resistance.

3.1.5.4.3 Epidemiologic Studies

A large body of epidemiologic studies reviewed in the 2013 Ozone ISA (U.S. EPA, 2013a) provides generally consistent evidence that increases in short-term ozone concentrations are associated with decreased lung function in children with asthma. Associations were observed across a range of ozone concentrations, daily averaging times (e.g., 24-hour avg, 8-hour avg, 8-hour max, and 1-hour max), and diverse geographic locations, including multicity U.S. studies (O'Connor et al., 2008; Mortimer et al.,

2002; Mortimer et al., 2000). In contrast to studies of lung function in healthy children (Section 3.1.4.1.3) that generally had reduced potential for exposure measurement error due to the use of ozone monitors at study sites, studies in children with asthma generally relied on central site monitors. The majority of the observed ozone-related decrements in FEV_1 and PEF ranged from <1 to 2%, but results were more variable for FEV_1. Additionally, in studies that observed increases in ozone-related respiratory symptoms and decreases in lung function, associations were generally reported at similar lags. No recent U.S. or Canadian studies examined ozone associations with lung function in children with asthma.

In addition to studies of children with asthma, the 2013 Ozone ISA evaluated a limited number of studies that examined lung function in adults with asthma (U.S. EPA, 2013a). In contrast to results from studies of children, short-term ozone concentrations were not consistently associated with lung function decrements in adults with asthma. Differences in exposure assignment techniques, including single fixed-site monitors, on-site monitoring during outdoor activity, and personal exposure monitoring, did not appear to explain the inconsistent results. No recent studies examined ozone associations with lung function in adults with asthma.

3.1.5.4.4 Integrated Summary for Lung Function

Based on studies reviewed in the 1996 and 2006 Ozone AQCDs (U.S. EPA, 2006, 1996a) and the 2013 Ozone ISA (U.S. EPA, 2013a), there is evidence that individuals with asthma were at least as sensitive to acute effects of ozone on lung function as healthy individuals. Several controlled human exposure studies evaluated in the 2013 Ozone ISA (U.S. EPA, 2013a) demonstrated that acute ozone exposures result in lung function decrements in individuals with asthma. However, the majority of these studies observed similar ozone-induced lung function changes in individuals with and without asthma. Consistent with prior findings, one recent study showed increasing ozone-induced decrements in lung function with decreasing baseline lung function. That is, the effect of ozone on lung function increased with increasing asthma severity. Beyond this, recent studies do little to inform potential differences in lung function responses to ozone among individuals with and without asthma. While one recent study observed similar lung function decrements in individuals with and without asthma, most recent studies do not examine a healthy comparison group. However, despite limited evidence demonstrating increased sensitivity to ozone in individuals with asthma compared to those without asthma, there is consistent evidence that asthmatic individuals experience lung function decrements in response to acute ozone exposures. A recent animal toxicological study also provides additional evidence of ozone-induced lung function changes. Changes in ventilator parameters (e.g., breathing frequencies) and increased airway resistance were more pronounced in allergic rats. Additionally, the recently available study provides mechanistic evidence that sensory nerve pathways play an important role in reflex bronchoconstriction to ozone.

Similar to controlled human exposure studies, few epidemiologic panel studies evaluated in the 2013 Ozone ISA (U.S. EPA, 2013a) compare lung function responses with ozone in individuals with and

without asthma. Nonetheless, many studies have reported that increases in ambient ozone concentrations are associated with decreases in lung function in children with asthma. This association is established from studies evaluated in the 2013 Ozone ISA, as recent epidemiologic studies in the U.S. or Canada have not examined ozone associations with lung function in study populations restricted to individuals with asthma.

3.1.5.5 Airway Responsiveness

3.1.5.5.1 Controlled Human Exposure Studies

As reviewed in the 2016 Oxides of Nitrogen ISA [see Section 5.2.2.1 of U.S. EPA (2016)], airway responsiveness is log-normally distributed in the general population, with individuals having airway hyperresponsiveness tending to be those with asthma. Along with symptoms, variable airway obstruction, and airway inflammation, airway hyperresponsiveness is a primary feature in the clinical definition and characterization of asthma severity. Thus, individuals with asthma generally have greater baseline airway responsiveness than those unaffected by asthma. Similar relative changes in airway responsiveness are seen in subjects with asthma and healthy control subjects exposed to ozone despite their markedly different baseline airway responsiveness [see Section 6.2.2.1 of U.S. EPA (2013a)]. Increased airway responsiveness can be an important consequence of exposure to ambient ozone in individuals with asthma because their airways are potentially predisposed to narrowing on inhalation of a variety of stimuli. An important aspect of ozone-induced increases in airway responsiveness is that this effect may provide biological plausibility for associations observed between increases in ambient ozone concentrations and increased respiratory symptoms in children with asthma and increased hospital admissions and ED visits for asthma.

3.1.5.5.2 Animal Toxicological Studies

The 2013 Ozone ISA summarized evidence of increased airway responsiveness in rodent models of allergic airway disease. Repeated ozone exposure (0.1−0.5 ppm) over 10 days increased nonspecific airway responsiveness in allergen-sensitized animals. Ozone exposure (1 ppm) increased airway responsiveness to inhaled allergens in allergen-sensitized animals. A recent study, detailed below, also found that allergic mice exhibited enhanced airway responsiveness compared with naïve mice. This effects was statistically significant. Sensory nerve pathways, specifically vagal afferents, were found to play an important role in increased airway responsiveness. Additional study-specific details are summarized in Table 3-18 in Section 3.3.1.

- Schelegle and Walby (2012) evaluated the role of vagal afferents in mediating ozone-induced increased airway responsiveness to allergen. Direct measurements of airway resistance were

made in naïve and allergic rats (sensitized and challenged with nDer f 1) following ozone exposure (1 ppm, 8 hours). Ozone exposure enhanced allergen-induced airway resistance (i.e., increase in specific airway responsiveness) in allergic rats to a greater degree than in naïve rats. This was an early airway response; no late airway response was observed. The mechanisms underlying this response were explored using vagotomy and pharmacological agents and demonstrated the involvement of vagal C-fibers, vagal myelinated fibers, and possibly neuropeptides released in the airway. Results indicated that vagal lung C-fibers mediated the enhanced specific airway reactivity (to the allergen). Neuropeptides (e.g., substance P) may also be involved in the bronchoconstrictive response to allergen.

3.1.5.5.3 Integrated Summary for Airway Responsiveness

Controlled human exposure studies previously evaluated in the 2013 Ozone ISA indicate that individuals with and without asthma exhibit similar relative increases in ozone-induced airway responsiveness. However, in general individuals with asthma having greater baseline airway responsiveness than individuals without asthma. Increased airway responsiveness can result in the narrowing of airways upon inhalation of a variety of stimuli, providing biological plausibility for epidemiologic associations observed between increases in ozone and asthma exacerbation (i.e., hospital admissions and ED visits for asthma and prevalence of respiratory symptoms in children with asthma). No recent controlled human exposure studies or epidemiologic studies were identified for review.

Consistent with previously reviewed experimental studies in humans, animal toxicological studies reviewed in the 2013 Ozone ISA observed increased airway responsiveness to inhaled allergens in allergen-sensitized animal models. A recent study also found that ozone exposure resulted in enhanced airway responsiveness in allergic mice compared to naïve mice.

3.1.5.6 Respiratory Tract Inflammation, Injury, and Oxidative Stress

3.1.5.6.1 Controlled Human Exposure Studies

Studies reviewed in Section AX6.9.3 of the 2006 Ozone AQCD (U.S. EPA, 2006) and carried forward into Section 6.2.3.1 starting on p. 6-77 of the 2013 Ozone ISA (U.S. EPA, 2013a) showed greater ozone-induced neutrophilic responses in lavage samples collected at 18 hours post-exposure from individuals with asthma than without asthma. Specifically, two studies showed that individuals with asthma exposed to 200 ppb ozone for 4−6 hours with exercise exhibited significantly more neutrophils in BALF (18 hours post-exposure) than similarly exposed healthy individuals. In another study, when lavage samples were collected at 6 hours following a 2-hour exposure with exercise to 200 ppb ozone, there were no observed differences in inflammatory responses between those with and without asthma. However, the subjects with asthma were on average 5 years older than the healthy subjects in this study, and it is still

not yet known how age affects inflammatory responses. It is also possible that the time course of neutrophil influx differs between healthy individuals and those with asthma.

Human studies described in the 2013 Ozone ISA (U.S. EPA, 2013a) contribute to the understanding of mechanisms underlying respiratory effects in individuals with atopy or asthma exposed to ozone. Indicating allergic skewing of responses, increases in airway eosinophils and the Th2 cytokine IL-5 were observed in subjects with atopy and mild asthma exposed to 160−400 ppb ozone. In addition, increased expression of high and low affinity IgE receptors on sputum macrophages, which may enhance IgE-dependent inflammation, was observed. Studies of subjects with allergic asthma also found increased expression of TLR4 and CD86. While TLR4 is an activator of innate immunity, CD86 is associated with Th2 responses. Prior allergen challenge enhanced nasal and airway eosinophilia in subjects with mild asthma exposed to ozone. Studies indicated that ozone exposure enhances components of allergic inflammation. In addition, controlled human exposure studies have shown increased airway responsiveness in subjects with mild allergic asthma and allergic rhinitis exposed to 120−250 ppb ozone. Study-specific details from recent studies, including exposure concentrations and durations, are summarized in Table 3-20 and Table 3-21 in Section 3.3.1.

- In a recent study, Arjomandi et al. (2015) exposed healthy adults (7 F, 9 M; 30.8 ± 6.9 years) and asthmatic adults (6 F, 4 M; 33.5 ± 8.8 years) to 0, 100, and 200 ppb ozone for 4 hours with intermittent exercise (30-minute intervals of rest and exercise at 20 L/minute per m^2 BSA). Sputum neutrophil and eosinophil concentrations increased significantly with the increasing ozone concentrations. Eosinophil effects remained significant after adjustment for asthma and atopy, suggesting the effect may be unrelated to the presence of asthma or atopy.
- Dokic and Trajkovska-Dokic (2013) exposed subjects with allergic rhinitis (5 F, 5 M; 27.9 ± 2.1 years) to 0 and 400 ppb ozone during and out of grass pollen season. Based on a greater statistical significance of increases in nasal mucus total protein, albumin, PMNs, and eosinophils following ozone exposures during pollen season, the authors concluded that allergens exaggerate the response to ozone. However, the statistical tests the authors used did not support their conclusions: the tests appeared to be relative to a baseline, were not adjusted to responses following air control, and were not performed across seasons.
- (Hernandez et al., 2012) examined inflammatory responses of healthy volunteers (20 F, 14 M; 24.2 ± 3.9 years) and atopic individuals with asthma (10 F, 7 M; 24.4 ± 5.5 years) exposed to 400 ppb ozone for 2 hours with moderate intermittent exercise. Induced sputum samples were collected 4 hours after exposure. This study is a continuation (i.e., an additional 15 subjects were included) of the Hernandez et al. (2010) study discussed in the 2013 Ozone ISA (U.S. EPA, 2013a). Although there was no filtered air control, it is possible to make comparisons between the healthy and asthmatic subjects. After ozone exposure, the proinflammatory cytokines IL6, IL8, IL18, and TNF-α, were significantly increased in asthmatic compared to healthy volunteers despite similar neutrophil and macrophages proportions between groups. The authors suggested that unlike healthy subjects, those with atopic asthma cannot limit epithelial cell proliferative responses due to oxidative stress from ozone exposures.

3.1.5.6.2 Animal Toxicological Studies

The 2013 Ozone ISA summarized evidence of increased injury, inflammation, and oxidative stress following ozone exposure in rodent models of allergic airway disease. Repeated ozone exposure (0.1−0.5 ppm) over 10 days enhanced goblet cell metaplasia in allergen-sensitized animals. In addition, ozone exposure (1 ppm for 2 days) enhanced inflammation and allergic responses to allergen challenge in allergen-sensitized animals. Further, treatment with the antioxidant γ tocopherol (but not α tocopherol) blunted ozone-induced inflammation in allergic rhinosinusitis and allergic inflammation of the lower airways, indicating a role for oxidative stress mediating these effects. Recent studies, detailed below and grouped by concentration-exposure profile, provide additional evidence for ozone exposure-induced respiratory effects in animal models of allergic airway disease. This includes injury, inflammation, and increased mucin/mucosubstance content. Allergic mice showed enhanced responses compared with naïve mice for some of these endpoints. These changes, which are described below, were statistically significant. Study-specific details are summarized in Table 3-22, Table 3-23, and Table 3-24 in Section 3.3.1.

- Allergic and inflammatory responses to acute ozone exposure (2 ppm, 3 hours) were evaluated in naïve and allergic mice, which were sensitized with ovalbumin. Hansen et al. (2016) found no enhancement of allergic responses such as serum IgE, bronchoalveolar cells, and lung tissue cytokines. Bao et al. (2013) found that allergic mice exhibited greater inflammatory responses to ozone compared with naïve mice, including enhancement of neutrophils, hyaluronan, and the Th2 cytokines Il-5 and IL-13 in BALF. Stored mucosubstance content and Muc5AC gene expression were also enhanced to a greater degree in allergic mice exposed to ozone.
- Schelegle and Walby (2012) found increased BALF protein, an injury marker, but no increase in BALF cells in allergic rats (sensitized and challenged with nDer f 1) following ozone exposure (1 ppm, 8 hours).

3.1.5.6.3 Epidemiologic Studies

The 2013 Ozone ISA described generally consistent epidemiologic evidence of an association between short-term ozone exposure and subclinical effects in children with asthma (U.S. EPA, 2013a). The most commonly studied respiratory biomarker was exhaled nitric oxide (eNO), an indicator of pulmonary inflammation. A link between eNO and asthma exacerbation is well supported in the literature (Jones et al., 2001; Kharitonov and Barnes, 2000). Increases in 8-hour daily max ozone concentrations were associated with increased eNO in a CHS study in southern California (Berhane et al., 2011) and a single-city panel study conducted in Mexico City that assigned exposure from monitors within 5 km of children's homes or schools (Barraza-Villarreal et al., 2008). Ozone was also associated with other subclinical markers of pulmonary inflammation and oxidative stress in children with asthma across a number of other single-city panel studies. Biomarkers examined included IL-6, IL-8, eosinophils, TBARS, 8-isoprostane, and malondialdehyde.

One recent epidemiologic study of ozone exposure examined subclinical effects in children with asthma. In a panel study in southern California, 8-hour max ozone concentrations measured at fixed-site monitors within 12 km of subjects' residences were not associated with increases in exhaled nitric oxide (eNO) (Delfino et al., 2013). See Table 3-25 in Section 3.3.1 for complete study details.

3.1.5.6.4 Integrated Summary for Respiratory Tract Inflammation, Injury, and Oxidative Stress

Controlled human exposure studies evaluated in the 2013 Ozone ISA (U.S. EPA, 2013a) established evidence of enhanced allergic inflammation to ozone in individuals with asthma. Specifically, markers of airway and lung inflammation, and innate immunity, were increased in response to short-term ozone exposures. As with the findings for lung function, there is limited evidence that ozone-induced inflammatory responses differ due to the presence of asthma. Results from experimental animal studies are coherent with evidence from humans. Recent studies expand on findings summarized in the 2013 Ozone ISA (U.S. EPA, 2013a), indicating inflammation, oxidative stress, injury, allergic skewing, goblet cell metaplasia, and upregulation of mucus synthesis and storage in allergic animals exposed to ozone. Allergic mice generally exhibited enhanced responses compared to naïve mice for these endpoints.

Epidemiologic studies evaluated in the 2013 Ozone ISA (U.S. EPA, 2013a) support findings from experimental studies. Short-term ozone concentrations were associated with markers of oxidative stress and pulmonary inflammation in panel studies of children with asthma. While the only recent study available for review reported a null association between ozone and FeNO in children with asthma, the results should be considered in the context of previously reviewed studies, the majority of which observed positive associations.

3.1.5.7 Overall Summary of Respiratory Effects in Populations with Asthma

In summary, evidence from recent epidemiologic and experimental studies continues to support an association between ozone and asthma exacerbation. Recent, large multicity epidemiologic studies conducted in the U.S. build on evidence from the 2013 Ozone ISA and provide further support for an association between ozone and ED visits and hospital admissions for asthma. Hospital admission and ED visit studies that presented age-stratified results generally reported the strongest associations in children between the ages of 5 and 18. Additionally, associations were observed across a range of ozone concentrations, and were consistent in models with measured or modeled concentrations. A limited number of recent epidemiologic studies in the U.S. or Canada have examined respiratory symptoms, medication use, lung function, and subclinical effects in people with asthma. However, a large body of evidence from the 2013 Ozone ISA (U.S. EPA, 2013a) demonstrates ozone associations with these less severe indicators of asthma exacerbation, providing support for the ozone-related increases in asthma hospital admissions and ED visits observed in recent studies.

Evidence from controlled human exposure and animal toxicological studies provide biological plausibility for the associations observed in epidemiologic studies of short-term ozone exposure and asthma exacerbation. Results from experimental studies in humans demonstrate that ozone exposures lead to increased respiratory symptoms, lung function decrements, increased airway responsiveness, and increased lung inflammation in individuals with asthma. However, observed responses across the range of endpoints did not generally differ due to the presence of asthma. Animal toxicological studies similarly found that ozone exposures altered ventilatory parameters, increased airway responsiveness, and increased pulmonary inflammation and bronchoconstriction in allergic animals. In contrast to controlled human exposure studies, there was some evidence from studies of rodents that the observed respiratory effects were enhanced in allergic animals compared to naïve animals.

3.1.6 Respiratory Effects in Other Populations with Pre-existing Conditions

3.1.6.1 Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) is a chronic lung disorder characterized by destruction of alveolar tissue, airway remodeling, and minimally reversible airflow limitation. Reduced airflow is associated with decreased lung function, and clinical symptoms demonstrating exacerbation of COPD include cough, sputum production, and shortness of breath. Severe exacerbation can lead to ED visits or hospital admissions.

3.1.6.1.1 Epidemiologic Studies

A limited number of epidemiologic studies evaluated in the 2013 Ozone ISA provided some evidence that short-term exposure to ozone is associated with increased ED visits for COPD (U.S. EPA, 2013a). A large multicity study in Canada reported a year-round association that was driven largely by ED visits in the warm season (Stieb et al., 2009). In a single-city study in São Paulo, Brazil, Arbex et al. (2009) also observed a positive association, but only in a stratified analysis of women. There was little supporting evidence from studies of lung function or respiratory symptoms in adults with COPD. Specifically, epidemiologic studies did not provide strong evidence that short-term increases in ozone exposure result in lung function decrements in adults with COPD, and a single study of adults with COPD found that ozone was both positively and inversely associated with a range of respiratory symptoms (Peacock et al., 2011).

Recent studies provide generally consistent evidence that short-term exposure to ozone is associated with ED visits for COPD, with the strongest evidence coming from large multicity studies. Supporting evidence from less severe manifestations of COPD is still lacking. The majority of the

evaluated studies use 8-hour daily max averaging times, although there are instances in which the 1-hour daily max (Malig et al., 2016) and the 24-hour avg (Szyszkowicz et al., 2018) are used. The averaging time used in each study, along with other study-specific details, including air quality characteristics and select effect estimates, are highlighted in Table 3-26 and Table 3-27 in Section 3.3.1. An overview of the evidence is provided below.

- Large case-crossover studies, including a statewide study in California (Malig et al., 2016) and a multicity study in Ontario, Canada (Szyszkowicz et al., 2018), reported positive associations between ozone and COPD ED visits. Malig et al. (2016) noted stronger associations in the warm season than in year-round analysis, and associations that were robust to adjustment for NO_2, SO_2, and CO in copollutant models. Further discussion of potential copollutant confounding and the role of season and temperature on ozone associations with respiratory health effects can be found in the Relevant Issues for Interpreting Epidemiologic Evidence section (Section 3.1.10). In an effort to reduce potential exposure misclassification, both studies assigned ozone exposure using the nearest monitor or the average of the nearest monitors within maximum distance buffers (Szyszkowicz et al., 2018; Malig et al., 2016). A large time-series study in five U.S. cities also observed ozone-related increases in COPD ED visits in three of the five cities (Barry et al., 2018).
- Another large case-crossover study in the state of Georgia observed increased ED visits for chronic bronchitis, a condition that can contribute to or occur independently of COPD, corresponding to increases in fused-CMAQ and ground-based ozone concentrations (Xiao et al., 2016).
- Notably smaller studies in Little Rock, AR (Rodopoulou et al., 2015) and St. Louis, MO (Sarnat et al., 2015) examined ozone-related ED visits for COPD and reported a positive, but imprecise association (i.e., wide 95% CIs) and a null association, respectively. Each study used one monitor for the entire study area, which may have introduced exposure measurement error. Additionally, the short length of the time-series (Sarnat et al., 2015) and the small mean number of daily hospital admissions (Rodopoulou et al., 2015) likely reduced the statistical power to detect an association.
- COPD exacerbation measured by frequency of short-term bronchodilator inhaler use was not associated with short-term exposure to ozone (Magzamen et al., 2018). See Table 3-26 in Section 3.3.1 for complete study details.

3.1.6.1.2 Animal Toxicological Studies

No animal toxicological studies evaluating respiratory effects in animal models of COPD were described in the 2013 Ozone ISA (U.S. EPA, 2013a). Two recent studies employed an animal model of progressive pulmonary inflammation as a surrogate for COPD. This model involves deficiency of surfactant protein D (sfpd), a collectin protein synthesized by lung type 2 cells. Results suggest that chronic inflammation enhanced sensitivity to short-term ozone exposure. This effect was statistically significant. Study-specific details are summarized in Table 3-28, Table 3-29, and Table 3-30 in Section 3.3.1.

- Groves et al. (2012) found that acute ozone exposure (0.8 ppm for 3 hours) leads to increased indicators of injury, inflammation, oxidative stress in sfpd-deficient mice that do not resolve by

72 hours. In contrast, resolution of responses occurred by 72 hours in sfpd-sufficient mice. Ozone exposure resulted in altered lung mechanics that is indicative of central airway and peripheral tissue involvement in sfpd-deficient mice. In sfpd-sufficient mice, ozone exposure resulted in altered lung mechanics that is indicative of only central airway involvement. These determinations were made by analysis of resistance and elastance spectra obtained from impedance data. In a second study, Groves et al. (2013) found age-related increases in enlarged vacuolated macrophages, alveolar wall rupture, type 2 hyperplasia, BALF protein and cell number, and changes in lung mechanics consistent with COPD are observed in sfpd-deficient mice. Acute ozone exposure (0.8 ppm, 3 hours) resulted in greater alveolar hyperplasia and classically activated macrophages in sfpd-deficient than in sfpd-sufficient mice. The effects of ozone on lung mechanics were dampened in 27-week-old mice compared with 8-week-old mice.

3.1.6.1.3 Integrated Summary for Chronic Obstructive Pulmonary Disease (COPD)

In summary, recent large multicity epidemiologic studies of ED visits support an association between short-term ozone exposure and COPD exacerbation. Associations are reported across a variety of study locations, exposure levels, and exposure assignment methods, including nearest monitor concentrations and CMAQ-fused models. In limited copollutant results, the observed association is robust to adjustment for other gaseous pollutants (NO_2, SO_2, and CO). While none of the experimental animal studies evaluated in the 2013 Ozone ISA examined acute ozone exposure in animals with chronic inflammation, results from recent studies suggest that chronic inflammation enhances sensitivity to ozone exposure, providing coherence with ozone-related COPD exacerbation observed in epidemiologic studies.

3.1.6.2 Obese Populations or Populations with Metabolic Syndrome

Metabolic syndrome is comprised of a cluster of metabolic abnormalities, including obesity, dyslipidemia, hypertension, and type 2 diabetes. There is growing evidence that components of metabolic syndrome, including obesity, may increase susceptibility to air pollution-related health effects (Jiu-Chiuan and Schwartz, 2008). The following section evaluates studies examining the relationship between short-term exposure to ozone and respiratory health effects in obese populations or populations with metabolic syndrome.

3.1.6.2.1 Lung Function

3.1.6.2.1.1 Controlled Human Exposure Studies

In the 2013 Ozone ISA (U.S. EPA, 2013a), two retrospective analyses of controlled human exposure studies showed ozone-induced FEV_1 decrements increased with increasing BMI. Since the 2013 Ozone ISA, there is a new controlled human exposure study and a larger retrospective analysis

demonstrating an effect of BMI on lung function responses to ozone. Study-specific details, including exposure concentrations and durations, are summarized in Table 3-4 and Table 3-31 in Section 3.3.1.

- Bennett et al. (2016) exposed obese (19 F; 27.7 ± 5.2 years) and normal-weight (19 F; 24 ± 3.7 years) women to 0 and 400 ppb ozone for 2 hours during intermittent exercise (15-minute periods of seated rest and exercise at 25 L/minute per m^2 BSA). The ozone-induced FVC decrement was significantly ($p < 0.05$) greater in the obese women (12.5%) than normal-weight women (8.0%). The FVC decrement also tended ($p = 0.08$) to be greatest in the obese African-Americans (15.7%) relative to other obese subjects (9.6%). There was also a tendency ($p = 0.11$) for greater ozone-induced FEV_1 decrements in obese women (15.9%) relative to the normal-weight women (11.7%). While respiratory function was diminished, respiratory symptoms in response to ozone exposure did not differ between obese and normal-weight women.
- The new retrospective analysis by McDonnell et al. (2013) includes data from prior studies of young healthy adults (104 F, 637 M; 18−36 years) exposed one or more times to ozone and/or filtered air. The prior analysis by McDonnell et al. (2010), discussed in the 2013 Ozone ISA in relation to BMI effects, used data from 541 healthy nonsmoking white males (18−35 years). The analysis based on a larger data set continues to show that the BMI effect is of the same order of magnitude but in the opposite direction of the age effect. Thus, the model predicts FEV_1 responses increase with increasing BMI and diminish with increasing age.

3.1.6.2.1.2 Animal Toxicological Studies

No studies evaluating the effects of ozone exposure on lung function in obese animals or animal models of metabolic syndrome were available in the 2013 Ozone ISA (U.S. EPA, 2013a). A recent study (Gordon et al., 2016b) involved male and female rats fed normal, high-fructose or high-fat diets prior to acute and subacute ozone exposure (0.8 ppm × 5 hours). While there were some differences in effects depending on duration of exposure, diet, and sex of rat, ozone exposure generally resulted in statistically significant increases in enhanced pause and tidal volume, which are ventilatory parameters that reflect a change in lung function. Study-specific details are summarized in Table 3-32 in Section 3.3.1. Findings related to behavior and metabolism are found elsewhere in Appendix 7 and Appendix 5, respectively.

3.1.6.2.1.3 Epidemiologic Studies

In a study evaluated in the 2013 Ozone ISA (U.S. EPA, 2013a), short-term ozone concentrations were associated with decreases in lung function in older adults with airway hyperresponsiveness (Alexeeff et al., 2007). The observed association was stronger among those who were obese. A recent analysis of the Offspring and Third Generation Framingham Heart Study cohorts also found that obese participants had significantly stronger associations between 8-hour max summertime ozone concentrations and reduced lung function. Study specific details, including effect estimates, are summarized in Table 3-7 in Section 3.3.1.

3.1.6.2.2 Airway Responsiveness

3.1.6.2.2.1 Controlled Human Exposure Studies

No controlled human exposure studies were available for review in the 2013 Ozone ISA that examined airway responsiveness in obese individuals or individuals with metabolic syndrome (U.S. EPA, 2013a).

- A recent study showed an increase in airway responsiveness after ozone exposure did not differ between normal-weight and obese women (Bennett et al., 2016). Study-specific details, including exposure concentrations and durations, are summarized in Table 3-31 in Section 3.3.1.

3.1.6.2.2.2 Animal Toxicological Studies

The 2013 Ozone ISA summarized the evidence of respiratory effects in obese animals resulting from exposure to ozone (U.S. EPA, 2013a). In mouse models of obesity, airways were innately more responsive and responded more vigorously to acute ozone exposure (2 ppm for 3 hours) than lean controls. Newly available information confirms and extends these findings.

Several recent studies evaluated the respiratory effects of acute ozone exposure (2 ppm, 3 hours) in mouse models of obesity (Mathews et al., 2018; Mathews et al., 2017a; Mathews et al., 2017b; Williams et al., 2015). These studies compared responses in obese mice with those of lean mice. Changes in airway responsiveness described below were statistically significant. Study-specific details are summarized in Table 3-32 in Section 3.3.1.

- Pulmonary mechanics were assessed by using the flexiVent system. Baseline and nonspecific (i.e., methacholine challenge) airway responsiveness were greater in obese mice than lean mice in the absence of ozone exposure. Acute ozone exposure increased baseline and nonspecific airway responsiveness in obese mice, but not in lean mice.
- Williams et al. (2015) probed the role of TNF-α and TNF-α receptor in the augmented responses to ozone exposure in obese mice and found that deficiency in these factors enhanced the increase in airway responsiveness.

3.1.6.2.3 Respiratory Tract Inflammation, Injury, and Oxidative Stress

3.1.6.2.3.1 Controlled Human Exposure Studies

No controlled human exposure studies were available for review in the 2013 Ozone ISA that examined pulmonary inflammation, injury, or oxidative stress in obese individuals or individuals with metabolic syndrome (U.S. EPA, 2013a). Study-specific details from recent studies, including exposure concentrations and durations, are summarized in Table 3-21 and Table 3-31 in Section 3.3.1.

- Bennett et al. (2016) recently investigated PMN responses in obese (19 F; 27.7 ± 5.2 years) and normal-weight (19 F; 24 ± 3.7 years) women exposed to 0 and 400 ppb for 2 hour during intermittent exercise. Although PMN were significantly increased after ozone exposure relative to air, the PMN response did not differ between groups.
- In their study of healthy adults (7 F, 9 M; 30.8 ± 6.9 years) and asthmatic adults (6 F, 4 M; 33.5 ± 8.8 years), Arjomandi et al. (2015) also found that adjustment for age, sex, and BMI did not affect the association between PMN responses and ozone exposure.

3.1.6.2.3.2 Animal Toxicological Studies

The 2013 Ozone ISA summarized the evidence of respiratory effects in obese animals resulting from exposure to ozone (U.S. EPA, 2013a). In mouse models of obesity, respiratory tract inflammation and injury responses to acute ozone exposure (2 ppm for 3 hours) were enhanced compared with lean controls. However, the inflammatory response to subacute ozone exposure (0.3 ppm for 72 hours) was dampened. Several recent studies have evaluated the respiratory effects of ozone exposure in animal models of obesity, high fructose/fat diet, and diabetes. Enhanced inflammatory and injury responses were found in obese compared with lean mice and in animals fed high-fat/high-fructose diets compared with those fed a normal diet. These effects, which are described below, were statistically significant. Study-specific details are summarized in Table 3-33 and Table 3-34 in Section 3.3.1.

- Four studies of acute exposure to ozone (2 ppm, 3 hours) were conducted in mouse models of obesity (Mathews et al., 2018; Mathews et al., 2017a; Mathews et al., 2017b; Williams et al., 2015). These studies compared responses in obese mice with those of lean mice. Taken together, these studies shed new light on mechanisms underlying the augmentation of ozone-exposure-induced effects in animal models of obesity. Study-specific details are summarized in Table 3-38 in Section 3.3.1. Acute ozone exposure increased BALF markers of injury and inflammation to a greater extent in obese than in lean mice. Williams et al. (2015) probed the role of TNF-α and TNF-α receptor in the augmented responses to ozone exposure in obese mice and found that deficiency in these proteins attenuated the inflammatory effect. Mathews et al. (2017b) provided evidence that IL-33 contributes to the augmented responses to ozone exposure in obese mice by acting on immune lymphoid cells 2 (ILC2) and on gamma delta T cells, which express the Th2 cytokines IL-5 and IL-13. Mathews et al. (2018) showed a role for IL-17A and gastrin-releasing peptide in the augmented responses to ozone exposure in obese mice. Mathews et al. (2018) noted differences in metabolism, antioxidants, and microbiome in obese and lean mice exposed to ozone. Levels of corticosterone were increased by ozone exposure in obese mice.
- Two studies of subacute ozone exposure (0.5 for 4 hour/day for 13 days) were conducted in a diabetes-prone mouse model (Ying et al., 2016; Zhong et al., 2016). Ozone exposure increased BALF inflammatory cells and upregulated proinflammatory genes in lung tissue. However, no change in the T-cell profiles was found in the pulmonary lymph nodes. Study-specific details are summarized in Table 3-40 in Section 3.3.1. Findings related to systemic inflammation and insulin resistance are reported in Appendix 5.
- Gordon et al. (2016b) fed male and female rats normal, high-fructose, or high-fat diets prior to acute and subacute ozone exposure (0.8 ppm × 5 hours). While there were some differences in effects depending on duration of exposure, diet, and sex of rat, in general ozone exposure resulted

in increased eosinophils and albumin (a marker of injury) in BALF. Findings related to metabolism and behavior are found in Appendix 5 and Appendix 7, respectively.

3.1.6.2.3.3 Epidemiologic Studies

No epidemiologic studies in the 2013 Ozone ISA examined potential associations between short-term exposure to ozone and respiratory health effects in people with pre-existing metabolic syndrome (U.S. EPA, 2013a). A recent panel study of adults with type 2 diabetes mellitus reported decreases in pulmonary inflammation corresponding to 6- (3 a.m. to 9 a.m.) and 24-hour avg ozone concentrations prior to FeNO measurement (Peng et al., 2016). The apparent protective association may be explained by negative correlations between ozone and NO_X, black carbon (BC), and particle number (PN), each of which demonstrated strong positive associations with pulmonary inflammation. Study-specific details, including air quality characteristics and select effect estimates, are highlighted in Table 3-35 in Section 3.3.1.

3.1.6.2.4 Overall Summary for Respiratory Effects in Obese Populations or Populations with Metabolic Syndrome

A recent controlled human exposure study reported evidence of ozone-related increases in pulmonary inflammation in both obese and normal weight adult women during exercise, but inflammatory responses did not differ between the groups. In contrast, epidemiologic studies provide some evidence that ozone-related lung function decrements are larger in obese individuals. Similarly, recent animal toxicological studies expand the body of evidence evaluated in the 2013 Ozone ISA and continue to indicate that, compared to lean mice, obese mice exhibit enhanced airway responsiveness and pulmonary inflammation in response to acute ozone exposures.

In studies of a diabetes-prone mouse model, subacute ozone exposure increased airway inflammation and proinflammatory genes in lung tissue. In contrast, an epidemiologic panel study observed a protective association between ozone and pulmonary inflammation in adults with type 2 diabetes mellitus. This inverse association may be explained by negative correlations with copollutants that demonstrated strong positive associations with pulmonary inflammation in the same population.

In summary, experimental animal studies provide evidence for enhanced respiratory tract inflammation in obese and diabetic models, but evidence from a limited number of controlled human exposure and epidemiologic studies do not demonstrate coherence.

3.1.6.3 Pre-existing Cardiovascular Disease

3.1.6.3.1 Animal Toxicological Studies

No animal toxicological studies evaluating respiratory effects in populations with cardiovascular disease were described in the 2013 Ozone ISA (U.S. EPA, 2013a). Several recent studies evaluated respiratory effects of acute ozone exposure (0.2−1 ppm, 3−6 hours) in rodent models of cardiovascular disease. Some of the studies provide evidence that cardiovascular disease exacerbates the respiratory effects of ozone exposure. Injury, inflammation, oxidative stress, lung function changes, and increased airway responsiveness were seen in animals with cardiovascular disease in response to ozone exposure. Acute ozone exposure in animal models of hypertension resulted in enhanced injury, inflammation, and airway responsiveness compared with healthy animals. These effects, which are described below, were statistically significant. Study-specific details are summarized in Table 3-36, Table 3-37, and Table 3-38 in Section 3.3.1.

- A group of investigators from the same institution examined the effects of a 4-hour ozone exposure in spontaneously hypertensive (SH), fawn-hooded hypertensive (FHH), stroke-prone spontaneously hypertensive (SPSH), obese spontaneously hypertensive heart failure (SHHF), and obese atherosclerosis prone JCR rats across a range of ozone exposure concentrations [0.25−1 ppm ozone; Dye et al. (2015); Hatch et al. (2015); Kodavanti et al. (2015); Ramot et al. (2015); Ward and Kodavanti (2015); Farraj et al. (2012)]. Histopathological lesions and indicators of inflammation and injury were seen in all strains at 1 ppm, but rats with cardiovascular disease were less sensitive than healthy rats to the lowest concentration tested (0.25 ppm). Decreases in lung antioxidants were seen only in response to 1 ppm ozone. Some of the rats with cardiovascular disease exposed to 0.25 ppm exhibited changes in ventilatory parameters, while all of the strains were responsive to 1.0 ppm ozone. Another study from this same group of investigators (Farraj et al., 2016) did not find any increased indicators of inflammation following a 3-hour exposure to 0.3 ppm ozone in SH rats.
- Histopathologic responses following a 6-hour exposure to 1 ppm ozone were evaluated in healthy (Wistar Kyoto) and SH rats (Wong et al., 2018). Ozone exposure induced lesions in terminal bronchioles and alveolar ducts in both strains, with lesions also seen in large airways of the SH rats. In addition, lesion scores were higher in the SH rats than healthy rats for edema, PMN infiltrate, tracheobronchiolar epithelial necrosis, exudate, and large airway cilia cell loss/necrosis. In contrast, Ramot et al. (2015) observed similar histopathologic lesions in SH and Wistar Kyoto rats following a 4-hour exposure to 1 ppm ozone. This apparent discrepancy may be attributed to differences between the age and disease status of the animals studied. Specifically, Wong et al. (2018) examined mature adult (~48.0 week old) SH rats that showed fully developed cardiovascular disease while Ramot et al. (2015) evaluated young (12 to 14 week old) SH rats that were just beginning to develop hypertension.
- Respiratory effects of 4-hour exposure to 1 ppm ozone were evaluated in a mouse model of pulmonary hypertension that had been induced using exposure to hypoxia (Zychowski et al., 2016). Ozone exposure increased lung weight and lung water weight in mice with pulmonary hypertension but not in control mice. Mice with pulmonary hypertension exhibited larger increases in BALF cells and airway responsiveness to methacholine (measured in terms of airway resistance) than control mice in response to ozone exposure.

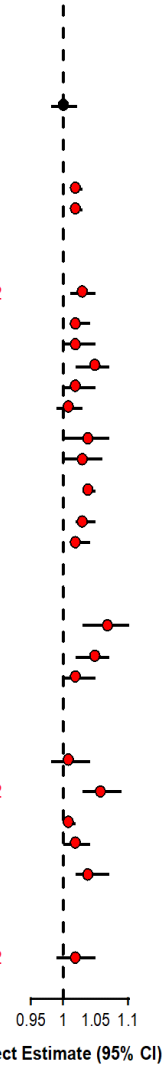
3.1.7 Respiratory Infection and other Associated Health Effects

3.1.7.1 Epidemiologic Studies

A single study evaluated in the 2013 Ozone ISA (U.S. EPA, 2013a) examined the association between ozone exposure and respiratory infection ED visits. Stieb et al. (2009) observed no evidence of an association between ozone exposure and respiratory infection ED visits at any lag examined (i.e., 0, 1, or 2 days) in an all-year analysis across seven Canadian cities. Several recent studies that have become available since the 2013 Ozone ISA provide generally consistent evidence of an association between short-term exposure to ozone and ED visits for a range of respiratory infection endpoints (Figure 3-6). Generally, the evaluated studies use 8-hour daily max averaging times, although there are instances in which the 1-hour daily max (Malig et al., 2016) and the 24-hour avg (Szyszkowicz et al., 2018) are used. The averaging times used in each study, along with other study-specific details, including air quality characteristics and select effect estimates, are highlighted in Table 3-39 in Section 3.3.1. The recently available multicity and single-city studies provide evidence of associations despite the implementation of various study designs, exposure assessment methods, and ozone averaging times. An overview of the evidence is provided below.

- Recent large multicity studies in the U.S. and Canada reported associations between ozone and ED visits for pneumonia (Malig et al., 2016; Xiao et al., 2016), acute respiratory infections (Malig et al., 2016), upper respiratory tract infections (Barry et al., 2018; Szyszkowicz et al., 2018; Malig et al., 2016; Xiao et al., 2016), and ear infections (Xiao et al., 2016). Increases in ED visits ranged from about 2 to 6% per standardized increase in 24-hour avg (Szyszkowicz et al., 2018), 8-hour max (Barry et al., 2018; Xiao et al., 2016), and 1-hour max (Malig et al., 2016) ozone concentrations.
- Large single-city studies in Atlanta (Darrow et al., 2014), Edmonton (Kousha and Rowe, 2014), and St. Louis (Sarnat et al., 2015; Winquist et al., 2012) also provided generally consistent evidence that ozone is associated with increases in ED visits for pneumonia (Sarnat et al., 2015; Darrow et al., 2014), upper respiratory tract infection (Darrow et al., 2014), and acute bronchitis (Kousha and Rowe, 2014). In contrast to results from Sarnat et al. (2015), another time-series study in St. Louis did not observe an association between ozone and ED visits for pneumonia (Winquist et al., 2012). The study periods overlapped, but Winquist et al. (2012) considered a longer time frame. Each study assigned exposure using one monitor for the entire study area, which may have introduced exposure measurement error.
- Notably smaller studies in Windsor, Canada (Kousha and Castner, 2016) and Little Rock, AR (Rodopoulou et al., 2015) did not observe associations between ozone and ED visits for acute respiratory infections, pneumonia, or ear infections. The observed effect estimates were imprecise (i.e., wide 95% CIs), likely due to the limited sample sizes.
- One recent multicity study evaluated copollutant confounding (Malig et al., 2016). These results are discussed in more detail in the Relevant Issues for Interpreting Epidemiologic Evidence Section (Section 3.1.10).

Study	Location	Ages	Mean Concentration[a]	Season	Lag
Combined Respiratory Infection					
Stieb et al. (2009)	Multicity, Canada	All Ages	10.3-22.1; 24-hr avg	Year-Round	1
Acute Respiratory Infection					
†Malig et al. (2016)	California	All	33-55; 1-hr max	Year-Round Warm	0-1
Upper Respiratory Infection					
†Darrow et al. (2014)	Atlanta, GA	0-4	45.9	Year-Round	Lag 0-2
†Barry et al. (2018)	Atlanta, GA Birmingham, AL Dallas, TX Pittsburgh, PA St. Louis, MO	All	37.5-42.2	Year-Round	0-2
†Malig et al. (2016)	California	All	33-55; 1-hr max	Year-Round Warm	0-1
†Xiao et al. (2016)	Georgia	2-18	42.1	Year-Round	0-3
†Szyszkowicz et al. (2018)	Multicity, Canada	<20; Females <20; Males	22.5-29.2; 24-hr avg	Year-Round Year-Round	1
Lower Respiratory Infection					
†Kousha et al. (2014)	Edmonton, Canada	All	18.6	Year-Round	0
†Szyszkowicz et al. (2018)	Multicity, Canada	<20; Females <20; Males	22.5-29.2; 24-hr avg	Year-Round Year-Round	1
Pneumonia					
†Winquist et al. (2012)	St. Louis, MO	All	NR	Year-Round	0-4 DL
†Darrow et al. (2014)	Atlanta, GA	0-4	45.9	Year-Round	Lag 0-2
†Malig et al. (2016)	California	All	33-55; 1-hr max	Year-Round Warm	0-1
†Xiao et al. (2016)	Georgia	2-18	42.1	Year-Round	0-3
Bronchitis					
†Darrow et al. (2014)	Atlanta, GA	0-4	45.9	Year-Round	Lag 0-2



DL = distributed lag.

Note: †Studies published since the 2013 Ozone ISA.

[a]Mean concentrations reported in ppb and are for 8-hour daily max averaging-times unless otherwise noted.

Results standardized to a 15-ppb increase in 24-hour avg, 20-ppb increase in 8-hour daily max, or 25-ppb increase in 1-hour daily max ozone concentrations. Corresponding quantitative results are reported in Table 3-38.

Figure 3-6 Summary of associations from studies of short-term ozone exposures and respiratory infection emergency department (ED) visits for a standardized increase in ozone concentrations.

3.1.7.2 Controlled Human Exposure

The inflammatory effects of ozone involve the innate immune system, as indicated by increases in airway neutrophils. The adaptive immune system may also be involved via alterations in antigen presentation and costimulation by innate immune cells such as macrophages and dendritic cells, which may lead to T-cell activation. Controlled human exposure studies described in the 2013 Ozone ISA (U.S. EPA, 2013a) show that ozone exposure results in airway neutrophilia, reflecting activation of the innate immune system, and altered antigen presentation in macrophages and dendritic cells. Subjects involved in these studies were exposed to 80−400 ppb ozone with moderate intermittent exercise. Enhanced adaptive immunity may bolster defenses against infection, as well as increase allergic responses via T-cell activation. In asthmatics, there is increased uptake of particles by airway macrophages that may also enhance the processing of particulate antigens and lead to greater progression of allergic airway disease and contribute to an increased risk of asthma exacerbation. There are no new controlled human exposure studies contributing to this evidence base.

3.1.7.3 Animal Toxicological Studies

The 2013 Ozone ISA (U.S. EPA, 2013a) summarized the animal toxicological evidence of impaired host defense resulting from exposure to ozone. Increased susceptibility to challenge with infectious agents was observed at ozone concentrations of 0.08−0.5 ppm. Decreases in mucociliary clearance occurred following exposure to 1 ppm ozone and altered macrophage phagocytosis or function following exposure to 0.1 ppm ozone. In addition, effects on adaptive immunity, such as altered T cell subsets in the spleen (0.6 ppm), decreased antibody response following influenza virus infection (0.5 ppm), and decreased mitogen activated T-cell proliferation (0.5 ppm), have been reported. Effects on natural killer cells, which are effectors of innate and adaptive immunity, have also been reported with decreased activity at concentrations of 0.6−1 ppm, and increased activity or no effect at lower concentrations. Acute exposures to 2 ppm ozone resulted in SP-A oxidation and impairment of SP-A dependent phagocytosis, which led to increased susceptibility to pneumonia.

Two recent studies provided evidence that acute ozone exposure (2 ppm, 3 hours) increased susceptibility to infectious disease. Effects, described below, were statistically significant. These studies build upon the investigators' previous work showing that the survival rate of mice infected with pneumonia was decreased by previous exposure to ozone (2 ppm, 3 hours). Study-specific details are summarized in Table 3-40 in Section 3.3.1.

- In one study Durrani et al. (2012) found that ozone exposure had different impacts on survival in male and female mice. To investigate sex-related differences in survival, mice were subjected to gonadectomy or gonadectomy plus hormone replacement. Survival was improved by gonadectomy and worsened by hormone treatment of gonadectomized mice. Mikerov et al. (2011) found that lung and spleen inflammation were evaluated in mice acutely exposed to ozone and later exposed to pneumonia. Ozone exposure increased the area and severity of lung

inflammation in both male and female mice, with a larger response observed in females. In addition, spleen red pulp congestion, indicating compromised spleen immune function, occurred in female mice.

3.1.7.4 Integrated Summary for Respiratory Infection and other Associated Health Effects

In summary, a large number of recently available epidemiologic studies expand the evidence base considerably, and provide consistent evidence of an association between short-term ozone exposure and ED visits for a variety of respiratory infection endpoints (Figure 3-6). The strongest evidence comes from large multicity studies, and the consistent associations observed across a variety of study designs and exposure assessment methods. Additionally, there was some limited evidence that the observed associations were robust, or attenuated, but still positive in copollutant models adjusting for gaseous pollutants (NO_2, SO_2, and CO) (Malig et al., 2016). Further discussion of potential copollutant confounding can be found in Section 3.1.10. The epidemiologic evidence is supported by animal toxicological studies evaluated in the 2013 Ozone ISA (U.S. EPA, 2013a) that demonstrate altered immunity following acute ozone exposure. Additionally, results from a limited number of recent experimental studies in mice were consistent with previous findings of ozone-induced infectious disease susceptibility.

3.1.8 Combinations of Respiratory Related Hospital Admissions and Emergency Department (ED) Visits

The 2013 Ozone ISA evaluated a limited number of studies conducted in the U.S., Canada, and Europe that examined ozone exposure and hospital admissions and/or ED visits for aggregated respiratory diseases (U.S. EPA, 2013a). The available studies added to existing evidence from the 2006 ozone AQCD, which concluded that there was strong evidence that short-term ozone exposures are associated with increased ED visits and hospital admissions in the warm season (U.S. EPA, 2006). The strongest evidence from the 2013 Ozone ISA came from multicity studies of hospital admissions (Katsouyanni et al., 2009; Cakmak et al., 2006) and large single-city studies examining ED visits (Darrow et al., 2011; Tolbert et al., 2007). Most studies examined hospital admissions or ED studies for individuals of all ages (Darrow et al., 2011; Tolbert et al., 2007; Cakmak et al., 2006), although Katsouyanni et al. (2009) restricted their analysis to older adults. While there was limited evaluation of potential copollutant confounding, Tolbert et al. (2007) observed an association between ozone and respiratory ED visits in Atlanta (March−October) that was robust to adjustment for CO and NO_2, and attenuated, but still positive, in a copollutant model adjusting for PM_{10}.

In a study of respiratory ED visits in Atlanta, GA, Darrow et al. (2011) compared a range of daily ozone averaging times, including 1-hour max, 8-hour max, 24-hour avg, 6-hour commute time avg

(7:00 a.m.−10:00 a.m., 4:00 p.m.−7:00 p.m.), 11-hour daytime avg (8:00 a.m.−7:00 p.m.), and 6-hour overnight avg (12:00 a.m.−6:00 a.m.) concentrations. Respiratory ED visits were most strongly associated with 1-hour max, 8-hour max, and 11-hour daytime avg ozone metrics. Associations with 6-hour commute time avg and 24-hour avg ozone were smaller in magnitude, but still positive, and 6-hour overnight avg ozone was inversely associated with increased ED visits.

In the following summary of recent studies, hospital admissions and ED visits are evaluated separately. ED visits for respiratory effects are more common and often less serious than hospital admissions. Generally, only a small fraction of respiratory ED visits result in a hospital admission. As such, the two outcomes may reflect different severities of respiratory effects and are best considered independently.

3.1.8.1 Hospital Admissions

A single recent study provides further evidence of an association between respiratory-related hospital admissions and ozone exposure (Figure 3-7). Study-specific details, including air quality characteristics and select effect estimates, are highlighted in Table 3-41 in Section 3.3.1. An overview of the evidence is provided below.

- A large time-series study in St. Louis, MO (Winquist et al., 2012) reported that 8-hour daily max ozone concentrations were associated with hospital admissions for respiratory disease in children ages 2 to 18 years old. Associations with other age groups were null. The study only used one monitor for the entire study area, which likely contributed exposure measurement error.

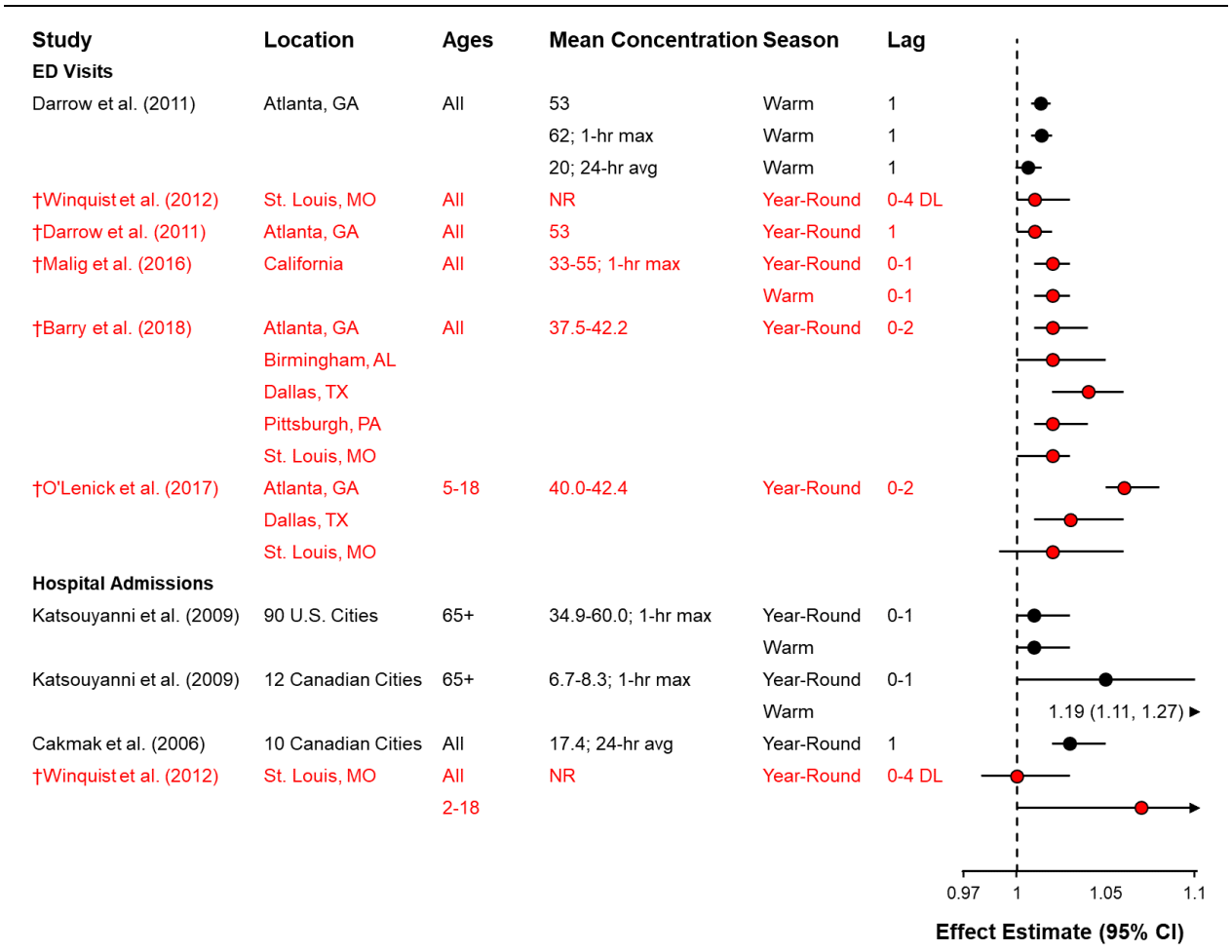
3.1.8.2 Emergency Department (ED) Visits

A larger evidence base exists for recent studies of ED visits for aggregated respiratory diseases. Generally, the evaluated studies use 8-hour daily max averaging times, although there is one study in which the 1-hour daily max (Malig et al., 2016) is used. The averaging-times used in each study, along with other study-specific details, including air quality characteristics and select effect estimates, are highlighted in Table 3-42 in Section 3.3.1. An overview of the evidence is provided below.

- Multicity studies provide consistent evidence of an association between ozone and ED visits for respiratory disease across diverse locations [Barry et al. (2018); O' Lenick et al. (2017); Malig et al. (2016), Figure 3-7]. Large single-city studies in St. Louis (Sarnat et al., 2015; Winquist et al., 2012) and Atlanta (Darrow et al., 2011) provide corroborating evidence.
- In addition to the diversity of locations examined in the above studies, the positive associations were observed across a number of exposure assignment techniques, including single monitors for an entire study area, nearest monitor within 20 km, and population-weighted city-wide averages from 12 km ozone concentration grids estimated using a fusion of observational data from monitors and pollutant concentration simulations from the CMAQ emissions-based chemical transport model.

- A limited number of studies evaluated lag structures (Malig et al., 2016; Darrow et al., 2011), seasonal differences in associations (Malig et al., 2016), and copollutant confounding (Malig et al., 2016). These results are discussed in more detail in the Relevant Issues for Interpreting Epidemiologic Evidence section (Section 3.1.10).

In summary, studies conducted in diverse locations with a variety of exposure assignment techniques continue to provide evidence of an association between ozone and both hospital admissions and ED visits for combined respiratory diseases. Additionally, there is some evidence, previously characterized in the 2013 Ozone ISA, that daily 8-hour max, 1-hour max, and daytime average ozone concentrations may be most strongly associated with respiratory ED visits (Darrow et al., 2011).



DL = distributed lag.

Note: †Studies published since the 2013 Ozone ISA. Black text = studies included in the 2013 Ozone ISA.

[a]Mean concentrations reported in ppb and are for 8-hour daily max averaging times unless otherwise noted.

Results standardized to a 15-ppb increase in 24-hour avg, 20-ppb increase in 8-hour daily max, or 25-ppb increase in 1-hour daily max ozone concentrations. Corresponding quantitative results are reported in Table 3-40 and Table 3-41.

Figure 3-7 Summary of associations from studies of short-term ozone exposures and respiratory-related hospital admissions and emergency department (ED) visits for a standardized increase in ozone concentrations.

3.1.9 Respiratory Mortality

Recent multicity studies have not extensively examined the relationship between short-term ozone exposure and respiratory mortality. The majority of evidence examining respiratory mortality consists of studies evaluated in the 2013 Ozone ISA, which reported positive associations for respiratory mortality in all-year and summer/warm season analyses. Of the recent multicity studies evaluated, only Vanos et al. (2014) examined respiratory mortality and reported positive associations in all-year and summer season analyses, which is consistent with the multicity studies previously evaluated. These studies are further characterized in Table 6-5. An additional single-city study examined respiratory mortality and reported results that are inconsistent with the large body of evidence from multicity studies:

- Klemm et al. (2011) conducted a study in Atlanta, GA that included 7.5 additional years of data compared to Klemm and Mason (2000) and Klemm et al. (2004). In analyses that examined respiratory mortality, the authors reported no evidence of an association with respiratory mortality (−0.44% change in mortality [95% CI: −6.06, 5.51] for a 20-ppb increase in 8-hour max ozone concentrations).

3.1.10 Relevant Issues for Interpreting Epidemiologic Evidence—Short-Term Ozone Exposure and Respiratory Effects

3.1.10.1 Potential Copollutant Confounding of the Ozone-Respiratory Relationship

The 2013 Ozone ISA (U.S. EPA, 2013a) evaluated a limited number of studies that examined potential copollutant confounding. The available studies observed ozone associations with respiratory hospital admissions and ED visits that were generally robust to the inclusion of gaseous pollutants and PM in copollutant models (Silverman and Ito, 2010; Ito et al., 2007; Tolbert et al., 2007). Along with some limited evidence from studies of respiratory mortality (Stafoggia et al., 2010; Katsouyanni et al., 2009), the 2013 Ozone ISA concluded that "copollutant-adjusted findings across respiratory endpoints provide support for the independent effects of short-term exposures to ambient ozone" (U.S. EPA, 2013a). A number of recent studies are available that provide further evidence that ozone-related respiratory effects persist in statistical models adjusting for copollutants. The following summary of the recent evidence is organized by copollutant.

3.1.10.1.1 Fine Particulate Matter ($PM_{2.5}$)

- Studies evaluated in the 2013 Ozone ISA observed single-pollutant associations between ozone and hospital admissions (Silverman and Ito, 2010) and ED visits (Ito et al., 2007) for asthma that were robust to statistical adjustment for $PM_{2.5}$. Additionally, Tolbert et al. (2007) reported

ozone-related increases in ED visits for combined respiratory diseases that were attenuated, but still positive, in copollutant models with $PM_{2.5}$.

- In recent studies, ozone correlations with $PM_{2.5}$ varied greatly across studies ($r = -0.19$ to 0.66; see Section 3.3.1). $PM_{2.5}$-adjusted ozone effect estimates for asthma- (Sarnat et al., 2015; Sacks et al., 2014) and COPD-related (Rodopoulou et al., 2015) ED visits were slightly attenuated, but still positive, compared to single-pollutant estimates. Wendt et al. (2014) used Medicaid claims to examine initial asthma diagnosis in children in Houston, TX. An association with warm-season ozone concentrations was similar in magnitude and precision when $PM_{2.5}$ was included in the model.
- In one of the few studies on subclinical respiratory effects to examine potential copollutant confounding, Peng et al. (2016) observed ozone-related increases in FeNO that were similar in magnitude, but less precise, in a copollutant model adjusting for $PM_{2.5}$.
- One study examined short-term exposure to ozone and asthma- and wheeze-related ED visits in copollutant models adjusting for various $PM_{2.5}$ components (Sarnat et al., 2015). In comparison to a single-pollutant model, models adjusting for SO_4^{2-} or NO_3^- were slightly attenuated but still positive. Effect estimates from copollutant models adjusting for OC, EC, *n*-Alkanes, hopanes, PAHs, Si, K, Ca, Fe, Cu, Zn, or Pb were similar to, or slightly larger than the single-pollutant estimate.

3.1.10.1.2 Sulfur Dioxide (SO_2)

- In recently evaluated studies of short-term exposure to ozone and respiratory health effects, ozone correlations with SO_2 were generally weak ($r = -0.06$ to 0.42; see Section 3.3.1).
- As evaluated in the 2013 Ozone ISA, Ito et al. (2007) reported similar associations between ozone and asthma ED visits in single pollutant models and copollutant models adjusting for SO_2.
- Similar to Ito et al. (2007), a statewide study in California observed single-pollutant associations between ozone and a range of respiratory-related ED visits, including asthma, ARI, pneumonia, COPD, URTI, and aggregated respiratory diseases, that were relatively unchanged in copollutant models with SO_2 (Malig et al., 2016).

3.1.10.1.3 Nitrogen Dioxide (NO_2)

- The associations between ozone and NO_2 in recent studies range from moderate negative correlations to moderate positive correlations ($r = -0.52$ to 0.54; see Section 3.3.1).
- Studies evaluated in the 2013 Ozone ISA observed single-pollutant associations between ozone and ED visits for asthma (Ito et al., 2007) and combined respiratory disease (Tolbert et al., 2007) that persisted in copollutant models adjusting for NO_2.
- In a recent study, Malig et al. (2016) reported single-pollutant ozone associations for a variety of respiratory-related ED visit outcomes that were persistent, although sometimes attenuated, in copollutant models adjusting for NO_2. Wendt et al. (2014) similarly reported in a Medicaid cohort an ozone association with childhood asthma incidence that was reduced in magnitude, but still positive in a model with NO_2.

3.1.10.1.4 Carbon Monoxide (CO)

- Except for Wendt et al. (2014), the same studies that evaluated potential confounding by NO_2 also examined models with CO. The within-study trends for NO_2 adjusted models were similar for CO.

3.1.10.1.5 Summary of Copollutant Confounding Evaluation

In summary, evidence from recent studies is consistent with the 2013 Ozone ISA in supporting an association between ozone concentrations and respiratory health effects independent of coexposures to correlated pollutants. Across pollutants, single-pollutant associations reported between ozone and a range of respiratory-related hospital admissions and ED visits were persistent, although sometimes attenuated, in copollutant models.

3.1.10.2 The Role of Season and Temperature on Ozone Associations with Respiratory Health Effects

The 2013 Ozone ISA concluded that stratified seasonal analyses provided evidence of stronger ozone-respiratory effect associations in the warm season or summer months than in the cold season (U.S. EPA, 2013a). Seasonal differences were particularly evident for asthma (Strickland et al., 2010; Ito et al., 2007; Villeneuve et al., 2007) and COPD (Stieb et al., 2009; Medina-Ramon et al., 2006) hospital admissions and ED visits. Recent studies are generally consistent with these findings. Seasonally stratified analyses of asthma hospital admissions and ED visits found warm season associations with ozone that were either similar to or stronger in magnitude than cold season or year-round associations (Goodman et al., 2017b; Goodman et al., 2017a; Malig et al., 2016; Byers et al., 2015; Sacks et al., 2014; Winquist et al., 2014). A few studies of COPD also reported a larger increase in ozone-related ED visits during the warm season (Malig et al., 2016; Rodopoulou et al., 2015). In contrast, results from recent studies of respiratory infection were reversed, with associations that were similar across seasons, or slightly stronger in magnitude during the cold season (Malig et al., 2016; Darrow et al., 2014; Kousha and Rowe, 2014).

While most studies adjust for temperature to account for potential confounding related to daily morbidity trends and time-activity patterns, no recent studies examined whether temperature modifies the relationship between short-term ozone exposure and respiratory morbidity. However, a recent study of asthma hospitalizations conducted in 10 Canadian cities assessed potential effect modification by synoptic weather type (Hebbern and Cakmak, 2015). Individual days were grouped into six weather types based on a range of meteorological variables, including temperature, dew point, wind speed, pressure, and cloud cover. Hebbern and Cakmak (2015) reported ozone-related increases in hospital admissions for asthma across all six synoptic weather types, including those corresponding to low ozone concentrations. The

associations were heterogeneous across weather types, but no statistically significant differences were observed.

In addition to seasonal analyses, a number of recent studies examined the influence of aeroallergens on the association between ozone and respiratory health. Like ozone, aeroallergens have seasonal patterns and have been found to exacerbate asthma. Consequently, aeroallergens may act as a potential confounder or modifier on the relationship between ozone and respiratory health effects. A few studies evaluated in the 2013 Ozone ISA (U.S. EPA, 2013a) reported increases in respiratory symptoms and asthma medication use in models adjusting for pollen (Just et al., 2002; Ross et al., 2002; Gielen et al., 1997). Several recent studies compared models with and without adjustment for pollen and provided further evidence supporting an association between ozone and respiratory effects that is independent of coexposure to pollen. Multicity studies of hospital admissions for asthma in Canada (Hebbern and Cakmak, 2015) and Texas (Goodman et al., 2017b; Goodman et al., 2017a), and a single-city study of respiratory infection in children in Atlanta (Darrow et al., 2014) observed single-pollutant associations with ozone that were at times attenuated (Goodman et al., 2017a; Hebbern and Cakmak, 2015), but still persisted in models adjusting for pollen. While most studies adjusted for pollen as a potential confounder, Gleason et al. (2014) examined pollen as a potential effect modifier on the relationship between ozone and pediatric asthma ED visits in New Jersey. The authors reported increases in ED visits that were only associated with same day ozone concentrations on high 3-day avg weed pollen days, indicating that weed pollen is a potential effect modifier in the relationship between ozone and pediatric asthma ED visits.

3.1.10.3 The Effect of Lag Structure on Associations of Short-Term Ozone Exposure and Respiratory Effects

The evaluation of lag structure is an important aspect of epidemiologic research on short-term exposure to air pollution. The examination of lags, along with experimental evidence, can help determine whether ozone elicits an immediate (lags ranging from 0 to 1 days), delayed (lags ranging from 2 to 5 days), or prolonged (lags averaged from 0 to 5+ days) effect on respiratory health endpoints. Many recent epidemiologic studies of short-term exposure to ozone and respiratory health effects use previous evidence established in epidemiologic and experimental literature to define a temporal metric of interest a priori. Most of these studies, particularly those examining the association between ozone and asthma, have used 0−2 day average ozone concentrations (Barry et al., 2018; O'Lenick et al., 2017; Alhanti et al., 2016; Xiao et al., 2016; Sarnat et al., 2015; Sacks et al., 2014; Strickland et al., 2014; Winquist et al., 2014; Sarnat et al., 2013). Other recent studies have evaluated associations across a range of single-day and multiday lags. Results from these studies are summarized below.

3.1.10.3.1 Asthma

- Associations between short-term ozone exposure and hospital admissions and ED visits for asthma are generally present across daily lags ranging from 0 to 6 days (Table 3-1). Although precision (e.g., 95% CIs) is not specified in the table, within-study precision was generally consistent across single-day lags.
- The strongest single-day associations were generally observed with ozone concentrations on the same day as the outcome, or within the first 3 days prior to the outcome.
- Studies that examined multiday average lag associations generally reported stronger, but less precise associations than single-day lags (Goodman et al., 2017b; Zu et al., 2017; Malig et al., 2016; Byers et al., 2015).

3.1.10.3.2 Other Respiratory Effects

- A limited number of studies examined the lag structure of associations between short-term exposure to ozone and COPD ED visits. In a statewide study in California, Malig et al. (2016) observed associations between ED visits for COPD and single-day lagged ozone on Days 0 through 3. The largest and most precise (i.e., smallest 95% CI) effect estimate was observed with ozone concentrations on the day prior to ED visit. Similarly, in a multicity study in Canada, Szyszkowicz et al. (2018) reported evidence of more immediate effects of ozone in males. The authors observed associations of similar magnitude and precision on lag Days 0 through 2. Results for females were more delayed, with associations between ozone and COPD ED visits noted on lag Days 2 through 4.
- In a study of combined respiratory-related ED visits in Atlanta, warm season associations with same-day ozone concentrations were strongest (i.e., of greatest magnitude), compared to 1-, 2-, and 3-day lags (Darrow et al., 2011). Malig et al. (2016) similarly observed consistent warm season associations on single-day lags from 0 to 3, but reported the strongest associations with 1 and 2 day lagged ozone. The authors additionally reported that moving average ozone concentrations were associated with larger increases in respiratory ED visits, but the estimates were less precise than single day lag estimates.

3.1.10.3.3 Summary of Evidence on Lag Structures

In summary, the largest evidence base for lag structure comes from studies examining the association between ozone exposure and hospital admissions or ED visits for asthma. Associations were generally observed across a range of lags, extending as far as 6 days prior to the health outcome of interest. This range indicates that ozone may elicit both immediate and prolonged effects, with additional evidence of potentially delayed respiratory effects in one study (Sheffield et al., 2015). Additionally, the strongest associations were observed with multiday averages of ozone that were indicative of more immediate effects. Notably, effect estimates derived from multiday average concentrations were less precise than effect estimates from single-day lag estimates. Finally, it is important to note that different lag responses may be observed across different population subgroups (e.g., age or sex groups), as seen in Szyszkowicz et al. (2018).

Table 3-1 Heat map of daily lag associations between short-term exposure to ozone and hospital admissions and Emergency Department (ED) visits for asthma.

Asthma - Hospital Admissions									
Reference	Age	Season	Daily Lag						
			0	1	2	3	4	5	6
Sheffield et al. (2015)	5-17	Warm					*		
Shmool et al. (2016)	5-17	Warm			*				
Goodman et al. (2017)	5-14	All Year	*						
Zu et al. (2017)	5-14	All Year		*					
Zu et al. (2017)	15-64	All Year		*					
Goodman et al. (2017)	15-64	All Year		*					
Asthma - ED Visits									
Reference	Age	Season	Daily Lag						
			0	1	2	3	4	5	6
Szyszkowicz et al. (2018)	<19 (Female)	Warm			*	*			
Szyszkowicz et al. (2018)	<19 (Male)	Warm					*		
Sheffield et al. (2015)	5-17	Warm				*			
Shmool et al. (2016)	5-17	Warm			*	*			
Gleason et al. (2014)	3-17	Warm	*						
Byers et al. (2015)	5-17	All Year			*				
Byers et al. (2015)	18-44	All Year	*						
Malig et al. (2016)	All	Warm			*				

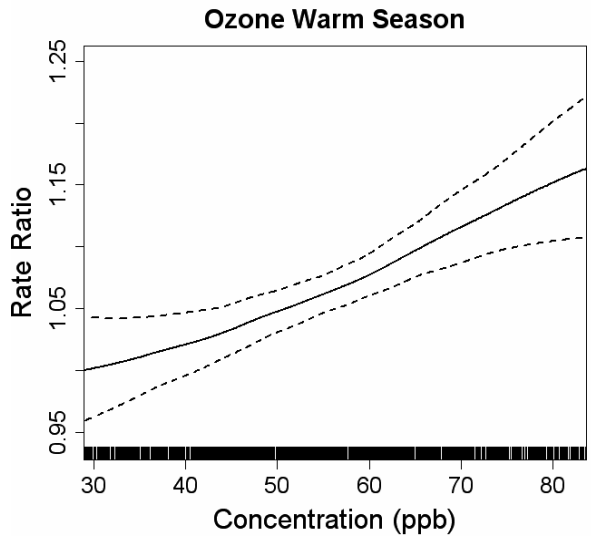
* = Lag at which the strongest association was observed (i.e., largest in magnitude).

Note: Dark blue = study reported statistically significant association ($p < 0.05$) between ozone and impaired respiratory health outcome; light blue = study reported association between ozone and impaired respiratory health outcome regardless of width of confidence intervals; light orange = study reported null or inverse association; red = study reported statistically significant association between ozone and improved respiratory health outcome; gray = study did not examine individual lags.

3.1.10.4 Shape of the Concentration-Response Function

The 2013 Ozone ISA evaluated a large body of epidemiologic evidence that provided evidence of an association between short-term exposure to ambient ozone and respiratory health effects. Of the evaluated studies, a limited number attempted to characterize the shape of the C-R relationship or determine the presence of a concentration threshold below which a positive association with health effects does not occur. Studies examining asthma-related hospital admissions (Silverman and Ito, 2010) and ED visits (Strickland et al., 2010) used natural splines and locally weighted smoothing functions, respectively, to examine the shape of the C-R relationship between ozone concentrations and

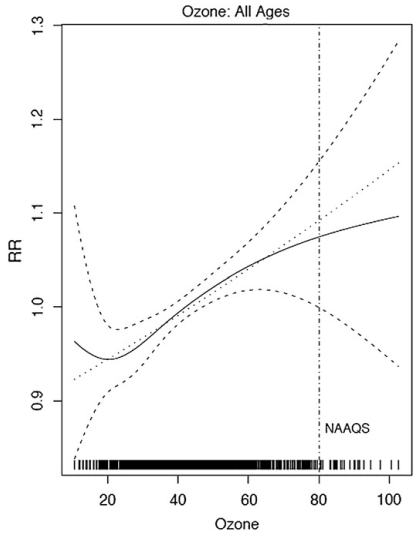
asthma-related hospital admissions or ED visits. Visual inspections of the plots revealed approximately linear associations and no evidence of a threshold with 8-hour daily max ozone concentrations as low as 30 ppb (Figure 3-8 and Figure 3-9). There is increased uncertainty in the shape of the C-R curve at the lower end of the distribution of ozone concentrations, starting around 30 ppb, due to the low density of data in this range.



Note: The reference for the rate ratio is the estimated rate at the 5th percentile of the 8-hour daily max ozone concentration. Estimates are presented for the 5th percentile through the 95th percentile of pollutant concentrations due to instability in the C-R estimates at the distribution tails. The solid lines are smoothed-fit data, with long broken lines indicating 95% confidence bands.

Source: Permission pending, Strickland et al. (2010).

Figure 3-8 Loess (locally estimated scatterplot smoothing) C-R estimates and twice-standard-error estimates from generalized additive models for associations between 8-hour max 3-day avg ozone concentrations and Emergency Department (ED) visits for pediatric asthma.

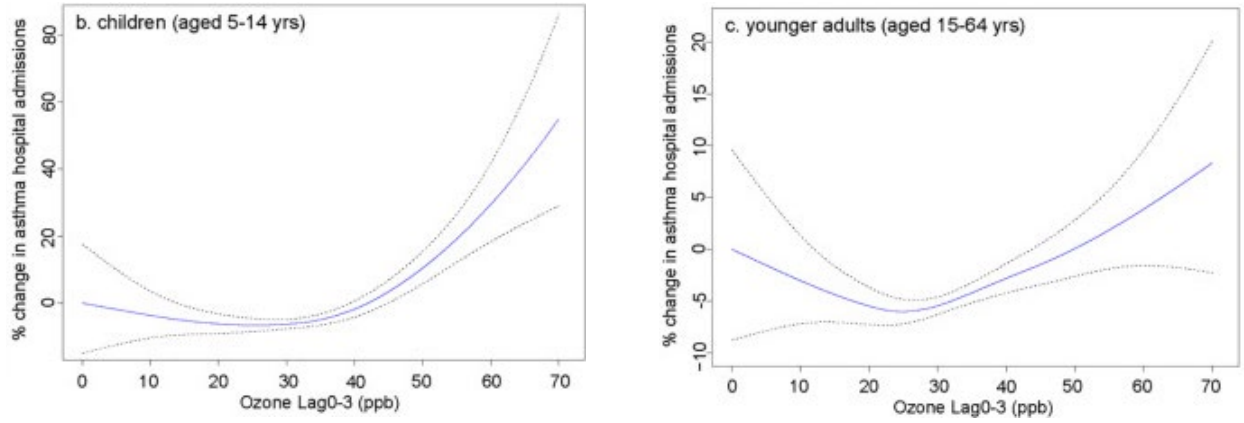


Note: The average of 0 day and 1 day lagged 8-hour daily max ozone was used in a two-pollutant model with $PM_{2.5}$ lag 0−1, adjusting for temporal trends, day of the week, and immediate and delayed weather effects. The solid lines are smoothed-fit data, with long broken lines indicating 95% confidence bands. The density of lines at the bottom of the figure indicates sample size. The NAAQS line indicated in the figure is reflective of a previous standard set in 1997. The form of this NAAQS was the 3-year avg of annual 4th highest daily max 8-hour concentrations.

Source: Permission pending, Silverman and Ito (2010).

Figure 3-9 Estimated relative risks (RRs) of asthma hospital admissions for 8-hour daily max ozone concentrations at lag 0-1 allowing for possible nonlinear relationships using natural splines.

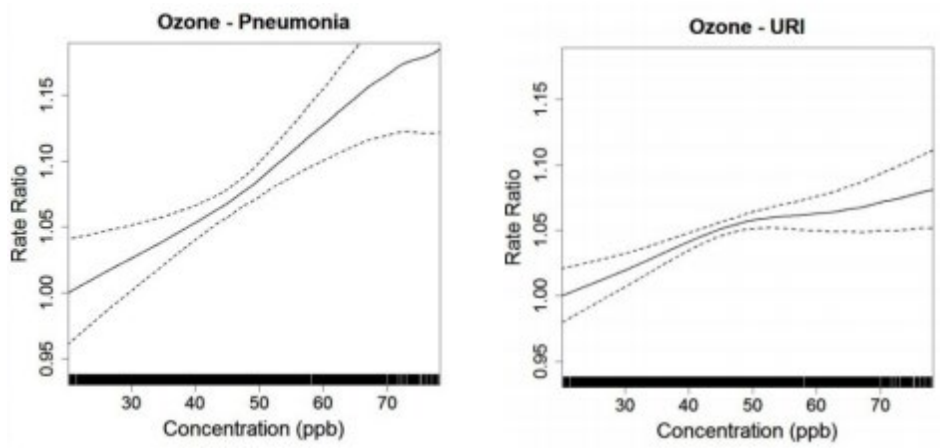
In addition, a small number of recent studies show conflicting evidence of C-R nonlinearity and the presence of a threshold. In contrast to evidence from the 2013 Ozone ISA, a multicity study in Texas estimated C-R curves using penalized spline models and observed evidence of nonlinearity in the relationship between 8-hour daily avg ozone and asthma hospital admissions (Zu et al., 2017). The C-R curves indicate the potential presence of a threshold between 30 and 40 ppb for children aged 5−14-years and adults aged 15−64-years (see Figure 3-10). The presence of a threshold in this range is supported by a recent statewide study in New Jersey that examined associations between pediatric asthma ED visits and quintiles of 8-hour daily max ozone exposure (Gleason et al., 2014). In comparison to the lowest quintile of ozone exposure, only quintiles 3 through 5 were associated with increased odds of ED visits. The third quintile exposure range started at 42.48 ppb.



Note: The solid lines are smoothed-fit data, with long broken lines indicating 95% confidence bands.
Source: Permission pending, Zu et al. (2017).

Figure 3-10 Estimated percent change in asthma hospital admissions for 8-hour daily avg ozone concentrations at lag 0−3 allowing for possible nonlinear relationships using penalized splines.

In contrast to recent results from studies of asthma hospital admissions, Darrow et al. (2014) reported evidence of approximately linear associations between ozone exposure and pneumonia and upper respiratory infection. Loess C-R curves provide evidence of an association down to 20 ppb, indicating that a threshold does not exist in the range of concentrations included in the study (Figure 3-11). Additionally, in a study of numerous respiratory outcomes in five U.S. cities, Barry et al. (2018) used maximum likelihood estimation to test for the presence of thresholds ranging from the minimum observed value (i.e., no threshold) to 50 ppb in 1 ppb increments. The presence of 8-hour daily max thresholds varied across cities and outcomes, but ranged from no threshold to 40 ppb, with the most commonly identified thresholds in the 20 to 30 ppb range. The authors also tested linearity by fitting a number of flexible models and comparing Akaike's Information Criteria values to determine the best fit. The best model fit also varied by city and outcome, with linear models and cubic spline models providing the best fit in an equal number of cases.



Note: The reference for the rate ratio is the estimated rate at the 5th percentile of the pollutant concentration. Estimates are presented for the 5th percentile through the 95th percentile of pollutant concentrations due to instability in the C-R estimates at the distribution tails. The solid lines are smoothed-fit data, with long broken lines indicating 95% confidence bands.

Source: Permission pending, Darrow et al. (2014).

Figure 3-11 Loess C-R estimates and twice-standard-error estimates from generalized additive models for associations between 3-day moving avg 8-hour daily max ozone concentrations and Emergency Department (ED) visits for pneumonia and upper respiratory infection.

3.1.11 Summary and Causality Determination

In the 2013 Ozone ISA, it was concluded that "there is a causal relationship between short-term ozone exposure and respiratory health effects" (U.S. EPA, 2013a). This causality determination was made on the basis of a strong body of evidence integrated across controlled human exposure, animal toxicological, and epidemiologic studies, in addition to established findings from previous AQCDs, demonstrating respiratory effects due to short-term exposure to ozone (U.S. EPA, 2006, 1996a). In particular, controlled human exposure studies provided evidence of lung function decrements, respiratory symptoms, and increased inflammation in young healthy adults exposed to ozone concentrations as low as 60 ppb. Dose-dependent increases in airway responsiveness were also noted after exposures to 0, 80, 100, and 120 ppb ozone. These studies were supported by epidemiologic studies that not only reported ozone-related respiratory effects in healthy populations, but also provided evidence of ozone associations with asthma exacerbation, COPD exacerbation, and hospital admissions and ED visits for combined respiratory disease. Additionally, there was consistent evidence of an association between short-term increases in ambient ozone concentrations and increases in respiratory mortality. Results observed in controlled human exposure and epidemiologic studies were supported by animal toxicological studies that indicated changes to ventilatory parameters, increased airway responsiveness, and lung injury and

inflammatory responses resulting from ozone exposures. Experimental studies also described the potential mechanistic pathways that underlie the respiratory effects observed in epidemiologic studies. Taken together, the synthesized results provided compelling evidence of a causal relationship between short-term exposure to ozone and respiratory effects.

Recent studies further expand the body of evidence regarding the relationship between short-term exposure to ozone and respiratory effects (Table 3-2). Evidence from a recent controlled human exposure study of respiratory effects in healthy adults is consistent with findings from prior assessments demonstrating post-exercise decrements in group mean pulmonary function after ozone exposures as low as 60 ppb in young adults (Section 3.1.4.1.1). There were no recent experimental studies in humans that examined respiratory symptoms in relation to short-term ozone exposures. However, ozone-induced respiratory symptoms in combination with FEV_1 decrements in young healthy adults at concentrations as low as 70 ppb were reported in the 2013 Ozone ISA (U.S. EPA, 2013a).

Controlled human exposure studies evaluated in the 2006 Ozone AQCD (U.S. EPA, 2006) and the 2013 Ozone ISA (U.S. EPA, 2013a) also provide consistent evidence of ozone-induced increases in airway responsiveness and inflammation in the respiratory tract and lungs. Recent studies expand on observed interindividual variability in inflammatory responses, providing additional evidence that GSTM1-null individuals are more susceptible to ozone-related inflammatory responses (Section 3.1.4.4.1). Recent animal toxicological studies are consistent with evidence summarized in the 2013 Ozone ISA (U.S. EPA, 2013a) and support the evidence observed in healthy humans. Specifically, recent studies demonstrated altered ventilatory parameters and increases in airway responsiveness, inflammation, injury, and oxidative stress following ozone exposures. Additionally, repeated exposure to ozone resulted in type 2 immune responses in upper and lower airways (Section 3.1.4.4.2).

Evidence from epidemiologic studies of healthy populations is generally coherent with experimental evidence, although the majority of the evidence comes from panel studies that were previously evaluated in the 2013 Ozone ISA (U.S. EPA, 2013a). A number of panel studies of children in summer camps observed decreases in FEV_1 and increases in markers of pulmonary inflammation associated with increases in short-term ozone exposure. In contrast to coherence of panel studies with experimental evidence of ozone-induced lung function decrements and respiratory tract inflammation, respiratory symptoms were not associated with ozone exposure in a limited number of panel studies. However, these studies of children generally relied on parental reported outcomes that may result in under- or over-reporting of respiratory symptoms.

Evidence from a large number of recent, large multicity epidemiologic studies conducted in the U.S. also expand upon evidence from the 2013 Ozone ISA (U.S. EPA, 2013a) to provide further support for an association between ozone and ED visits and hospital admissions for asthma (Section 3.1.5.1 and Section 3.1.5.2). Observed associations were generally of greatest magnitude for children between the ages of 5 and 18 years. Additionally, associations were observed across models implementing measured and modeled ozone concentrations. While there is a lack of recent epidemiologic studies conducted in the

U.S. or Canada that examine respiratory symptoms and medication use, lung function, and subclinical effects in people with asthma, a large body of evidence from the 2013 Ozone ISA (U.S. EPA, 2013a) reported ozone associations with these less severe markers of asthma exacerbation that provide support for the ozone-related increases in asthma hospital admissions and ED visits observed in recent studies. Recent experimental studies in animals, along with similar studies summarized in the 2013 Ozone ISA (U.S. EPA, 2013a), provide coherence with the epidemiologic evidence of asthma exacerbation, indicating respiratory tract inflammation, oxidative stress, injury, allergic skewing, goblet cell metaplasia, and upregulation of mucus synthesis and storage in allergic mice exposed to ozone (Section 3.1.5.4, Section 3.1.5.5, and Section 3.1.5.6).

In addition to epidemiologic evidence of asthma exacerbation, and consistent with studies reviewed in the 2013 Ozone ISA (U.S. EPA, 2013a), several recent epidemiologic studies provide evidence of an association between ozone and hospital admissions and ED visits for combined respiratory diseases (Section 3.1.8), ED visits for respiratory infection (Section 3.1.7.1), and ED visits for COPD (Section 3.1.6.1.1). A limited number of recent epidemiologic studies examining respiratory mortality were inconsistent (Section 3.1.9), but should be considered in the context of studies evaluated in the 2013 Ozone ISA that provided consistent evidence of an association between short-term ozone exposure and respiratory mortality(U.S. EPA, 2013a). A limited number of recent controlled human exposure and animal toxicological studies are consistent with studies evaluated in the 2013 Ozone ISA (U.S. EPA, 2013a) that demonstrate altered immunity and impaired lung host defense following acute ozone exposure (Section 3.1.7.3). These findings support the epidemiologic evidence of an association between ozone concentrations and respiratory infection. Additionally, results from recent animal toxicological studies provide new evidence that chronic inflammation enhances sensitivity to ozone exposure, providing coherence for ozone-related increases in ED visits for COPD (Section 3.1.6.1.2).

Recent mechanistic studies in humans and animals have expanded on findings from previous assessments (U.S. EPA, 2013a, 2006, 1996a) and improved the understanding of plausible pathways that may underlie the observed respiratory health effects resulting from short-term exposure to ozone. Notably, changes in lung function may be attributed to activation of sensory nerves in the respiratory tract that trigger local and autonomic reflex responses. Modest increases in airway resistance may occur due to activation of parasympathetic pathways. Mechanistic studies also present a plausible pathway by which ozone reacts with respiratory tract components to produce oxidized species that injure barrier function and activate innate immunity, resulting in a cycle of inflammation, injury, and oxidative stress. A recent animal toxicological study has also demonstrated that vagal C-fibers, vagal myelinated fibers, and possibly neuropeptides released in the airway are involved in increased airway responsiveness and bronchoconstriction in allergic animals. Together, results from mechanistic studies may provide biological plausibility for evidence of ozone-related lung function decrements and increased asthma symptoms from epidemiologic panel studies in healthy children and in children with asthma. Furthermore, they support the results of epidemiologic studies showing associations between ozone exposure and asthma-related ED visits and hospital admissions.

Copollutant analyses were limited in epidemiologic studies evauated in the 2013 Ozone ISA, but did not indicate that associations between ozone concentrations and respiratory effects were confounded by copollutants or aeroallergens (U.S. EPA, 2013a). Copollutant analyses have been more prevalent in recent studies and continue to suggest that observed associations are independent of coexposures to correlated pollutants or aeroallergens (Section 3.1.10.1 and Section 3.1.10.2). Despite expanded copollutant analyses in recent studies, determining the independent effects of ozone in epidemiologic studies is complicated by the high copollutant correlations observed in some studies, and the possibility for effect estimates to be overestimated for the better measured pollutant in copollutant models (Section 2.5). Nonetheless, the consistency of associations observed across studies with different copollutant correlations, the generally robust associations observed in copollutant models, and evidence from controlled human exposure studies demonstrating respiratory effects in response to ozone exposure in the absence of other pollutants, provide compelling evidence for the independent effect of short-term ozone exposure on respiratory symptoms.

Epidemiologic studies have also attempted to inform our understanding of the lag structure (Section 3.1.10.3) and the shape of the C-R relationship (Section 3.1.10.4) for associations between short-term exposure to ozone and respiratory effects. The largest evidence base for lag structure comes from studies of ozone exposure and hospital admissions or ED visits for asthma. The strongest single-day associations were generally observed with ozone concentrations on the same day as the outcome, but positive associations were present across a range of lags, extending as far as 6 days prior to the health outcome of interest. This range indicates that ozone may elicit both immediate and prolonged respiratory effects. Studies examining the shape of the C-R relationship and/or the presence of a threshold have been inconsistent. While most studies assume a no-threshold, log-linear C-R shape, a limited number of studies have used more flexible models to test this assumption. Results from some of these studies indicate approximately linear associations between ozone concentrations and hospital admissions for asthma, while others indicate the presence of a threshold ranging from 20 to 40 ppb 8-hour max ozone concentrations.

In summary, recent studies evaluated since the completion of the 2013 Ozone ISA support and expand upon the strong body of evidence that indicated a causal relationship between short-term ozone exposure and respiratory health effects. Controlled human exposure studies demonstrate ozone-induced decreases in FEV_1 and pulmonary inflammation at concentrations as low as 60 ppb after 6.6 hours of exposure. The combination of lung function decrements and respiratory symptoms has been observed following 70 ppb and greater ozone concentrations following 6.6 hour exposures. Epidemiologic studies continue to provide evidence that increased ozone concentrations are associated with a range of respiratory effects, including asthma exacerbation, COPD exacerbation, respiratory infection, and hospital admissions and ED visits for combined respiratory diseases. A large body of toxicological studies demonstrate ozone-induced changes in ventilatory parameters, inflammation, increased airway responsiveness, and impaired lung host defense. Additionally, mouse models indicate enchanced ozone-induced inflammation, oxidative stress, injury, allergic skewing, goblet cell metaplasia, and

upregulation of mucus synthesis and storage in allergic mice compared to naïve mice. These toxicological results further inform the potential mechanistic pathways that underlie downstream respiratory effects, providing continued support for the biological plausibility of the observed epidemiologic results. Thus, the recent evidence integrated across disciplines, along with the total body of evidence evaluated in previous integrated reviews, **is sufficient to conclude that there is a causal relationship between short-term ozone exposure and respiratory health effects.**

Table 3-2 Summary of evidence indicating a causal relationship between short-term ozone exposure and respiratory effects.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Respiratory Effects in Healthy Populations			
Consistent evidence from controlled human exposure studies at relevant concentrations	Studies show:		
	Decrements in lung function	Section 3.1.4.1.1	60−400 ppb
	Increased respiratory symptoms	Section 3.1.4.2.1	70−400 ppb
	Increased airway responsiveness	Section 3.1.4.3.1	80−1,000 ppb
	Inflammation, injury, and oxidative stress	Section 3.1.4.4.1	60−600 ppb
Consistent evidence from toxicological studies at relevant concentrations	Studies show:		
	Altered ventilatory parameters	Section 3.1.4.1.2	0.1−2 ppm
	Cough response	Clay et al. (2016)	2 ppm
	Increased airway responsiveness	Section 3.1.4.3.2	0.3−2 ppm
	Inflammation, injury, and oxidative stress	Section 3.1.4.4.2	0.15−2 ppm
	Type 2 immune responses—upper and lower airways	Harkema et al. (2017); Kumagai et al. (2017); Ong et al. (2016); Kumagai et al. (2016).	0.5−0.8 ppm

Table 3 2 (Continued): Summary of evidence indicating a causal relationship between short term ozone exposure and respiratory effects.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Coherence in epidemiologic studies of respiratory effects in healthy children	Panel studies provide support for experimental studies with consistent associations for lung function and pulmonary inflammation in healthy children	Section 3.1.4.1.3	32.6 ppb (8- h moving avg) 53−123 ppb (1- h avg)
		Section 3.1.4.4.3	31.6 ppb 8- h moving avg
Evidence supporting biological plausibility	Controlled human exposure studies provide evidence showing involvement of vagal C-fibers in pain on inspiration, decreased forced vital capacity, and altered breathing frequency. In addition, there is involvement of parasympathetic pathways leading to increased airway resistance	Section 3.1.4.1.1	400−420 ppb
	Animal toxicological studies provide evidence that changes in lung function may be attributed to activation of sensory nerves and involvement of parasympathetic pathways	Section 3.1.4.1.2 Section 3.1.4.3.2	
		Clay et al. (2016) Verhein et al. (2013)	2 ppm
Respiratory Effects in Populations with Asthma			
Consistent epidemiologic evidence from multiple, high-quality studies at relevant concentrations	Increases in asthma-related hospital admissions and ED visits in children, and all ages combined in studies conducted in the U.S. and Canada	Section 3.1.5.1 Section 3.1.5.2	8- h max/avg: 30.7−53.9 ppb 24- h avg: 22.5−41.9 ppb
Consistent evidence from controlled human exposure studies at relevant concentrations	Studies show that individuals with asthma experience all the ozone-induced respiratory outcomes (e.g., lung function decrements) observed in individuals without asthma. However, studies are not available at concentrations below 125 ppb	Section 3.1.5.3.1 Section 3.1.5.4.1 Section 3.1.5.5.1 Section 3.1.5.6.1	≥125 ppb
Consistent evidence from toxicological studies at relevant concentrations	Studies show enhanced allergic responses, bronchoconstriction, airway responsiveness, and altered ventilatory parameters in animal models of allergic airway disease	Section 3.1.5.4.2 Section 3.1.5.5.2 Section 3.1.5.6.2	0.1−2 ppm

Table 3 2 (Continued): Summary of evidence indicating a causal relationship between short term ozone exposure and respiratory effects.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Coherence in epidemiologic studies across the continuum of effects	Panel studies in children with asthma provide support for asthma exacerbation in children, with consistent associations for respiratory symptoms, lung function decrements, and pulmonary inflammation	Section 3.1.5.3.2 Section 3.1.5.4.3 Section 3.1.5.6.3	1- h max: 43.0−65.8 ppb 8- h max: 31.6−52.9 ppb
Epidemiologic evidence from copollutant models provide some support for an independent ozone association	Potential copollutant confounding is examined in a number of studies with evidence that associations persist in models with gaseous pollutants and $PM_{2.5}$	Section 3.1.10.1	
Evidence supporting biological plausibility	Evidence from animal toxicological study demonstrates involvement of vagal C-fibers in increased airway resistance and airway responsiveness in a model of allergic airway disease, providing biological plausibility for epidemiologic findings for exacerbation of allergic asthma, the most common asthma phenotype in children	Schelegle and Walby (2012)	1 ppm
Respiratory Effects in Populations with COPD			
Consistent epidemiologic evidence from a limited number of high-quality multicity studies at relevant concentrations	Increases in ED visits for COPD in studies conducted in the U.S. and Canada	Stieb et al. (2009)	24- h avg: 18.4 ppb
		Malig et al. (2016)	1- h max: 33−55 ppb
		Szyszkowicz et al. (2018)	24- h avg: 22.5−29.2 ppb
Consistent evidence from a limited number of toxicological studies at relevant concentrations	Results show enhanced injury, inflammation, oxidative stress, and altered morphology and lung mechanics in animal model of COPD	Groves et al. (2012) Groves et al. (2013)	0.8 ppm
But, lack of coherence in epidemiologic studies across the continuum of effects	Panel studies in adults with COPD do not observe ozone associations with lung function or respiratory symptoms in adults with COPD	Peacock et al. (2011); Magzamen et al. (2018)	
Also, limited evaluation of confounding by copollutants	Potential copollutant confounding is examined in a single study, with evidence that associations remain robust in copollutant models adjusted for gaseous pollutants	Malig et al. (2016)	

Table 3 2 (Continued): Summary of evidence indicating a causal relationship between short term ozone exposure and respiratory effects.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Respiratory Infection			
Generally consistent epidemiologic evidence from multiple, high-quality studies at relevant concentrations	Increases in ED visits for:		
	Pneumonia	Malig et al. (2016); Xiao et al. (2016)	1- h max: 33−55 ppb 8- h max: 42.1 ppb
	Acute respiratory infections	Malig et al. (2016)	1- h max: 33−55 ppb
	Upper respiratory tract infections	Malig et al. (2016); Xiao et al. (2016); Szyszkowicz et al. (2018); Barry et al. (2018).	1- h max: 33−55 ppb 8- h max: 37.5−42.2 ppb 24- h avg: 22.5−29.2 ppb
Coherence in toxicological studies at relevant concentrations	Increased susceptibility to infectious disease	Section 3.1.7.3	0.08−2 ppm
	Increased inflammatory response to infectious disease	Mikerov et al. (2011)	2 ppm
Evidence of biological plausibility	Animal toxicological studies show increased susceptibility to infections	Section 3.1.7.3	0.08−2 ppm
Combinations of Respiratory-Related Hospital Admissions and ED Visits			
Epidemiologic studies provide consistent evidence of positive associations when examining combined respiratory-related diseases	Increases in hospital admissions and ED visits for combined respiratory-related diseases in multicity studies	Section 3.1.8	1- h max: 33−55 ppb 8- h max: 30.7−50.3 ppb
But, limited evaluation of confounding by copollutants	Potential copollutant confounding is examined in a limited number studies, with evidence that associations generally remain robust in models with gaseous pollutants	Section 3.1.10.1	
Respiratory Mortality			
Generally consistent epidemiologic evidence from multiple, high-quality studies at relevant concentrations	Generally consistent evidence of increases in mortality in response to short-term ozone exposure in multicity studies in the U.S. and Canada. Evidence of effects within the first 2 days of exposure (lag 0 to 2 days)	Section 6.1.4	1- h max: 6.7−38.4 ppb 8- h avg/max: 15.1−62.8 ppb 24- h avg: 19.3 ppb

Table 3 2 (Continued): Summary of evidence indicating a causal relationship between short term ozone exposure and respiratory effects.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
But, limited evaluation of confounding by copollutants	Potential copollutant confounding for is examined in a single study, with evidence that associations remain robust in copollutant models adjusted PM_{10}	Katsouyanni et al. (2009)	

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble to the ISAs (U.S. EPA, 2015).

[b]Describes the key evidence and references, supporting or contradicting, contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where full body of evidence is described.

[c]Describes the ozone concentrations with which the evidence is substantiated. For epidemiologic studies, the study area mean or median ozone concentrations from the relevant studies are reported. Study-specific ozone concentrations are presented in the evidence inventories (Section 3.3.1).

3.2 Long-Term Ozone Exposure

3.2.1 Introduction, Summary from the 2013 Ozone ISA, and Scope for Current Review

The 2013 Ozone ISA concluded that "*there is likely to be a causal relationship between long-term exposure to ozone and respiratory health effects*" (U.S. EPA, 2013a). The epidemiologic evidence for a relationship between long-term ozone exposure and respiratory health effects was provided by studies of new-onset asthma, respiratory symptoms in children with asthma, and respiratory mortality. Associations between long-term exposure to ozone and new-onset asthma in children and increased respiratory symptoms in individuals with asthma were primarily observed in studies that examined interactions between ozone and exercise or different genetic variants. The evidence relating new-onset asthma to long-term ozone exposure was supported by toxicological studies of allergic airways disease in infant monkeys. This nonhuman primate evidence that ozone exposure altered airway development supported the biological plausibility of early-life exposure to ozone contributing to asthma development in children. Generally, the epidemiologic and toxicological evidence provided a compelling case that supported the causality determination for long-term exposure to ambient ozone and measures of respiratory health effects. Results from a limited number of epidemiologic studies examining potential copollutant confounding suggested that the observed associations were robust to adjustment for other pollutants, including $PM_{2.5}$ in a study of long-term ozone exposure and respiratory mortality. Additionally, the evidence for short-term exposure to ozone and effects on respiratory endpoints provided support for the observed respiratory health associations with long-term exposure to ozone. Building upon

that evidence, the more recent epidemiologic evidence, combined with toxicological studies in rodents and nonhuman primates, provides biologically plausible evidence of a *likely to be causal* relationship between long-term exposure to ozone and respiratory effects.

The following section on long-term ozone exposure and respiratory effects begins with an overview of study inclusion criteria (Section 3.2.2) that defines the scope of the literature to be considered for inclusion in the section. The ensuing section presents a discussion of biological plausibility (Section 3.2.3) that provides background for the subsequent sections in which groups of related endpoints are presented in the context of relevant disease pathways. The respiratory effects subsections are organized by outcome group and aim to clearly characterize the extent of coherence among related endpoints and biological plausibility of ozone effects. These outcome groups include development of asthma (Section 3.2.4.1), lung function and development (Section 3.2.4.2), development of COPD (Section 3.2.4.3), respiratory infection (Section 3.2.4.4), severity of respiratory disease (Section 3.2.4.5), allergic responses (Section 3.2.4.6), respiratory effects in healthy pregnancy (Section 3.2.4.7), respiratory effects in populations with metabolic syndrome (Section 3.2.4.8), and respiratory mortality (Section 3.2.4.9). Finally, Section 3.2.5 comprises an integrated discussion of relevant issues for interpreting the epidemiologic evidence discussed in Section 3.2.4. Throughout the sections on respiratory health effects, results from recent studies are evaluated in the context of the evidence provided by previous studies in the 2013 Ozone ISA (U.S. EPA, 2013a). Study-specific details, including exposure time periods and exertion levels in experimental studies, and study design, averaging times, and select results in epidemiologic studies are presented in evidence inventories in Section 3.3.

3.2.2 Population, Exposure, Comparison, Outcome, and Study Design (PECOS) Tool

The scope of this section is defined by a scoping tool that generally describes the relevant Population, Exposure, Comparison, Outcome, and Study Design (PECOS). The PECOS tool defines the parameters and provides a framework to help identify the relevant literature to inform the draft 2019 Ozone ISA. Because the 2013 Ozone ISA concluded there is *a likely to be causal relationship* between long-term ozone exposure and respiratory health effects, the recent epidemiologic studies evaluated in this ISA are limited to study locations in the U.S. and Canada to provide a focus on study populations and air quality characteristics that are most relevant to circumstances in the U.S. The studies evaluated and subsequently discussed within this section were included if they satisfied all of the components of the following PECOS tool:

Experimental studies:

- Population: Study population of any animal toxicological study of mammals at any lifestage
- Exposure: Long-term (on the order of months to years) or perinatal inhalation exposure to relevant ozone concentrations (i.e., ≤2 ppm)

- Comparison: Appropriate comparison group exposed to a negative control (i.e., clean air or filtered air control)
- Outcome: Respiratory effects
- Study Design: Studies in mammals meeting the above criteria

Epidemiologic studies:

- Population: Any U.S. or Canadian population, including populations or lifestages that might be at increased risk
- Exposure: Long-term exposure (months to years) to ambient concentration of ozone
- Comparison: Per unit increase (in ppb), or humans exposed to lower levels of ozone compared with humans exposed to higher levels
- Outcome: Change in risk (incidence/prevalence) of respiratory effects
- Study Design: Epidemiologic studies consisting of panel, case-crossover, time-series studies, and case-control studies, as well as cross-sectional studies with appropriate timing of exposure for the health endpoint of interest

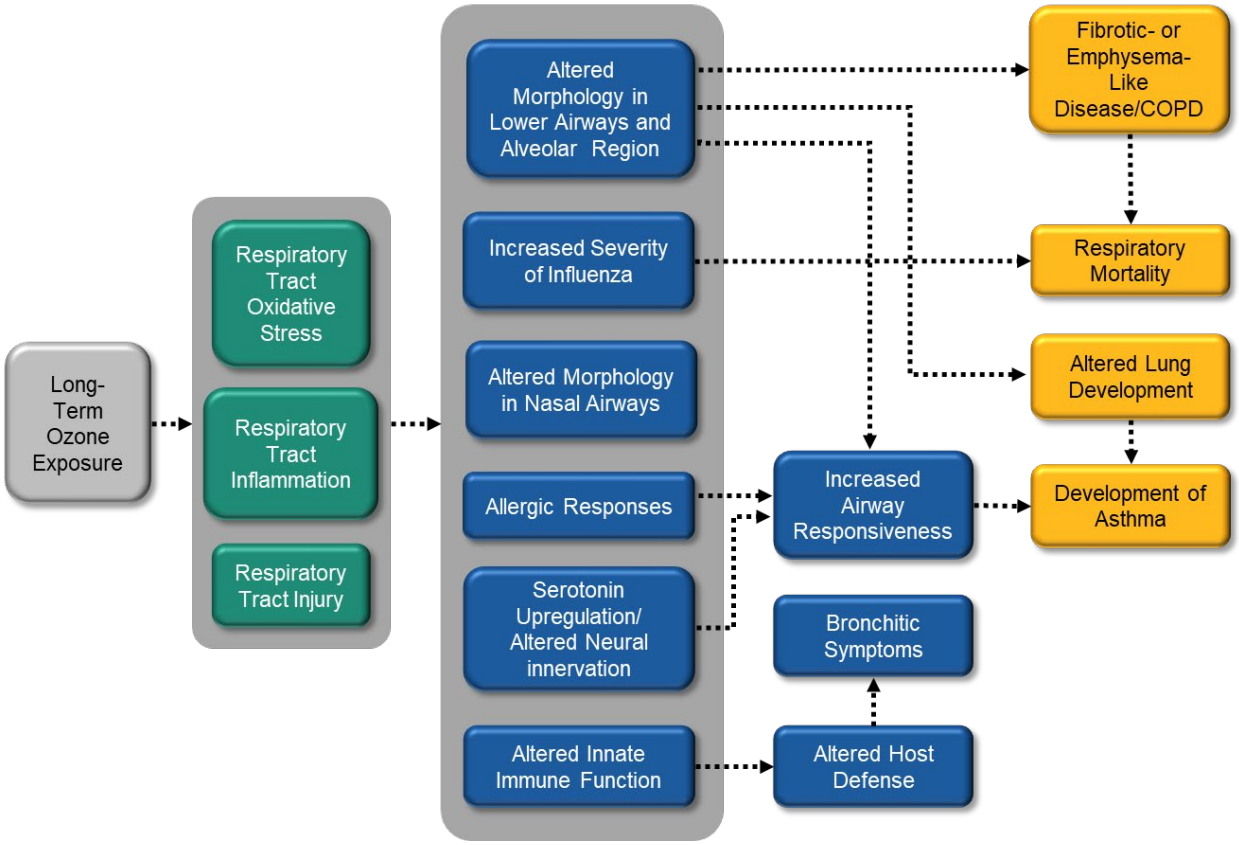
3.2.3 Biological Plausibility

This section describes biological pathways that potentially underlie respiratory health effects resulting from long-term exposure to ozone. Figure 3-12 graphically depicts the proposed pathways as a continuum of upstream events, connected by arrows, that lead to downstream events observed in epidemiologic studies. This discussion of how long-term exposure to ozone may lead to respiratory health effects contributes to an understanding of the biological plausibility of epidemiologic results evaluated later in Section 3.2.4.

Evidence that long-term exposure to ozone may affect the respiratory tract generally informs one proposed pathway (Figure 3-12). It begins with oxidative stress, inflammation, and injury in the respiratory tract, as demonstrated by studies in rodents described in the 2013 Ozone ISA (U.S. EPA, 2013a) and more recently. These responses, which are difficult to disentangle, were also observed in some studies of short-term exposure to ozone. Prolonged or intermittent exposure of adult rodents to ozone over months to years led to persistent inflammation and morphologic alterations, including fibrotic- and emphysematous-like changes (U.S. EPA, 2013a). Also discussed in the 2013 Ozone ISA was an increase in the severity of post-influenza alveolitis and injury to nasal airways that resulted in altered structure and function of the nose (U.S. EPA, 2013a). In an infant monkey model of allergic airway disease, postnatal ozone exposure compromised airway growth and development and resulted in changes that favor allergic airways responses and persistent effects on the immune system (U.S. EPA, 2013a). Baseline airway responsiveness and nonspecific airway responsiveness were increased, morphologic changes occurred that were consistent with increased airway responsiveness, airway neural innervation was altered, and a

host defense response was diminished. These types of alterations in structure and function in the developing lung may underlie the development of asthma.

Recent studies include those conducted in adult and neonatal rodents and those conducted in infant monkeys. Studies in adult rodents found that long-term exposure to ozone results in respiratory tract oxidative stress, inflammation, and injury (Gordon et al., 2016b; Gordon et al., 2016a; Miller et al., 2016a; Snow et al., 2016). Similar findings were reported in the developing lungs of rodents exposed postnatally to ozone (Dye et al., 2017; Gabehart et al., 2015; Gabehart et al., 2014). In addition, secretion and upregulation of mucus expression, which can offer protection against injury, were increased, while cell proliferation was decreased in the neonatal rodents.



Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence related to ozone exposure, and the arrows indicate a proposed relationship between those effects. Solid arrows denote evidence of essentiality as provided, for example, by an inhibitor of the pathway or a genetic knockout model used in an experimental study involving ozone exposure. Shading around multiple boxes is used to denote a grouping of these effects. Arrows may connect individual boxes, groupings of boxes, and individual boxes within groupings of boxes. Progression of effects is generally depicted from left to right and color-coded (gray, exposure; green, initial effect; blue, intermediate effect; orange, effect at the population level or a key clinical effect). Here, population level effects generally reflect results of epidemiologic studies. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below.

Figure 3-12 Potential biological pathways for respiratory effects following long-term ozone exposure.

Postnatal ozone exposure had morphological effects. For example, decreased sensory neuron development (Zellner et al., 2011) and altered airway architecture (Lee et al., 2011) were demonstrated in rodents. Studies in infant monkeys found altered components of a cell death pathway, altered expression of serotonin, which is a neurotransmitter involved in airway smooth muscle contraction, and altered innate immune function (Clay et al., 2014; Murphy et al., 2014; Murphy et al., 2013). Alterations in cell growth and cell death pathways observed in these long-term studies may underlie changes in structure (i.e., airway architecture) in the developing lung. Effects on serotonin could potentially underlie changes in function in the developing lung (i.e., increased airway responsiveness), while effects on innate immune function may lead to altered immune response. Studies in the infant model of allergic airway disease model found impaired alveolar morphogenesis (Herring et al., 2015; Avdalovic et al., 2012), airway

smooth muscle hyperreactivity (Moore et al., 2012), an enhanced allergic phenotype (Crowley et al., 2017; Chou et al., 2011), and priming of responses to oxidant stress (Murphy et al., 2012).

As described here, there is one main pathway, with many branches, by which long-term exposure to ozone could lead to respiratory health effects. It involves respiratory tract oxidative stress, inflammation, and injury as early events resulting from prolonged exposure. Respiratory tract inflammation may also lead to morphologic and immune system-related changes that may affect the structure and function of the respiratory tract. In adult animals these changes may underlie the progression and development of chronic lung disease. In developing lungs, these changes may underlie impaired lung development or the development of asthma. The multibranched pathway described here may provide biological plausibility for the epidemiologic evidence of COPD- and influenza-related mortality in adults. Increased severity of infection-related lung disease may also underlie mortality. In addition, ozone-related effects on the developing lung may provide biological plausibility for epidemiologic evidence for new-onset asthma and increased respiratory symptoms in children with asthma. These pathways will be used to inform a causality determination, which is discussed later in the Appendix (Section 3.2.6).

3.2.4 Respiratory Health Effects

3.2.4.1 Development of Asthma

Asthma is a chronic inflammatory disease of the airways that develops over time (NHLBI, 2017). Pulmonary inflammation can increase airway responsiveness and induce airway remodeling, resulting in bronchoconstriction (bronchial smooth muscle contraction), and in turn, episodes of shortness of breath, coughing, wheezing, and chest tightness. When the pathophysiology of asthma advances to the stage at which symptoms lead people to seek medical treatment, a diagnosis of asthma can result.

3.2.4.1.1 Epidemiologic Studies

The 2013 Ozone ISA reported evidence of an association between long-term exposure to ozone and asthma incidence in adults from two epidemiologic studies of the same cohort (U.S. EPA, 2013a). Evidence was available from a cohort study and a subsequent extended follow-up of nonsmoking, non-Hispanic, white, Seventh-Day Adventist adults in California (McDonnell et al., 1999a; Greer et al., 1993). The association, which was only observed in stratified analyses of male participants, was robust to the inclusion of PM_{10}, SO_4^{2-}, SO_2, and NO_2 in copollutant models (McDonnell et al., 1999a). Notably, the results provide limited generalizability, given the restricted cohort demographics.

Studies evaluated in the 2013 Ozone ISA did not provide evidence of a main effect of long-term ozone exposure on asthma incidence in children, but they did indicate potential interactions between ozone and exercise or different genetic variants on childhood asthma (U.S. EPA, 2013a). In analyses of the Children's Health Study (CHS) cohort in southern California, Islam et al. (2008) and Salam et al. (2009) observed evidence of interaction between ozone concentrations and functional polymorphisms of the heme oxygenase-1 gene and variants in genes for arginase, respectively, related to the risk of new-onset asthma in children. In the same cohort, McConnell et al. (2002) reported increased asthma incidence in children who played three or more sports in high-ozone communities, compared with those who played no sports. In contrast, no such association was observed in low-ozone communities, indicating that ozone concentrations may modify the effect of exercise and asthma development.

A limited number of recent studies provide evidence of an association between long-term exposure to ozone and asthma development in children. Only a few recent epidemiologic studies in the U.S. or Canada examine asthma development or subclinical effects underlying asthma development in children, while none focus on asthma development in adults. Study specific details, including air quality characteristics and select effect estimates, are highlighted in Table 3-43 in Section 3.3.2. An overview of the evidence is provided below.

- A recent CHS analysis examined asthma incidence in relation to improved air quality in nine southern California communities (Garcia et al., 2019). Decreases in baseline ozone concentrations in three CHS cohorts, enrolled in 1993, 1996, and 2006, were associated with decreased asthma incidence. The findings indicate that improved air quality is associated with lower asthma incidence. The magnitude and precision of the observed association was comparable in a model adjusting for local near-road pollution. Due to modeling constraints, the authors used 1 year ozone concentrations at baseline.
- In analyses of a large administrative database birth cohort in Quebec, an increase in average summertime ozone concentrations at participants' birth addresses were associated with a 19% increase (95% CI: 16, 23%) in asthma onset in children of all ages (Tétreault et al., 2016a). Notably, the associations were present at low concentrations, and were robust in sensitivity analyses that used time-varying ozone concentrations or relied on a more stringent case definition for children under five, an age group with less reliable asthma diagnoses.
- In contrast, a pooled retrospective case-control analysis of minority children in the U.S. reported null associations between early-life ozone exposure and asthma incidence (Nishimura et al., 2013). The study was much smaller than Tétreault et al. (2016a) and consequently had less precision (i.e., wider 95% CIs).
- Results from a previous CHS analysis (Bastain et al., 2011) showed that elevated eNO was associated with increased risk of asthma development in children. However, Berhane et al. (2014) examined airway inflammation in response to long-term ozone exposure in the CHS cohort and observed a null association between ozone and changes in eNO in children.

In summary, recent studies provide support for an association between long-term ozone exposure and asthma development in children. While one study presented contrasting evidence, the authors focused on a specific at-risk population and the study included fewer participants (Nishimura et al., 2013).

3.2.4.1.2 Animal Toxicological Studies

The 2013 Ozone ISA summarized the animal toxicological evidence of the development of asthma resulting from ozone exposure during the early postnatal period (U.S. EPA, 2013a). Several studies found that cyclic challenge of infant rhesus monkeys to allergen and ozone during the postnatal period compromised airway growth and development and resulted in changes that favor allergic airways responses and persistent effects on the immune system. Rhesus monkeys were chosen as a model because the branching pattern and distribution of airways in rhesus monkeys are more similar to humans than those of rodents. In addition, a model of allergic airways disease, which exhibits the main features of human asthma, had already been established in the adult rhesus monkey. Studies in infant monkeys were designed to determine whether repeated exposure to ozone altered postnatal lung growth and development, and if so, whether such effects were reversible. In addition, exposure to ozone was evaluated for its potential to increase the development of allergic airways disease. The animals were exposed episodically to ozone beginning at 1 month of age. The exposure regimen involved biweekly cycles of alternating filtered air and ozone (i.e., 9 consecutive days of filtered air and 5 consecutive days of 0.5 ppm ozone, 8 hour/day)and to house dust mite allergen (HDMA) for 2 hours per day for 3 days on the last 3 days of ozone exposure, followed by 11 days of filtered air. In most of these studies, infant monkeys were sensitized to HDMA before the start of the cyclical exposures. These animals exhibited the hallmarks of allergic asthma for humans including a positive skin test for HDMA with elevated levels of IgE in serum and IgE-positive cells within the tracheobronchial airway walls; impaired airflow which was reversible by treatment with aerosolized albuterol; increased abundance of immune cells, especially eosinophils in airway exudates and bronchial lavage; and development of nonspecific airway responsiveness.

The infant monkey studies reported numerous key findings (U.S. EPA, 2013a). Baseline airway resistance and airway responsiveness to inhaled histamine were dramatically increased by combined exposure to ozone plus HDMA. This finding suggests that long-term ozone exposure may contribute to the effects of asthma in children. A follow-up study assessing ex vivo airway responsiveness of the infant monkeys found that ozone plus HDMA exposure resulted in increased airway responsiveness in the respiratory bronchioles, where dosimetric models indicated that the dose would be higher. In another study, the growth pattern of distal airways was changed to a large extent by exposure to ozone alone and in combination with HDMA. More specifically, the airways became longer and narrower and the number of conducting airway generations between the trachea and the gas exchange area was decreased. This effect was not ameliorated by a recovery period of 6 months in filtered air. Other structural changes included increases in mucus goblet cell mass and alterations in smooth muscle orientation in the respiratory bronchioles, epithelial nerve fiber distribution, and basement membrane zone morphometry, all of which could potentially contribute to airway obstruction and increased airway responsiveness. Additional effects on neural innervation in the epithelium of the conducting airways were observed in response to ozone alone or ozone plus HDMA, including decreased nerve fiber density and altered nerve

bundle morphology. Six months of recovery in filtered air led to reversal of some, but not all, of these structural and functional effects.

As described in the 2013 Ozone ISA (U.S. EPA, 2013a), exposure to ozone also resulted in increased number and proportion of eosinophils and decreased number of neutrophils and lymphocytes in BALF and the blood of infant monkeys. These effects were not evident after a 6-month recovery period in filtered air. Challenge with LPS, which activates monocytes and other innate immune cells, elicited a lower response in ozone-exposed animals. While increased airway eosinophilia suggests an increased allergic profile, the decreased response to LPS suggests diminished host defenses. Other effects on the developing immune system of exposure to ozone plus HDMA included increased CD4+ and CD8+ lymphocytes in blood and BALF and activated lymphocytes (CD25+ cells) in airway mucosa.

The effect of cyclic episodic ozone exposure on nasal airways was also studied in the infant rhesus monkey (U.S. EPA, 2013a). The three-dimensional detail of the nasal passages was analyzed for developing predictive dosimetry models and exposure dose-response relationships. The relative amounts of the five epithelial cell types in the nasal airways remained consistent between infancy and adulthood. Ozone exposure resulted in 50−80% decreases in epithelial thickness and epithelial cell volume of the ciliated respiratory and transitional epithelium, confirming that these cell types in the nasal cavity were the most sensitive to ozone exposure. The character and location of nasal lesions were similar in infant and adult monkeys that were similarly exposed. However, the nasal epithelium of infant monkeys did not undergo nasal airway epithelial remodeling or adaptation which occurs in adult animals following ozone-mediated injury and which may protect against subsequent ozone challenge. This lack of remodeling suggests the potential for developing persistent necrotizing rhinitis following longer term exposure.

Recent studies have examined a wide range of effects of postnatal ozone exposure. Several studies were conducted in infant monkey model of allergic airway in which monkeys were sensitized to HDMA. Several other studies were conducted in nonallergic infant monkeys and neonatal rodents. A brief discussion of their findings is found below, with studies grouped according to the animal model employed. Study-specific details are summarized in Table 3-44, Table 3-45, Table 3-46, and Table 3-47 in Section 3.3.2. All of the effects described below were statistically significant. Recent studies add to previous evidence that postnatal ozone exposure may lead to the development of asthma by compromising airway growth and development, promoting the development of an allergic phenotype and increased airway responsiveness, and causing persistent alterations of the immune system.

Recent studies in the infant monkey model of allergic airway disease demonstrated airway smooth muscle hyperreactivity, an enhanced allergic phenotype, and priming of responses to oxidant stress as a result of postnatal ozone exposure.

- Postnatal ozone exposure increased airway smooth muscle contraction mediated by serotonin (Moore et al., 2012). Exposures were to episodic ozone beginning at 1 month of age. This involved biweekly cycles of alternating filtered air and ozone(i.e., 9 consecutive days of filtered

air and 5 consecutive days of 0.5 ppm ozone, 8 hour/day)and to house dust mite allergen (HDMA) for 2 hours per day for 3 days on the last 3 days of ozone exposure, followed by 11 days of filtered air. Eleven cycles of episodic ozone exposure (0.5 ppm) did not increase airway responsiveness to histamine in vivo or airway responsiveness to acetylcholine in an in vitro study using tracheal rings. In fact, responsiveness to acetylcholine was decreased by postnatal ozone exposure and exogenous serotonin. When electrical field stimulation, which causes the release of acetylcholine from airway nerves, was used to test the properties of the airway smooth muscle in the tracheal rings model, the response was not increased in the ozone or ozone/HDMA groups. But when exogenous serotonin was added, the electrical field stimulation response was enhanced in the ozone and ozone/HDMA groups. Airway smooth muscle contraction to electrical field stimulation is a measure of post-ganglionic and parasympathetic-mediated processes. Experiments with receptor agonists and antagonists found that postnatal ozone exposure enhanced the excitatory parasympathetic pathway in the presence of serotonin. Postnatal exposure to HDMA also had this effect and it enhanced intrinsic airway smooth muscle contractility. The result of postnatal exposure to ozone and HDMA was a hyperresponsive airway. This study provides evidence of a functional change in the airways due to postnatal ozone exposure. The presence of the neurotransmitter serotonin is required for the enhanced airway smooth muscle contraction. Given that previous work from this laboratory showed increased serotonin positive cells in the airway resulting from postnatal ozone exposure, the development of asthma in this model could be due to increased serotonin acting on post-ganglionic parasympathetic fibers to increase airway responsiveness.

- Two other recent studies found that postnatal exposure to ozone and HDMA altered immune system development, including effects on eosinophils that are characteristic of an allergic phenotype. In one study (Chou et al., 2011), episodic ozone exposure (five biweekly cycles, 0.5 ppm) resulted in decreased total white blood cells and blood eosinophils and increased BALF eosinophils. However, ozone exposure did not lead to an increase in airway mucosa eosinophils. Ozone/HDMA exposure increased BALF eosinophils and eotaxin, but no increase in airway mucosa eosinophils was found. In the study by Crowley et al. (2017), episodic ozone exposure (11 biweekly cycles, 0.5 ppm ozone) decreased numbers of monocytes and frequency of eosinophils in peripheral blood and increased the frequency of eosinophils in BALF. Exposure to ozone and HDMA had similar effects on blood monocytes, immune system development, including effects on eosinophils which are characteristic of an allergic phenotype.

- Postnatal ozone exposure primed the airway for an enhanced response to oxidant stress (Murphy et al., 2012). In this study, episodic ozone exposure (11 biweekly cycles, 0.5 ppm) enhanced responses of airway explant tissue to an exogenous oxidant, including increased expression of IL-8, a neutrophil chemokine, and increased expression of neurokinin-1 receptor, which is involved in a nonapoptotic pathway of cell death.

Recent studies in nonallergic infant monkeys demonstrated increased serotonin-positive airway cells and immunomodulation as a result of postnatal ozone exposure.

- Acute exposure to ozone (0.5 ppm, 8 hours) altered serotonin expression in a specific region of the developing lung (Murphy et al., 2013). Serotonin positive cells were increased in midlevel airways in 2-month-old monkeys. Serotonin is a neurotransmitter involved in airway smooth muscle contraction.

- Episodic exposure to ozone (11 biweekly cycles of 0.5 ppm) altered innate immune function in airway epithelia (Clay et al., 2014). Ozone exposure attenuated the inflammatory response to LPS, measured in an in vitro assay as gene expression of the cytokines IL-6 and IL-8. In contrast, ozone-exposed infant monkeys subsequently challenged with LPS in vivo exhibited increased

IL-6 and IL-8 gene expression in response to LPS in the vitro assay. These results suggest that early life exposure to ozone is immunomodulatory.

- Episodic ozone exposure decreased expression of components of a nonapoptotic cell death pathway that had been increased by a single acute ozone exposure (Murphy et al., 2014). In this study, ozone exposure consisted of 1 or 11 biweekly cycles (0.5 ppm) plus or minus an acute ozone exposure of 0.5 ppm for 8 hours. Results were compared with exposure to filtered air plus or minus acute ozone exposure. Components that were upregulated by acute ozone exposure included TAC1, which is the substance P precursor, neurokinin-1 receptor, and nuclear receptor 77. These same components were downregulated by episodic/acute ozone exposure in the same specific regions of the developing lung in which they were upregulated by a single acute ozone exposure.

Recent studies in rats demonstrated impaired airway growth and altered airway sensory nerve innervation as a result of postnatal ozone exposure.

- Persistent changes in airway architecture resulted from early postnatal exposure to ozone (Lee et al., 2011). Rats were exposed for 3 weeks to ozone (0.5 ppm × 6 hour/day) beginning on Postnatal Day (PND) 7. After a 56 days recovery period, diameter, length, and branching angle of conducting airways were examined. Decreased diameter and airway length were demonstrated for airway generations 7−22 and 16−20, respectively. These changes occurred when ozone exposures consisted of 5 days on and 2 days off per week, but not when ozone exposures consisted of 2 days on and 5 days off per week. This study suggests that early postnatal ozone exposure may impact airway resistance through effects on airway architecture.
- Two studies from the same laboratory examined effects of early postnatal ozone exposure on airway innervation in rats. Zellner et al. (2011) provides evidence of altered sensory neuron development. In rats, exposure to ozone (2 ppm for 3 hours) on Postnatal Day 5 resulted in decreased total neuron number at Postnatal Day 21, but no effect on substance P-containing neurons in the developing airway. Hunter et al. (2011) demonstrated that ozone exposure (2 ppm for 3 hours) on Postnatal Day 6 enhances the production of nerve growth factor by airways in response to a later ozone exposure on Postnatal Day 28. Postnatal ozone exposure also increased numbers of BALF neutrophils. Nerve growth factor may link ozone exposure to an increase in substance P-containing nerve fibers in the airways. Taken together, these two studies indicate that early postnatal ozone exposure affects the number, and possibly the type, of neurons in the developing airway.

3.2.4.1.3 Integrated Summary for Development of Asthma

The 2013 Ozone ISA (U.S. EPA, 2013a) presented evidence of ozone modified associations between exercise and asthma incidence in children, and interactions between ozone and different genetic variants on associations with childhood asthma. A recent large administrative cohort study provides evidence of an association between long-term ozone exposure and asthma development in children. This finding is supported by a CHS analysis that reported a decrease in childhood asthma incidence associated with decreases in ozone concentrations. A smaller cohort study focusing on minority children found a null association, but the larger studies provide compelling evidence of a positive association. Recent animal toxicological studies in rodents and monkeys support the epidemiologic results and findings from previous toxicological studies that postnatal ozone exposure may lead to the development of asthma by

compromising airway growth and development, promoting the development of an allergic phenotype, and causing persistent alterations to the immune system.

3.2.4.2 Lung Function and Development

After organogenesis in the embryonic stage, the development of the human lung continues throughout the fetal period and into early adulthood (Schittny, 2017). This continued development comprises an extended window of potential vulnerability to environmental stressors, such as ozone. To characterize lung health, lung function metrics capture the cumulative effects of pulmonary growth, damage, and repair (Wang et al., 1993). As such, measures of lung function are effective indicators of pulmonary effects related to exposure to environmental stressors.

3.2.4.2.1 Epidemiologic Studies

Epidemiologic studies evaluated in the 2013 Ozone ISA provided inconsistent evidence of an association between long-term exposure to ozone and lung development in children (U.S. EPA, 2013a). In an 8-year follow-up of the CHS cohort, Gauderman et al. (2004) observed a null association between mean annual 8-hour ozone concentrations and deficits in lung function growth (FEV_1). In contrast, in a subsequent CHS analysis, Breton et al. (2011) reported ozone-related deficits in 8-year lung function growth among children without a particular GSS glutathione gene haplotype. Cross-sectional studies of ozone and lung function in children or adults were similarly inconsistent.

A limited number of recent studies in the U.S. continue to provide inconsistent evidence of an association between ozone and lung development or lung function. Study-specific details, including air quality characteristics and select effect estimates, are highlighted in Table 3-48 in Section 3.3.2. An overview of the evidence is provided below.

- An extended follow-up of the CHS combined data obtained from three separate cohorts to examine the association between long-term reductions in air pollution and lung development in children between the ages of 11 and 15 (Gilliland et al., 2017; Gauderman et al., 2015). The authors did not observe a notable change in lung function growth or cross-sectional lung function corresponding to decreasing ozone concentrations.
- Other cross-sectional studies reported modest decreases in lung function metrics associated with ozone, including a pooled retrospective case-control analysis of minority children with asthma in the U.S. Neophytou et al. (2016) and another analysis of a recent CHS cohort that overlaps with one of the cohorts included in the Gauderman et al. (2015) study (Urman et al., 2014).
- While cross-sectional studies of adult lung function evaluated in the 2013 Ozone ISA provided inconsistent evidence of an association with ozone (Forbes et al., 2009; Qian et al., 2005), a recent longitudinal study of lung function in older adults in the U.S. reported decrements in FEV_1 and FVC relative to ozone concentrations (Eckel et al., 2012).

In summary, a limited number of recent studies continue to provide inconsistent evidence of an association between long-term ozone exposure and lung development or lung function in children. While the only recent study that examined lung function in adults observed evidence of an association, this result should be considered in the context of inconsistent evidence presented in the 2013 Ozone ISA (U.S. EPA, 2013a).

3.2.4.2.2 Animal Toxicological Studies

The 2013 Ozone ISA summarized the animal toxicological evidence of altered lung function and development resulting from ozone exposure during both the prenatal and early postnatal periods. These studies are described above in Section 3.2.4.1.2 because they found evidence for compromised airway development in the infant rhesus monkey exposed episodically to ozone. In addition, maternal exposure to 0.8−1.2 ppm ozone during gestation resulted in developmental health effects, mainly related to immune function and allergic lung disease, in the respiratory tract of offspring mice. Recent studies include several in the infant monkey model of allergic airway disease in which monkeys were sensitized to HDMA and several in rodents of varying ages. These studies examined a wide range of effects of postnatal ozone exposure. A brief discussion of their findings is found below, with studies grouped according to the animal model employed. Postnatal ozone exposure resulted in altered lung development in the infant monkeys and increased oxidative stress, inflammation, and injury in neonatal rodents. Effects on lung function parameters were found in rodents of different ages following long-term exposure to ozone.

Recent studies examined alveolar morphogenesis in a model of allergic airways disease using infant monkeys that were sensitized to HDMA. This model shares many features with childhood asthma. Alveolar morphogenesis is the process by which alveoli are formed de novo in the lower respiratory tract during lung development. Results of these studies demonstrating statistically significant effects provide evidence that postnatal ozone exposure leads to impairment of alveolar morphogenesis. Study-specific details are summarized in Table 3-49 in Section 3.3.2.

- In Avdalovic et al. (2012), episodic ozone exposure resulted in altered alveolar morphogenesis. Exposures to episodic ozone began at 1 month of age. This involved biweekly cycles of alternating filtered air and ozone(i.e., 9 consecutive days of filtered air and 5 consecutive days of 0.5 ppm ozone, 8 hours/day)and to house dust mite allergen (HDMA) for 2 hours per day for 3 days on the last 3 days of ozone exposure, followed by 11 days of filtered air. Five cycles of episodic ozone exposure (0.5 ppm) resulted in decreased alveolar number, increased alveolar volume, decreased distribution of alveolar volume, and decreased capillary surface density in infant monkeys that were sensitized to HDMA. These changes reflect reduced alveolarization, which was also seen in infant monkeys exposed to 5 cycles of episodic ozone and HDMA challenge (ozone/HDMA). However, these changes were not seen after 11 cycles of episodic ozone exposure. Instead, increases in lobe volume were seen in the HDMA/ozone group, suggesting that a “catch up” phase of alveolarization had occurred. Changes in TGF-β gene

expression in lung parenchyma occurred between 5 and 11 cycles of episodic ozone exposure in ozone and ozone/HDMA groups, suggesting that TGF-β played a role in the later alveolarization.

- In a follow-up study, Herring et al. (2015) demonstrated additional effects on alveolar morphogenesis. Eleven cycles of episodic ozone exposure (0.5 ppm) resulted in decreased numbers of alveoli in the right middle lobe in ozone/HDMA group. After a 30-month recovery period, the number of alveoli in the right middle lobe was increased in the ozone/HDMA group compared with controls. The coefficient of variation of distribution of mean number-weighted alveolar volumes and ratio of pulmonary capillary to inter-alveolar septal surface in the left cranial lobe was also increased after a 30-month recovery period in the ozone/HDMA group compared with controls. This indicates that alveoli that formed during the 30-month recovery period were smaller and had a greater capillary-to-alveolar gas-exchange surface and suggests a potentially greater susceptibility to obstructive lung disease.

In addition, recent studies found respiratory tract oxidative stress, inflammation and injury in neonatal rodents exposed to 1 ppm ozone during the early postnatal period. Effects described below were statistically significant. Study-specific details are summarized in Table 3-46 in Section 3.3.2.

- Dye et al. (2017) demonstrated injury and oxidative stress-related responses to ozone exposure (1 ppm, 2 hours) in rats at Postnatal Days 14, 21, and 28. These changes included increased lung wet weight:body weight ratio, altered levels of the antioxidants uric acid and glutathione, and altered activities of the antioxidant enzymes superoxide dismutase, glutathione peroxidase, and glucose-6-phosphate dehydrogenase. These changes were dependent on sex, age, and strain, with activities of antioxidants and antioxidant enzymes decreasing in younger animals and increasing in older animals in response to ozone exposure.
- Two studies from the same laboratory (Gabehart et al., 2015; Gabehart et al., 2014) found inflammation, injury, and oxidative stress-related responses to ozone exposure (1 ppm, 3 hours) in mice at Postnatal Day 1 and 7. This included increases in metallothionein I, heme oxygenase 1, and chemokine gene expression and increases in BALF neutrophils. In addition, secretion and expression of mucus, which can offer protection against injury, were increased. Neutrophil and chemokine responses to ozone exposure were inhibited in toll receptor 4-deficient mice. Cell proliferation was decreased by ozone exposure. Responses to ozone exposure in 2-, 3-, and 6-week-old mice (i.e., juvenile, weanling, and adult lifestages) are reported elsewhere in this document. In general, responses were smallest in 1-week-old and greatest in 6-week-old mice, except for effects on mucus, which were found only in the 1-week-old mice. Toll receptor 4 expression was found to increase with age, suggesting that toll receptor 4 pathway may underlie responses to ozone that were more pronounced in adult compared with neonatal mice.

Two other recent studies examined the effects of long-term ozone exposure on lung function in rodents of varying lifestages. Results of these studies demonstrate that subchronic exposure to 0.5−1.0 ppm ozone alters ventilatory parameters. Effects described below were statistically significant. Study-specific details are summarized in Table 3-50 in Section 3.3.2.

- In Snow et al. (2016), adolescent, young adult, adult, and senescent rats were exposed for 13 weeks to ozone (0.25 and 1.0 ppm for 6 hours/day, twice a week). No effects on ventilatory parameters were observed at 0.25 ppm. Relaxation time was decreased in senescent rats. Minute volume and enhanced pause were increased in young adult rats; these effects were largely resolved after 5 recovery days.

- In Gordon et al. (2016a), rats that were exercise trained or sedentary were exposed for 6 weeks to ozone (0.25, 0.5, and 1.0 ppm for 5 hours/day, once a week). Exposure to 1.0 ppm ozone increased enhanced pause in sedentary but not exercise-trained rats.

3.2.4.2.3 Integrated Summary for Lung Function and Development

The 2013 Ozone ISA (U.S. EPA, 2013a) described inconsistent epidemiologic evidence that long-term exposure to ozone is associated with lung function development in children. Recent epidemiologic studies continue to provide limited support for an association between long-term ozone exposure and lung function development in children. A CHS cohort study in the 2013 Ozone ISA reported ozone-related impairment in lung function development in children without a particular GSS glutathione gene haplotype; however, recent studies have not examined similar genetic variants. Additionally, while a limited number of recent epidemiologic studies of long-term ozone exposure and lung function development in children are consistently null, cross-sectional studies of children and adults have observed some evidence of an association between long-term ozone concentrations and lung function.

In contrast to the limited and inconsistent evidence from epidemiologic studies, recent experimental studies in animals provide evidence that postnatal ozone exposure may affect the developing lung. Results from studies of neonatal rodents demonstrate ozone-induced injury and changes in inflammatory and oxidative stress responses during lung development. In an infant monkey model with similarities to childhood asthma, postnatal ozone exposure resulted in impaired alveolar morphogenesis, a key step in lung development. Notably, these studies indicated some capacity for repair. Additional studies in adult rats suggest that chronic ozone exposure may alter ventilatory parameters.

3.2.4.3 Development of Chronic Obstructive Pulmonary Disease and Other Associated Respiratory Effects

Chronic obstructive pulmonary disease (COPD) is a lung disease characterized by persistent respiratory symptoms and airflow limitation due to destruction of alveolar tissue and airway remodeling. Reduced airflow is associated with decreased lung function, and clinical symptoms demonstrating exacerbation of COPD include cough, sputum production, and shortness of breath.

3.2.4.3.1 Epidemiologic Studies

There were no epidemiologic studies examining the association between long-term exposure to ozone and COPD available for inclusion in the 2013 Ozone ISA (U.S. EPA, 2013a). One recent study used the Ontario Asthma Surveillance Information System to identify adults with asthma, and found that ozone was associated with an increase in the odds of COPD incidence in this population (To et al., 2016).

The association was attenuated and less precise (i.e., wider 95% CIs) but still positive in copollutant models adjusted for $PM_{2.5}$. Further discussion of potential copollutant confounding of the relationship between respiratory effects and long-term exposure to ozone can be found in the "Relevant Issues for Interpreting Epidemiologic Evidence" section (Section 3.2.5). Additional study-specific details from To et al. (2016), including air quality characteristics, are highlighted in Table 3-51 in Section 3.3.2.

3.2.4.3.2 Animal Toxicological Studies

The 2013 Ozone ISA (U.S. EPA, 2013a) summarized the animal toxicological evidence of respiratory effects in healthy populations resulting from exposure to ozone. While most of the studies were conducted in rodents, a few involved chronic exposures to ozone in adult or young adult monkeys. Chronic ozone exposure (0.12−1 ppm) resulted in damage to the distal airways and proximal alveoli, resulting in persistent inflammation and lung tissue remodeling, leading to irreversible changes. Some studies demonstrated increased collagen synthesis and deposition, inducing fibrotic-like changes in the lung. Changes in other components of the extracellular matrix, such as glycosoaminoglycans, were reported. Some of these effects were found to be dependent on the TGF-β signaling pathway. Other studies demonstrated emphysematous-like changes or attenuation of inflammation. Thus, chronic ozone exposure may lead to persistent inflammation and interstitial remodeling that may contribute to the progression and development of chronic lung disease, such as pulmonary fibrosis and COPD. In addition, chronic ozone exposure (0.12−0.5 ppm) is capable of damaging nasal airways resulting in altered structure and function, as demonstrated by increased mucus flow and goblet cell metaplasia.

Several recent studies examined the effects of repeated ozone exposure on airway inflammation and injury in rodents of varying lifestages. These studies demonstrate that subchronic exposure to 0.5−1.0 ppm ozone resulted in airway injury and inflammation. All of the changes described below were statistically significant. While most studies were conducted in male rats, one study found injury and inflammatory effects in both male and female rats. Some of these effects were dependent on the age of the animal or whether it was exercise-trained and some of these effects resolved following a 5-day recovery period. Study-specific details are summarized in Table 3-46 in Section 3.3.2.

- In Gordon et al. (2016b), male and female rats were exposed for 4 weeks to ozone (0.8 ppm for 5 hours/day, once a week). Ozone exposure increased a marker of injury (albumin) and a marker of inflammation (eosinophils) in BALF of male and female rats.
- In Miller et al. (2016a), rats were exposed for 13 weeks to ozone (0.25 and 1.0 ppm for 5 hours/day, three times/week). No effects on inflammation or injury were observed in response to 0.25 ppm ozone. Markers of injury (protein, albumin, *N*-acetyl-glutaminidase) and inflammation (neutrophils and alveolar macrophages) were increased in the BALF in response to 1.0 ppm ozone. Most of these effects were lost after 5 recovery days.
- In Snow et al. (2016), adolescent, young adult, adult, and senescent rats were exposed for 13 weeks to ozone (0.25 and 1.0 ppm for 6 hours/day, twice a week). No effects on inflammation

or injury were observed at 0.25 ppm. A marker of inflammation (BALF total cell number) was increased in young adult rats exposed to 1 ppm ozone.

- In Gordon et al. (2016a), rats that were exercise-trained or sedentary were exposed for 6 weeks to ozone (0.25, 0.5, and 1.0 ppm for 5 hours/day, once a week). No effects on inflammation or injury were observed at 0.25 ppm. Exposure to 0.5 ppm ozone increased markers of injury (BALF protein and albumin) in exercise-trained rats. Exposure to 1.0 ppm ozone increased inflammatory markers in sedentary and exercise-trained rats, with more pronounced effects in sedentary rats.

3.2.4.3.3 Integrated Summary for Development of Chronic Obstructive Pulmonary Disease and Other Associated Respiratory Effects

The 2013 Ozone ISA did not evaluate any epidemiologic studies that examined the relationship between long-term exposure to ozone and the development of COPD. One recent epidemiologic study provides evidence of an association between long-term ozone concentrations and incident COPD hospitalizations. Animal toxicological studies reviewed in the 2013 Ozone ISA found that chronic ozone exposure can damage the distal airways and proximal alveoli, resulting in persistent inflammation and lung tissue remodeling that leads to irreversible changes including fibrotic- and emphysematous-like changes in the lung. Additionally, recent animal toxicological studies provide consistent evidence that subchronic ozone exposure can lead to airway injury and inflammation. In adult animals these changes may underlie the progression and development of chronic lung disease and provide biological plausibility for ozone-induced development of COPD.

3.2.4.4 Respiratory Infection and other Associated Respiratory Effects

3.2.4.4.1 Epidemiologic Studies

There were no epidemiologic studies examining the association between long-term exposure to ozone and respiratory infection available for inclusion in the 2013 Ozone ISA (U.S. EPA, 2013a). Two recent studies observed inverse associations between ozone and respiratory infection. Smith et al. (2016) reported an inverse association between 2-year avg ozone concentrations and pulmonary tuberculosis in a nested case-control study of adults in northern California. The authors did observe a strong positive association with NO_2 and a negative correlation between ozone and NO_2, which may explain the inverse association. In a study of otitis media in the first 2 years of life, 2-month avg ozone concentrations were associated with decreased risk of infection (MacIntyre et al., 2011). Study-specific details, including air quality characteristics and select effect estimates, are highlighted in Table 3-52 in Section 3.3.2.

3.2.4.5 Severity of Respiratory Disease

3.2.4.5.1 Epidemiologic Studies

Respiratory symptoms and increased medication use are often indicators of disease severity. Symptom frequency is used as a measure of asthma severity, in particular. Additionally, more severe symptoms, potentially resulting in hospitalization or ED visits for asthma, are indicative of exacerbation severity, but may also suggest greater underlying disease severity (NAEPP, 2008). While the 2013 Ozone ISA did not have a delineated discussion of epidemiologic studies that examined severity of respiratory diseases, a limited number of relevant studies were evaluated and provided evidence of associations between long-term ozone concentrations and respiratory hospital admissions or symptoms (U.S. EPA, 2013a). Specifically, two cross-sectional studies of asthma hospital admissions in children (Moore et al., 2008) and adults (Meng et al., 2010) in California observed associations with long-term exposure to ozone. Notably, Meng et al. (2010) also reported an association between ozone concentrations and self-reported asthma symptoms. Similarly, McConnell et al. (2003) observed that bronchitic symptoms in children with asthma were associated with yearly variation in ozone within CHS communities, but not with 4-year avg ozone across communities. The longitudinal nature of the within-community estimate makes it more informative than the cross-sectional between-group estimate because it establishes a temporal relationship between the exposure and outcome. Another cross-sectional study, in the U.K., reported that ozone concentrations (annual accumulated ozone over 40 ppb per daylight hour) were associated with more severe emphysema, as measured by a density mask analysis of a CT Scan (Wood et al., 2009).

Recent studies further support a relationship between long-term exposure to ozone and severity of respiratory disease, although some uncertainties remain. Study-specific details, including air quality characteristics and select effect estimates, are highlighted in Table 3-53 in Section 3.3.2. An overview of the evidence is provided below.

- In a large administrative database cohort of adults with asthma in Quebec, summertime average ozone was associated with aggregated hospital admissions and ED visits for asthma (Tétreault et al., 2016b). The hazard ratio (HR) was positive (1.17; 95% CI: 1.12, 1.22) when time-dependent ozone concentrations were used to estimate exposure, but not when ozone exposure was assigned at birth residences (0.99; 95% CI: 0.96, 1.11). This may indicate that asthma exacerbation is related to continued exposure to ozone, rather than prenatal and 1st year of life exposure. However, as the authors do not adjust for short-term exposures to ozone, the time-dependent model could be capturing acute responses to ozone.
- Like McConnell et al. (2003), Berhane et al. (2016) and Gilliland et al. (2017) observed decreased prevalence of bronchitic symptoms in children with asthma associated with decreased ozone exposure over two decades of follow-up of CHS cohorts. The association was attenuated but still present in copollutant models with NO_2 or $PM_{2.5}$. Further discussion of potential copollutant confounding of the relationship between respiratory effects and long-term exposure to

ozone can be found in the "Relevant Issues for Interpreting Epidemiologic Evidence" section (Section 3.2.5).

In summary, recent studies continue to provide support for an association between ozone and respiratory disease severity. However, notable uncertainties remain. Some studies examine hospital admissions or ED visits as a measure of disease severity but do not control for short-term exposures to ozone. Given the acute nature of the health endpoint, the observed effects could be confounded by short-term increases in air pollution. There is also a lack of available studies that examine potential copollutant confounding. However, one study observed an association between ozone and bronchitic symptoms in children with asthma that is robust to adjustment for $PM_{2.5}$ and NO_2.

3.2.4.5.2 Animal Toxicological Studies

The 2013 Ozone ISA (U.S. EPA, 2013a) summarized the animal toxicological evidence related to severity of disease resulting from exposure to ozone. A 4-week exposure to ozone (0.5 ppm for 5 hours, once a week) enhanced injury, inflammation, and allergic responses in a rodent model of allergic airway disease. In addition, 4 months exposure (0.5 ppm) resulted in increased severity of post-influenza alveolitis and lung parenchymal changes. No additional studies have become available since then.

3.2.4.5.3 Integrated Summary for Severity of Respiratory Disease

Results from recent epidemiologic studies are consistent with evidence evaluated in the 2013 Ozone ISA that provides support for an association between ozone and respiratory disease severity. Specifically, there is consistent evidence that long-term exposure to ozone is associated with hospital admissions and ED visits for asthma and prevalence of bronchitic symptoms in children with asthma. There is some uncertainty due to the acute nature of some of these outcomes. Additionally, while there are no recent animal toxicological studies available for review, a previously evaluated study provides biological plausibility for enhanced respiratory effects in populations with pre-existing respiratory conditions.

3.2.4.6 Allergic Responses

3.2.4.6.1 Epidemiologic Studies

The 2013 Ozone ISA reviewed a limited number of epidemiologic studies examining a range of allergic indicators that found generally positive associations with long-term exposure to ozone (U.S. EPA, 2013a). Cross-sectional studies reported increases in prevalence of hay fever (Parker et al., 2009) and rhinitis (Hwang et al., 2006; Penard-Morand et al., 2005), and increased total serum IgE levels (Rage et

al., 2009) associated with ozone concentrations. In copollutant models adjusting for NO_2, the observed association between ozone and rhinitis was persistent (Penard-Morand et al., 2005), while the association with IgE levels was attenuated, but still positive (Rage et al., 2009). In contrast to generally consistent evidence of an association, one study reported null associations between ozone and hay fever (Ramadour et al., 2000).

One recent cross-sectional study provides additional support for an association between long-term exposure to ozone and allergic response. A 2005−2006 NHANES analysis, comprising a nationally representative sample of the U.S. population, examined allergic sensitization measured by detectable allergen-specific IgE levels (Weir et al., 2013). Weir et al. (2013) found that annual average ozone concentrations were associated with increased odds of sensitization to indoor allergens and inhalants. The observed ORs were comparable for exposure assigned from monitors within 20 miles of the participants' home address and using geocoded CMAQ ozone concentration estimates. The authors did not present models adjusted for copollutants, and while limited evidence from the previous ozone ISA indicated that associations were persistent to adjustment for NO_2, potential copollutant confounding remains an uncertainty, specifically regarding potential confounding by pollen levels. Complete study details, including air quality characteristics, are highlighted in Table 3-54 in Section 3.3.2.

3.2.4.6.2 Animal Toxicological Studies

The 2013 Ozone ISA (U.S. EPA, 2013a) summarized the animal toxicological evidence of allergic responses resulting from exposure to ozone. A 4-week exposure to ozone (0.5 ppm for 5 hours, once a week) increased injury, inflammation, and allergic responses in a rodent model of allergic airway disease. Newly available evidence shows that repeated subchronic exposure to 0.1 ppm ozone promoted eosinophilic airway inflammation in a model of allergic sensitization.

A recent study Hansen et al. (2013) was conducted in mice exposed for 12 weeks to ozone (0.1 ppm for 20 minutes/day for 5 days a week for 2 weeks and once weekly for 12 weeks). Mice were also exposed to a low dose of ovalbumin which produced minimal sensitization because levels of serum ovalbumin-specific IgE were minimally affected. After 14 weeks, mice were challenged with a high dose of ovalbumin. As mentioned above in Section 3.2.4.1.2, no increases in ventilatory parameters or indicators of bronchoconstriction were observed. In addition, ozone exposure did not increase ovalbumin-specific IgE levels indicating that ozone did not act as an adjuvant. However, ozone exposure resulted in a statistically significant increase in BALF eosinophils. Study-specific details are summarized in Table 3-55 in Section 3.3.2.

3.2.4.6.3 Integrated Summary for Allergic Response

Cross-sectional epidemiologic studies provide generally consistent evidence that ozone concentrations are associated with hay fever/rhinitis and serum-markers of allergic response. However, in addition to uncertainties regarding cross-sectional associations, potential confounding by pollen concentrations also remains a considerable uncertainty. There is supporting evidence from recent and previously evaluated toxicological studies that provides biological plausibility for some of the observed epidemiologic associations. Specifically, ozone exposure induced airway eosinophilia in a rodent model of allergic sensitization and enhanced allergic responses in a rodent model of allergic airway disease. In contrast to the epidemiologic evidence, one recent experimental study did not observe ozone-related changes in allergen-specific IgE levels in mice.

3.2.4.7 Respiratory Effects in Pregnancy

3.2.4.7.1 Animal Toxicological Studies

No animal toxicological studies evaluating respiratory effects in pregnancy were described in the 2013 Ozone ISA (U.S. EPA, 2013a). Newly available evidence shows that pregnant rats responded to ozone exposure with immediate effects on ventilatory parameters and later effects reflecting airway injury.

A recent study in pregnant rats Miller et al. (2017) demonstrated that exposure to 0.4 and 0.8 ppm ozone on Gestational Days 5 and 6 resulted in altered ventilatory parameters (decreased minute volume and increased enhanced pause) immediately post-exposure and increased markers of injury (gamma glutamyl transferase and *N*-acetyl-glutaminidase) in BALF on Gestational Day 21. The observed alterations in enhanced pause were dose dependent. These effects were statistically significant. Study-specific details are summarized in Table 3-50 in Section 3.3.2. Nonrespiratory endpoints evaluated in this study are discussed elsewhere in this document.

3.2.4.8 Respiratory Effects in Populations with Metabolic Syndrome

3.2.4.8.1 Animal Toxicological Studies

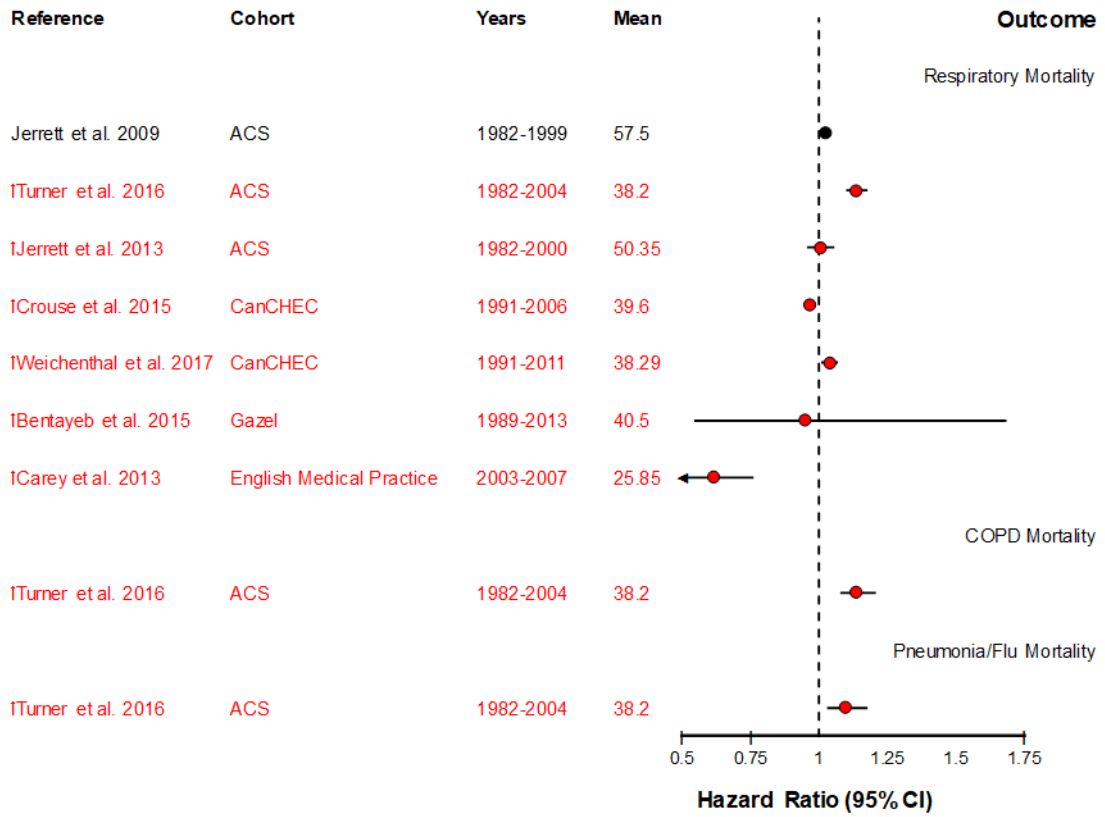
No animal toxicological studies evaluating respiratory effects in populations with diabetes or metabolic syndrome were described in the 2013 Ozone ISA (U.S. EPA, 2013a). Newly available evidence shows that male and female rats had different responses to subchronic ozone exposure that were dependent on diet. These effects, described below, were statistically significant. Study-Specific details are summarized in Table 3-46 in Section 3.3.2.

- A recent study Gordon et al. (2016b) was conducted in male and female rats fed high-fructose and high-fat diets prior to and during 4 weeks of ozone exposure (0.8 ppm for 5 hours/day, once a week). Ozone exposure increased a marker of injury (albumin) and a marker of inflammation (eosinophils) in BALF of males on the high-fructose and high-fat diets. Females on the high-fat diet had increased albumin and females on the high-fructose diet had increased eosinophils in response to ozone exposure.

3.2.4.9 Respiratory Mortality

When considering the entire body of evidence, there is limited support for an association with long-term ozone exposure and respiratory mortality. Recent studies use a variety of both fixed-site (i.e., monitors) and models (e.g., CMAQ, dispersion models) to measure or estimate ozone concentrations for use in assigning long-term ozone exposure in epidemiologic studies (Section 2.6.2). The strongest evidence comes from analyses of the ACS cohort data, including studies observing positive associations between long-term ozone exposure and respiratory mortality (Jerrett et al., 2009) included in the 2013 Ozone ISA, and a recent analysis of respiratory, COPD, and pneumonia mortality (Turner et al., 2016). Results from other recent studies are less consistent, with analyses of U.S., Canadian, and European cohorts reporting inconsistent associations between long-term ozone exposure and respiratory mortality. The differences in how ozone exposure was assessed do not explain the heterogeneity in the observed associations. The results from studies evaluating long-term ozone exposure and respiratory mortality are presented in Figure 3-13. Overall, there is some evidence that long-term ozone exposure is associated with respiratory mortality, but the evidence is not consistent across studies. Specifically:

- The strongest evidence for an association between long-term ozone exposure and respiratory mortality comes from nationwide analyses of the ACS cohort, demonstrating positive associations with respiratory mortality (Turner et al., 2016; Jerrett et al., 2009) and COPD, and pneumonia/flu (Turner et al., 2016). In contrast, Jerrett et al. (2013) reported a null association between long-term ozone exposure and respiratory mortality in an analysis of the ACS cohort limited to participants from California.
- Several recent analyses of the CanCHEC cohort in Canada provide inconsistent evidence for an association between long-term ozone exposure and respiratory mortality, with one reporting a positive association (Weichenthal et al., 2017) and the other reporting a negative association (Crouse et al., 2015). Cohort studies conducted in France (Bentayeb et al., 2015) and the U.K. (Carey et al., 2013) also report negative associations between long-term ozone exposure and respiratory mortality.



ACS = American Cancer Society; CanCHEC = Canadian Census Health and Environment Cohort.

Note: †Studies published since the 2013 Ozone ISA. Associations are presented per 10 ppb increase in pollutant concentration. Circles represent point estimates; horizontal lines represent 95% confidence intervals for ozone. Black text and circles represent evidence included in the 2013 Ozone ISA; red text and circles represent recent evidence not considered in previous ISAs or AQCDs.

Figure 3-13 Associations between long-term exposure to ozone and respiratory mortality in recent cohort studies.

3.2.5 Relevant Issues for Interpreting Epidemiologic Evidence—Long-Term Ozone Exposure and Respiratory Effects

3.2.5.1 Potential Copollutant Confounding of the Ozone-Respiratory Relationship

Potential copollutant confounding is a recurrent issue in epidemiologic studies of the health effects of air pollutants. Pollutant concentrations are often correlated, such that it can be difficult to distinguish the effect of one pollutant from another. In the recently evaluated studies of long-term exposure to ozone and respiratory health effects, ozone correlations with other pollutants have varied greatly across studies ($PM_{2.5}$: $r = -0.21$ to 0.66; NO_2: $r = -0.42$ to 0.38; SO_2: −0.24 to 0.15). Limited evaluation of copollutant models in recent studies provides some evidence that ozone associations may be attenuated, but still positive in copollutant models with NO_2 and $PM_{2.5}$ (Gilliland et al., 2017; Berhane et al., 2016; To et al., 2016). Additionally, because many studies report modest copollutant correlations, strong copollutant confounding from any of the measured copollutants is unlikely. However, given the limited amount of available evidence, including a lack of measurement of noncriteria pollutants, the potential confounding effect of copollutants remains a notable source of uncertainty in the relationship between long-term ozone exposure and respiratory health effects.

3.2.5.2 The Role of Season and Temperature on Ozone Associations with Respiratory Health Effects

A number of epidemiologic studies of short-term ozone concentrations and respiratory health effects have conducted seasonal analyses, comparing associations observed in the warm season to cold season or year-round estimates (Section 3.1.10.2). Results from these studies have generally supported stronger associations in the warm season. Despite this line of evidence, there have not been seasonal comparisons of long-term exposures to ozone. While one study in Quebec, Canada used summertime average ozone concentrations as a surrogate for annual exposure, the results are not informative to seasonal differences given the evaluation of year-round health outcomes (Tétreault et al., 2016a).

3.2.5.3 Shape of the Concentration-Response Function

There are no recent epidemiologic studies or studies previously considered in the 2013 Ozone ISA (U.S. EPA, 2013a) that examine the C-R relationship of the association between long-term exposure to ozone and respiratory health effects. While most studies use linear or log-linear models to characterize health effects, there are a lack of studies that empirically assess deviations from linearity, or use alternative models that allow for nonlinearity. Thus, there is some uncertainty regarding the shape of the C-R relationship and existence of a threshold.

3.2.6 Summary and Causality Determination

The 2013 Ozone ISA concluded that a "causal relationship is likely to exist" between long-term ozone exposure and respiratory effects (U.S. EPA, 2013a). This conclusion was based on epidemiologic evidence of associations between long-term ozone exposure and new-onset asthma, respiratory symptoms in children with asthma, and respiratory mortality. Notably, associations between long-term exposure to ozone and new-onset asthma in children were primarily evident in longitudinal studies that examined interactions between ozone and exercise or different genetic variants, including HMOX-1, ARG, and GSTP1. The observed gene-environment interactions were supported by evidence that the specific enzymes corresponding to these genetic variants have antioxidant and/or anti-inflammatory properties, providing biological plausibility for the observed interactions. Additionally, the evidence relating new-onset asthma to long-term ozone exposure was supported by toxicological studies in infant monkeys, which indicate that postnatal ozone exposures can lead to the development of asthma. This nonhuman primate evidence of ozone-induced respiratory effects supported the biological plausibility of long-term exposure to ozone contributing to the development of asthma in children. Specifically, these studies indicate that early-life ozone exposure can cause structural and functional changes that could potentially contribute to airway obstruction and increased airway responsiveness. Some uncertainties were acknowledged in the previous causality determination, specifically regarding the limited number of epidemiologic studies examining potential copollutant confounding. However, in general, the epidemiologic and toxicological evidence provided evidence of a likely to be causal relationship between long-term exposure to ozone and respiratory effects.

Recent studies continue to examine the relationship between long-term exposure to ozone and respiratory effects. Key studies that inform the causality determination are presented in Table 3-3. A limited number of recent epidemiologic studies provide generally consistent evidence that long-term ozone exposure is associated with the development of asthma in children (Section 3.2.4.1.1). A large administrative cohort study, following over one million children from birth, observed an association between long-term exposure to ozone and asthma onset. This finding was consistent with a recent CHS analysis that reported a decrease in childhood asthma incidence associated with decreases in ozone concentrations across nine southern California communities. While a smaller study restricted to minority children did not find evidence of an association between long-term ozone concentrations and asthma development, the two larger studies provide compelling evidence of a positive association. In addition to the development of asthma, epidemiologic studies have also evaluated the relationship between ozone and asthma severity (Section 3.2.4.5). Consistent with results from the 2013 Ozone ISA, recent studies have presented consistent evidence that long-term exposure to ozone is associated with hospital admissions and ED visits for asthma and prevalence of bronchitic symptoms in children with asthma. Notably, there is some uncertainty regarding the results from studies of hospital admissions and ED visits for asthma, which typically represent an acute outcome. Most of these studies do not adjust for short-term ozone concentrations, despite there being an established association between short-term exposure and asthma exacerbation (Section 3.1.4.2).

In support of the evidence from recent epidemiologic studies, there are a number of recent animal toxicological studies that expand the evidence for long-term ozone exposure-induced effects that may lead to asthma development (Section 3.2.4.1.2). Specifically, studies in nonhuman primates have shown that postnatal ozone exposure can compromise airway growth and development, promote the development of an allergic phenotype, and cause persistent alterations to the immune system. In addition, findings that ozone exposure enhances injury, inflammation, and allergic responses in allergic rodents provide biological plausibility for the relationship between ozone exposure and the exacerbation of allergic asthma.

In addition to studies of asthma, there is new and/or expanded evidence from epidemiologic and animal toxicological studies published since the completion of the 2013 Ozone ISA that provide evidence of associations between long-term ozone exposure and the development of COPD (Section 3.2.4.3), allergic responses (Section 3.2.4.6). A recently available epidemiologic study provides limited evidence that long-term ozone exposure is associated with incident COPD hospitalizations in adults with asthma. This finding is supported by recent animal toxicological studies that provide consistent evidence of airway injury and inflammation resulting from subchronic ozone exposures. These results are coherent with animal toxicological studies reviewed in the 2013 Ozone ISA, which demonstrated that chronic ozone exposure damages distal airways and proximal alveoli, resulting in persistent inflammation and lung tissue remodeling that leads to irreversible changes, including fibrotic- and emphysematous-like changes in the lung. Respiratory tract inflammation and morphologic and immune system-related changes may underlie the progression and development of chronic lung disease, such as COPD.

A larger body of epidemiologic studies also provides support for an association between long-term ozone exposure and allergic responses, including hay fever/rhinitis and serum allergen-specific IgE. While recent studies demonstrate generally consistent results, potential confounding by pollen exposure remains an uncertainty. However, there is supporting evidence from animal toxicological studies demonstrating enhanced responses in ozone-exposed allergic rodents (Section 3.2.4.6.2). In addition, animal toxicological studies reviewed in the short-term exposure section show type 2 immune responses in nasal airways of rodents exposed repeatedly to ozone (Section 3.1.4.4.2). These findings are characteristic of induced nonatopic asthma and rhinitis and provide biological plausibility for the observed epidemiologic associations with hay fever/rhinitis.

Taken together, previous and more recent animal toxicological studies of long-term exposure to ozone demonstrate biological plausibility for many of the associations observed in recent epidemiologic studies. Specifically, there is strong evidence of ozone-induced inflammation, injury, and oxidative stress in adult animals. These effects represent initial events through which ozone may lead to a number of downstream respiratory endpoints, including altered morphology in the lower respiratory tract and the development of COPD. Further, there is evidence of a range of ozone-induced effects on lung development in neonatal rodents and infant monkeys, including altered airway architecture, airway sensory nerve innervation, airway cell death pathways, increased serotonin-positive airway cells, and

immunomodulation. An infant monkey model of allergic airway disease also demonstrated effects on lung development, including compromised airway growth, impaired alveolar morphogenesis, airway smooth muscle hyperreactivity, an enhanced allergic phenotype, priming of responses to oxidant stress, and persistent effects on the immune system. These various upstream effects provide a plausible pathway through which ozone may act on downstream events, such as altered immune function leading to altered host defense and allergic responses, as well as morphologic changes leading to the development of asthma. A more thorough discussion of the biological pathways that potentially underlie respiratory health effects resulting from long term exposure to ozone can be found in Section 3.2.3.

Recent epidemiologic studies provide some evidence that long-term ozone exposure is associated with respiratory mortality, but the evidence is not consistent across studies (Section 3.2.4.9). A recent nationwide study in the U.S. observed associations between ozone and underlying causes of respiratory mortality, including COPD. This finding is supported by the new lines of evidence from animal toxicological and epidemiologic studies on the development of COPD, as discussed previously. Results from epidemiologic studies of ozone-related respiratory mortality in populations outside the U.S are inconsistent.

A notable source of uncertainty across the reviewed epidemiologic studies is the lack of examination of potential copollutant confounding. A limited number of studies that include results from copollutant models suggest that ozone associations may be attenuated but still positive after adjustment for NO_2 or $PM_{2.5}$. However, the few studies that include copollutant models examine different outcomes, making it difficult to draw strong conclusions about the nature of potential copollutant confounding for any given outcome. Importantly, in addition to studies that explicitly address potential copollutant confounding through modeling adjustments, many studies report modest copollutant correlations, which suggests that strong confounding due to copollutants is unlikely. Another source of uncertainty common to epidemiologic studies of air pollution is the potential for exposure measurement error. The majority of recent epidemiologic studies of long-term ozone exposure use concentrations from fixed-site monitors as exposure surrogates. Exposure measurement error relating to exposure assignment from fixed-site monitors has the potential to bias effect estimates in either direction, although it is more common that effect estimates are underestimated, and the magnitude of the bias is likely small given that ozone concentrations do not vary over space as much as other criteria pollutants, such as NO_X or SO_2 (Section 2.3.1.1)

Despite some uncertainties in the epidemiologic literature, there is coherence from animal toxicological studies that provides support for the observed epidemiologic associations. Experimental evidence also provides biologically plausible pathways through which long-term ozone exposure may lead to respiratory effects. **Overall, the collective evidence is sufficient to conclude that a likely to be causal relationship exists between long-term ozone exposure and respiratory effects**.

Table 3-3 Summary of evidence for a likely to be causal relationship between long-term ozone exposure and respiratory effects.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Development of Asthma			
Consistent evidence from toxicological studies at relevant concentrations	Animal toxicological studies show postnatal exposure results in compromised airway growth and development	Section 3.2.4.1.2 (infant monkeys)	0.5 ppm
		Lee et al. (2011) (rats)	0.5 ppm
	Animal toxicological studies show postnatal exposure promotes an allergic phenotype in the developing lung	Section 3.2.4.1.2 (infant monkeys)	0.5 ppm
	Animal toxicological studies show postnatal exposure alters sensory nerve innervation in the developing lung	Section 3.2.4.1.2 (infant monkeys)	0.5 ppm
	Animal toxicological studies show postnatal exposure alters airway responsiveness	Section 3.2.4.1.2 (infant monkeys)	0.5 ppm
		Moore et al. (2012)	0.5 ppm
Generally consistent evidence from a limited number of epidemiologic studies of asthma development in children	Cohort studies demonstrating an association with asthma development in children	Tétreault et al. (2016a); Garcia et al. (2019)	32.1 ppb mean summer ozone concentration, based on 8-h midday avg
	Longitudinal studies provide evidence of associations with asthma development in populations with specific genetic variants	Islam et al. (2008); Salam et al. (2009)	38.4 ppb mean annual ozone concentration in low ozone communities; 55.2 ppb in high ozone communities, based on 8-h avg (10:00 a.m.−6:00 p.m.)
Uncertainty regarding confounding by copollutants	No examination of copollutant confounding in models of gene-environment interaction. Available studies report low to moderate copollutant correlations	-	

Table 3-3 (Continued): Summary of evidence for a likely to be causal relationship between long-term ozone exposure and respiratory effects.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Severity of Respiratory Disease			
Limited, but consistent epidemiologic evidence from studies of respiratory disease severity	Longitudinal studies provide consistent evidence of an association between long-term ozone concentrations and bronchitic symptoms in children with asthma	McConnell et al. (2003); Berhane et al. (2016); Gilliland et al. (2017)	44.8−47.7 ppb annual average, across cohorts. based on 8- h avg (10:00 a.m.−6:00 p.m.)
	Consistent evidence of an association between long-term ozone concentrations and hospital admissions and ED visits for asthma	Moore et al. (2008); Meng et al. (2010); Tétreault et al. (2016b)	32.1 ppb mean summer ozone concentration, based on 8-h midday avg (Tétreault et al., 2016b) 87.8 ppb quarterly 1 h daily max (Moore et al., 2008)
Uncertainty regarding confounding by copollutants	Limited evidence that observed associations were attenuated but still positive in copollutant models adjusting for NO_2 or $PM_{2.5}$	Berhane et al. (2016); Gilliland et al. (2017)	
Other uncertainties	Studies of hospital admissions and ED visits for asthma do not account for the potential effect of short-term exposures leading to these acute events	Section 3.2.4.5.1	
Biological plausibility	Evidence that ozone exposure enhances injury, inflammation, and allergic responses in allergic rodents provide biological plausibility for the relationship between ozone exposure and the exacerbation of allergic asthma	Section 3.2.4.5.2	
Development of Chronic Obstructive Pulmonary Disease			
Consistent evidence from toxicological studies at relevant concentrations	Animal toxicological evidence of morphologic changes in distal airways and proximal alveoli leading to lung tissue remodeling and fibrotic/emphysematous-like changes in rodents and monkeys	Section 3.2.4.3.2	0.12−1 ppm

Table 3-3 (Continued): Summary of evidence for a likely to be causal relationship between long-term ozone exposure and respiratory effects.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Limited epidemiologic evidence from study of COPD incidence	The only study evaluated indicates an association between ozone concentrations and COPD incidence in adults with asthma	To et al. (2016)	38.4 ppb mean ozone concentration, based on average of monthly 24- h max from time of enrollment
Uncertainty regarding confounding by copollutants	Limited evidence from a single study reported an association between ozone and COPD incidence that was attenuated, but still positive in a copollutant model adjusting for $PM_{2.5}$	To et al. (2016)	
Allergic Response			
Limited, but consistent epidemiologic evidence from studies of allergic response	Epidemiologic studies provide consistent evidence of ozone associations with hay fever/rhinitis and allergen-specific IgE levels	Section 3.2.4.6.1	51.5 ppb annual average, based on 8- h max
Uncertainty regarding confounding by copollutants	Limited evidence from a single study reported an association between ozone and rhinitis that was persistent in a copollutant model adjusting for NO_2.	Penard-Morand et al. (2005)	
Other uncertainties	All available studies were cross-sectional. Additionally, potential confounding by pollen concentrations also remains a considerable uncertainty	Section 3.2.4.6.1	
Coherent evidence from toxicological studies at relevant concentrations	Animal toxicological evidence for enhanced allergic responses	Section 3.2.4.6.2	0.1−0.5 ppm
	Animal toxicological evidence from short-term studies show type 2 immune responses in nasal airways of rodents repeatedly exposed	Section 3.1.4.4.2	0.5−0.8 ppm
Respiratory Mortality			
Inconsistent epidemiologic evidence from multiple, high-quality studies	Recent epidemiologic studies provide some evidence of an association with respiratory mortality, but the evidence is not consistent. New evidence from one study demonstrating an association with COPD mortality	Section 3.2.4.9	

Table 3-3 (Continued): Summary of evidence for a likely to be causal relationship between long-term ozone exposure and respiratory effects.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Some coherence with underlying causes of mortality	Studies of COPD development provide coherence with COPD mortality	Section 3.2.4.3	
Biological plausibility	Animal toxicological studies show the development of emphysematous-like disease and increased severity of infection-related alveolitis	Section 3.2.4.3.2 Section 3.2.4.5.2	

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble to the ISAs (U.S. EPA, 2015).

[b]Describes the key evidence and references, supporting or contradicting, contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where full body of evidence is described.

[c]Describes the ozone concentrations with which the evidence is substantiated.

3.3 Evidence Inventories—Data Tables to Summarize Study Details

1

3.3.1 Short-Term Exposure

Table 3-4 Study-specific details from controlled human exposure studies of lung function in healthy populations.

Study	Population n, Sex, Age (Range or Mean ± SD)	Exposure Details (Concentration, Duration)	Endpoints Examined
Madden et al. (2014)	Healthy adults n = 11 males, 4 females Age: 27 ± 4 yr	0 (Day 1) ppb, 2 h 300 (Day 1) ppb, 2 h 300 (Day 2) ppb, 2 h	Spirometry (before, immediately PE, and once per hour for 4 h PE)
Ghio et al. (2014)	Healthy adults n = 14 males, 5 females Age: 25 ± 3 yr	0 ppb, 2 h 300 ppb, 2 h	FEV_1 (immediately before and PE)
Hoffmeyer et al. (2013)	Healthy adults n = 8 males, 7 females Age: 26 yr	40 ppb, 4 h 240 ppb, 4 h	Spirometry (before and immediately PE) Plethysmograph (before and immediately PE)
Frampton et al. (2015)	Healthy adults n = 12 males, 12 females Age: 26.4 yr	0 ppb, 3 h 100 ppb, 3 h 200 ppb, 3 h	FEV_1, FVC (30 min before and immediately PE and 4 h PE)
Kahle et al. (2015)	Healthy adults n = 14 males, 2 females Age: 27 yr	0 ppb at 22°C, 2 h 0 ppb at 32.5°C, 2 h 300 ppb at 22°C, 2 h 300 ppb at 32.5°C, 2 h	FEV_1/FVC (before and immediately PE)
Bates et al. (2014)	Healthy adult nonsmokers n = 17 males, 13 females Age: 25 ± 6 yr Healthy adult smokers n = 19 males, 11 females Age: 24 ± 4 yr	300 ppb, 1 h	FEV_1, FVC, dead space, alveolar slope, spirometry (before and PE)

Table 3-4 (Continued): Study-specific details from controlled human exposure studies of lung function in healthy populations.

Study	Population n, Sex, Age (Range or Mean ± SD)	Exposure Details (Concentration, Duration)	Endpoints Examined
Bennett et al. (2016)	Healthy adults n = 19 normal weight females Age: 24 ± 4 yr n = 19 obese females Age: 28 ± 5 yr	0 ppb, 2 h 400 ppb, 2 h	Airway responsiveness (3 h PE) FEV_1, FVC, sGaw (before and PE) PFT, PMN, airway responsiveness, symptoms (train day, before and immediately and 3 h PE)
Stiegel et al. (2017)	Healthy adults n = 11 males, 4 females Age: 27 yr	0 ppb, 2 h 300 ppb, 2 h	FEV_1, FVC (before and immediate PE)
Arjomandi et al. (2018) Frampton et al. (2017)	Healthy adults n = 35 males, 52 females Age: 59.9 ± 4.5 yr	0 ppb, 3 h 70 ppb, 3 h 120 ppb, 3 h	Spirometry (30 min before, 5 min PE, 22 h PE)
Biller et al. (2011)	Healthy adults n = 11 males, 3 females Age: 33.1 ± 9.5 yr	0 ppb, 3 h 250 ppb, 3 h	FEV_1, FVC (before and 0, 3, and 21 h PE)
Tank et al. (2011)	Healthy adults n = 11 males, 3 females Age: 34 ± 10 yr	0 ppb, 3 h 250 ppb, 3 h	FEV_1, FVC (before and immediate PE)

Table 3-5 Study-specific details from animal toxicological studies of short-term ozone exposure and lung function—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Schelegle and Walby (2012)	Rats (BN) n = 5−11 males Age: 8−10 weeks	1 ppm, 8 h	Lung function, breathing pattern, (immediately PE)
Wolkoff et al. (2012)	Mice (BALB/cA) n = 9−20 males Age: NR but mean weight was 24 g	0.1 ppm, 1 h/day for 10 days	Lung function (during exposure)
Lee et al. (2013)	Mice (BALB/c) n = 6 females Age: 5−6 weeks	2 ppm, 3 h	Enhanced pause (immediately PE)
Sunil et al. (2013)	Rats (WS) n = 3−6 females Age: NR but weight was 200−225 g	2 ppm, 3 h	Pulmonary mechanics (48 and 96 h PE)
Groves et al. (2012)	Mice (C57BL/6J) n = 4−9 males Age: 8 weeks	0.8 ppm, 3 h	Pulmonary mechanics (72 h PE)
Cho et al. (2013)	Mice (ICR) WT and NRF2 deficient n = 3−12 Sex and age: NR	2 ppm, 3 h	Pulmonary mechanics, acetylcholine challenge (24 h PE)
Barreno et al. (2013)	Mice (C57BL/6) WT and osteopontin deficient n = 6−10 females Age: 8 weeks	2 ppm, 3 h	Pulmonary mechanics, MCh challenge (24 h PE)
Groves et al. (2013)	Mice (C57BL/6J) n = 3−10 males Age: 8, 27, 80 weeks	0.8 ppm, 3 h	Pulmonary mechanics, resistance and elastance spectra (72 h PE)
Ghio et al. (2014)	Mice (CD-1) n = 6 females Age: 4 weeks	2 ppm, 3 h	Enhanced pause, MCh challenge (24 h PE)

Table 3-5 (Continued): Study-specific details from animal toxicological studies of short-term ozone exposure and lung function—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Razvi et al. (2015)	Mice (C57BL/6J WT and resistin deficient) n = 6−8 males and females Age: 4−8 weeks	2 ppm, 3 h	Pulmonary mechanics, MCh challenge (24 h PE)
Dye et al. (2015)	Rats (WKY, WS, SD) n = 4−8 males Age: 12−14 weeks	0.25 ppm, 4 h 0.5 ppm, 4 h 1.0 ppm, 4 h	Whole body plethysmography (0 and 20 h PE)
Clay et al. (2016)	Guinea pigs (Dunkin-Hartley) n = 4−32 males Age: NR but weight was 300−500 g Rabbits (New Zealand white) n = 4−16 males Age: NR but weight was 2.5−4 g	2 ppm, 1 h 2 ppm, 30 min	Cough response, pulmonary mechanics, challenge with Mch (4 h or 3 days PE)
Snow et al. (2016)	Rats (BN) n = 6−8 males Age: 1, 4, 12, 24 mo	0.25 ppm, 6 h/day for 2 days 1 ppm, 6 h/day for 2 days	Ventilatory parameters (18 h PE)
Gordon et al. (2016b)	Rats (BN) n = 9−10 males and females Age: 20 weeks	0.8 ppm, 5 h	Ventilatory parameters (18 h PE)
Kasahara et al. (2015)	Mice (C57BL/6 WT, ROCK1 insufficient, ROCK2 insufficient) n = 4−12 males Age: 20−25 weeks	2 ppm, 3 h	Pulmonary mechanics, challenge with MCh (24 h PE)
Verhein et al. (2013)	Guinea pigs (Dunkin-Hartley) n = 3−7 females Age: NR but weight was 300−470 g	2 ppm, 4 h	Pulmonary inflation pressure, challenge with i.v. of acetylcholine and electrical stimulation of the vagal nerve (24 h PE)
Williams et al. (2015)	Mice (C57BL/6, TNF-α sufficient and deficient) n = 5−9 females Age: 10−12 weeks	2 ppm, 3 h	Pulmonary mechanics, challenge with MCh (24 h PE)
Hansen et al. (2016)	Mice (BALB/cJ) n = 5 females Age: 6 weeks	2 ppm, 1 h/day for 3 days	Breathing frequency, tidal volume, time of brake, time of pause (during exposure)

Table 3-5 (Continued): Study-specific details from animal toxicological studies of short-term ozone exposure and lung function—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Zychowski et al. (2016)	Mice (C57BL/6) n = 4−8 males Age: 6−8 weeks	1 ppm, 4 h	Pulmonary mechanics, challenge with MCh (18−20 h PE)
Miller et al. (2016b)	Rats (WKY) n = 4−6 males Age: 12−13 weeks	1 ppm, 4 h/day for 1−2 days	Ventilatory parameters (immediately post-exposure Day 1 and about 12 h later)
Zhu et al. (2016)	Mice (BALB/c) n = 3−5 males Age: 5−6 weeks	0.1 ppm, 3 h/day for 7 days 0.5 ppm, 3 h/day for 7 days 1 ppm, 3 h/day for 7 days	Pulmonary mechanics, challenge with MCh (24 h PE)
Gordon et al. (2017b)	Rats (LE) n = 10 females Age: 13 weeks	0.25 ppm, 5 h/day for 2 days 0.5 ppm, 5 h/day for 2 days 1 ppm, 5 h/day for 2 days	Ventilatory parameters (immediately PE)
Mathews et al. (2017b)	Mice (C57BL/6J WT and TCR gamma delta deficient) n = 6 males, 4−10 females Age: 10 weeks	2 ppm, 3 h	Pulmonary mechanics, challenge with MCh (24 h PE)
Henriquez et al. (2017)	Rats (WKY) n = 6−8 males Age: 12 weeks	0.8 ppm, 4 h/day for 1−2 days	Ventilatory parameters (immediately PE)
Malik et al. (2017)	Mice (C57BL/6J WT, Ccrl2 deficient) n = 8−13 females Age: 8 weeks	2 ppm, 3 h	Pulmonary mechanics, challenge with MCh (4 and 24 h PE)
Michaudel et al. (2018)	Mice (C57BL/6J WT, ST2 deficient) n = 4−6 females Age: 8−10 weeks	1 ppm, 1 h	Ventilatory parameters, challenge with MCh (24 h PE)
Stober et al. (2017)	Mice (BALB/cByJ WT, TSG-6 deficient) n = 4−8 Sex and age: NR	2 ppm, 3 h	Pulmonary mechanics, challenge with MCh (24 h PE)
Mathews et al. (2018)	Mice (C57BL/6J) n = 4−14 females Age: 10 weeks	2 ppm, 3 h	Pulmonary mechanics, challenge with MCh (24 h PE)
Liu et al. (2016)	Mice (BALB/c) n = 6 Sex and age: NR but weight was 20 g	1.5 ppm, 0.5 h/day for 5 days	Ventilatory parameters, challenge with MCh (immediately PE)

Table 3-5 (Continued): Study-specific details from animal toxicological studies of short-term ozone exposure and lung function—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Cho et al. (2018)	Mice (C57BL/6) Specific pathogen free and germ free n = 6−14 males Age: 10 weeks	2 ppm, 3 h	Pulmonary mechanics, challenge with MCh (24 h PE)

BN = brown Norway; LE = Long-Evans; MCh = methacholine; PE = post-exposure; S-D = Sprague-Dawley; WKY = Wistar Kyoto; WS = Wistar; WT = wild type.

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Table 3-6 Epidemiologic studies of short-term exposure to ozone and lung function in healthy populations.

Study	Study Population	Exposure Assessment	Mean ppb	Copollutant Examination	Effect Estimates 95% CI[a]
Berry et al. (1991) Hamilton, NJ, U.S. July 1988 Panel study	n = 14 Campers without asthma Age: <14 yr	Regional monitor for part of study (<8 miles from camps) Mobile trailer monitor onsite at one camp 1- h max	Mean: NR Maximum: 204	Correlation (*r*): NR Copollutant models with: NR	FEV_1 (mL): 20.5 (4.3, 36.7) PEF (mL/sec): −25.3 (−82.6, 32.1)
Spektor and Lippmann (1991) Fairview Lake, NJ, U.S. July−August 1988 Panel study	n = 46 Campers without asthma Age: 8−14 yr	On-site monitor 1- h avg	Mean: 69 Maximum: 137	Correlation (*r*): NR Copollutant models with: NR	Percent increase FEV_1: −1.4 (−1.9, −0.8)
Avol et al. (1990) Pine Springs, CA, U.S. June−August 1988 Panel study	n = 295 Campers without asthma Age: 8−17 yr	On-site monitoring 1- h avg	Mean: 94 Maximum: 161	Correlation (*r*): NR Copollutant models with: NR	Percent increase FEV_1: −0.4 (−0.6, −0.1) PEF: 1.2 (0.4, 1.9)
Burnett et al. (1990) Lake Couchiching, Ontario, Canada June−July 1983 Panel study	n = 29 Campers without asthma Age: 7−15 yr	On-site monitoring 1- h avg	Mean: 59 Maximum: 95	Correlation (*r*): NR Copollutant models with: NR	Percent increase FEV_1: −0.2 (−1.1, 0.7) PEF: −1.19 (−2.38, −0.03)
Higgins et al. (1990) San Bernardino, CA, U.S. June−July 1987 Panel study	n = 43 Campers without asthma Age: 7−13 yr	On-site monitoring 1- h avg	Mean: 103 Maximum: 245	Correlation (*r*): NR Copollutant models with: NR	Percent increase FEV_1: −1.0 (−1.5, −0.5) PEF: −0.5 (−1.3, 0.2)
Raizenne et al. (1989) Lake Erie, Ontario, Canada June−August 1986 Panel study	n = 112 Campers without asthma Age: mean 11.6 yr	On-site monitoring 1- h avg	Mean: 71 Maximum: 143	Correlation (*r*): NR Copollutant models with: NR	Percent increase FEV_1: −0.3 (−0.5, −0.1) PEF: −0.04 (−0.35, 0.26)
Spektor et al. (1988) Fairview Lake, NJ, U.S. July−August 1984 Panel study	n = 91 Campers without asthma Age: 8−15 yr	On-site monitoring 1- h avg	Mean: 53 Maximum: 113	Correlation (*r*): $PM_{2.5}$: 0.78; SO_4^{2-}: 0.82 Copollutant models with: NR	Percent Increase FEV_1: −0.6 (−0.9, −0.2) PEF: −1.1 (−2.1, −0.3)

Table 3-6 (Continued): Epidemiologic studies of short-term exposure to ozone and lung function in healthy populations.

Study	Study Population	Exposure Assessment	Mean ppb	Copollutant Examination	Effect Estimates 95% CI[a]
†Dales et al. (2013) Sault Ste. Marie, Ontario, Canada Ozone: May−August, 2010 Follow-up: May−August, 2010 Panel study	n = 61 Age: 24 ± 6 yr 8 h near steel plant or college campus for 5 consecutive days at each site with 9-day period between. Outcomes 0 h after exposure period	Portable monitor at site 8- h avg (8- h period between 7:50 a.m.−5:50 p.m.) Summer days	Mean: college campus: 32.6; steel plant: 29.7	Correlation (*r*): NR Copollutant models with: NR	Percent increase FEV_1: −0.47 (−1.00, 0.06) FEV_1/FVC: −0.48 (−0.90, −0.05) FVC: −0.56 (−1.39, 0.26) TLC: −0.97 (−2.71, 0.76) FEF_{25-75}: −1.46 (−3.46, 0.53) RV: −6.48 (−12.47, 0.50)

Note: †Studies published since the 2013 Ozone ISA.

[a]Results standardized to a 15-ppb increase in 24-hour avg, 20-ppb increase in 8-hour daily max, or 25-ppb increase in 1-hour daily max ozone concentrations.

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Table 3-7 Epidemiologic studies of short-term exposure to ozone and lung function, airway inflammation, and oxidative stress in general populations.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI[a]
†Lepeule et al. (2014) Boston, MA, U.S. 1999−2009	n = 776 Adult men Age: 72.3 (mean); 6.8 (SD) 5.9% asthma prevalence, 6.8% chronic bronchitis prevalence	City-wide monitor average (median monitor distance from participant homes: 22.3) 4-h avg (4 a.m. to 8 a.m.) 24-h avg	Mean: 47 (24-h avg) 95th: 60	Correlation (*r*): CO: −0.29; NO_2: −0.31; $PM_{2.5}$: 0.04; BC: −0.21 Copollutant models with: NR	Results presented graphically. 1-day lag ozone concentrations, as well as longer moving averages (3, 4, 5, 6, 7, 14, 21, and 28 days), were associated with decreased FEV_1 and FVC. DNA methylation did not significantly modify the effect of ozone on lung function.
†Rice et al. (2013) Boston, MA, U.S. 1995−2011	n = 3,362 Adults Age: 51.8 (mean); 12.7 (SD) 20.7% asthma or COPD prevalence	City-wide monitor average 8-h max Warm season (April−September)	Mean: 28.7 75th: 35.3 Max: 59.6	Correlation (*r*): NO_2: 0.01; $PM_{2.5}$: 0.33 Copollutant models with: NR	Lag 1 FEV_1 (mL): −34.8 (−61.8, −8.0) Obese participants FEV_1 (mL): −60.8 (−94.0, −27.4) Nonobese participants FEV_1 (mL): −24.8 (−52.8, 3.4)
†Patel et al. (2013) New York City, NY, U.S. 2005 Panel Study	n = 36 Schoolchildren ages 14-19 50% asthma prevalence	Single monitor within 14 km of schools 8- h max May-June	Median: 38.8	Correlation (*r*): BC: 0.02 Copollutant models with: NR	Lag 0 Unit change in exhaled breath condensate pH -0.14 (-0.33, 0.05) Unit change in 8-isoprostane -0.41 (-0.72, -0.10)
†Salam et al. (2012) Multicity, southern California, U.S. 2004−2006	n = 940 Schoolchildren ages 6−11 14.2% asthma prevalence	Single monitor in each study community. 8-h avg (10 a.m. to 6 p.m.)	Mean: 35.1 Max: 63.7	Correlation (*r*): $PM_{2.5}$: 0.07; PM_{10}: 0.34; NO_2: −0.41 Copollutant models with: NR	7-day avg Inducible nitric oxide synthase (iNOS) % methylation −0.08 (−1.40, 1.28)

Note: †Studies published since the 2013 Ozone ISA.

[a]Results standardized to a 15-ppb increase in 24-hour avg, 20-ppb increase in 8-hour daily max, or 25-ppb increase in 1-hour daily max ozone concentrations.

Table 3-8 Study-specific details from controlled human exposure studies of respiratory symptoms in healthy populations.

Study	Population n, Sex, Age (Range or Mean ± SD)	Exposure Details (Concentration, Duration)	Endpoints Examined
Bennett et al. (2016)	Healthy adults n = 19 normal weight females Age: 24 ± 4 yr n = 19 obese females Age: 28 ± 5 yr	0 ppb, 2 h 400 ppb, 2 h	Symptoms (immediately PE)

Table 3-9 Study-specific details from controlled human exposure studies of inflammation, oxidative stress, and injury in healthy populations.

Study	Population n, Sex, Age (Range or Mean ± SD)	Exposure Details (Concentration, Duration)	Endpoints Examined
Bosson et al. (2013)	Healthy adults n = 8 males, 5 females Age: 24.6 yr	0 ppb, 2 h 200 ppb, 2 h	BALF PMNs (1.5 h PE)
	Healthy adults n = 9 males, 6 females Age: 24.5 yr		BALF PMNs (6 h PE)
	Healthy adults n = 10 males, 5 females Age: 23 yr		BALF PMNs (18 h PE)
Holland et al. (2014)	Healthy adults n = 10 males, 12 females Age: 33.0 ± 7.4 yr	0 ppb, 4 h 100 ppb, 4 h 200 ppb, 4 h	BALF (20 h PE)
Gomes et al. (2011a)	Healthy adults n = 9 males, 0 females Age: 30 ± 2.6 yr	100 ppb with heat, 0.5 h	Nasal lavage (0 and 6 h PE)
Alexis et al. (2013)	Healthy adults n = 24 Age: 20−33yr	60 ppb, 6.6 h	Sputum PMN (18 h PE)
Hoffmeyer et al. (2015)	Healthy adults n = 5 males, 5 females Age: 25.6 ± 2.5yr	40 ppb, 4 h 240 ppb, 4 h	EBC-pH (before and immediately after and 16 h PE) FeNO (before and immediately after and 16 h PE)
Holz et al. (2015)	Healthy adults n = 12 males, 12 females; only 18 subjects completed study Age: 35 yr (median)	250 ppb, 3 h	Sputum (3 h PE)
Speen et al. (2016)	Healthy adults n = 9−11 Age: 18−35 yr	0 ppb, 2 h 300 ppb, 2 h	BALF Oxysterols (1 and 24 h PE)

Table 3-9 (Continued): Study-specific details from controlled human exposure studies of inflammation, oxidative stress, and injury in healthy populations.

Study	Population n, Sex, Age (Range or Mean ± SD)	Exposure Details (Concentration, Duration)	Endpoints Examined
Bennett et al. (2016)	Healthy adults n = 19 normal weight females Age: 24 ± 4 yr n = 19 obese females Age: 28 ± 5 yr	0 ppm, 2 h 400 ppb, 2 h	Sputum PMN (4 h PE)
Cheng et al. (2018) Devlin et al. (2012)	Healthy adults n = 20 males, 3 females Age: 28.8 yr (median)	0 ppb, 2 h 300 ppb, 2 h	BALF samples (1 or 24 h PE)
Arjomandi et al. (2018) Frampton et al. (2017)	Healthy adults n = 35 males, 52 females Age: 59.9 ± 4.5 yr	0 ppb, 3 h 70 ppb, 3 h 120 ppb, 3 h	Sputum protein and PMNs (22.5 h PE)
Lazaar et al. (2011)	Healthy adults n = 24 males, 0 females Age: 35.5 yr	250 ppb, 3 h	Sputum PMN (3 h PE)
Biller et al. (2011)	Healthy adults n = 11 males, 3 females Age: 33.1 ± 9.5 yr	0 ppb, 3 h 250 ppb, 3 h	Sputum (screening and 3 h PE)
Tank et al. (2011)	Healthy adults n = 11 males, 3 females Age: 34 ± 10 yr	0 ppb, 3 h 250 ppb, 3 h	Sputum (3 h PE)
Kirsten et al. (2011)	Healthy adults n = 15 males, 3 females Age: 43.9 ± 7.4 yr	250 ppb, 3 h	Sputum PMN (3 h PE)
Gomes et al. (2011b)	Healthy adults n = 9 males, 0 females Age: 24 ± 6 yr	0 ppb with control, 0.5 h 0 ppb with heat, 0.5 h 100 ppb with control, 0.5 h 100 ppb with heat, 0.5 h	Sputum markers (15 min PE)

Table 3-10 Study-specific details from animal toxicological studies of short-term ozone exposure and allergic sensitization—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Brand et al. (2012)	Mice (C57BL/6) n = NR males Age: 8−12 weeks	0.8 ppm, 8 h/day for 3 days	Histopathology, BALF total cells and differentials, dendritic cell number and activation in specific sites, T cell number in MLN (immediately PE)
Zhu et al. (2016)	Mice (BALB/c) n = 3−5 males Age: 5−6 weeks	0.1 ppm, 3 h/day for 7 days 0.5 ppm, 3 h/day for 7 days 1 ppm, 3 h/day for 7 days	IgE, Th2 cytokines, mast cell degranulation (24 h PE)

BALF = bronchoalveolar lavage fluid; IgE = immunoglobulin E; MLN = mediastinal lymph node; PE = post-exposure; Th2 = T helper 2.

Table 3-11 Study-specific details from animal toxicological studies of short-term ozone exposure and inflammation, oxidative stress, and injury—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Hulo et al. (2011)	Mice (C57BL6/SV129 WT and AMPK-α deficient) n = 3−10 males Age: 20−24 weeks	2 ppm, 3 h	Markers of oxidative stress, inflammation, injury; AMPK activation; Na/K-ATPase abundance (24 h PE)
Connor et al. (2012)	Mice (C3H/HeOuJ and TLR4 mutant C3H/HeJ) n = 3−18 males Age: 11−12 weeks	0.8 ppm, 3 h	Markers of oxidative stress, injury, and inflammation; surfactant protein D (0.5−48 h PE)
Kasahara et al. (2012)	Mice (C57BL/6J WT and adiponectin deficient) n = 3−10 (sex and age matched) males, females Age: 11−13 weeks	0.3 ppm, up to 72 h	Markers of injury and inflammation (PE)
Shore et al. (2011)	Mice (C57BL/6 WT and TNRF1 deficient) n = 3−6 males or females Age: 7 and 39 weeks	2 ppm, 3 h	BALF total and differential cell count, cytokines, chemokines and tissue mRNA MT, HO-1, claudin-4 and amphiregulin (4 h PE)
Schelegle and Walby (2012)	Rats (BN) n = 5−11 males Age: 8−10 weeks	1 ppm, 8 h	BALF markers of injury and inflammation (immediately PE)
Wolkoff et al. (2012)	Mice (BALB/cA) n = 9−20 males Age: NR, but mean weight was 24 g	0.1 ppm, 1 h/day for 10 days	BALF cells (immediately PE)
Tankersley et al. (2013)	Mice (C57BL/6J WT and atrial natriuretic peptide-deficient) n = 5−6 males Age: 11 weeks	0.5 ppm, 3 h	BALF cell counts and cell differentials, total protein (8−10 h PE)
Lee et al. (2013)	Mice (BALB/c) n = 6 females Age: 5−6 weeks	2 ppm, 3 h	BALF cells, MDA, antioxidants, RNS (immediately PE)
Sunil et al. (2013)	Rats (WS) n = 3−6 females Age: NR but weight was 200−225 g	2 ppm, 3 h	BALF protein and CCSP (3−72 h PE)

Table 3-11 (Continued): Study-specific details from animal toxicological studies of short-term ozone exposure and inflammation, oxidative stress, and injury—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Groves et al. (2012)	Mice (C57BL/6J WT) n = 4−9 males Age: 8 weeks	0.8 ppm, 3 h	BALF protein, RNS, macrophage number, chemotactic activity s (24−72 h PE)
Sunil et al. (2012)	Rats (WS) 3−11 females Age: NR but weight was 200−225 g	2 ppm, 3 h	BALF protein, cell number, differentials, immunohistochemistry—markers of oxidative stress, apoptosis and autophagy, BALF macrophages—markers of classical and alternative activation pathways, (3−72 h PE)
Bhoopalan et al. (2013)	Rats (S-D) n = 6 males Age: 8−9 weeks	0.8 ppm, 3 h	BALF total cells and differentials, protein, albumin, LDH, total antioxidant capacity, lung tissue SODs, catalase, β-actin (18−24 h PE)
Cho et al. (2013)	Mice (ICR WT and NRF2 deficient) n = 3−12 Sex and age: NR	0.3 ppm, 6−72 h 2 ppm, 3 h	BALF total protein and cell differentials, mucin, glutathione, lung tissue redox measurements, histopathology (immediately and 3−24 h PE)
Robertson et al. (2013)	Mice (C57BL/6 WT and CD36-deficient) n = 3−8 females Age: 8−10 weeks	1 ppm, 4 h	BALF protein, cell number and cell differentials (24 h PE)
Thomson et al. (2013)	Rats (F344) n = 4−6 males Weight NR but age was 200−250 g	0.4 ppm, 4 h 0.8 ppm, 4 h	mRNA expression in tissue (immediately and 24 h PE)
Yanagisawa et al. (2012)	Mice (C57BL/6J WT and peroxiredoxin-1 deficient) n = 4−8 males Age: 18 weeks	2 ppm, 6 h	BALFtotal cell count and cell differentials, total protein, mediators, Prxd1 tissue HO-1 and GST mRNA, NRF2 protein, histopathology (0, 4, 18 h PE)
Barreno et al. (2013)	Mice (C57BL/6 WT and osteopontin deficient) n = 6−10 females Age: 8 weeks	2 ppm, 3 h	BALF and serum osteopontin, cytokines, total cells and cell differentials, total protein, soluble collagen, epithelial cells (6 and 24 h PE)
McIntosh-Kastrinsky et al. (2013)	Mice (C57BL/6) n = 8 females Age: 7 mo	0.245 ppm, 4 h	BALF cells (12 h PE)

Table 3-11 (Continued): Study-specific details from animal toxicological studies of short-term ozone exposure and inflammation, oxidative stress, and injury—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Groves et al. (2013)	Mice (C57BL/6J WT) n = 3−10 males Age: 8, 27, 80 weeks	0.8 ppm, 3 h	BALF total cells and differential cell counts, protein (72 h PE)
Brand et al. (2012)	Mice (C57BL/6) n = NR males Age: 8−12 weeks	0.8 ppm, 8 h/day for 3 days	Histopathology, BALF total cells and differentials, dendritic cell number and activation in specific sites, T cell number in MLN (immediately PE)
Wang et al. (2013)	Rats (WS) n = 6 males Age NR but weight was 150−180 g	0.8 ppm, 4 h/day, 2 days per week for 3 weeks	BALF total cells and cell differentials, LDH, protein, albumin, alkaline phosphatase; histopathology; lung tissue activity of GPx and SOD, MDA levels, mRNA eNOS, iNOS, ICAM-1 (24 h after 6th exposure)
Gonzalez-Guevara et al. (2014)	Rats (WS) n = 3−6 males Age: NR but weight was 250−300 g	1 ppm, 1 or 3 h/day for 5 days 1 ppm, 1, 3, and 6 h	Tissue IL-6 and TNF-α (immediately PE)
Kurhanewicz et al. (2014)	Mice (C57BL/6) n = 6 females Age: 10−12 weeks	0.3 ppm, 4 h	BALF LDH, microalbumin, NAG, total protein (24 h PE)
Ghio et al. (2014)	Mice (CD-1) n = 6 females Age: 4 weeks	2 ppm, 3 h	BALF and liver nonheme iron, BALF ferritin; BALF injury markers and neutrophils and cytokines (24 h PE)
Paffett et al. (2015)	Rats (SD) n = 3−5 males Age: 8−12 weeks	1 ppm, 4 h	BALF total cell and cell differential counts, total protein (24 h PE)
Sunil et al. (2015)	Mice (C57BL/6J WT and galectin-deficient) n = 3−14 females Age: 8−11 weeks	0.8 ppm, 3 h	BALF protein, tissue cytochrome b5 as injury markers; macrophage subpopulations in tissue and BAL (2,472 h PE)
Gabehart et al. (2015)	Mice (BALB/c) n = 3−14 females Age: 6 weeks	1 ppm, 3 h	BAFL total cell number and differential cell counts, albumin, Muc5AC; gene expression chemokines, antioxidants, TLR4, neuropeptides (6, 24, and 48 h PE)

Table 3-11 (Continued): Study-specific details from animal toxicological studies of short-term ozone exposure and inflammation, oxidative stress, and injury—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Razvi et al. (2015)	Mice (C57BL/6J WT and resistin-deficient) n = 6−8 males/females Age: 4−8 weeks	2 ppm, 3 h	BALf cells, protein, and mediators, tissue injury and inflammation (24 h PE)
Kumarathasan et al. (2015)	Rats (F344) n = 8−17 males Age: NR but weight was 200−250 g	0.4 ppm, 4 h 0.8 ppm, 4 h	BALF cells and cell differentials, BALF markers of oxidative stress and injury (24 h PE)
Verhein et al. (2015)	Mice (B6129SF1/J WT and Notch3 and Notch4 deficient) n = 4−10 males Age: 7−13 weeks	0.3 ppm, 6−72 h	BALF total protein, total cell count, and cell differentials; tissue NFκB activation, mRNA for tnf and Notch-related genes, microarray analysis (immediately PE)
Cabello et al. (2015)	Mice (C57BL/6J) n = 6 males, 6 females Age: 8 weeks	2 ppm, 3 h	BALF total cells and cell differential counts, protein, albumin (24 and 72 h PE); lung tissue mRNA array of 84 inflammatory gene; lung tissue PCR of proinflammatory cytokines and chemokines, pattern recognition receptors, transcription factors, STAT3 phosphorylation (4 h PE)
Ward et al. (2015)	Rats (WKY) n = 3−4 males Age: 10−12 weeks	0.25 ppm, 4 h 0.5 ppm, 4 h 1 ppm, 4 h	BALF protein and neutrophils, lung gene expression (0 and 20 h PE)
Kodavanti et al. (2015)	Rats (WKY, WS, SD) n = 4−8 males Age: 12−14 weeks	0.25 ppm, 4 h 0.5 ppm, 4 h 1 ppm, 4 h	BALF total cell counts and cell differentials, total protein, albumin, LDH, NAG, GGT; lung tissue mRNA for HO-1, MIP-2, TNF-α, IL-6, IL-10 (0 and 20 h PE)
Ramot et al. (2015)	Rats (WKY, WS, S-D) n = 4−8 males Age: 12−14 weeks	0.25 ppm, 4 h 0.5 ppm, 4 h 1 ppm, 4 h	Lung histopathology (0 and 20 h PE)
Ward and Kodavanti (2015)	Rats (WKY) n = 3−4 males Age: 12−14 weeks	1 ppm, 4 h	Lung gene expression profiling (immediately PE)
Ong et al. (2016)	Mice (C57BL/6 WT and lymphoid cell-deficient) n = 6 males Age: 6−8 weeks	0.5 ppm, 4 h for up to 9 days	Histopathology, immunochemistry, mRNA expression (2−24 h after exposure)

Table 3-11 (Continued): Study-specific details from animal toxicological studies of short-term ozone exposure and inflammation, oxidative stress, and injury—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Hatch et al. (2015)	Rats (WKY, WS) n = 8 males Age: 12−14 weeks	1 ppm, 4 h	BALF and tissue antioxidants (0 and 20 h PE)
Mishra et al. (2016)	Mice (C57BL/6) n = 6−8 males, 6−8 females with females at different stages of estrous cycle Age: 8 weeks	2 ppm, 3 h	Lung tissue inflammatory mediators and transcription factors (4 h PE)
Che et al. (2016)	Mice (C57BL/6 WT and Il-17a and Il-1r1 deficient) n = 6 females Age: 6−8 weeks	0.7 ppm, 72 h	BALF total cells and cell differentials, protein; lung tissue cytokines and chemokines, mRNA, flow cytometry of lymphocyte subpopulations; flow cytometry of lung macrophage ROS; lung macrophage mtDNA (24 h PE)
Snow et al. (2016)	Rats (BN) n = 6−8 males Age: 1, 4, 12, 24 mo	0.25 ppm, 6 h/day for 2 days 1 ppm, 6 h/day for 2 days	BALF total cells, cell differentials, protein, albumin, GGT, NAG (18 h PE)
Gordon et al. (2016b)	Rats (BN) n = 9−10 males, 9−10 females Age: 16 weeks	0.8 ppm, 5 h	BALF total cells, cell differentials, albumin (18 h PE)
Kasahara et al. (2015)	Mice (C57BL/6 WT, ROCK1 insufficient, ROCK2 insufficient) n = 4−12 males Age: 20−25 weeks	2 ppm, 3 h	BALF total cells, cell differentials, albumin, epithelial cells, protein, cytokines, chemokines, hyaluronan; lung tissue GRPR mRNA, ROCK, and rho protein (24 h PE)
Verhein et al. (2013)	Guinea pigs (Dunkin-Hartley) n = 3−7 females Age: NR but weight was 300−470 g	2 ppm, 4 h	BALF total cells and cell differentials (24 h PE)
Mathews et al. (2015)	Mice (C57BL/6 WT, gamma delta T cell deficient) n = 4−14 males Age: 10−13 weeks	0.3 ppm, 24−72 h	BALF total cells and cell differentials, cytokines, protein; lung tissue mRNA; lung tissue macrophage subpopulations, histopathology (0, 1, 3, 5 days PE)

Table 3-11 (Continued): Study-specific details from animal toxicological studies of short-term ozone exposure and inflammation, oxidative stress, and injury—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Williams et al. (2015)	Mice (C57BL/6J WT or Cpe fat, TNF-α sufficient and deficient) 5−9 females Age: 10−12 weeks	2 ppm, 3 h	BALF total cells and cell differentials, MCP-1, G-CSF, hyaluronan, osteopontin, IL-13, protein carbonyls; lung tissue mRNA for antioxidant proteins and Il17a (24 h PE)
Zychowski et al. (2016)	Mice (C57BL/6) n = 4−8 males Age: 6−8 weeks	1 ppm, 4 h	Lung weight:body weight ratios, BAL total cells (18−20 h PE)
Thomson et al. (2016)	Rats (F344) n = 5 males Age: NR but weight was 200−250 g	0.8 ppm, 4 h	BALF total cells and cytokines; lung tissue mRNA for cytokines and antioxidant proteins and glucocorticoid inducible proteins (immediately PE)
Miller et al. (2016b)	Rats (WKY) n = 4−6 males Age: 12−13 weeks	1 ppm, 4 h/day for 1−2 days	BALF total cells and differential cells, protein, albumin, LDH (immediately PE Day 1 and Day 2)
Zhu et al. (2016)	Mice (BALB/c) n = 3−5 males Age: 5−6 weeks	0.1 ppm, 3 h/day for 7 days 0.5 ppm, 3 h/day for 7 days 1 ppm, 3 h/day for 7 days	Oxidative stress and upregulation of antioxidants (24 h PE)
Kasahara et al. (2013)	Mice (C57BL/6 WT, adiponectin, and T-cadherin deficient) n = 3−16 (sex matched males and females) Age: 11−13 weeks	0.3 ppm, 72 h	BALF total cells and cell differentials, cytokines, chemokines; lung tissue mRNA for IL-17A, serum amyloid A3 and Ki67 (immediately PE)
Kasahara et al. (2014)	Mice (C57BL/6 WT, adiponectin deficient, IL-6 deficient) n = 3−13 (sex matched males and females) Age: 11−13 weeks	0.3 ppm, 24−72 h	BALF total cells and differentials, cytokines, adiponectin; lung tissue mRNA for serum amyloid A3, TIMP1, Il17a, microarray analysis; flow cytometry (immediately PE)
Brand et al. (2016)	Mice (C57BL/6 WT, IL-23 deficient, Flt3l deficient (lacking conventional dendritic cells) n = 5−12 (sex matched males and females) Age: 8−12 weeks	0.3 ppm, 24−72 h	BALF total cells and cell differentials, cytokines, chemokines, protein, lung tissue mRNA for Il17a and Il23a; flow cytometry for cDC macrophages (immediately PE)

Table 3-11 (Continued): Study-specific details from animal toxicological studies of short-term ozone exposure and inflammation, oxidative stress, and injury—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Kumagai et al. (2016)	Mice (C57BL/6 WT, Rag2 deficient, Il2rg deficient) n = 6 males Age: 6−8 weeks	0.5 ppm, 4 h/day for 1 or 9 days	Quantitative immunochemistry for markers of nasal epithelial remodeling and eosinophilic rhinitis; upregulation of Th2-related genes (24 h after last exposure)
Elkhidir et al. (2016)	Mice (C57BL/6 WT, PAI-1 deficient) n = 6−10 females Age: NR but WT and deficient mice were age matched	2 ppm, 3 h	BALF total cells and cell differential, epithelial cells, protein, Il-6, KC, MIP-2, PAI-1 (4 and 24 h PE)
Gordon et al. (2017b)	Rats (LE) n = 10 females Age: 13 weeks	0.25 ppm, 5 h/day for 2 days 0.5 ppm, 5 h/day for 2 days 1 ppm, 5 h/day for 2 days	BALF total cells and cell differentials, total protein, albumin, NAG, GGT (immediately PE)
Ciencewicki et al. (2016)	Mice (C57BL/6 WT, mannose binding lectin deficient) n = 6−12 males Age: 6 weeks	0.3 ppm; 24, 48, 72 h	BALF total cell counts and cell differentials, protein; lung tissue mRNA Il6, tnf, cxcl2, cxcl5, microarray (immediately PE)
Feng et al. (2015)	Mice (BALB/c) n = 3−7 males, 3−7 females Age: 6−8 weeks	0.25 ppm, 3 h/day for 7 days 0.5 ppm, 3 h/day for 7 days 1 ppm, 3 h/day for 7 days	BALF total cells and cell differentials, protein, ROS, EGF, TGF-α (20−24 h PE)
Francis et al. (2017b)	Mice (C57BL/6J) n = 3−10 females Age: 11−14 weeks	0.8 ppm, 3 h	BALF total cells and cell differentials, total proteins; lung tissue immunohistochemistry for macrophage markers and 4-HNE, western blotting for SP-D, mRNA for chemokines and ligands; flow cytometry of lung tissue and BALF cells for monocyte subpopulations (24−72 h PE)
Harkema et al. (2017)	Mice (C57BL/6NTac and BALB/cNTac) n = 6 males Age: 6−8 weeks	0.8 ppm, 4 h/day for 9 days	BALF total cells and cell differentials; lung tissue mRNA for MUC5AC, MUC5B, Clca1/Gob5, Il33, Il25, Tslp, Il5, Il13, Chia, Chil4/Ymw (24−72 h PE)

Table 3-11 (Continued): Study-specific details from animal toxicological studies of short-term ozone exposure and inflammation, oxidative stress, and injury—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Mathews et al. (2017b)	Mice (C57BL/6J T, TCR gamma delta deficient) n = 4−10 females Age: 10 weeks	2 ppm, 3 h	BALF total cells and cell differentials, cytokines, chemokines; flow cytometry of isolated cells from lung tissue (24 h PE)
Xiang et al. (2012)	Mice (BALB/c) n = 5 Sex and age: NR	2 ppm, 0.5 h for 1−8 days	mRNA for transcription factor
Mathews et al. (2017a)	Mice (C57BL/6J) n = 5−8 females Age: 10 weeks	2 ppm, 3 h	Markers of oxidative stress (24 h PE)
Malik et al. (2017)	Mice (C57BL/6J WT, Ccrl2 deficient) n = 8−13 females Age: 8 weeks	2 ppm, 3 h	BALF total cell number and differential cell counts, total protein, epithelial cells, chemerin, adiponectin, eotaxin, hyaluronan, IL-6, KC, MIP-2, MIP-3α; lung tissue mRNA for Ccrl2 (4 and 24 h PE)
Tighe et al. (2018)	Mice (C57BL/6J) n = 5 males Age: 8−10 weeks	2 ppm, 3 h	BALF total cells and cell differentials, total protein, albumin (24 h PE)
Holze et al. (2018)	Mice (C57BL/6 WT, Nlrp deficient, caspase deficient, Asc deficient, and Pgam5 deficient) n = 5 Sex and age: NR	1 ppm, 1 h	BALF cells, protein, MPO, cytokines, (4 or 24 h PE)
Michaudel et al. (2018)	Mice (C57BL/6J WT, ST2 deficient, IL-33 deficient, and IL-33 citrine reporter) n = 8−12 males, 4−6 females Age: 8−10 weeks	0.3 ppm, 1 h 1 ppm, 1 h	BALF total cells and cell differentials, proinflammatory cytokines, chemokines, and remodeling parameters, ROS producing cells, total protein, vascular leak, epithelial desquamation marker, tissue IL-33, ST2, tight junction proteins and mRNA, cell death marker, FACS (1−48 h PE)
Snow et al. (2018)	Rats (WKY) n = 6−8 males Age: 12 weeks	0.8 ppm, 4 h/day for 2 days	BALF total cell counts and cell differentials, injury markers, cytokines; lung tissue mRNA for cholesterol transporters, cholesterol receptors, nuclear receptors (2 h PE)

Table 3-11 (Continued): Study-specific details from animal toxicological studies of short-term ozone exposure and inflammation, oxidative stress, and injury—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Mathews et al. (2018)	Mice (C57BL/6J) n = 4−14 females Age: 10 weeks	2 ppm, 3 h	BALF total cells and cell differentials, IL-17A, IL-23, IL-33, CCL20, CXCL1, CXCL2, IL-6, G-CSF, GRP; lung tissue flow cytometry for IL-17A producing cells, microarray analysis, mRNA for GRPR, NQO1 (24 h PE)
Kumagai et al. (2017)	Mice (C57BL/6 WT, Rag2 deficient, and IL2rg deficient) n = 3−6 males Age: 7−9 weeks	0.8 ppm, 4 h/day for 1 or 9 days	BALF total cells and cell differentials; lung tissue mRNA for type 2 immunity-related transcripts, flow cytometry for ILCs. (24 h and 2 weeks PE)
Yonchuk et al. (2017)	Rats (Han Wistar) n = 5 Sex and age: NR but weight was 290−370 g	1 ppm, 3 h	BALF total cells and cell differential counts; lung tissue glutathione (immediately PE)
Zhang et al. (2017)	Rats (S-D) n = 4−5 Sex and age: NR but weight was 180−220 g	2 ppm, 0.5 h/day for up to 12 days	BALF TNF-α, TGFβ1, IL10, MPO; lung tissue 8-oxoguanine, OGG1, NOS and arginase activity/protein level, ROS (immediately PE)
Francis et al. (2017a)	Mice (C57BL/6J)WT and CCR2 deficient n = 3−10 females Age: 8−11 weeks	0.8 ppm, 1 h	BALF inflammatory cell subpopulations, BAL total protein; lung tissue iNOS, MR-1, ADAM17, Cypb5, 4-HNE, HO-1, CCR2, mRNA for TNF-α, IL-β, iNOS, CX3CR1, CX3CL1, NUR77 (24−72 h PE)
Liu et al. (2016)	Mice (BALB/c) n = 6 Sex and age: NR but weight was 20 g	1.5 ppm, 0.5 h/day for 5 days	BALF inflammatory cell subpopulations, IFN-γ, IL-4, IL-17, TGFβ, PGE2 (immediately PE)

Table 3-11 (Continued): Study-specific details from animal toxicological studies of short-term ozone exposure and inflammation, oxidative stress, and injury—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Cho et al. (2018)	Mice (C57BL/6) Specific pathogen free and germ free n = 6−14 males Age: 10 weeks	2 ppm, 3 h	BALF total cell counts and cell differential counts, total protein, IL-17A, osteopontin, IL-33, IL-5, GRP, G-CSF, eotaxin, IL-6, IP-10, LIF, MCP-1, KC MIP-2, MIP-1α, LTB4 (24 h PE)

4-HNE = 4-hydroxynonenol; ADAM = a disintegrin and metalloproteinase; AMPK = AMP-activated protein kinase; BALF = bronchoalveolar lavage fluid; BN = brown Norway; CCL = chemokine ligand; CCR2 = C-C chemokine receptor type 2; CCSP = club cell secretory protein; CD36 = cluster of differentiation 36; CXCL = chemokine family of cytokines with highly conserved motif: cys-xxx-cys (CXC) ligand; CXCR = receptor for chemokine family of receptors; EGF = epidermal growth factor; eNOS = endothelial nitric oxide synthase; F344 = Fischer 344; FACS = fluorescence activated cell sorting; GGT = gamma glutamyl transferase; G-CSF = granulocyte colony-stimulating factor; GPx = glutathione peroxidase; GRP = gastrin releasing peptide; GRPR = gastrin-releasing peptide receptor; GST = glutathione S-transferase; HO-1 = heme oxygenase 1; ICAM-1 = inter-cellular adhesion molecule 1; IFN-γ= interferon gamma; IL = interleukin; ILC = immune lymphoid cell; iNOS = inducible nitric oxide synthase; KC = keratinocyte-derived chemokine; LDH = lactate dehydrogenase; LE = Long-Evans; LTB4 = leukotriene B4; MCP-1 = monocyte chemotactic protein 1; MIP = macrophage inflammatory protein; MLN = mediastinal lymph node; MPO = myeloperoxidase; MR-1 = major histocompatibility complex class I-related gene protein; mtDNA = mitochondrial DNA; MUC = mucin; NAG = *N*-acetyl-glucosaminidase; Na/K-ATPase = sodium-potassium adenosine 5′-triphosphatase; NFκB = nuclear factor kappa-light-chain-enhancer of activated B cells; NQO1 = NADPH quinone oxidoreductase 1; NRF2 = nuclear factor (erythroid-derived 2)-like 2; NUR = nuclear receptor subfamily; OGG1 = 8-oxoguanine glycosylase; PAI-1 = plasminogen activator inhibitor 1; PE = post-exposure; PGE2 = prostaglandin E2; Prxd1 = peroxiredoxin 1; RNS = reactive nitrogen species; ROCK = rho-associated coiled-coil-containing protein kinase; ROS = reactive oxygen species; S-D = Sprague-Dawley; SOD = superoxide dismutase; SP-D = surfactant protein D; STAT3 = signal transducer and activator of transcription 3; TGF = transforming growth factor; Th2 = T helper 2; TIMP1 = TIMP metalloprotease inhibitor 1; TLR = toll receptor; TNF = tumor necrosis factor; TSLP = thymic stromal lymphopoietin; WKY = Wistar Kyoto; WS = Wistar; WT = wild type.

Table 3-12 Study-specific details from animal toxicological studies of short-term ozone exposure and morphology—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Connor et al. (2012)	Mice (C3H/HeOuJ and C3H/HeJ TLR4 mutant) n = 3−18 males Age: 11−12 weeks	0.8 ppm, 3 h	Type 2 cell proliferation (12−72 h PE)
Groves et al. (2012)	Mice (C57BL/6J) n = 4−9 males Age: 8 weeks	0.8 ppm, 3 h	Histopathology, immunohistochemistry (24−72 h PE)
Cho et al. (2013)	Mice (ICR WT and NRF2 deficient) n = 12 Sex and age: NR	0.3 ppm, 6−72 h 2 ppm, 3 h	Histopathology (3−24 h PE—acute) Histopathology (immediately PE—subacute)
Groves et al. (2013)	Mice (C57BL/6J WT) n = 3−10 males Age: 8, 27, 80 weeks	0.8 ppm, 3 h	Markers of cell proliferation, radial alveolar counts, lesion scores (72 h PE)
Brand et al. (2012)	Mice (C57BL/6) Males n = NR Age: 8−12 weeks	0.8 ppm, 8 h/day for 3 days	Histopathology and flow cytometry (immediately PE)
Wang et al. (2013)	Rats (WS) n = 6 males Age NR but weight was 150−180 g	0.8 ppm, 4 h day for 2 days per week for 3 weeks	Histopathology (24 h PE)
Gabehart et al. (2015)	Mice (BALB/c)WT and TLR4 deficient n = 3−14 females Age: 6 weeks	1 ppm, 3 h	Tissue: quantitative immunochemistry—Muc-5AC (6, 24, and 48 h PE)
Cabello et al. (2015)	Mice (C57BL/6J) n = 4−6 males, 4−6 females Age: 8 weeks	2 ppm, 3 h	Histopathology (24 and 72 h PE)
Ramot et al. (2015)	Rats (WKY, WS, S-D) n = 4−8 males Age: 12−14 weeks	0.25 ppm, 4 h 0.5 ppm, 4 h 1 ppm, 4 h	Lung histopathology (0 and 20 h PE)

Table 3-12 (Continued): Study-specific details from animal toxicological studies of short-term ozone exposure and morphology—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Ong et al. (2016)	Mice (C57BL/6J WT, Rag2 deficient, and Il2rg deficient) n = 6 males Age: 6−8 weeks	0.5 ppm, 4 h for 1−9 days	Histopathology, immunochemistry, mRNA expression (2−24 h PE)
Zhu et al. (2016)	Mice (BALB/c) n = 3−5 males Age: 5−6 weeks	0.1 ppm, 3 h/day for 7 days	Histopathology scores (24 h PE)
Kasahara et al. (2013)	Mice (C57BL/6 WT, adiponectin, and T-cadherin deficient) n = 3−16 males and females (age and sex matched) Age: 11−13 weeks	0.3 ppm, 72 h	Histopathology (immediately PE)
Kumagai et al. (2016)	Mice (C57BL/6 WT, Rag2 deficient, Il2rg deficient) n = 6 males Age: 6−8 week	0.5 ppm, 4 h/day for 1 or 9 days	Histopathology, quantitative histochemistry and immunochemistry for markers of nasal epithelial remodeling and eosinophilic rhinitis (24 h PE)
Feng et al. (2015)	Mice (BALB/cJ) n = 3−7, half males and half females Age: 6−8 weeks	0.25 ppm, 3 h/day for 7 days 0.5 ppm, 3 h/day for 7 days 1 ppm, 3 h/day for 7 days	Lung tissue immunohistochemistry, inflammation scores, mean linear intercept (20−24 h PE)
Harkema et al. (2017)	Mice (C57BL/6NTac and BALB/cNTac) n = 6 males Age: 6−8 weeks	0.8 ppm, 4 h/day for 9 days	Lung tissue histochemistry and immunochemistry for mucosubstances and myelin basic protein (24 h PE)
Wong et al. (2018)	Rats (WKY) n = 8−12 males Age: 44−48 weeks	1 ppm, 6 h	Histopathology lesion scores (8 h PE)
Michaudel et al. (2018)	Mice (C57BL/6J WT, ST2 deficient, IL-33 deficient, and IL-33 citrine reporter) n = 4−6 females Age: 8−10 weeks	0.3 ppm, 1 h 1 ppm, 1 h	Histopathological lesion scores, immunofluorescence, and confocal microscopy of specific proteins (1−48 h PE)
Kumagai et al. (2017)	Mice (C57BL/6 WT, Rag2 deficient, and IL2rg deficient) n = 3−6 males Age: 7−9 weeks	0.8 ppm, 4 h/day for 1 or 9 days	Quantitative histopathology, Histochemistry for mucosubstances, immunochemistry for BrdU (24 h or 2 weeks PE)

Table 3-12 (Continued): Study-specific details from animal toxicological studies of short-term ozone exposure and morphology—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Liu et al. (2016)	Mice (BALB/c) n = 6 Sex and age: NR but weight was 20 g	1.5 ppm, 0.5 h/day for 5 days	Lung histopathology scores

BALF = bronchoalveolar lavage fluid; MLN = mediastinal lymph node; PE = post-exposure; SD = Sprague-Dawley; WKY = Wistar Kyoto; WS = Wistar; WT = wild type.

Table 3-13 Epidemiologic studies of short-term exposure to ozone and hospital admission for asthma.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)[a]
Silverman and Ito (2010) New York, NY, U.S. Ozone: 1999−2006 Follow-up: 1999−2006 Time-series study	n = 75,383 All ages	Average of 13 monitors within 20 miles of the geographic city center 8-h max Warm season (April−August)	Mean: 41.0 75th: 53 90th: 68	Correlation (*r*): $PM_{2.5}$: 0.59 Copollutant models with: $PM_{2.5}$	RR All ages: 1.05 (0.99, 1.10) <6 yr: 1.03 (0.92, 1.15) 6−18 yr: 1.23 (1.09, 1.39) 19−49 yr: 0.97 (0.89, 1.07) 50+ yr: 1.04 (0.96, 1.12)
†Winquist et al. (2012) St. Louis, MO, U.S. Ozone: 2001−2007 Follow-up: 2001−2007 Time-series study	All ages	One monitor 8-h max Year-round		Correlation (*r*): NR Copollutant models with: NR	RR 0−4 DL: 1.05 (0.99, 1.11)
†Sheffield et al. (2015) New York City, NY, U.S. Ozone: May−September 2005−2012 Follow-up: May−September 2005−2011 Case-crossover study	n = 8,009 Age: 5−17 yr	Average of city monitors 24-h avg Warm season (May−September)	Max: 60	Correlation (*r*): NR Copollutant models with: NR	No quantitative results. Results presented graphically
†Shmool et al. (2016) New York City, NY, U.S. Ozone: June−August 2005−2011 Follow-up: June−August 2005−2011 Case-crossover study	n = 2,353 Age: 5−17 yr	Temporal estimates: Average of city monitors Spatiotemporal estimates: Fusion of monitors and LUR 24-h avg Warm season (May−September)	Mean: Temporal: 30.4 Spatio-temporal: 29.0 Max: Temporal: 60.0 Spatio-temporal: 60.3	Correlation (*r*): NR Copollutant models with: NR	No quantitative results. Results presented graphically

Table 3-13 (Continued): Epidemiologic studies of short-term exposure to ozone and hospital admission for asthma.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)[a]
†Goodman et al. (2017a) New York City, NY, U.S. Ozone: 1999−2009 Follow-up: 1999−2009 Time-series study	n = 295,497 All ages	Average of monitors within 20 miles of the geographic city center 8-h max Seasonal: Warm season (April−August) and year-round estimates	Mean: 30.7 Median: 28 75th: 39.9 Max: 105.4	Correlation (*r*): $PM_{2.5}$: 0.2 Copollutant models with: NR	Lag 0−1 RRs Warm season All ages: 1.01 (0.99, 1.03) <6 yr: 0.99 (0.96, 1.03) 6−18 yr: 1.05 (1.01, 1.10) 19−49 yr: 1.03 (1.00, 1.06) 50+ yr: 1.00 (0.97, 1.03)
†Zu et al. (2017) Six Texas cities, U.S. Ozone: 2001−2013 Follow-up: 2001−2013 Time-series study	n = 1,552,432 Age: 5+ yr	Average of monitors in each city 8-h avg Year-round	Mean: 32.2 Median: 31 75th: 40.1 90th: 48.6 Max: 82.8	Correlation (*r*): NR Copollutant models with: NR	Lag 0−3 RRs All ages (5+ yr): 1.05 (1.03, 1.07) 5−14 yr: 1.10 (1.05, 1.14) 15−64 yr: 1.04 (1.01, 1.07) 65+ yr: 1.00 (0.96, 1.05)
†Goodman et al. (2017b) Houston, Dallas, and Austin, TX, U.S. Ozone: 2003−2011 Follow-up: 2003−2011 Time-series study	n = 74,824 All ages	Average of monitors within each city 8-h max Year-round	Mean: 41.8 Median: 39.7 75th: 51.3 90th: 62.3 Max: 107	Correlation (*r*): NR Copollutant models with: NR	Lag 0 RRs All ages: 1.01 (1.00, 1.03) 5−14 yr: 1.05 (1.02, 1.10) 15−64 yr: 1.01 (0.99, 1.03) 65+ yr: 0.99 (0.95, 1.03)

Note: †Studies published since the 2013 Ozone ISA.

[a]Results standardized to a 15-ppb increase in 24-hour avg, 20-ppb increase in 8-hour daily max, or 25-ppb increase in 1-hour daily max ozone concentrations.

Table 3-14 Epidemiologic studies of short-term exposure to ozone and emergency department (ED) visits for asthma.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
Stieb et al. (2009) Seven Canadian cities Ozone: 1992−2003 Follow-up: 1992−2003 Time-series study	All ages	Average of monitors in each city. 24-h avg Year-round	Mean: 18.4 75th: 19.3−28.6 across cities	Correlation (*r*): Warm season (across cities): $PM_{2.5}$: −0.05, 0.62; NO_2: −0.17, 0.10; SO_2: -0.24, 0.21; CO: −0.34, 0.17 Cold season: $PM_{2.5}$: −0.65, 0.06; NO_2: −0.57, −0.35; SO_2: −0.52, −0.18; CO: −0.67, −0.16 Copollutant models with: NR	Percent increase Lag 1: 2.6 (0.2, 5.0)
Villeneuve et al. (2007) Alberta, Canada Ozone: 1992−2002 Follow-up: 1992−2002 Time-series study	n = 57,912 All ages	Average of three monitors. 8-h max Year-round and seasonal (April−September, October−March)	Summer: Mean: 38.0 75th: 46.0 Winter: Mean: 24.3 75th: 31.5	Correlation (*r*): NR Copollutant models with: NR	Lag 1 OR All ages Year-round: 1.04 (1.02, 1.07) Winter: 1.02 (0.98, 1.06) Summer: 1.07 (1.04, 1.10)
Ito et al. (2007) New York City, NY, U.S. Ozone: 1999−2002 Follow-up: 1999−2002 Time-series study	All ages	Average of 16 monitors within 20 miles of the geographic city center 8-h max Year-round and seasonal (April−September, October−March)	All year: Mean: 30.4 95th: 68.0 Warm: Mean: 42.7 95th: 77.0 Cold: Mean: 18.0 95th: 33.0	Correlation (*r*): NR Copollutant models with: $PM_{2.5}$, NO_2, SO_2, CO	Percent increase Lag 0−1 Warm season: 11.0 (7.1, 15.0)

Table 3-14 (Continued): Epidemiologic studies of short-term exposure to ozone and emergency department (ED) visits for asthma.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Sarnat et al. (2013) Atlanta, GA, U.S. Ozone: 1999−2002 Follow-up: 1999−2002 Time-series study	n = 270,816 All ages	Zip-code centroid estimates from a hybrid model fusing spatially interpolated background O_3 concentrations with local-scale AERMOD output 24-h avg Year-round	Mean: 41.9 Median: 39.3 75th: 53.8 95th: 76.2 Max: 132.7	Correlation (*r*): $PM_{2.5}$: 0.51; NO_X: −0.03 Copollutant models with: NR	Lag 0−2 RRs Overall: 1.03 (1.01, 1.04) High AER: 1.02 (1.00, 1.04) Low AER: 1.04 (1.02, 1.06)
†Winquist et al. (2012) St. Louis, MO, U.S. Oznoe: 2001−2007 Follow-up: 2001−2007 Time-series study	All ages	One monitor 8-h max Year-round		Correlation (*r*): NR Copollutant models with: NR	RR 0−4 DL: 1.05 (1.02, 1.08)
†Sacks et al. (2014) North Carolina (statewide), U.S. Ozone: 2006−2008 Follow-up: 2006−2008 Case-crossover study	n = 122,607 All ages	CMAQ model estimates predicted to census tract centroids and aggregated to the county-level using area-weighted average of census tract centroids 8-h max Seasonal: Warm season (April−October) and year-round estimates	Mean: All-year: 43.6; warm season: 50.1 75th: All-year: 54.3; warm season: 59.2 Max: All-year: 108.1; warm season: 108.1	Correlation (*r*): $PM_{2.5}$: 0.54 Copollutant models with: $PM_{2.5}$	Lag 0−2 ORs All-year: 1.02 (1.00, 1.04) Warm season: 1.02 (1.00, 1.04)
†Winquist et al. (2014) Atlanta, GA, U.S. Ozone: 1998−2004 Follow-up: 1998−2004 Time-series study	Age: 5−17 yr	Population-weighted monitor averages 8-h max Seasonal: Cold season (November−April) and warm season (May−October) estimates	Mean: 53.9 Median: 53.3 75th: 67.7	Correlation (*r*): $PM_{2.5}$: 0.66; NO_2: 0.54; SO_2: 0.27 Copollutant models with: NR	Lag 0−2 RRs Cold season (November−April): 1.05 (1.01, 1.09) Warm season (May−October): 1.05 (1.02, 1.09)
†Barry et al. (2018) Five U.S. cities Ozone: 2002−2008 Follow-up: 2002−2008 Time-series study	All ages	Fusion of CMAQ model estimates and ground-based measurements; population-weighted average of 12-km grid cells for each city 8-h max Year-round	Mean: 37.5−42.2 75th: 50.1−54.4 90th: 59.3−63.5 Max: 80.2−106.3	Correlation (*r*): NR Copollutant models with: NR	RR Lag 0−2 Atlanta: 1.03 (1.01, 1.05) Birmingham: 1.03 (0.98, 1.08) Dallas: 1.03 (1.00, 1.06) Pittsburgh: 1.03 (1.00, 1.06) St. Louis: 1.06 (1.02, 1.09)

Table 3-14 (Continued): Epidemiologic studies of short-term exposure to ozone and emergency department (ED) visits for asthma.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Gleason et al. (2014) New Jersey (statewide), U.S. Ozone: April−September, 2004−2007 Follow-up: April−September, 2004−2007 Case-crossover study	n = 21,854 Age: 3−17 yr	Fusion of monitor and CMAQ modeling 8-h max Warm season (April−September)		Correlation (*r*): $PM_{2.5}$: 0.56 Copollutant models with: $PM_{2.5}$	OR Lag 0−2: 1.06 (1.05, 1.08)
†Strickland et al. (2014) Atlanta, GA, U.S. Ozone: 2002−2010 Follow-up: 2002−2010 Time-series study	n = 109,758 Age: 2−16 yr	Population-weighted monitor averages 8-h max Year-round	Mean: 42.22	Correlation (*r*): NR Copollutant models with: NR	RR Lag 0−2: 1.07 (1.04, 1.09)
†Sarnat et al. (2015) St. Louis, MO, U.S. Ozone: 2001−2004 Follow-up: 2001−2003 Time-series study	n = 34,086 All ages	One monitor 8-h max Year-round	Mean: 36.2	Correlation (*r*): $PM_{2.5}$: 0.23; NO_2: 0.37; SO_2: −0.04; SO_4^{2-}: 0.49; NO_3^-: −0.57; OC: 0.30; EC: −0.09 Copollutant models with: $PM_{2.5}$, SO_4^{2-}, EC, OC, NO_2	RR 0−2 DL: 1.05 (1.00, 1.09)
†Byers et al. (2015) Indianapolis, IN, U.S. Ozone: 2007−2011 Follow-up: 2007−2011 Time-series study	n = 165,056 Age: ≥5 yr	Inverse-distance and population-weighted average of 11 monitors 8-h max Seasonal: Cold season (October−March) and warm season (April−September) estimates	Mean: 48.5	Correlation (*r*): $PM_{2.5}$: 0.54; SO_2: 0.42 Copollutant models with: NR	Lag 0−2 RRs Warm season All ages: 1.03 (0.99, 1.07) 5−17 yr: 1.05 (0.97, 1.14) 18−44 yr: 1.06 (1.00, 1.12) 45+ yr: 0.97 (0.91, 1.04)

Table 3-14 (Continued): Epidemiologic studies of short-term exposure to ozone and emergency department (ED) visits for asthma.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Alhanti et al. (2016) Three U.S. cities, U.S. Ozone: 1993−2009 Follow-up: 1993−2009 Time-series study	n = 611,970 All ages	Population weighted monitor averages, using all monitors in each city 8-h max Year-round	Mean: Range across cities: 37.3 to 43.7	Correlation (*r*): NR Copollutant models with: NR	Lag 0−2 RRs 0−4 yr: 1.02 (1.01, 1.04) 5−18 yr: 1.05 (1.03, 1.07) 19−39 yr: 1.02 (1.00, 1.04) 40−64 yr: 1.01 (0.99, 1.03) 65+ yr: 1.02 (0.98, 1.06)
†Sheffield et al. (2015) New York City, NY, U.S. Ozone: May−September 2005−2012 Follow-up: May−September 2005−2011 Case-crossover study	n = 8,009 Age: 5−17 yr	Average of city monitors 24-h avg Warm season (May−September)	Max: 60	Correlation (*r*): NR Copollutant models with: NR	Percent increase Lag 0−3: 10.81 (6.84, 15.03)
†Malig et al. (2016) California (statewide), U.S. Ozone: 2005−2009 Follow-up: 2005−2008 Case-crossover study	All ages	Nearest monitor within 20 km of population weighted zip-code centroid 1-h max Seasonal: Warm season (May−October) and year-round estimates	Mean: 33−55 across climate zones	Correlation (*r*): NO_2: −0.01 YR; 0.26 warm; SO_2: −0.06 YR; 0.02 warm; CO: −0.28 YR; 0.02 warm Copollutant models with: NO_2, CO, SO_2	Percent increase Lag 0−1 Year-round: 3.85 (2.17, 5.56) Warm season: 4.18 (1.93, 6.48)

Table 3-14 (Continued): Epidemiologic studies of short-term exposure to ozone and emergency department (ED) visits for asthma.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Shmool et al. (2016) New York City, NY, U.S. Ozone: June−August 2005−2011 Follow-up: June−August 2005−2011 Case-crossover study	n = 11,719 Age: 5−17 yr	Temporal estimates: Average of city monitors; spatiotemporal estimates: Fusion of monitors and LUR 24-h avg Warm season (May−September)	Mean: Temporal: 30.4; spatio-temporal: 29.0 Max: Temporal: 60.0; spatio-temporal: 60.3	Correlation (*r*): NR Copollutant models with: NR	No quantitative results. Results presented graphically
†O'Lenick et al. (2017) Atlanta, GA, U.S. Ozone: 2002−2008 Follow-up: 2002−2008 Case-crossover study	n = 128,758 Age: 5−17 yr	Fusion of CMAQ model estimates and ground-based measurements; 12-km grid cells 8-h max Year-round		Correlation (*r*): NR Copollutant models with: NR	OR Lag 0−2: 1.06 (1.03, 1.08)
†Xiao et al. (2016) Georgia (statewide), U.S. Ozone: 2002−2008 Follow-up: 2002−2008 Case-crossover study	n = 148,256 Age: 2−18 yr	Fusion of CMAQ model estimates and ground-based measurements; 12-km grid cells 8-h max Year-round	Mean: 42.1 75th: 50.9 Max: 106.1	Correlation (*r*): $PM_{2.5}$: 0.61; NO_2: −0.12; SO_2: −0.03; SO_4^{2-}: 0.61; NO_3^-: −0.39; OC: 0.35; EC: 0.01 Copollutant models with: NR	OR Lag 0−3: 1.03 (1.01, 1.05)
†Szyszkowicz et al. (2018) Multicity, Canada Ozone: 2004−2011 Follow-up: 2004−2011 Case-crossover study	n = 223,845 Age: 0−19 yr	Average of all monitors within 35 km 24-h avg Year-round	Mean: 22.5−29.2 across cities Max: 80	Correlation (*r*): NR Copollutant models with: NR	OR Lag 1: Males: 1.03 (1.00, 1.06) Females: 1.04 (1.00, 1.08)

Note: †Studies published since the 2013 Ozone ISA.

Results standardized to a 15-ppb increase in 24-hour avg, 20-ppb increase in 8-hour daily max, or 25-ppb increase in 1-hour daily max ozone concentrations.

Table 3-15 Epidemiologic studies of short-term exposure to ozone and respiratory symptoms in children with asthma.

Study	Study Population	Exposure Assessment	Mean ppb	Copollutant Examination	Effect Estimates 95% CI
†Lewis et al. (2013) Detroit, MI, U.S. Ozone: 1999−2002 Follow-up: 1999−2002 Panel study	n = 298 Children with asthma, primarily African American and Latino, living in low-income communities Age: 5−12 yr	One rooftop school monitor for each of two communities. 95% of participants lived within 5 km of one of the monitors 8-h max Year-round	Mean: 41.8	Correlation (*r*): $PM_{2.5}$: 0.55 Copollutant models with: NR	No quantitative results. Results presented graphically.

Note: †Studies published since the 2013 Ozone ISA.

Table 3-16 Study-specific details from controlled human exposure studies of lung function in adults with asthma.

Study	Population n, Sex, Age (Range or Mean ± SD)	Exposure Details (Concentration, Duration)	Endpoints Examined
Bartoli et al. (2013)	Adults with asthma n = 86 males, 34 females Age: 32.9 ± 12.9 yr	0 ppb, 2 h 300 ppb, 2 h	FEV_1 (2 days before exposure) FEV_1 (before and PE)

Table 3-17 Study-specific details from controlled human exposure studies of lung function in healthy adults and adults with asthma.

Study	Population n, Sex, Age (Range or Mean ± SD)	Exposure Details (Concentration, Duration)	Endpoints Examined
Fry et al. (2012)	Healthy adults and adults with asthma n = 12 males, 15 females Age: 21−35 yr	400 ppb, 2 h	FEV_1 (immediately before and PE)
Arjomandi et al. (2015)	Healthy adults and adults with asthma n = 13 males, 13 females Age: 31.8 ± 7.6 yr	0 ppb, 4 h 150 ppb, 4 h 200 ppb, 4 h	FEV_1, FVC, FEV_1/FVC (before, after, 20 h PE)
Leroy et al. (2015)	Asthmatic adults n = 3 males, 4 females Age: 33.7 ± 10.1 yr Nonasthmatic adults n = 7 males, 5 females Age: 31.8 ± 6.0 yr	0 ppb, 4 h 100 ppb, 4 h 200 ppb, 4 h	FEV_1, FVC (before, immediately after, and 20 h PE)

Table 3-18 Study-specific details from animal toxicological studies of short-term ozone exposure and lung function—allergy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Schelegle and Walby (2012)	Rats (BN) naïve and sensitized/challenged with allergen n = 5−11 males Age: 8−10 weeks	1 ppm, 8 h	Lung function, breathing pattern (immediately PE)

BN = brown Norway; PE = post-exposure.

Table 3-19 Study-specific details from animal toxicological studies of short-term ozone exposure and lung function—asthma.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Bao et al. (2013)	Mice (BALB/c naïve and ovalbumin sensitized/challenged) n = 6−7 females Age: 6−8 weeks	2 ppm, 3 h	Enhanced pause, MCh challenge (24 h PE)
Hansen et al. (2016)	Mice (BALB/cJ) naïve and ovalbumin sensitized n = 5 females Age: 6 weeks	2 ppm, 1 h/day for 3 days	Breathing frequency, tidal volume, time of brake, time of pause (during exposure)

MCh = methacholine. PE = post-exposure.

Table 3-20 Study-specific details from controlled human exposure studies of inflammation, oxidative stress, and injury in healthy adults and adults with asthma.

Study	Population n, Sex, Age (Range or Mean ± SD)	Exposure Details (Concentration, Duration)	Endpoints Examined
Fry et al. (2012)	Healthy adults and adults with asthma n = 12 males, 15 females Age: 21−35 yr	400 ppb, 2 h	Induced sputum PMN (48 h before and 5 h PE)
Hernandez et al. (2012)	Healthy adults N = 14 males, 20 females Age: 24.2 ± 3.9 yr Atopic adults with asthma N = 7 males, 10 females Age: 24.4 ± 5.5 yr	400 ppb, 2 h	Induced sputum (screening visit and 4 h PE)
Arjomandi et al. (2015)	Healthy adults and adults with asthma n = 13 males, 13 females Age: 31.8 ± 7.6 yr	0 ppb, 4 h 100 ppb, 4 h 200 ppb, 4 h	BALF protein, PMNs, and eosinophils (20 h PE)
Leroy et al. (2015)	Healthy adults and adults with asthma n = 14 males, 2 females Age: 32.5 ± 7.6 yr	0 ppb, 4 h 100 ppb, 4 h 200 ppb, 4 h	BALF (20 h PE)

Table 3-21 Study-specific details from controlled human exposure studies of allergic sensitization—atopy.

Study	Population n, Sex, Age (Range or Mean ± SD)	Exposure Details (Concentration, Duration)	Endpoints Examined
Dokic and Trajkovska-Dokic (2013)	Adults with atopy n = 5 males, 5 females Age: 27.9 ± 6.6 yr	0 ppb before pollen season, 2 h 0 ppb pollen season, 2 h 400 ppb before pollen season, 2 h 400 ppb pollen season, 2 h	Nasal lavage (2 h before, immediately after, and 6 h PE)

Table 3-22 Study-specific details from animal toxicological studies of short-term ozone exposure and inflammation, oxidative stress, and injury—allergy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Schelegle and Walby (2012)	Rats (BN) naïve and sensitized/ challenged with allergen n = 5−11 males Age: 8−10 weeks	1 ppm, 8 h	BALF markers of injury, inflammation (immediately PE)

BALf = bronchoalveolar lavage fluid; BN = brown Norway; PE = post-exposure.

Table 3-23 Study-specific details from animal toxicological studies of short-term ozone exposure and inflammation, oxidative stress, and injury—asthma.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Bao et al. (2013)	Mice (BALB/c naïve and ovalbumin sensitized/challenged) n = 6−7 females Age: 6−8 weeks	2 ppm, 3 h	BALF total cells and cell differentials, mediators (24 h PE)

BALF = bronchoalveolar lavage fluid; PE = post-exposure.

Table 3-24 Study-specific details from animal toxicological studies of short-term ozone exposure and morphology—asthma.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Bao et al. (2013)	Mice (BALB/c naïve and ovalbumin sensitized/challenged) n = 6−7 females Age: 6−8 weeks	2 ppm, 3 h	Mucosubstance secretion and Muc5AC, epithelial cell density (24 h PE)

MUC5AC = mucin 5AC glycoprotein; PE = post-exposure.

Table 3-25 Epidemiologic studies of short-term exposure to ozone and inflammation, oxidative stress, and injury in children with asthma.

Study	Study Population	Exposure Assessment	Mean ppb	Copollutant Examination	Effect Estimates HR (95% CI)[a]
†Delfino et al. (2013) Riverside and Whittier, CA, U.S. Ozone: August−December 2003 (Riverside); July−November 2004 (Whittier) Follow-up: August−December 2003 (Riverside); July−November 2004 (Whittier) Panel study	n = 45 Children with asthma Age: 9−18 yr	One monitor (Riverside); average of two monitors (Whittier) 8-h max Year-round	Mean: 52.9 Median: 46.8 Max: 120.8	Correlation (r): $PM_{2.5}$: 0.39; NO_2: 0.07; EC: 0.55; OC: 0.71 Copollutant models with: NR	Lag 0: 0.42 (−1.33, 2.19) Lag 1: 0.63 (−1.05, 2.35) Lag 0−2: 1.24 (−1.04, 3.57)

Note: †Studies published since the 2013 Ozone ISA.

[a]Results standardized to a 15-ppb increase in 24-hour avg, 20-ppb increase in 8-hour daily max, or 25-ppb increase in 1-hour daily max ozone concentrations.

Table 3-26 Epidemiologic studies of short-term exposure to ozone and emergency department (ED) visits for chronic obstructive pulmonary disease (COPD).

Study	Study Population	Exposure Assessment	Mean ppb	Copollutant Examination	Effect Estimates HR 95% CI[a]
Arbex et al. (2009) São Paulo, Brazil Ozone: 2001−2003 Follow-up: 2001−2003 Time-series study	n = 1,769 All ages	Average of four monitors 1-h max Year-round	Mean: 48.8 75th: 61.0 Max: 143.8	Correlation (*r*): NR Copollutant models with: NR	Percent increase Women Lag 0: 1.0 (0.0, 2.0)
†Rodopoulou et al. (2015) Little Rock, AR, U.S. Ozone: 2002−2012 Follow-up: 2002−2012 Time-series study	n = 12,511 Ages 15+	One monitor 8-h max Seasonal: cold season (October−March) and warm season (April−September) estimates	Mean: 40 Median: 39 75th: 50	Correlation (*r*): $PM_{2.5}$: 0.33 Copollutant models with: $PM_{2.5}$	Percent increase Lag 2: 2.29 (−2.07, 6.85)
†Sarnat et al. (2015) St. Louis, MO, U.S. Ozone: 2001−2004 Follow-up: 2001−2003 Time-series study	n = 34,086 All ages	One monitor 8-h max Year-round	Mean: 36.2	Correlation (*r*): $PM_{2.5}$: 0.23; NO_2: 0.37; SO_2: −0.04; SO_4^{2-}: 0.49; NO_3^-: −0.57; OC: 0.30; EC: −0.09 Copollutant models with: $PM_{2.5}$, SO_4^{2-}, EC, OC, NO_2	RR Lag 0−2 0.98 (0.92, 1.06)
†Malig et al. (2016) California (statewide), U.S. Ozone: 2005−2009 Follow-up: 2005−2008 Case-crossover study	All ages	Nearest monitor within 20 km of population-weighted zip-code centroid 1-h max Seasonal: warm season (May−October) and year-round estimates	Mean: 33−55 across climate zones	Correlation (*r*): NO_2: −0.01 YR; 0.26 warm; SO_2: −0.06 YR; 0.02 warm; CO: −0.28 YR; 0.02 warm Copollutant models with: NO_2, CO, SO_2	Percent increase Lag 0−1 Year-round: 0.89 (−0.26, 2.06) Warm season: 2.02 (0.46, 3.61)

Table 3-26 (Continued): Epidemiologic studies of short-term exposure to ozone and emergency department (ED) visits for chronic obstructive pulmonary disease (COPD).

Study	Study Population	Exposure Assessment	Mean ppb	Copollutant Examination	Effect Estimates HR 95% CI[a]
†Xiao et al. (2016) Georgia (statewide), U.S. Ozone: 2002−2008 Follow-up: 2002−2008 Case-crossover study	n = 84,597 Ages: 2−18 yr	Fusion of CMAQ model estimates and ground-based measurements; 12-km grid cells 8-h max Year-round	Mean: 42.1 75th: 50.9 Max: 106.1	Correlation (*r*): $PM_{2.5}$: 0.61; NO_2: −0.12; SO_2: −0.03; SO_4^{2-}: 0.61; NO_3^-: −0.39; OC: 0.35; EC: 0.01 Copollutant models with: NR	OR Lag 0−3: 1.03 (1.00, 1.06)
†Barry et al. (2018) Five U.S. cities Ozone: 2002−2008 Follow-up: 2002−2008 Time-series study	All ages	Fusion of CMAQ model estimates and ground-based measurements; population-weighted average of 12-km grid cells for each city 8-h max Year-round	Mean: 37.5−42.2 75th: 50.1−54.4 90th: 59.3−63.5 Max: 80.2−106.3	Correlation (*r*): NR Copollutant models with: NR	RR Lag 0−2 Atlanta: 1.00 (0.97, 1.03) Birmingham: 0.99 (0.93, 1.05) Dallas: 1.04 (0.99, 1.09) Pittsburgh: 1.02 (0.98, 1.07) St. Louis: 1.03 (0.99, 1.08)
†Szyszkowicz et al. (2018) Multicity, Canada Ozone: 2004−2011 Follow-up: 2004−2011 Case-crossover Study	n = 183,544 Age: 55+ yr	Average of all monitors within 35 km 24-h avg Year-round	Mean: 22.5−29.2 across cities Max: 80	Correlation (*r*): NR Copollutant models with: NR	OR Lag 1; females: 1.01 (0.99, 1.03) Lag 0; males: 1.01 (0.99, 1.03)

Note: †Studies published since the 2013 Ozone ISA.

[a]Results standardized to a 15-ppb increase in 24-hour avg, 20-ppb increase in 8-hour daily max, or 25-ppb increase in 1-hour daily max ozone concentrations.

Table 3-27 Epidemiologic studies of short-term exposure to ozone and medication use in adults with chronic obstructive pulmonary disease (COPD).

Study	Study Population	Exposure Assessment	Mean ppb	Copollutant Examination	Effect Estimates 95% CI[a]
†Magzamen et al. (2018) Seattle and Tacoma, WA, U.S. Ozone: December 2011 to October 2012 Follow-up: December 2011 to October 2012 Panel study	n = 35 Age: 40+ yr Former smokers with COPD but not asthma	Monitors 8-h max Year-round	Median: 17.21 75th: 24.37 Max: 40.86	Correlation (*r*): NR Copollutant models with: NR	RR Lag 0: 0.98 (0.93, 1.45)

Note: †Studies published since the 2013 Ozone ISA.

[a]Results standardized to a 15-ppb increase in 24-hour avg, 20-ppb increase in 8-hour daily max, or 25-ppb increase in 1-hour daily max ozone concentrations.

Table 3-28 Study-specific details from animal toxicological studies of short-term ozone exposure and lung function—chronic obstructive pulmonary disease (COPD).

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Groves et al. (2012)	Mice (C57BL/6J WT and surfactant protein D deficient) n = 4−9 males Age: 8 weeks	0.8 ppm, 3 h	Pulmonary mechanics (72 h PE)
Groves et al. (2013)	Mice (C57BL/6J WT and surfactant protein D deficient) n = 3−10 males Age: 8, 27, 80 weeks	0.8 ppm, 3 h	Resistance and elastance spectra (72 h PE)

PE = post-exposure; WT = wild type.

Table 3-29 Study-specific details from animal toxicological studies of short-term ozone exposure and inflammation, oxidative stress, and injury—chronic obstructive pulmonary disease (COPD).

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Groves et al. (2012)	Mice (C57BL/6J WT and surfactant protein D deficient) n = 4−9 males Age: 8 weeks	0.8 ppm, 3 h	BALF protein, RNS, macrophage number, chemotactic activity (24−72 h PE)
Groves et al. (2013)	Mice (C57BL/6J WT and surfactant protein D deficient) n = 3−10 males Age: 8, 27, 80 weeks	0.8 ppm, 3 h	BALF total cells and differential cell counts, protein (72 h PE)

BALF = bronchoalveolar lavage fluid; PE = post-exposure; RNS = reactive nitrogen species; WT = wild type.

Table 3-30 Study-specific details from animal toxicological studies of short-term ozone exposure and morphology—chronic obstructive pulmonary disease (COPD).

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Groves et al. (2012)	Mice (C57BL/6J WT and surfactant protein D deficient) n = 4−9 males Age: 8 weeks	0.8 ppm, 3 h	Histopathology, immunohistochemistry (72 h PE)
Groves et al. (2013)	Mice (C57BL/6J WT and surfactant protein D deficient) n = 3−10 males Age: 8, 27, 80 weeks	0.8 ppm, 3 h	Markers of cell proliferation, radial alveolar counts, lesion scores (72 h PE)

PE = post-exposure, WT = wild type.

Table 3-31 Study-specific details from controlled human exposure studies of respiratory effects in obese adults.

Study	Population n, Sex, Age (Range or Mean ± SD)	Exposure Details (Concentration, Duration)	Endpoints Examined
Bennett et al. (2016)	Healthy adult women n = 19 obese Age: 28 ± 5 yr n = 19 normal weight Age: 24 ± 4 yr	0 ppb, 2 h 400 ppb, 2 h	FEV_1, FVC, sGaw (before and PE); airway responsiveness (3 h PE) PFT, PMN, airway responsiveness, symptoms (train day, before, immediately after, and 3 h PE); symptoms (immediately PE)

Table 3-32 Study-specific details from animal toxicological studies of short-term ozone exposure and lung function—obesity.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Williams et al. (2015)	Mice (C57BL/6) WT and Cpe fat, TNF-α sufficient and deficient n = 5−9 females Age: 10−12 weeks	2 ppm, 3 h	Pulmonary mechanics, challenge with MCh (24 h PE)
Mathews et al. (2017b)	Mice (C57BL/6J) WT and db/db, TCR gamma delta deficient mice on high-fat diet for 24 weeks n = 4−10 females Age: 10 weeks and greater than 24 weeks	2 ppm, 3 h	Pulmonary mechanics, challenge with MCh (24 h PE)
Mathews et al. (2018)	Mice (C57BL/6J) WT and db/db, Cpe fat/TNFR2 deficient mice; some animals on high-fat diet for 24 weeks n = 4−14 females Age: 10 weeks and older than 24 weeks	2 ppm, 3 h	Pulmonary mechanics, challenge with MCh (24 h PE)

MCh = methacholine; PE = post-exposure; TCR = T cell receptor; TNF = tumor necrosis factor.

Table 3-33 Study-specific details from animal toxicological studies of short-term ozone exposure and inflammation, oxidative stress, and injury—obesity.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Ying et al. (2016)	Mice (Kkay) n = 8 males Age: 6−7 weeks	0.5 ppm, 4 h/day for 13 days	Lung tissue mRNA for proinflammatory genes; T cell subpopulations in lymph nodes (about 2 h PE)
Zhong et al. (2016)	Mice (Kkay) n = 8 Sex and age: NR	0.5 ppm, 4 h/day for 13 days	BALF total cells and cell differentials (22 h PE)
Mathews et al. (2017b)	Mice (C57BL/6J) WT and db/db, WT and TCR gamma delta deficient mice on high fat diet for 24 weeks n = 4−10 females Age: 10 weeks and greater than 24 weeks	2 ppm, 3 h	BALF total cells and cell differentials, cytokines, chemokines; flow cytometry of isolated cells from lung tissue (24 h PE)
Mathews et al. (2017a)	Mice (C57BL/6J) WT and db/db n = 5−8 females Age: 10 weeks	2 ppm, 3 h	Markers of oxidative stress (24 h PE)
Mathews et al. (2018)	Mice (C57BL/6J) WT and db/db, and Cpe fat/TNFR2 deficient mice, WT and TCR gamma delta-deficient mice; some animals on high fat diet for 24 weeks n = 4−14 females Age: 10−12 weeks and older than 24 weeks	2 ppm, 3 h	BALF total cells and cell differentials, IL-17A, IL-23, IL-33, CCL20, CXCL1, CXCL2, IL-6, G-CSF, GRP; lung tissue flow cytometry for IL-17A producing cells, (24 h PE)

BALF = bronchoalveolar lavage fluid; CCL20 = C-C motif chemokine ligand 20; CXCL = chemokine family of cytokines with highly conserved motif: cys-xxx-cys (CXC) ligand; G-CSF = granulocyte colony-stimulating factor (receptor); GRP = gastrin-releasing peptide; IL = interleukin; PE = post-exposure; TCR = T cell receptor.

Table 3-34 Study-specific details from animal toxicological studies of short-term ozone exposure and morphology—obesity.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Zhong et al. (2016)	Mice (Kkay) n = 8 Sex and age: NR	0.5 ppm, 4 h/day for 13 days	Qualitative histopathology (22 h PE)

PE = post-exposure.

Table 3-35 Epidemiologic studies of short-term exposure to ozone and pulmonary inflammation in populations with metabolic syndrome.

Study	Study Population	Exposure Assessment	Mean ppb	Copollutant Examination	Effect Estimates HR (95% CI)[a]
†Peng et al. (2016) Boston, MA, U.S. Ozone: 2006−2010 Follow-up: 2006−2010 Panel study	Adults with type 2 diabetes mellitus. Mostly white population (83%) n = 69 Age: 44–85 yr	One monitor 24-h avg Year-round	Median: 26.76 75th: 32.57	Correlation (*r*): $PM_{2.5}$: 0.22; Other: NO_X: −0.35; BC: 0.28; OC: 0.24; sulfate: 0.32; PN: −0.78 Copollutant models with: $PM_{2.5}$	Estimated change in FeNO (ppb); Lag 1: −5.95 (−10.79, −0.90)

Note: †Studies published since the 2013 Ozone ISA.

[a]Results standardized to a 15-ppb increase in 24-hour avg, 20-ppb increase in 8-hour daily max, or 25-ppb increase in 1-hour daily max ozone concentrations.

Table 3-36 Study-specific details from animal toxicological studies of short-term ozone exposure and lung function—cardiovascular disease.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Dye et al. (2015)	Rats (WKY, WS, S-D, SH, FHH, SPSH, obese SHHF, obese atherosclerosis prone JCR rats) n = 4−8 males Age: 12−14 weeks	0.25 ppm, 4 h 0.5 ppm, 4 h 1 ppm, 4 h	Whole body plethysmography (0 and 20 h PE)
Zychowski et al. (2016)	Mice (C57BL/6) control and mice with induced pulmonary hypertension n = 4−8 males Age: 6−8 weeks	1 ppm, 4 h	Airway responsiveness to MCh (18−20 h PE)

FHH = fawn-hooded hypertensive; MCh = methacholine; PE = post-exposure; S-D = Sprague-Dawley; SH = spontaneously hypertensive; SHHF = spontaneously hypertensive heart failure; SPSH = stroke-prone spontaneously hypertensive; WKY = Wistar Kyoto; WS = Wistar.

Table 3-37 Study-specific details from animal toxicological studies of short-term ozone exposure and inflammation, oxidative stress, and injury—cardiovascular disease.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Farraj et al. (2012)	Rats (SH) n = 6 males Age: 12 weeks	0.2 ppm, 4 h 0.8 ppm, 4 h	BALF total cells and differential cell counts, total protein, LDH, NAG, SOD, GPx, GST (1 and 18 h PE)
Kodavanti et al. (2015)	Rats (WKY, WS, SD, SH, FHH, SPSH, obese SHHF, obese atherosclerosis prone JCR rats) n = 4−8 males Age: 12−14 weeks	0.25 ppm, 4 h 0.5 ppm, 4 h 1 ppm, 4 h	BALF total cell counts and cell differentials, BALF total protein, albumin, LDH, NAG, GGT; lung tissue mRNA for HO-1, MIP-2, TNF-α, IL-6, IL-10 (BALF 0 and 20 h PE, tissue 0 h PE)
Ramot et al. (2015)	Rats (WKY, WS, SD, SH, FHH, SPSH, obese SHHF, obese atherosclerosis prone JCR rats) n = 4−8 males Age: 12−14 weeks	0.25 ppm, 4 h 0.5 ppm, 4 h 1 ppm, 4 h	Lung histopathology (0 and 20 h PE)
Ward and Kodavanti (2015)	Rats (WKY, SH, SPSH, obese SHHF, obese atherosclerosis prone JCR) n = 3−4 males Age: 10−12 weeks	1 ppm, 4 h	Lung gene expression profiling (immediately PE)
Hatch et al. (2015)	Rats (WKY, WS, SD, SH, FHH, SPSH, SHHF, obese atherosclerosis prone JCR) n = 8 males Age: 12−14 weeks	1 ppm, 4 h	BALF and tissue antioxidants (0 and 20 h PE)
Zychowski et al. (2016)	Mice (C57BL/6) control and mice with induced pulmonary hypertension n = 4−8 males Age: 6−8 weeks	1 ppm, 4 h	BALF cells and lung tissue indicators of injury (18−20 h PE)

BALF = bronchoalveolar lavage fluid; FHH = fawn-hooded hypertensive; GGT = gamma glutamyl transferase; GPx = glutathione peroxidase; GST = glutathione S-transferase; HO-1 = heme oxygenase 1; IL = interleukin; LDH = lactate dehydrogenase; MIP-2 = macrophage inflammatory protein 2; NAG = *N*-acetyl-glucosaminidase; PE = post-exposure; S-D = Sprague-Dawley; SH = spontaneously hypertensive; SHHF = spontaneously hypertensive heart failure; SPSH = stroke-prone spontaneously hypertensive; SOD = superoxide dismutase; TNF-α = tumor necrosis factor α; WKY = Wistar Kyoto, WS = Wistar.

Table 3-38 Study-specific details from animal toxicological studies of short-term ozone exposure and morphology—cardiovascular disease.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Ramot et al. (2015)	Rats (WKY, WS, SD, SH, FHH, SPSH, obese SHHF, obese atherosclerosis prone JCR rats) n = 4−8 males Age: 12−14 weeks	0.25 ppm, 4 h 0.5 ppm, 4 h 1 ppm, 4 h	Lung histopathology (0 and 20 h PE)
Wong et al. (2018)	Rats (WKY, SH) n = 8−12 males, Age: 44−48 weeks	1 ppm, 6 h	Histopathology scores (8 h PE)

FHH = fawn-hooded hypertensive; PE = post-exposure; S-D = Sprague-Dawley; SH = spontaneously hypertensive; SHHF = spontaneously hypertensive heart failure ; SPSH = stroke-prone spontaneously hypertensive; WKY = Wistar Kyoto; WS = Wistar.

Table 3-39 Epidemiologic studies of short-term exposure to ozone and emergency department (ED) visits for respiratory infection.

Study	Study Population	Exposure Assessment	Mean ppb	Copollutant Examination	Effect Estimates HR (95% CI)
Stieb et al. (2009) Seven Canadian cities Ozone: 1992−2003 Follow-up: 1992−2003 Time-series study	All ages	Average of monitors in each city 24-h avg Year-round	Mean: 18.4 75th: 19.3−28.6 across cities	Correlation (*r*): Warm season (across cities): $PM_{2.5}$: −0.05, 0.62; NO_2: −0.17, 0.10; SO_2: −0.24, 0.21; CO: −0.34, 0.17 Cold season: $PM_{2.5}$: −0.65, 0.06; NO_2: −0.57, −0.35; SO_2: −0.52, −0.18; CO: −0.67, −0.16 Copollutant models with: NR	Percent increase Lag 1: 1.00 (0.98, 1.02)
†Winquist et al. (2012) St. Louis, MO, U.S. Ozone: 2001−2007 Follow-up: 2001−2007 Time-series study	All Ages	One monitor 8-h max Year-round		Correlation (*r*): NR Copollutant models with: NR	RR Pneumonia 0−4 DL: 1.01 (0.98, 1.04)
†Kousha and Rowe (2014) Edmonton, Canada Ozone: 1999−2002 Follow-up: 1999−2002 Case-crossover study	n = 48,252 All ages	Three monitors 8-h max Seasonal: cold season (October−March) and warm season (April−September) estimates	Mean: 18.6 Median: 17.8	Correlation (*r*): NR Copollutant models with: NR	OR Lower respiratory disease Lag 0; Year-round: 1.07 (1.03, 1.10)

Table 3-39 (Continued): Epidemiologic studies of short-term exposure to ozone and emergency department (ED) visits for respiratory infection.

Study	Study Population	Exposure Assessment	Mean ppb	Copollutant Examination	Effect Estimates HR (95% CI)
†Darrow et al. (2014) Atlanta, GA, U.S. Ozone: 1993−2010 Follow-up: 1993−2010 Time-series study	n = 80,399 Age: 0−4 yr	Population-weighted monitor averages 8-h max Seasonal: cold season (November−February) and warm season (March−October) estimates	Mean: 45.9 Median: 43.8 75th: 58.7 95th: 80.6 Max: 127.1	Correlation (*r*): $PM_{2.5}$: 0.3; NO_2: 0.37; CO: 0.21 Copollutant models with: NR	Lag 0−2 RRs Year-round Bronchitis: 1.02 (0.99, 1.05) URI: 1.03 (1.01, 1.05) Pneumonia: 1.06 (1.03, 1.09)
†Rodopoulou et al. (2015) Little Rock, AR U.S. Ozone: 2002−2012 Follow-up: 2002−2012 Time-series study	n = 13,650 Age: 15+ yr	One monitor 8-h max Seasonal: cold season (October−March) and warm season (April−September) estimates	Mean: 40 Median: 39 75th: 50	Correlation (*r*): $PM_{2.5}$: 0.33 Copollutant models with: NR	Percent increase Acute RI; Lag 2: −1.49 (−5.79, 3.00) Pneumonia; Lag 2: −8.19 (−16.64, 1.16)
†Barry et al. (2018) Five U.S. cities Ozone: 2002−2008 Follow-up: 2002−2008 Time-series study	All ages	Fusion of CMAQ model estimates and ground-based measurements; population-weighted average of 12-km grid cells for each city 8-h max Year-round	Mean: 37.5−42.2 75th: 50.1−54.4 90th: 59.3−63.5 Max: 80.2−106.3	Correlation (r): NR Copollutant models with: NR	RR URI—Lag 0−2 Atlanta: 1.02 (1.01, 1.04) Birmingham: 1.02 (1.00, 1.05) Dallas: 1.05 (1.02, 1.07) Pittsburgh: 1.02 (1.00, 1.05) St. Louis: 1.01 (0.99, 1.03)
†Kousha and Castner (2016) Windsor, Canada Ozone: 2004−2010 Follow-up: 2004−2010 Case-crossover study	n = 4,815 Age: 0−3 yr	Monitors in city 8-h max Year-round	Mean: 25.3	Correlation (*r*): NR Copollutant models with: NR	OR Otitis Media Lag 0; Year-round: 1.04 (0.86, 1.21)

Table 3-39 (Continued): Epidemiologic studies of short-term exposure to ozone and emergency department (ED) visits for respiratory infection.

Study	Study Population	Exposure Assessment	Mean ppb	Copollutant Examination	Effect Estimates HR (95% CI)
†Malig et al. (2016) California (statewide), U.S. Ozone: 2005−2009 Follow-up: 2005−2008 Case-crossover study	All ages	Nearest monitor within 20 km of population-weighted zip-code centroid 1-h max Seasonal: warm season (May−October) and year-round estimates	Mean: 33−55 across climate zones	Correlation (*r*): NO_2: −0.01 YR; 0.26 warm; SO_2: −0.06 YR; 0.02 warm; CO: −0.28 YR; 0.02 warm Copollutant models with: NO_2, CO, SO_2	Percent increase Lag 0−1 Pneumonia Year-round: 1.32 (0.20, 2.46) Warm season: 2.27 (0.32, 4.26) ARI Year-round: 2.15 (1.45, 2.86) Warm Season: 2.30 (1.24, 3.37) URTI Year-round: 3.77 (0.40, 7.26) Warm season: 3.14 (0.16, 6.21)
†Xiao et al. (2016) Georgia (statewide), U.S. Ozone: 2002−2008 Follow-up: 2002−2008 Case-crossover study	n = 90,063 Age: 2−18 yr	Fusion of CMAQ model estimates and ground-based measurements; 12-km grid cells 8-h max Year-round	Mean: 42.1 75th: 50.9 Max: 106.1	Correlation (*r*): $PM_{2.5}$: 0.61; NO_2: −0.12; SO_2: −0.03; SO_4^{2-}: 0.61; NO_3^-: −0.39; OC: 0.35; EC: 0.01 Copollutant models with: NR	Lag 0−3 ORs Otitis Media: 1.02 (1.01, 1.03) Pneumonia: 1.04 (1.02, 1.07) URI: 1.04 (1.03, 1.05)

Table 3-39 (Continued): Epidemiologic studies of short-term exposure to ozone and emergency department (ED) visits for respiratory infection.

Study	Study Population	Exposure Assessment	Mean ppb	Copollutant Examination	Effect Estimates HR (95% CI)
†Szyszkowicz et al. (2018) Multicity, Canada Ozone: 2004−2011 Follow-up: 2004−2011 Case-crossover study	n = 717,676 All ages	Average of all monitors within 35 km 24-h avg Year-round	Mean: 22.5−29.2 across cities Max: 80	Correlation (*r*): NR Copollutant models with: NR	OR URI—Lag 0 Females: 1.03 (1.02, 1.05) Males: 1.02 (1.01, 1.04) ALR—Lag 0 Females: 1.05 (1.02, 1.07) Males: 1.02 (1.00, 1.05)

Note: †Studies published since the 2013 Ozone ISA.

[a]Results standardized to a 15-ppb increase in 24-hour avg, 20-ppb increase in 8-hour daily max, or 25-ppb increase in 1-hour daily max ozone concentrations.

Table 3-40 Study-specific details from animal toxicological studies of short-term ozone exposure and host defense/infection—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Durrani et al. (2012)	Mice (C57BL/6) n = 5 males, 5 females Age: 10 weeks	2 ppm, 3 h	Survival after infection (14 days PE)
Mikerov et al. (2011)	Mice (C57BL/6J) n = 14 males, 11−14 females Age: 8−12 weeks	2 ppm, 3 h	Lung, liver, and spleen histopathology (48 h PE)

PE = post-exposure.

Table 3-41 Epidemiologic studies of short-term exposure to ozone and hospital admissions for aggregate respiratory diseases.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)[a]
Katsouyanni et al. (2009) 90 U.S. cities 32 European cities 12 Canadian cities	NMMAPS APHEA All ages	Average of monitors in each city. 1-h max Year-round and warm season (April−September)	NMMAPS: 50th: 34.9−60.0 75th: 46.8−68.8 APHEA: 50th: 11.0−38.1 75th: 15.3−49.4 12 Canadian cities: 50th: 6.7 8.3 75th: 8.4−12.4	Correlation (*r*): No quantitative results. Results presented graphically. Copollutant models with: NR	Percent Increase Lag 0−1 Year-round U.S.: 1.5 (0.0, 3.0) Canada: 5.0 (0.1, 10.1) Warm season: U.S.: 1.3 (−0.4, 3.1) Canada: 18.9 (11.1, 27.4)
Cakmak et al. (2006) 10 Canadian cities	All ages	Average of monitors in each city. 24-h avg Year-round	Mean: 17.4 Max (across cities): 38.0−79.0	Correlation (*r*): NR Copollutant models with: NR	Percent increase Lag 1 3.3 (1.7, 4.9)
†Winquist et al. (2012) St. Louis, MO, U.S. Ozone: 2001−2007 Follow-up: 2001−2007 Time-series study	All ages	One monitor 8-h max Year-round		Correlation (*r*): NR Copollutant models with: NR	RR All ages 0−4 DL: 1.00 (0.98, 1.03) 2−18 yr 0−4 DL: 1.07 (1.00, 1.16)

Note: †Studies published since the 2013 Ozone ISA.

[a]Results standardized to a 15-ppb increase in 24-hour avg, 20-ppb increase in 8-hour daily max, or 25-ppb increase in 1-hour daily max ozone concentrations.

Table 3-42 Epidemiologic studies of short-term exposure to ozone and emergency department (ED) visits for aggregate respiratory diseases.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)[a]
Tolbert et al. (2007) Atlanta, GA, U.S. Ozone: 1993−2004 Follow-up: 1993−2004 Time-series study	n = 1,072,429 All ages	Average of monitors in city. 8-h max Warm season (March−October)	Mean: 53.0 75th: 67.0 90th: 82.1 Max: 147.5	Correlation (*r*): $PM_{2.5}$: 0.62; NO_2: 0.44; SO_2: 0.21; CO: 0.27 SO_4^{2-}: 0.56; TC: 0.52; OC: 0.54; EC: 0.40 Copollutant models with: CO, NO_2, $PM_{2.5}$	RR Lag 0−1: 1.03 (1.02, 1.03)
Darrow et al. (2011) Atlanta, GA, U.S. Ozone: 1993−2004 Follow-up: 1993−2004 Time-series study	All ages	One monitor 1-h max, 24-h avg, 8-h max Warm season (March−October)	1-h max: Mean: 62 75th: 76 Max: 180 24-h avg: Mean: 30 75th: 37 Max: 81 8-h max: Mean: 53 75th: 67 Max: 148	Correlation (*r*): 1-h max O_3: $PM_{2.5}$: 0.49; NO_2: 0.33; CO: 0.21; 24-h avg O_3: $PM_{2.5}$: 0.25; NO_2: −0.15; CO: −0.17; 8-h max O_3: $PM_{2.5}$: 0.46; NO_2: 0.24; CO: 0.15 Copollutant models with: NR	Lag 1 RR 1-h max: 1.01 (1.01, 1.02) 24-h avg: 1.01 (1.00, 1.01) 8-h max: 1.01 (1.01, 1.02)
†Winquist et al. (2012) St. Louis, MO, U.S. Ozone: 2001−2007 Follow-up: 2001−2007 Time-series study	All ages	One monitor 8-h max Year-round		Correlation (*r*): NR Copollutant models with: NR	RR 0−4 DL: 1.01 (1.00, 1.03)

Table 3-42 (Continued): Epidemiologic studies of short-term exposure to ozone and emergency department (ED) visits for aggregate respiratory diseases.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)[a]
†Darrow et al. (2011) Atlanta, GA, U.S. Ozone: March−October, 1993−2004 Follow-up: March−October, 1993−2004 Time-series study	n = 1,068,525 All ages	One monitor 8-h max Year-round	Mean: 53 Median: 51 75th: 67 Max: 148	Correlation (*r*): $PM_{2.5}$: 0.46; NO_2: 0.24 Copollutant models with: NR	Lag 1 RRs Commute (7:00 a.m.−10:00 a.m.; 4:00 p.m.−7:00 p.m.): 1.01 (1.00, 1.02) per 25 ppb increase Daytime (8:00 a.m.−7:00 p.m.): 1.01 (1.01, 1.02) per 20 ppb increase Nighttime (12:00 a.m.−6:00 a.m.): 0.99 (0.98, 1.00) per 25 ppb 1-h max: 1.01 (1.01, 1.02) 24-h avg: 1.01 (1.00, 1.01) 8-h max: 1.01 (1.01, 1.02)
†Malig et al. (2016) California (statewide), U.S. Ozone: 2005−2009 Follow-up: 2005−2008 Case-crossover study	All ages	Nearest monitor within 20 km of population weighted zip-code centroid 1-h max Seasonal: warm season (May−October) and year-round estimates	Mean: 33−55 across climate zones	Correlation (*r*): NO_2: −0.01 YR; 0.26 warm; SO_2: −0.06 YR; 0.02 warm; CO: −0.28 YR; 0.02 warm Copollutant models with: NO_2, CO, SO_2	Percent increase Lag 0−1 Year-round: 2.07 (1.63, 2.52) Warm season: 2.39 (1.56, 3.23)
†Barry et al. (2018) Five U.S. cities Ozone: 2002−2008 Follow-up: 2002−2008 Time-series study	All ages	Fusion of CMAQ model estimates and ground-based measurements; population weighted average of 12-km grid cells for each city 8-h max Year-round	Mean: 37.5−42.2 75th: 50.1−54.4 90th: 59.3−63.5 Max: 80.2−106.3	Correlation (*r*): NR Copollutant models with: NR	RR Lag 0−2 Atlanta: 1.02 (1.01, 1.04) Birmingham: 1.02 (1.00, 1.05) Dallas: 1.04 (1.02, 1.06) Pittsburgh 1.02 (1.01, 1.04) St. Louis 1.02 (1.00, 1.03)

Table 3-42 (Continued): Epidemiologic studies of short-term exposure to ozone and emergency department (ED) visits for aggregate respiratory diseases.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)[a]
†O' Lenick et al. (2017) Atlanta, GA; Dallas, TX; and St. Louis, MO, U.S. Ozone: 2002−2008 Follow-up: 2002−2008 Case-crossover study	n = 421,798 Age: 5−18 yr	Fusion of CMAQ model estimates and ground-based measurements; 12-km grid cells area weighted to ZCTAs 8-h max Year-round	Mean: 40.0−42.2 across cities Max: 125	Correlation (*r*): NR Copollutant models with: NR	Lag 0−2 ORs St. Louis: 1.02 (0.99, 1.06) Dallas: 1.03 (1.01, 1.06) Atlanta: 1.06 (1.05, 1.09)

Note: †Studies published since the 2013 Ozone ISA.

[a]Results standardized to a 15-ppb increase in 24-hour avg, 20-ppb increase in 8-hour daily max, or 25-ppb increase in 1-hour daily max ozone concentrations.

3.3.2 Long-Term Exposure

Table 3-43 Epidemiologic studies of long-term exposure to ozone and development of asthma.

Study	Study Population	Exposure Assessment	Mean ppb	Copollutant Examination	Effect Estimates (95% CI)[a]
†Garcia et al. (2019) Multicity, southern California Ozone: 1993, 1996, and 2006 Follow-up: 1993−2001; 1996−2004; 2006−2014 Cohort study	Children's Health Study n = 4,140 Age: 4th grade at enrollment to 12th grade at end of follow up. 8 yr follow-up for three different cohorts spanning 21 yr	Ozone measure at one monitor in each of nine communities. Community-specific mean annual concentration (10 a.m. to 6 p.m. avg) measured at baseline in each community.	Mean: NR; Ozone concentrations depicted graphically. Annual average ozone concentrations ranged from about 26 ppb to 76 ppb. Median decrease across communities from baseline to end of follow-up was 8.9 ppb	Correlation (*r*): NO_2: 0.54; $PM_{2.5}$: 0.62 Copollutant models with: NR	Asthma Incidence (per 10 ppb decrease) Fully adjusted model: 0.83 (0.68, 1.02) Adjusted for traffic with local near roadway pollution term: 0.84 (0.68, 1.05)
†Tétreault et al. (2016a) Quebec, Canada Ozone: 1999−2011 Follow-up: 1999−2011 Cohort study	Quebec Integrated Chronic Disease Surveillance System n = 1,183,865 Children born in Quebec	Average summer (June−August) concentrations of 8-h midday O_3 estimated using a BME-LUR model.	Mean: 32.07 Median: 32.19 75th: 33.76 Max: 43.12	Correlation (*r*): NR Copollutant models with: NR	Asthma onset HRs Birth address: 1.20 (1.16, 1.23) Time-varying exposure: 1.23 (1.20, 1.27)
†Nishimura et al. (2013) Multicity, U.S. Ozone: NR Follow-up: Case-control study	Gala II and Sage II n = 1,968 African American and Latino American children and young adults. Case subjects had physician-diagnosed asthma, while control subjects, matched 1:1 by age, had no history of asthma or other respiratory disease. Age: 8−21 yr	IDW from up to four monitors within 50 km of residence. First year of life and first 3 yr of life exposures estimated. 1-h max; 8-h max	Mean: 27.6 Median: 27.3 75th: 30.9	Correlation (*r*): NR Copollutant models with: NR	ORs First 3 yr of life, 8-h max: 0.90 (0.66, 1.23) First year of life, 1-h max: 0.94 (0.81, 1.12) First 3 yr of life, 1-h max: 0.96 (0.71, 1.28)

Note: †Studies published since the 2013 Ozone ISA.

[a]Results standardized to a 10-ppb increase in long-term ozone concentrations.

Table 3-44 Study-specific details from animal toxicological studies of long-term ozone exposure and inflammation, oxidative stress, and injury—allergy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Chou et al. (2011)	Rhesus macaque (*Macaca mulatta*) Sensitized and challenged with house dust mite n = 6 males Age: 1 mo	0.5 ppm, 8 h/day for 5 days followed by 9 days of FA—5 cycles	BALF differential cell counts, eotaxins/airway mucosa eosinophil number; lung tissue immunofluorescence of major basic protein, eotaxin and eotaxin receptor; lung tissue mRNA for eotaxin (4−5 days PE, at 90 days of age)
Zellner et al. (2011)	Rats (F344) n = 3−14 Sex: NR Age: PND 5	2 ppm, 3 h	Numbers of airway neurons (PNDs 10−28)
Crowley et al. (2017)	Rhesus macaque (*Macaca mulatta*) Sensitized and challenged with house dust mite n = 5−6 males Age: 1 mo	0.5 ppm, 8 h/day for 5 days followed by 9 days of FA—11 cycles	BALF total cell and differential cell counts and immune cell phenotypes; mRNA for T-lymphocyte markers, cytokines, and CCR3 (3−5 h PE, at 25 weeks of age)
Murphy et al. (2012)	Rhesus macaque (*Macaca mulatta*) Sensitized and challenged with house dust mite n = 4−6 males Age: 6 mo	0.5 ppm, 8 h/day for 5 days followed by 9 days of FA—11 cycles	Neurokinin pathway components (at 12 mo of age)

BALF = bronchoalveolar lavage fluid; CCR3 = C-C motif chemokine receptor 3; FA = filtered air, PND = post-natal day; PE = post-exposure.

Table 3-45 Study-specific details from animal toxicological studies of long-term ozone exposure and lung function—allergy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration Duration)	Endpoints Examined
Moore et al. (2012)	Rhesus macaque (*Macaca mulatta*) Sensitized and challenged with house dust mite n = 6−9 males Age: 1 mo	0.5 ppm, 8 h/day for 5 days followed by 9 days of FA—11 cycles	Pulmonary mechanics, challenge with histamine; airway smooth muscle contraction to electrical field stimulation (PE, at 6 mo of age)

FA = filtered air; PE = post-exposure.

Table 3-46 Study-specific details from animal toxicological studies of long-term ozone exposure and inflammation, oxidative stress, and injury—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Hunter et al. (2011)	Rats n = 4−6 Strain and sex: NR Age: PND 6−28	2 ppm, 3 h	BALF neutrophils and NGF; NGF mRNA in tracheal epithelial cells, SP+ airway neurons in vagal ganglia, airway SP-nerve fiber density (12−24 h PE)
Gabehart et al. (2014)	Mice (BALB/c) n = NR Sex: NR Age: 3 days	1 ppm, 3 h	BALF total and differential cell counts, albumin; lung tissue mRNA for chemokine and antioxidant genes, transcriptome analysis, histology, cell proliferation (6 and 24 h PE)
Clay et al. (2014)	Rhesus macaque (*Macaca mulatta*) n = 3−5 males Age: 1 mo	0.5 ppm, 8 h/day for 5 days followed by 9 days of FA—11 cycles followed by FA until 12 mo	IL-6 and IL-8 mRNA and protein in airway epithelial cells in vitro and cell culture apical supernatant following LPS challenge; micro RNA gene expression in airway epithelial cells in vitro following LPS challenge (12 mo of age)
Gabehart et al. (2015)	Mice (BALB/c), wild type and TLR4 deficient n = 3−14 females Age: 1, 2, 3 weeks	1 ppm, 3 h	BALF total cell number and differential cell counts, albumin, Muc-5AC; lung tissue mRNA for chemokines, antioxidants, TLR4, neuropeptides (6, 24, 48 h PE)
Snow et al. (2016)	Rats (BN) n = 8−10 males Age: 1, 4, 12, 24 mo	0.25 ppm, 6 h/day, 2 days/week for 13 weeks 1 ppm, 6 h/day, 2 days/week for 13 weeks	BALF total cells, cell differentials, protein, albumin, GGT, NAG (18 h PE)
Gordon et al. (2016b)	Rats (BN) n = 9−10 males, 9−10 females Age: 20 weeks	0.8 ppm, 5 h/day for 1 day/week for 4 weeks	BALF total cells, cell differentials, albumin (18 h PE)
Gordon et al. (2016a)	Rats (S-D) n = 10 females Age: 20 weeks	0.25 ppm, 5 h/day for 1 day/week for 6 weeks 0.5 ppm, 5 h/day for 1 day/week for 6 weeks 1 ppm, 5 h/day for 1 day/week for 6 weeks	BALF total cells, cell differentials, albumin, NAG, GGT (24 h PE)

Table 3-46 (Continued): Study-specific details from animal toxicological studies of long term ozone exposure and inflammation, oxidative stress, and injury—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Miller et al. (2016a)	Rats (WKY) n = 8−10 males Age: 10 weeks	0.25 ppm, 5 h/day for 3 days/week for 13 weeks 0.5 ppm, 5 h/day for 3 days/week for 13 weeks 1 ppm, 5 h/day for 3 days/week for 13 weeks	BALF total cells, cell differentials, albumin, NAG, GGT (immediately PE or following 1 week recovery)
Dye et al. (2017)	Rats (F344) n = 3−4 males, 3−4 females Age: PND 14, 21, 28 Rats (S-D) n = 7−8 males, 7−8 females Age: PND 14, 21, 28 Rats (WS) n = 7−8 males, 7−8 females Age: PND 14, 21, 28	1 ppm, 2 h	Lung tissue antioxidants (immediately PE)
Miller et al. (2017)	Rats (LE) n = 9−10 females Age : NR but weight was 200 g, pregnant	0.4 ppm, 4 h/day for 2 days; GDs 5-6 0.8 ppm, 4 h/day for 2 days; GDs 5-6	BALF total protein, albumin, LDH, NAG, GGT, total cell, and differential cell count (GD 21)

BALF = bronchoalveolar lavage fluid; BN = brown Norway; FA = filtered air; GGT = gamma glutamyl transferase; IL = interleukin; LE = Long-Evans; LPS = lipopolysaccharide; NAG = *N*-acetyl-glucosaminidase; NGF = nerve growth factor; PE = post-exposure; TLR4 = toll receptor 4; WKY = Wistar Kyoto; WS = Wistar.

Table 3-47 Study-specific details from animal toxicological studies of long-term ozone exposure and morphology and other endpoints—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Lee et al. (2011)	Rats (S-D) n = 7−9 males Age: 7 days	0.5 ppm, 6 h/day for 3 weekly cycles either 5 days ozone and 2 days recovery or 2 days ozone and 5 days recovery	Airway architectural parameters—diameter, length, branching angles of conducting airways (56 days PE)
Murphy et al. (2013)	Rhesus macaque (*Macaca mulatta*) n = 3−4 males Age: 1 mo	0.5 ppm, 8 h 0.5 ppm, 8 h/day for 5 days followed by 9 days of FA—1 or 11 cycles	Serotonin pathway components (PE, at 2 and 6 mo of age)
Murphy et al. (2014)	Rhesus macaque (*Macaca mulatta*) n = 3−4 males Age: 1 mo	0.5 ppm, 8 h/day for 5 days followed by 9 days of FA—1 or 11 cycles	Lung tissue mRNA and immunostaining for NK-1R, TAC1/SP, Nur77 (PE, up to 25 weeks)

Table 3-48 Epidemiologic studies of long-term exposure to ozone and lung function and development.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates (95% CI)[a]
†Eckel et al. (2012) Multicity, U.S. Ozone: 1990−1997 Follow-up: 1990−1997 Cohort study	Cardiovascular Health Study n = 3,382 Age: ≥65 yr	Cumulative sum of monthly averages calculated using IDW from up to three monitors within 50 km of residences. Cumulative exposures estimated for time on study. 8-h avg	Mean: 39.7 Median: 39.7 75th: 51.3 95th: 64.1 Max: 79.6	Correlation (*r*): PM_{10}: 0.96 Copollutant models with: NR	Difference in FEV_1 (mL) Women: −0.17 (−0.24, −0.10) Men: −0.34 (−0.47, −0.21) Difference in FVC (mL) Women: −0.76 (−0.86, −0.66) Men: −1.24 (−1.40, −1.09)
†Urman et al. (2014) Multicity, southern California, U.S. Ozone: 2002−2007 Follow-up: 2007−2008 Cross-sectional study	Children's Health Study n = 1,811 Age: 11–12 yr	6-yr avg of 10 a.m. to 6 p.m. Ozone measured at one monitor in each of the eight communities Other	Mean: 22.7	Correlation (*r*): $PM_{2.5}$: 0.66; NO_2: 0.12 Copollutant models with: NR	FVC (percent increase): −0.14 (−1.38, 1.12) FEV_1 (percent increase): −1.38 (−2.34, −0.40)
†Gauderman et al. (2015) Multicity, southern California, U.S. Ozone: 1994−1997; 1997−2000; 2007−2010 Follow-up: 1994−1997; 1997−2000; 2007−2010 Cohort study	Children's Health Study n = 2,120 Age: 11–15 yr 4 yr follow-up for three different cohorts spanning 19 yr	4-yr avg of 10 a.m. to 6 p.m. Ozone measured at one monitor in each of the five communities Other	Mean: Range across communities: 28.6 to 61.9	Correlation (*r*): $PM_{2.5}$: 0.39; NO_2: 0.02 Copollutant models with: NR	4-yr FEV_1 growth (mL) per decrease in O_3: −12.18 (−92.73, 68.18) 4-yr FVC growth (mL) per decrease in O_3: −13.27 (−144.18, 117.45)
†Neophytou et al. (2016) Multicity, U.S. Ozone: NR Follow-up: Cross-sectional study	Gala II and Sage II n = 1,968 Age: 8−21 yr African American and Latino American children and young adults with asthma	IDW from up to four monitors within 50 km of residence. First year of life and lifetime exposures estimated. 8-h max	Mean: NR Median: Results presented graphically. Median average lifetime concentrations range from approximately 20 to 37 across study sites	Correlation (*r*): $PM_{2.5}$: First year: 0.19; Lifetime: 0.73; NO_2: First year: 0.02; Lifetime: 0.49; SO_2: First year: 0.15; Lifetime: 0.04 Copollutant models with: NR	FEV_1 (percent increase) First year of life exposure: −1.12 (−2.60, 0.40) Average lifetime exposure: −1.30 (−3.88, 1.36)

Note: †Studies published since the 2013 Ozone ISA.

[a]Results standardized to a 10-ppb increase in long-term ozone concentrations.

Table 3-49 Study-specific details from animal toxicological studies of long-term ozone exposure and morphology—allergy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Avdalovic et al. (2012)	Rhesus macaque (*Macaca mulatta*), sensitized and challenged with house dust mite n = 12 males Age: 30 days	0.5 ppm, 8 h/day for 5 days followed by 9 days of FA—5 or 11 cycles	Alveolar volume and number, distribution of alveolar size, and capillary surface density per alveolar septa; mRNA of candidate genes (PE, at 3 or 6 mo of age)
Herring et al. (2015)	Rhesus macaque (*Macaca mulatta*), sensitized and challenged with house dust mite n = 6 males Age: 30 days	0.5 ppm, 8 h/day for 5 days followed by 9 days of FA—11 cycles followed by 30 mo recovery in FA	Alveolar number and size, alveolar capillary surface density, length and volume of terminal and respiratory bronchioles (PE at 6 and 36 mo)

FA = filtered air; PE = post-exposure.

Table 3-50 Study-specific details from animal toxicological studies of long-term ozone exposure and lung function—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Snow et al. (2016)	Rats (BN) n = 6−10 males Age: 1, 4, 12, 24 mo	0.25 ppm, 6 h/day, 2 days/week for 13 weeks 1 ppm, 6 h/day, 2 days/week for 13 weeks	Ventilatory parameters (1−5 days PE each week)
Gordon et al. (2016a)	Rats (S-D) n = 10 females Age: 20 weeks	0.25 ppm, 5 h/day for 1 day/week for 6 weeks 0.5 ppm, 5 h/day for 1 day/week for 6 weeks 1 ppm, 5 h/day for 1 day/week for 6 weeks	Ventilatory parameters (24 h post 5th week of exposure)
Miller et al. (2017)	Rats (LE) n = 9−10 females Age: NR but weight was 200 g, pregnant	0.4 ppm, 4 h/day for 2 days; GDs 5-6 0.8 ppm, 4 h/day for 2 days; GDs 5-6	Ventilatory parameters (immediately PE)

BALF = bronchoalveolar lavage fluid; BN = brown Norway; GD = gestational day; GGT = gamma glutamyl transferase; LDH = lactate dehydrogenase; LE = Long-Evans; NAG = *N*-acetyl-glucosaminidase; PE = post-exposure, S-D = Sprague-Dawley.

Table 3-51 Epidemiologic studies of long-term exposure to ozone and development of chronic obstructive pulmonary disease (COPD).

Study	Study Population	Exposure Assessment	Mean ppb	Copollutant Examination	Effect Estimates 95% CI[a]
†To et al. (2016) Ontario, Canada Ozone: 1996−2013 Follow-up: 1996−2014 Cohort study	Ontario Asthma Surveillance Information System and the Canadian Community Health Survey n = 6,040 Age: ≥18 yr Adults with asthma	Interpolated surface using IDW of 49 monitors across the province. Average of monthly 24-h max from time of asthma incidence to time of COPD incidence or end of follow-up. Other	Mean: 39.3 Median: 39.2 75th: 40.4	Correlation (*r*): $PM_{2.5}$: NR Copollutant models with: $PM_{2.5}$	COPD in adults with asthma: HR 2.05 (1.17, 3.60)

Note: †Studies published since the 2013 Ozone ISA.

[a]Results standardized to a 10-ppb increase in long-term ozone concentrations.

Table 3-52 Epidemiologic studies of long-term exposure to ozone and respiratory infection.

Study	Study Population	Exposure Assessment	Mean ppb	Copollutant Examination	Effect Estimates HR (95% CI)[a]
†MacIntyre et al. (2011) Georgia Basin Airshed (including Vancouver and Victoria, British Columbia), Canada Ozone: 1999−2002 Follow-up: 1999−2002 Cohort study	n = 45,513 All singleton live births in the Georgia Basin Airshed, followed for the first 2 yr of life	IDW average of three closest monitors within 50 km	Mean: 28.2 Median: 26.1 Max: 71.8	Correlation (*r*): NR Copollutant models with: NR	Otitis media HR 0.96 (0.95, 0.97)
†Smith et al. (2016) Multicity, northern California, U.S. Ozone: 1996−2010 Follow-up: 1996−2010 Case-control study	n = 6,913 Cases are adult members of Kaiser Permanente Northern California with a clinical diagnosis of TB and a corresponding anti-TB prescription or a positive TB culture. Controls were matched 2-1 on age, sex, and race/ethnicity. Age: ≥21 yr	2-yr avg from the nearest monitor 8-h avg	Median: 31.5 Max: 67	Correlation (*r*): $PM_{2.5}$: 0.25; NO_2: −0.33; SO_2: −0.24; Other: CO: −0.28 Copollutant models with: NR	Pulmonary tuberculosis ORs 1st quintile: Ref 2nd quintile: 0.92 (0.78, 1.10) 3rd quintile: 0.95 (0.80, 1.14) 4th quintile: 0.71 (0.59, 0.85) 5th quintile: 0.66 (0.55, 0.79)

Note: †Studies published since the 2013 Ozone ISA.

[a]Results standardized to a 10-ppb increase in long-term ozone concentrations.

Table 3-53 Epidemiologic studies of long-term exposure to ozone and severity of respiratory disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI[a]
†Tétreault et al. (2016b) Quebec, Canada Ozone: 1990−2006 Follow-up: 1996−2011 Cohort study	Quebec Integrated Chronic Disease Surveillance System n = 1,183,865 Children born in Quebec	Average summer (June−August) concentrations of 8-h midday O_3 estimated using a BME-LUR model. Other	Mean: 30.57 Median: 30.8 75th: 32.42 Max: 38.92	Correlation (*r*): NR Copollutant models with: NR	Hospital/ED visits HRs Birth residence: 0.99 (0.96, 1.11) Time-Dependent: 1.17 (1.12, 1.22)
†To et al. (2016) Ontario, Canada Ozone: 1996−2013 Follow-up: 1996−2014 Cohort study	Ontario Asthma Surveillance Information System and the Canadian Community Health Survey n = 6,040 Age: ≥18 yr Adults with asthma	Interpolated surface using IDW of 49 monitors across the province. Average of monthly 24-h max from time of asthma incidence to time of COPD incidence or end of follow-up. Other	Mean: 39.3 Median: 39.2 75th: 40.4	Correlation (*r*): $PM_{2.5}$: NR Copollutant models with: $PM_{2.5}$	COPD in adults with asthma: 2.05 (1.17, 3.60)
†Berhane et al. (2016) Multicity, southern California, U.S. O_3: 1992−2011 Follow-up: 1992−2000; 1995−2003; 2002−2011 Cohort study	Children's Health Study n = 4,602 Age: 10 and 15 yr-olds	9- or 10-yr avg of 10 a.m. to 6 p.m. Ozone measured at one monitor in each of the eight communities Other	Mean: Range across cohorts: 44.8−47.7	Correlation (*r*): $PM_{2.5}$: 0.54; NO_2: 0.38 Copollutant models with: NO_2, $PM_{2.5}$	Absolute (percent) changes in bronchitis symptoms 15-yr-olds with asthma: −29.22 (−40.80, −12.77) 10-yr-olds with asthma: −39.00 (−53.34, −17.52)

Note: †Studies published since the 2013 Ozone ISA.

[a]Results standardized to a 10-ppb increase in long-term ozone concentrations.

Table 3-54 Epidemiologic studies of long-term exposure to ozone and allergic sensitization.

Study	Study Population	Exposure Assessment	Mean ppb	Copollutant Examination	Effect Estimates 95% CI[a]
†Weir et al. (2013) Multicity, U.S. Ozone: 2005−2006 Follow-up: 2005−2006 Cross-sectional study	NHANES n = 6,227 (CMAQ); 5,201 (IDW monitors) Age: ≥6 yr	Annual average CMAQ estimates and estimates derived from inverse distance weighting for participants living within 20 miles of a monitor 8-h max	Mean: 51.5 (IDW); 57.2 (CMAQ) Median: 52 (IDW); 57.0 (CMAQ) 75th: 55.3 (IDW); 61.2 (CMAQ) 95th: 60.3 (IDW); 70.8 (CMAQ)	Correlation (*r*): $PM_{2.5}$: 0.08 (IDW); −0.21 (CMAQ); NO_2: −0.25 (IDW); −0.42 (CMAQ) Copollutant models with: NR	ORs IDW Food allergens: 0.80 (0.54, 1.19) Indoor allergens: 0.91 (0.78, 1.06) Outdoor allergens: 1.17 (0.99, 1.38) Inhalant allergens: 1.06 (0.93, 1.20) Any allergens: 1.07 (0.94, 1.21) CMAQ Food allergens: 1.01 (0.77, 1.32) Indoor allergens: 1.02 (0.86, 1.22) Outdoor allergens: 1.14 (0.90, 1.43) Inhalant allergens: 1.11 (0.93, 1.32) Any allergens: 1.10 (0.93, 1.29)

Note: †Studies published since the 2013 Ozone ISA.

[a]Results standardized to a 10-ppb increase in long-term ozone concentrations.

Table 3-55 Study-specific details from animal toxicological studies of long-term ozone exposure and allergic sensitization—healthy.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Hansen et al. (2013)	Mice (BALB/cJ), minimally sensitized by low dose ovalbumin n = 8−10 females Age: 6−7 weeks	0.1 ppm, 0.33 h/day for 5 days/week for 2 weeks and once weekly for 12 weeks	Development of allergy (IgE), BALF total and differential cell counts, ventilatory parameters

BALF = bronchoalveolar lavage fluid; IgE = immunoglobulin E.

Annex for Appendix 3: Evaluation of Studies on Health Effects of Ozone

This annex describes the approach used in the Integrated Science Assessment (ISA) for Ozone and Related Photochemical Oxidants to evaluate study quality in the available health effects literature. As described in the Preamble to the ISA (U.S. EPA, 2015), causality determinations were informed by the integration of evidence across scientific disciplines (e.g., exposure, animal toxicology, epidemiology) and related outcomes and by judgments of the strength of inference in individual studies. Table Annex 3-1 describes aspects considered in evaluating study quality of controlled human exposure, animal toxicological, and epidemiologic studies. The aspects found in Table Annex 3-1 are consistent with current best practices for reporting or evaluating health science data.1 Additionally, the aspects are compatible with published U.S. EPA guidelines related to cancer, neurotoxicity, reproductive toxicity, and developmental toxicity (U.S. EPA, 2005, 1998, 1996b, 1991).

These aspects were not used as a checklist, and judgments were made without considering the results of a study. The presence or absence of particular features in a study did not necessarily lead to the conclusion that a study was less informative or should be excluded from consideration in the ISA. Further, these aspects were not used as criteria for determining causality in the five-level hierarchy. As described in the Preamble, causality determinations were based on judgments of the overall strengths and limitations of the collective body of available studies and the coherence of evidence across scientific disciplines and related outcomes. Table Annex 3-1 is not intended to be a complete list of aspects that define a study's ability to inform the relationship between ozone and health effects, but it describes the major aspects considered in this ISA to evaluate studies. Where possible, study elements, such as exposure assessment and confounding (i.e., bias due to a relationship with the outcome and correlation with exposures to ozone), are considered specifically for ozone. Thus, judgments on the ability of a study to inform the relationship between an air pollutant and health can vary depending on the specific pollutant being assessed.

1 For example, NTP OHAT approach (Rooney et al., 2014), IRIS Preamble (U.S. EPA, 2013b), ToxRTool (Klimisch et al., 1997), STROBE guidelines (von Elm et al., 2007), and ARRIVE guidelines (Kilkenny et al., 2010).

Table Annex 3-1 Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Study Design
Controlled Human Exposure:
Studies should clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested. Study subjects should be randomly exposed without knowledge of the exposure condition. Preference is given to balanced crossover (repeated measures) or parallel design studies which include control exposures (e.g., to clean filtered air). In crossover studies, a sufficient and specified time between exposure days should be provided to avoid carry over effects from prior exposure days. In parallel design studies, all arms should be matched for individual characteristics, such as age, sex, race, anthropometric properties, and health status. In studies evaluating effects of disease, appropriately matched healthy controls are desired for interpretative purposes.
Animal Toxicology:
Studies should clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested. Studies should include appropriately matched control exposures (e.g., to clean filtered air, time matched). Studies should use methods to limit differences in baseline characteristics of control and exposure groups. Studies should randomize assignment to exposure groups and where possible conceal allocation to research personnel. Groups should be subjected to identical experimental procedures and conditions; animal care including housing, husbandry, etc. should be identical between groups. Blinding of research personnel to study group may not be possible due to animal welfare and experimental considerations; however, differences in the monitoring or handling of animals in all groups by research personnel should be minimized.
Epidemiology:
Inference is stronger for studies that clearly describe the primary and any secondary aims of the study, or specific hypotheses being tested. For short-term exposure, time-series, case-crossover, and panel studies are emphasized over cross-sectional studies because they examine temporal correlations and are less prone to confounding by factors that differ between individuals (e.g., SES, age). Panel studies with scripted exposures, in particular, can contribute to inference because they have consistent, well-defined exposure durations across subjects, measure personal ambient pollutant exposures, and measure outcomes at consistent, well-defined lags after exposures. Studies with large sample sizes and conducted over multiple years are considered to produce more reliable results. Additionally, multicity studies are preferred over single-city studies because they examine associations for large diverse geographic areas using a consistent statistical methodology, avoiding the publication bias often associated with single-city studies.[a] If other quality parameters are equal, multicity studies carry more weight than single-city studies because they tend to have larger sample sizes and lower potential for publication bias. For long-term exposure, inference is considered to be stronger for prospective cohort studies and case-control studies nested within a cohort (e.g., for rare diseases) than cross-sectional, other case-control, or ecologic studies. Cohort studies can better inform the temporality of exposure and effect. Other designs can have uncertainty related to the appropriateness of the control group or validity of inference about individuals from group-level data. Study design limitations can bias health effect associations in either direction.

Table Annex 3-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Study Population/Test Model
Controlled Human Exposure:
In general, the subjects recruited into study groups should be similarly matched for age, sex, race, anthropometric properties, and health status. In studies evaluating effects of specific subject characteristics (e.g., disease, genetic polymorphism, etc.), appropriately matched healthy controls are preferred. Relevant characteristics and health status should be reported for each experimental group. Criteria for including and excluding subjects should be clearly indicated. For the examination of populations with an underlying health condition (e.g., asthma), independent, clinical assessment of the health condition is ideal, but self-report of physician diagnosis generally is considered to be reliable for respiratory and cardiovascular disease outcomes.[b] The loss or withdrawal of recruited subjects during the course of a study should be reported. Specific rationale for excluding subject(s) from any portion of a protocol should be explained.
Animal Toxicology:
Ideally, studies should report species, strain, substrain, genetic background, age, sex, and weight. Unless data indicate otherwise, all animal species and strains are considered appropriate for evaluating effects of ozone exposure. It is preferred that the authors test for effects in both sexes and multiple lifestages, and report the result for each group separately. All animals used in a study should be accounted for, and rationale for exclusion of animals or data should be specified.
Epidemiology:
There is greater confidence in results for study populations that are recruited from and representative of the target population. Studies with high participation and low dropout over time that is not dependent on exposure or health status are considered to have low potential for selection bias. Clearly specified criteria for including and excluding subjects can aid assessment of selection bias. For populations with an underlying health condition, independent, clinical assessment of the health condition is valuable, but self-report of physician diagnosis generally is considered to be reliable for respiratory and cardiovascular diseases.[b] Comparisons of groups with and without an underlying health condition are more informative if groups are from the same source population. Selection bias can influence results in either direction or may not affect the validity of results but rather reduce the generalizability of findings to the target population.
Pollutant
Controlled Human Exposure:
The focus is on studies testing ozone exposure.
Animal Toxicology:
The focus is on studies testing ozone exposure.
Epidemiology:
The focus is on studies evaluating ozone exposure.

Table Annex 3-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Exposure Assessment or Assignment
Controlled Human Exposure:
For this assessment, the focus is on studies that use ozone concentrations <0.4 ppm. Studies that use higher exposure concentrations may provide information relevant to biological plausibility, dosimetry, or inter-species variation. Studies should have well-characterized pollutant concentration, temperature, and relative humidity and/or have measures in place to adequately control the exposure conditions. Preference is given to balanced crossover or parallel design studies that include control exposures (e.g., to clean filtered air). Study subjects should be randomly exposed without knowledge of the exposure condition. Method of exposure (e.g., chamber, facemask, etc.) should be specified and activity level of subjects during exposures should be well characterized.
Animal Toxicology:
For this assessment, the focus is on studies that use ozone concentrations <2 ppm. Studies that use higher exposure concentrations may provide information relevant to biological plausibility, dosimetry, or inter-species variation. Studies should characterize pollutant concentration, temperature, and relative humidity and/or have measures in place to adequately control the exposure conditions. The focus is on inhalation exposure. Noninhalation exposure experiments (i.e., intra-tracheal instillation [IT]) are informative for size fractions that cannot penetrate the airway of a study animal and may provide information relevant to biological plausibility and dosimetry. In vitro studies may be included if they provide mechanistic insight or examine similar effects as in vivo studies, but are generally not included. All studies should include exposure control groups (e.g., clean filtered air).
Epidemiology:
Of primary relevance are relationships of health effects with the ambient component of ozone exposure. However, information about ambient exposure rarely is available for individual subjects; most often, inference is based on ambient concentrations. Studies that compare exposure assessment methods are considered to be particularly informative. Inference is stronger when the duration or lag of the exposure metric corresponds with the time course for physiological changes in the outcome (e.g., up to a few days for symptoms) or latency of disease (e.g., several years for cancer). Ambient ozone concentration tends to have low spatial heterogeneity at the urban scale, except near roads where ozone concentration is lower because ozone reacts with nitric oxide emitted from vehicles. For studies involving individuals with near-road or on-road exposures to ozone, in which ambient ozone concentrations are more spatially heterogeneous and relationships between personal exposures and ambient concentrations are potentially more variable, validated methods that capture the extent of variability for the epidemiologic study design (temporal vs. spatial contrasts) and location carry greater weight. Fixed-site measurements, whether averaged across multiple monitors or assigned from the nearest or single available monitor, typically have smaller biases and smaller reductions in precision compared with spatially heterogeneous air pollutants. Concentrations reported from fixed-site measurements can be informative if correlated with personal exposures, closely located to study subjects, highly correlated across monitors within a location, or combined with time-activity information. Atmospheric models may be used for exposure assessment in place of or to supplement ozone measurements in epidemiologic analyses. For example, grid-scale models (e.g., CMAQ) that represent ozone exposure over relatively large spatial scales (e.g., typically greater than 4- × 4-km grid size) often do provide adequate spatial resolution to capture acute ozone peaks that influence short-term health outcomes. Uncertainty in exposure predictions from these models is largely influenced by model formulations and the quality of model input data pertaining to precursor emissions or meteorology, which tends to vary on a study-by-study basis. In studies of short-term exposure, temporal variability of the exposure metric is of primary interest. For long-term exposures, models that capture within-community spatial variation in individual exposure may be given more weight for spatially variable ambient ozone. Given the low spatial variability of ozone at the urban scale, exposure measurement error typically causes health effect estimates to be underestimated for studies of either short-term or long-term exposure. Biases and decreases in the precision of the association (i.e., wider 95% CIs) tend to be small. Even when spatial variability is higher near roads, the reduction in ozone exposure would cause the exposure to be overestimated at a monitor distant from the road or when averaged across a model grid cell, so that health effects would likely be underestimated.

Table Annex 3-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Outcome Assessment/Evaluation
Controlled Human Exposure:
Endpoints should be assessed in the same manner for control and exposure groups (e.g., time after exposure, methods, endpoint evaluator) using valid, reliable methods. Blinding of endpoint evaluators is ideal, especially for qualitative endpoints (e.g., histopathology). For each experiment and each experimental group, including controls, precise details of all procedures carried out should be provided including how, when, and where. Time of the endpoint evaluations is a key consideration that will vary depending on endpoint evaluated. Endpoints should be assessed at time points that are appropriate for the research questions.
Animal Toxicology:
Endpoints should be assessed in the same manner for control and exposure groups (e.g., time after exposure, methods, endpoint evaluator) using valid, reliable methods. Blinding of endpoint evaluators is ideal, especially for qualitative endpoints (e.g., histopathology). For each experiment and each experimental group, including controls, precise details of all procedures carried out should be provided including how, when, and where. Time of the endpoint evaluations is a key consideration that will vary depending on endpoint evaluated. Endpoints should be assessed at time points that are appropriate for the research questions.
Epidemiology:
Inference is stronger when outcomes are assessed or reported without knowledge of exposure status. Knowledge of exposure status could produce artifactual associations. Confidence is greater when outcomes assessed by interview, self-report, clinical examination, or analysis of biological indicators are defined by consistent criteria and collected by validated, reliable methods. Independent, clinical assessment is valuable for outcomes like lung function or incidence of disease, but report of physician diagnosis has shown good reliability.[b] When examining short-term exposures, evaluation of the evidence focuses on specific lags based on the evidence presented in individual studies. Specifically, the following hierarchy is used in the process of selecting results from individual studies to assess in the context of results across all studies for a specific health effect or outcome: i. Distributed lag models; ii. Average of multiple days (e.g., 0−2); iii. If a priori lag days were used by the study authors these are the effect estimates presented; or iv. If a study focuses on only a series of individual lag days, expert judgment is applied to select the appropriate result to focus on considering the time course for physiologic changes for the health effect or outcome being evaluated. When health effects of long-term exposure are assessed by acute events such as symptoms or hospital admissions, inference is strengthened when results are adjusted for short-term exposure. Validated questionnaires for subjective outcomes such as symptoms are regarded to be reliable,[c] particularly when collected frequently and not subject to long recall. For biological samples, the stability of the compound of interest and the sensitivity and precision of the analytical method is considered. If not based on knowledge of exposure status, errors in outcome assessment tend to bias results toward the null.
Potential Copollutant Confounding
Controlled Human Exposure:
Exposure should be well characterized to evaluate independent effects of ozone.
Animal Toxicology:
Exposure should be well characterized to evaluate independent effects of ozone.

Table Annex 3-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Epidemiology:
Not accounting for potential copollutant confounding can produce artifactual associations; thus, studies that examine copollutant confounding carry greater weight. The predominant method is copollutant modeling (i.e., two-pollutant models), which is especially informative when correlations are not high. However, when correlations are high ($r > 0.7$), such as those often encountered for UFP and other traffic-related copollutants, copollutant modeling is less informative. Although the use of single-pollutant models to examine the association between ozone and a health effect or outcome are informative, ideally studies should also include copollutant analyses. Copollutant confounding is evaluated on an individual study basis considering the extent of correlations observed between the copollutant and ozone, and relationships observed with ozone and health effects in copollutant models.
Other Potential Confounding Factors[d]
Controlled Human Exposure:
Preference is given to studies using experimental and control groups that are matched for individual level characteristics (e.g., race/ethnicity, sex, body weight, smoking history, age) and time varying factors (e.g., seasonal and diurnal patterns).
Animal Toxicology:
Preference is given to studies using experimental and control groups that are matched for individual level characteristics (e.g., strain, sex, body weight, litter size, food and water consumption) and time varying factors (e.g., seasonal and diurnal patterns).
Epidemiology:
Factors are considered to be potential confounders if demonstrated in the scientific literature to be related to health effects and correlated with ozone. Not accounting for confounders can produce artifactual associations; thus, studies that statistically adjust for multiple factors or control for them in the study design are emphasized. Less weight is placed on studies that adjust for factors that mediate the relationship between ozone and health effects, which can bias results toward the null. Confounders vary according to study design, exposure duration, and health effect and may include, but are not limited to the following: Short-term exposure studies: Meteorology, day of week, season, medication use, allergen exposure, and long-term temporal trends. Long-term exposure studies: Socioeconomic status, race, age, medication use, smoking status, stress, noise, and occupational exposures.
Statistical Methodology
Controlled Human Exposure:
Statistical methods should be clearly described and appropriate for the study design and research question (e.g., correction for multiple comparisons). Generally, statistical significance is used to evaluate the findings of controlled human exposure studies. However, consistent trends are also informative. Detection of statistical significance is influenced by a variety of factors including, but not limited to, the size of the study, exposure and outcome measurement error, and statistical model specifications. Sample size is not a criterion for exclusion; ideally, the sample size should provide adequate power to detect hypothesized effects (e.g., sample sizes less than three are considered less informative). Because statistical tests have limitations, consideration is given to both trends in data and reproducibility of results.

Table Annex 3-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Animal Toxicology:
Statistical methods should be clearly described and appropriate for the study design and research question (e.g., correction for multiple comparisons). Generally, statistical significance is used to evaluate the findings of animal toxicology studies. However, consistent trends are also informative. Detection of statistical significance is influenced by a variety of factors including, but not limited to, the size of the study, exposure and outcome measurement error, and statistical model specifications. Sample size is not a criterion for exclusion; ideally, the sample size should provide adequate power to detect hypothesized effects (e.g., sample sizes less than three are considered less informative). Because statistical tests have limitations, consideration is given to both trends in data and reproducibility of results.
Epidemiology:
Multivariable regression models that include potential confounding factors are emphasized. However, multipollutant models (more than two pollutants) are considered to produce too much uncertainty due to copollutant collinearity to be informative. Models with interaction terms aid in the evaluation of potential confounding as well as effect modification. Sensitivity analyses with alternate specifications for potential confounding inform the stability of findings and aid in judgments of the strength of inference from results. In the case of multiple comparisons, consistency in the pattern of association can increase confidence that associations were not found by chance alone. Statistical methods that are appropriate for the power of the study carry greater weight. For example, categorical analyses with small sample sizes can be prone to bias results toward or away from the null. Statistical tests such as *t*-tests and chi-squared tests are not considered sensitive enough for adequate inferences regarding ozone-health effect associations. For all methods, the effect estimate and precision of the estimate (i.e., width of 95% CI) are important considerations rather than statistical significance.

[a](U.S. EPA, 2008).

[b]Murgia et al. (2014); Weakley et al. (2013); Yang et al. (2011); Heckbert et al. (2004); Barr et al. (2002); Muhajarine et al. (1997); Toren et al. (1993).

[c]Burney et al. (1989).

[d]Many factors evaluated as potential confounders can be effect measure modifiers (e.g., season, comorbid health condition) or mediators of health effects related to ozone (comorbid health condition).

3.4 References

Adams, WC. (2000). Ozone dose-response effects of varied equivalent minute ventilation rates. J Expo Anal Environ Epidemiol 10: 217-226. http://dx.doi.org/10.1038/sj.jea.7500086.

Adams, WC. (2002). Comparison of chamber and face-mask 6.6-hour exposures to ozone on pulmonary function and symptoms responses. Inhal Toxicol 14: 745-764. http://dx.doi.org/10.1080/08958370290084610.

Adams, WC. (2003a). Comparison of chamber and face mask 6.6-hour exposure to 0.08 ppm ozone via square-wave and triangular profiles on pulmonary responses. Inhal Toxicol 15: 265-281. http://dx.doi.org/10.1080/08958370390168283.

Adams, WC. (2003b). Relation of pulmonary responses induced by 66-h exposures to 0.08 ppm ozone and 2-h exposures to 0.30 ppm ozone via chamber and face-mask inhalation. Inhal Toxicol 15: 745-759. http://dx.doi.org/10.1080/08958370390217828.

Adams, WC. (2006). Comparison of chamber 6.6-h exposures to 0.04-0.08 ppm ozone via square-wave and triangular profiles on pulmonary responses. Inhal Toxicol 18: 127-136. http://dx.doi.org/10.1080/08958370500306107.

Adams, WC; Ollison, WM. (1997). Effects of prolonged simulated ambient ozone dosing patterns on human pulmonary function and symptomatology. Pittsburgh, PA: Air & Waste Management Association.

Alexeeff, SE; Litonjua, AA; Suh, H; Sparrow, D; Vokonas, PS; Schwartz, J. (2007). Ozone exposure and lung function: Effect modified by obesity and airways hyperresponsiveness in the VA Normative Aging Study. Chest 132: 1890-1897. http://dx.doi.org/10.1378/chest.07-1126.

Alexis, NE; Lay, JC; Zhou, H; Kim, CS; Hernandez, ML; Kehrl, H; Hazucha, MJ; Devlin, RB; Diaz-Sanchez, D; Peden, DB. (2013). The glutathione-S-transferase mu 1 (GSTM1) null genotype and increased neutrophil response to low-level ozone (0.06 ppm) [Letter]. J Allergy Clin Immunol 131: 610-612. http://dx.doi.org/10.1016/j.jaci.2012.07.005.

Alexis, NE; Zhou, H; Lay, JC; Harris, B; Hernandez, ML; Lu, TS; Bromberg, PA; Diaz-Sanchez, D; Devlin, RB; Kleeberger, SR; Peden, DB. (2009). The glutathione-S-transferase Mu 1 null genotype modulates ozone-induced airway inflammation in human subjects. J Allergy Clin Immunol 124: 1222-1228. http://dx.doi.org/10.1016/j.jaci.2009.07.036.

Alhanti, BA; Chang, HH; Winquist, A; Mulholland, JA; Darrow, LA; Sarnat, SE. (2016). Ambient air pollution and emergency department visits for asthma: A multi-city assessment of effect modification by age. J Expo Sci Environ Epidemiol 26: 180-188. http://dx.doi.org/10.1038/jes.2015.57.

Arbex, MA; de Souza Conceição, GM; Cendon, SP; Arbex, FF; Lopes, AC; Moysés, EP; Santiago, SL; Saldiva, PHN; Pereira, LAA; Braga, ALF. (2009). Urban air pollution and chronic obstructive pulmonary disease-related emergency department visits. J Epidemiol Community Health 63: 777-783. http://dx.doi.org/10.1136/jech.2008.078360.

Aris, RM; Tager, I; Christian, D; Kelly, T; Balmes, JR. (1995). Methacholine responsiveness is not associated with O3-induced decreases in FEV1. Chest 107: 621-628.

Arjomandi, M; Balmes, JR; Frampton, MW; Bromberg, P; Rich, DQ; Stark, P; Alexis, NE; Costantini, M; Hollenbeck-Pringle, D; Dagincourt, N; Hazucha, MJ. (2018). Respiratory responses to ozone exposure: the multicenter ozone study in older subjects (moses). Am J Respir Crit Care Med 197: 13191327. http://dx.doi.org/10.1164/rccm.201708-1613OC.

Arjomandi, M; Wong, H; Donde, A; Frelinger, J; Dalton, S; Ching, W; Power, K; Balmes, JR. (2015). Exposure to medium and high ambient levels of ozone causes adverse systemic inflammatory and cardiac autonomic effects. Am J Physiol Heart Circ Physiol 308: H1499-1509. http://dx.doi.org/10.1152/ajpheart.00849.2014.

Avdalovic, MV; Tyler, NK; Putney, L; Nishio, SJ; Quesenberry, S; Singh, PJ; Miller, LA; Schelegle, ES; Plopper, CG; Vu, T; Hyde, DM. (2012). Ozone exposure during the early postnatal period alters the timing and pattern of alveolar growth and development in nonhuman primates. Anat Rec 295: 1707-1716. http://dx.doi.org/10.1002/ar.22545.

Avol, EL; Trim, SC; Little, DE; Spier, CE; Smith, MN; Peng, RC; Linn, WS; Hackney, JD; Gross, KB; D'Arcy, JB; Gibbons, D; Higgins, ITT. (1990). Ozone exposure and lung function in children attending a southern California summer camp. In Proceedings of the 83rd A&WMA Annual Meeting. Pittsburgh, PA: Air & Waste Management Association.

Balmes, JR; Chen, LL; Scannell, C; Tager, I; Christian, D; Hearne, PQ; Kelly, T; Aris, RM. (1996). Ozone-induced decrements in FEV1 and FVC do not correlate with measures of inflammation. Am J Respir Crit Care Med 153: 904-909. http://dx.doi.org/10.1164/ajrccm.153.3.8630571.

Bao, A; Liang, L, i; Li, F; Zhang, M, in; Zhou, X, in. (2013). Effects of acute ozone exposure on lung peak allergic inflammation of mice. Front Biosci 18: 838-851. http://dx.doi.org/10.2741/4147.

Barker, JS; Wu, Z; Hunter, DD; Dey, RD. (2015). Ozone exposure initiates a sequential signaling cascade in airways involving interleukin-1beta release, nerve growth factor secretion, and substance P upregulation. J Toxicol Environ Health A 78: 397-407. http://dx.doi.org/10.1080/15287394.2014.971924.

Barr, RG; Herbstman, J; Speizer, FE; Camargo, CA, Jr. (2002). Validation of self-reported chronic obstructive pulmonary disease in a cohort study of nurses. Am J Epidemiol 155: 965-971. http://dx.doi.org/10.1093/aje/155.10.965.

Barraza-Villarreal, A; Sunyer, J; Hernandez-Cadena, L; Escamilla-Nuñez, MC; Sienra-Monge, JJ; Ramírez-Aguilar, M; Cortez-Lugo, M; Holguin, F; Diaz-Sánchez, D; Olin, AC; Romieu, I. (2008). Air pollution, airway inflammation, and lung function in a cohort study of Mexico City schoolchildren. Environ Health Perspect 116: 832-838. http://dx.doi.org/10.1289/ehp.10926.

Barreno, RX; Richards, JB; Schneider, DJ; Cromar, KR; Nadas, AJ; Hernandez, CB; Hallberg, LM; Price, RE; Hashmi, SS; Blackburn, MR; Haque, IU; Johnston, RA. (2013). Endogenous osteopontin promotes ozone-induced neutrophil recruitment to the lungs and airway hyperresponsiveness to methacholine. Am J Physiol Lung Cell Mol Physiol 305: L118-L129. http://dx.doi.org/10.1152/ajplung.00080.2013.

Barry, V; Klein, M; Winquist, A; Chang, HH; Mulholland, JA; Talbott, EO; Rager, JR; Tolbert, PE; Sarnat, SE. (2018). Characterization of the concentration-response curve for ambient ozone and acute respiratory morbidity in 5 US cities. J Expo Sci Environ Epidemiol. http://dx.doi.org/10.1038/s41370-018-0048-7.

Bartoli, ML; Vagaggini, B; Malagrino, L; Bacci, E; Cianchetti, S; Dente, FL; Novelli, F; Costa, F; Paggiaro, P. (2013). Baseline airway inflammation may be a determinant of the response to ozone exposure in asthmatic patients. Inhal Toxicol 25: 127-133. http://dx.doi.org/10.3109/08958378.2013.763313.

Bastain, T; Islam, T; Berhane, K; McConnell, R; Rappaport, E; Salam, M; Linn, W; Avol, E; Zhang, Y; Gilliland, F. (2011). Exhaled nitric oxide, susceptibility and new-onset asthma in the Children's Health Study. Eur Respir J 37: 523-531. http://dx.doi.org/10.1183/09031936.00021210.

Bates, ML; Brenza, TM; Ben-Jebria, A; Bascom, R; Eldridge, MW; Ultman, JS. (2014). Pulmonary function responses to ozone in smokers with a limited smoking history. Toxicol Appl Pharmacol 278: 85-90. http://dx.doi.org/10.1016/j.taap.2014.04.011.

Bennett, WD; Ivins, S; Alexis, NE; Wu, J; Bromberg, PA; Brar, SS; Travlos, G; London, SJ. (2016). Effect of obesity on acute ozone-induced changes in airway function, reactivity, and inflammation in adult females. PLoS ONE 11: e0160030. http://dx.doi.org/10.1371/journal.pone.0160030.

Bentayeb, M; Wagner, V; Stempfelet, M; Zins, M; Goldberg, M; Pascal, M; Larrieu, S; Beaudeau, P; Cassadou, S; Eilstein, D; Filleul, L; Le Tertre, A; Medina, S; Pascal, L; Prouvost, H; Quénel, P; Zeghnoun, A; Lefranc, A. (2015). Association between long-term exposure to air pollution and mortality in France: A 25-year follow-up study. Environ Int 85: 5-14. http://dx.doi.org/10.1016/j.envint.2015.08.006.

Berhane, K; Chang, CC; McConnell, R; Gauderman, WJ; Avol, E; Rapapport, E; Urman, R; Lurmann, F; Gilliland, F. (2016). Association of changes in air quality with bronchitic symptoms in children in California, 1993-2012. JAMA 315: 1491-1501. http://dx.doi.org/10.1001/jama.2016.3444.

Berhane, K; Zhang, Y; Linn, WS; Rappaport, EB; Bastain, TM; Salam, MT; Islam, T; Lurmann, F; Gilliland, FD. (2011). The effect of ambient air pollution on exhaled nitric oxide in the Children's Health Study. Eur Respir J 37: 1029-1036. http://dx.doi.org/10.1183/09031936.00081410.

Berhane, K; Zhang, Y; Salam, MT; Eckel, SP; Linn, WS; Rappaport, EB; Bastain, TM; Lurmann, F; Gilliland, FD. (2014). Longitudinal effects of air pollution on exhaled nitric oxide: the Children's Health Study. Occup Environ Med 71: 507-513. http://dx.doi.org/10.1136/oemed-2013-101874.

Berry, M; Lioy, PJ; Gelperin, K; Buckler, G; Klotz, J. (1991). Accumulated exposure to ozone and measurement of health effects in children and counselors at two summer camps. Environ Res 54: 135-150.

Bhoopalan, V; Han, SG; Shah, MM; Thomas, DM; Bhalla, DK. (2013). Tobacco smoke modulates ozone-induced toxicity in rat lungs and central nervous system. Inhal Toxicol 25: 21-28. http://dx.doi.org/10.3109/08958378.2012.751143.

Biller, H; Holz, O; Windt, H; Koch, W; Müller, M; Jörres, RA; Krug, N; Hohlfeld, JM. (2011). Breath profiles by electronic nose correlate with systemic markers but not ozone response. Respir Med 105: 1352-1363. http://dx.doi.org/10.1016/j.rmed.2011.03.002.

Bosson, JA; Blomberg, A; Stenfors, N; Helleday, R; Kelly, FJ; Behndig, AF; Mudway, I. (2013). Peripheral blood neutrophilia as a biomarker of ozone-induced pulmonary inflammation. PLoS ONE 8: e81816. http://dx.doi.org/10.1371/journal.pone.0081816.

Brand, JD; Ballinger, CA; Tuggle, KL; Fanucchi, MV; Schwiebert, LM; Postlethwait, EM. (2012). Site-specific dynamics of CD11b(+) and CD103(+) dendritic cell accumulations following ozone exposure. Am J Physiol Lung Cell Mol Physiol 303: L1079-L1086. http://dx.doi.org/10.1152/ajplung.00185.2012.

Brand, JD; Mathews, JA; Kasahara, DI; Wurmbrand, AP; Shore, SA. (2016). Regulation of IL-17A expression in mice following subacute ozone exposure. J Immunotoxicol 13: 428-438. http://dx.doi.org/10.3109/1547691X.2015.1120829.

Breton, CV; Salam, MT; Vora, H; Gauderman, WJ; Gilliland, FD. (2011). Genetic variation in the glutathione synthesis pathway, air pollution, and children's lung function growth. Am J Respir Crit Care Med 183: 243-248. http://dx.doi.org/10.1164/rccm.201006-0849OC.

Brown, JS; Bateson, TF; Mcdonnell, WF. (2008). Effects of exposure to 0.06 ppm ozone on FEV1 in humans: A secondary analysis of existing data. Environ Health Perspect 116: 1023-1026. http://dx.doi.org/10.1289/ehp.11396.

Burnett, R; Raizenne, M; Krewski, D. (1990). Acute health effects of transported air pollution: A study of children attending a residential summer camp. Can J Stat 18: 367-373. http://dx.doi.org/10.2307/3315843.

Burney, PG; Laitinen, LA; Perdrizet, S; Huckauf, H; Tattersfield, AE; Chinn, S; Poisson, N; Heeren, A; Britton, JR; Jones, T. (1989). Validity and repeatability of the IUATLD (1984) Bronchial Symptoms Questionnaire: an international comparison. Eur Respir J 2: 940-945.

Byers, N; Ritchey, M; Vaidyanathan, A; Brandt, AJ; Yip, F. (2015). Short-term effects of ambient air pollutants on asthma-related emergency department visits in Indianapolis, Indiana, 2007-2011. J Asthma 53: 1-8. http://dx.doi.org/10.3109/02770903.2015.1091006.

Cabello, N; Mishra, V; Sinha, U; Diangelo, SL; Chroneos, ZC; Ekpa, NA; Cooper, TK; Caruso, CR; Silveyra, P. (2015). Sex differences in the expression of lung inflammatory mediators in response to ozone. Am J Physiol Lung Cell Mol Physiol 309: ajplung.00018.02015. http://dx.doi.org/10.1152/ajplung.00018.2015.

Cakmak, S; Dales, RE; Judek, S. (2006). Respiratory health effects of air pollution gases: Modification by education and income. Arch Environ Occup Health 61: 5-10. http://dx.doi.org/10.3200/AEOH.61.1.5-10.

Carey, IM; Atkinson, RW; Kent, AJ; van Staa, T; Cook, DG; Anderson, HR. (2013). Mortality associations with long-term exposure to outdoor air pollution in a national English cohort. Am J Respir Crit Care Med 187: 1226-1233. http://dx.doi.org/10.1164/rccm.201210-1758OC.

Che, L; Jin, Y; Zhang, C; Lai, T; Zhou, H; Xia, L; Tian, B; Zhao, Y; Liu, J; Wu, Y; Wu, Y; Du, J; Li, W; Ying, S; Chen, Z; Shen, H. (2016). Ozone-induced IL-17A and neutrophilic airway inflammation is orchestrated by the caspase-1-IL-1 cascade. Sci Rep 6: 18680. http://dx.doi.org/10.1038/srep18680.

Cheng, W; Duncan, KE; Ghio, AJ; Ward-Caviness, C; Karoly, ED; Diaz-Sanchez, D; Conolly, RB; Devlin, RB. (2018). Changes in metabolites present in lung lining fluid following exposure of humans to ozone. Toxicol Sci 163: 430439. http://dx.doi.org/10.1093/toxsci/kfy043.

Cho, H; Gladwell, W; Yamamoto, M; Kleeberger, SR. (2013). Exacerbated airway toxicity of environmental oxidant ozone in mice deficient in Nrf2. Oxid Med Cell Longev 2013: Article #254069. http://dx.doi.org/10.1155/2013/254069.

Cho, Y; Abu-Ali, G; Tashiro, H; Kasahara, DI; Brown, TA; Brand, JD; Mathews, JA; Huttenhower, C; Shore, SA. (2018). The microbiome regulates pulmonary responses to ozone in mice. Am J Respir Cell Mol Biol 59: 346-354. http://dx.doi.org/10.1165/rcmb.2017-0404OC.

Chou, DL; Gerriets, JE; Schelegle, ES; Hyde, DM; Miller, LA. (2011). Increased CCL24/eotaxin-2 with postnatal ozone exposure in allergen-sensitized infant monkeys is not associated with recruitment of eosinophils to airway mucosa. Toxicol Appl Pharmacol 257: 309-318. http://dx.doi.org/10.1016/j.taap.2011.09.001.

Ciencewicki, JM; Verhein, KC; Gerrish, K; Mccaw, ZR; Li, J; Bushel, PR; Kleeberger, SR. (2016). Effects of mannose-binding lectin on pulmonary gene expression and innate immune inflammatory response to ozone. Am J Physiol Lung Cell Mol Physiol 311: L280-L291. http://dx.doi.org/10.1152/ajplung.00205.2015.

Clay, CC; Maniar-Hew, K; Gerriets, JE; Wang, TT; Postlethwait, EM; Evans, MJ; Fontaine, JH; Miller, LA. (2014). Early life ozone exposure results in dysregulated innate immune function and altered microRNA expression in airway epithelium. PLoS ONE 9: e90401. http://dx.doi.org/10.1371/journal.pone.0090401.

Clay, E; Patacchini, R; Trevisani, M; Preti, D; Brana, M; Spina, D; Page, C. (2016). Ozone-induced hypertussive responses in rabbits and guinea pigs. J Pharmacol Exp Ther 357: 73-83. http://dx.doi.org/10.1124/jpet.115.230227.

Connor, AJ; Laskin, JD; Laskin, DL. (2012). Ozone-induced lung injury and sterile inflammation. Role of toll-like receptor 4. Exp Mol Pathol 92: 229-235. http://dx.doi.org/10.1016/j.yexmp.2012.01.004.

Crouse, DL; Peters, PA; Hystad, P; Brook, JR; van Donkelaar, A; Martin, RV; Villeneuve, PJ; Jerrett, M; Goldberg, MS; Pope, CA; Brauer, M; Brook, RD; Robichaud, A; Menard, R; Burnett, RT. (2015). Ambient PM 2.5, O 3, and NO 2 exposures and associations with mortality over 16 years of follow-up in the Canadian Census Health and Environment Cohort (CanCHEC). Environ Health Perspect 123: 1180-1186. http://dx.doi.org/10.1289/ehp.1409276.

Crowley, CM; Fontaine, JH; Gerriets, JE; Schelegle, ES; Hyde, DM; Miller, LA. (2017). Early life allergen and air pollutant exposures alter longitudinal blood immune profiles in infant rhesus monkeys. Toxicol Appl Pharmacol 328: 60-69. http://dx.doi.org/10.1016/j.taap.2017.05.006.

Dales, R; Kauri, LM; Cakmak, S; Mahmud, M; Weichenthal, SA; Van Ryswyk, K; Kumarathasan, P; Thomson, E; Vincent, R; Broad, G; Liu, L. (2013). Acute changes in lung function associated with proximity to a steel plant: A randomized study. Environ Int 55: 15-19. http://dx.doi.org/10.1016/j.envint.2013.01.014.

Darrow, LA; Klein, M; Flanders, WD; Mulholland, JA; Tolbert, PE; Strickland, MJ. (2014). Air pollution and acute respiratory infections among children 0-4 years of age: an 18-year time-series study. Am J Epidemiol 180: 968-977. http://dx.doi.org/10.1093/aje/kwu234.

Darrow, LA; Klein, M; Sarnat, JA; Mulholland, JA; Strickland, MJ; Sarnat, SE; Russell, AG; Tolbert, PE. (2011). The use of alternative pollutant metrics in time-series studies of ambient air pollution and respiratory emergency department visits. J Expo Sci Environ Epidemiol 21: 10-19. http://dx.doi.org/10.1038/jes.2009.49.

Delfino, RJ; Staimer, N; Tjoa, T; Gillen, DL; Schauer, JJ; Shafer, MM. (2013). Airway inflammation and oxidative potential of air pollutant particles in a pediatric asthma panel. J Expo Sci Environ Epidemiol 23: 466-473. http://dx.doi.org/10.1038/jes.2013.25.

Devlin, RB; Duncan, KE; Jardim, M; Schmitt, MT; Rappold, AG; Diaz-Sanchez, D. (2012). Controlled exposure of healthy young volunteers to ozone causes cardiovascular effects. Circulation 126: 104-111. http://dx.doi.org/10.1161/CIRCULATIONAHA.112.094359.

Dokic, D; Trajkovska-Dokic, E. (2013). Ozone exaggerates nasal allergic inflammation. Prilozi 34: 131-141.

Durrani, F; Phelps, DS; Weisz, J; Silveyra, P; Hu, SM; Mikerov, AN; Floros, J. (2012). Gonadal hormones and oxidative stress interaction differentially affects survival of male and female mice after lung Klebsiella Pneumoniae infection. Exp Lung Res 38: 165-172. http://dx.doi.org/10.3109/01902148.2011.654045.

Dye, JA; Gibbs-Flournoy, EA; Richards, JH; Norwood, J; Kraft, K; Hatch, GE. (2017). Neonatal rat age, sex and strain modify acute antioxidant response to ozone. Inhal Toxicol 29: 291-303. http://dx.doi.org/10.1080/08958378.2017.1369602.

Dye, JA; Ledbetter, AD; Schladweiler, MC; Costa, DL; Kodavanti, UP. (2015). Whole body plethysmography reveals differential ventilatory responses to ozone in rat models of cardiovascular disease. Inhal Toxicol 27: 14-25. http://dx.doi.org/10.3109/08958378.2014.954167.

Eckel, SP; Louis, TA; Chaves, PH; Fried, LP; Margolis, AH. (2012). Modification of the association between ambient air pollution and lung function by frailty status among older adults in the cardiovascular health study. Am J Epidemiol 176: 214-223. http://dx.doi.org/10.1093/aje/kws001.

Elkhidir, HS; Richards, JB; Cromar, KR; Bell, CS; Price, RE; Atkins, CL; Spencer, CY; Malik, F; Alexander, AL; Cockerill, KJ; Haque, IU; Johnston, RA. (2016). Plasminogen activator inhibitor-1 does not contribute to the pulmonary pathology induced by acute exposure to ozone. 4. http://dx.doi.org/10.14814/phy2.12983.

Farraj, AK; Hazari, MS; Winsett, DW; Kulukulualani, A; Carll, AP; Haykal-Coates, N; Lamb, CM; Lappi, E; Terrell, D; Cascio, WE; Costa, DL. (2012). Overt and latent cardiac effects of ozone inhalation in rats: evidence for autonomic modulation and increased myocardial vulnerability. Environ Health Perspect 120: 348-354. http://dx.doi.org/10.1289/ehp.1104244.

Farraj, AK; Malik, F; Haykal-Coates, N; Walsh, L; Winsett, D; Terrell, D; Thompson, LC; Cascio, WE; Hazari, MS. (2016). Morning NO2 exposure sensitizes hypertensive rats to the cardiovascular effects of same day O3 exposure in the afternoon. Inhal Toxicol 28: 170-179. http://dx.doi.org/10.3109/08958378.2016.1148088.

Feng, F; Jin, Y; Duan, L; Yan, Z; Wang, S; Li, F; Liu, Y; Samet, JM; Wu, W. (2015). Regulation of ozone-induced lung inflammation by the epidermal growth factor receptor in mice. Environ Toxicol 31: 2016-2027. http://dx.doi.org/10.1002/tox.22202.

Folinsbee, LJ; Horstman, DH; Kehrl, HR; Harder, S; Abdul-Salaam, S; Ives, PJ. (1994). Respiratory responses to repeated prolonged exposure to 0.12 ppm ozone. Am J Respir Crit Care Med 149: 98-105. http://dx.doi.org/10.1164/ajrccm.149.1.8111607.

Folinsbee, LJ; Mcdonnell, WF; Horstman, DH. (1988). Pulmonary function and symptom responses after 6.6-hour exposure to 0.12 ppm ozone with moderate exercise. J Air Waste Manag Assoc 38: 28-35. http://dx.doi.org/10.1080/08940630.1988.10466349.

Forbes, LJL; Kapetanakis, V; Rudnicka, AR; Cook, DG; Bush, T; Stedman, JR; Whincup, PH; Strachan, DP; Anderson, HR. (2009). Chronic exposure to outdoor air pollution and lung function in adults. Thorax 64: 657-663. http://dx.doi.org/10.1136/thx.2008.109389.

Foster, WM; Brown, RH; Macri, K; Mitchell, CS. (2000). Bronchial reactivity of healthy subjects: 18-20 h postexposure to ozone. J Appl Physiol (1985) 89: 1804-1810.

Foster, WM; Weinmann, GG; Menkes, E; Macri, K. (1997). Acute exposure of humans to ozone impairs small airway function. Ann Occup Hyg 1: 659-666. http://dx.doi.org/10.1093/annhyg/41.inhaled_particles_VIII.659.

Frampton, MW; Balmes, JR; Bromberg, PA; Stark, P; Arjomandi, M; Hazucha, MJ; Rich, DQ; Hollenbeck-Pringle, D; Dagincourt, N; Alexis, N; Ganz, P; Zareba, W; Costantini, MG. (2017). Multicenter Ozone Study in oldEr Subjects (MOSES: Part 1. Effects of exposure to low concentrations of ozone on respiratory and cardiovascular outcomes [HEI]. (Research Report 192, Pt 1). Boston, MA: Health Effects Institute.

Frampton, MW; Pietropaoli, A; Dentler, M; Chalupa, D; Little, EL; Stewart, J; Frasier, L; Oakes, D; Wiltshire, J; Vora, R; Utell, MJ. (2015). Cardiovascular effects of ozone in healthy subjects with and without deletion of glutathione-S-transferase M1. Inhal Toxicol 27: 113-119. http://dx.doi.org/10.3109/08958378.2014.996272.

Francis, M; Groves, AM; Sun, R; Cervelli, JA; Choi, H; Laskin, JD; Laskin, DL. (2017a). Editor's highlight: CCR2 regulates inflammatory cell accumulation in the lung and tissue injury following ozone exposure. Toxicol Sci 155: 474-484. http://dx.doi.org/10.1093/toxsci/kfw226.

Francis, M; Sun, R; Cervelli, JA; Choi, H; Mandal, M; Abramova, EV; Gow, AJ; Laskin, JD; Laskin, DL. (2017b). Editor's highlight: role of spleen-derived macrophages in ozone-induced lung inflammation and injury. Toxicol Sci 155: 182-195. http://dx.doi.org/10.1093/toxsci/kfw192.

Freijer, JI; Van Eijkeren, JCH; Van Bree, L. (2002). A model for the effect on health of repeated exposure to ozone. Environ Modell Softw 17: 553-562.

Fry, RC; Rager, JE; Bauer, R; Sebastian, E; Peden, DB; Jaspers, I; Alexis, NE. (2014). Air toxics and epigenetic effects: ozone altered microRNAs in the sputum of human subjects. Am J Physiol Lung Cell Mol Physiol 306: L1129-L1137. http://dx.doi.org/10.1152/ajplung.00348.2013.

Fry, RC; Rager, JE; Zhou, H; Zou, B; Brickey, JW; Ting, J; Lay, JC; Peden, DB; Alexis, NE. (2012). Individuals with increased inflammatory response to ozone demonstrate muted signaling of immune cell trafficking pathways. Respir Res 13: 89. http://dx.doi.org/10.1186/1465-9921-13-89.

Gabehart, K; Correll, KA; Loader, JE; White, CW; Dakhama, A. (2015). The lung response to ozone is determined by age and is partially dependent on toll-Like receptor 4. Respir Res 16: 117. http://dx.doi.org/10.1186/s12931-015-0279-2.

Gabehart, K; Correll, KA; Yang, J; Collins, ML; Loader, JE; Leach, S; White, CW; Dakhama, A. (2014). Transcriptome profiling of the newborn mouse lung response to acute ozone exposure. Toxicol Sci 138: 175-190. http://dx.doi.org/10.1093/toxsci/kft276.

Garcia, E; Berhane, KT; Islam, T; Mcconnell, R; Urman, R; Chen, Z; Gilliland, FD. (2019). Association of changes in air quality with incident asthma in children in California, 1993-2014. JAMA 321: 1906-1915. http://dx.doi.org/10.1001/jama.2019.5357.

Gauderman, WJ; Avol, E; Gilliland, F; Vora, H; Thomas, D; Berhane, K; McConnell, R; Kuenzli, N; Lurmann, F; Rappaport, E; Margolis, H; Bates, D; Peters, J. (2004). The effect of air pollution on lung development from 10 to 18 years of age. N Engl J Med 351: 1057-1067. http://dx.doi.org/10.1056/NEJMoa040610.

Gauderman, WJ; Urman, R; Avol, E; Berhane, K; McConnell, R; Rappaport, E; Chang, R; Lurmann, F; Gilliland, F. (2015). Association of improved air quality with lung development in children. N Engl J Med 372: 905-913. http://dx.doi.org/10.1056/NEJMoa1414123.

Ghio, AJ; Soukup, JM; Dailey, LA; Richards, JH; Duncan, KE; Lehmann, J. (2014). Iron decreases biological effects of ozone exposure. Inhal Toxicol 26: 391-399. http://dx.doi.org/10.3109/08958378.2014.908330.

Gielen, MH; Van Der Zee, SC; Van Wijnen, JH; Van Steen, CJ; Brunekreef, B. (1997). Acute effects of summer air pollution on respiratory health of asthmatic children. Am J Respir Crit Care Med 155: 2105-2108. http://dx.doi.org/10.1164/ajrccm.155.6.9196122.

Gilliland, F; Avol, E; McConnell, R; K, B; Gauderman, WJ; Lurmann, FW; Urnam, R; Change, R; Rappaport, EB; Howland, S. (2017). The effects of policy-driven air quality improvements on childrens respiratory health [HEI]. (Research Report 190). Boston, MA: Health Effects Institute. https://www.healtheffects.org/system/files/GillilandRR190.pdf.

Gleason, JA; Bielory, L; Fagliano, JA. (2014). Associations between ozone, PM2.5, and four pollen types on emergency department pediatric asthma events during the warm season in New Jersey: a case-crossover study. Environ Res 132: 421-429. http://dx.doi.org/10.1016/j.envres.2014.03.035.

Gomes, EC; Allgrove, JE; Florida-James, G; Stone, V. (2011a). Effect of vitamin supplementation on lung injury and running performance in a hot, humid, and ozone-polluted environment. Scand J Med Sci Sports 21: e452-e460. http://dx.doi.org/10.1111/j.1600-0838.2011.01366.x.

Gomes, EC; Stone, V; Florida-James, G. (2011b). Impact of heat and pollution on oxidative stress and CC16 secretion after 8km run. Eur J Appl Physiol 111: 2089-2097. http://dx.doi.org/10.1007/s00421-011-1839-x.

Gonzalez-Guevara, E; Carlos Martinez-Lazcano, J; Custodio, V; Hernandez-Ceron, M; Rubio, C; Paz, C. (2014). Exposure to ozone induces a systemic inflammatory response: possible source of the neurological alterations induced by this gas. Inhal Toxicol 26: 485-491. http://dx.doi.org/10.3109/08958378.2014.922648.

Goodman, JE; Loftus, CT; Liu, X; Zu, K. (2017a). Impact of respiratory infections, outdoor pollen, and socioeconomic status on associations between air pollutants and pediatric asthma hospital admissions. PLoS ONE 12: e0180522. http://dx.doi.org/10.1371/journal.pone.0180522.

Goodman, JE; Zu, K; Loftus, CT; Tao, G; Liu, X; Lange, S. (2017b). Ambient ozone and asthma hospital admissions in Texas: a time-series analysis. 3: 6. http://dx.doi.org/10.1186/s40733-017-0034-1.

Gordon, CJ; Phillips, PM; Beasley, TE; Ledbetter, A; Aydin, C; Snow, SJ; Kodavanti, UP; Johnstone, AF. (2016a). Pulmonary sensitivity to ozone exposure in sedentary versus chronically trained, female rats. Inhal Toxicol 28: 293-302. http://dx.doi.org/10.3109/08958378.2016.1163441.

Gordon, CJ; Phillips, PM; Johnstone, AFM; Beasley, TE; Ledbetter, AD; Schladweiler, MC; Snow, SJ; Kodavanti, UP. (2016b). Effect of high-fructose and high-fat diets on pulmonary sensitivity, motor activity, and body composition of brown Norway rats exposed to ozone. Inhal Toxicol 28: 203-215. http://dx.doi.org/10.3109/08958378.2015.1134730.

Gordon, CJ; Phillips, PM; Johnstone, AFM; Schmid, J; Schladweiler, MC; Ledbetter, A; Snow, SJ; Kodavanti, UP. (2017a). Effects of maternal high-fat diet and sedentary lifestyle on susceptibility of adult offspring to ozone exposure in rats. Inhal Toxicol 29: 239-254. http://dx.doi.org/10.1080/08958378.2017.1342719.

Gordon, CJ; Phillips, PM; Ledbetter, A; Snow, SJ; Schladweiler, MC; Johnstone, AF; Kodavanti, UP. (2017b). Active vs. sedentary lifestyle from weaning to adulthood and susceptibility to ozone in rats. Am J Physiol Lung Cell Mol Physiol 312: L100-L109. http://dx.doi.org/10.1152/ajplung.00415.2016.

Greer, JR; Abbey, DE; Burchette, RJ. (1993). Asthma related to occupational and ambient air pollutants in nonsmokers. J Occup Environ Med 35: 909-915. http://dx.doi.org/10.1097/00043764-199309000-00014.

Groves, AM; Gow, AJ; Massa, CB; Hall, L; Laskin, JD; Laskin, DL. (2013). Age-related increases in ozone-induced injury and altered pulmonary mechanics in mice with progressive lung inflammation. Am J Physiol Lung Cell Mol Physiol 305: L555-L568. http://dx.doi.org/10.1152/ajplung.00027.2013.

Groves, AM; Gow, AJ; Massa, CB; Laskin, JD; Laskin, DL. (2012). Prolonged injury and altered lung function after ozone inhalation in mice with chronic lung inflammation. Am J Respir Cell Mol Biol 47: 776-783. http://dx.doi.org/10.1165/rcmb.2011-0433OC.

Hansen, JS; Nielsen, GD; Sorli, JB; Clausen, P; Wolkoff, P; Larsen, ST. (2013). Adjuvant and inflammatory effects in mice after subchronic inhalation of allergen and ozone-initiated limonene reaction products. J Toxicol Environ Health A 76: 1085-1095. http://dx.doi.org/10.1080/15287394.2013.838915.

Hansen, JS; Nørgaard, AW; Koponen, IK; Sørli, JB; Paidi, MD; Hansen, SW; Clausen, PA; Nielsen, GD; Wolkoff, P; Larsen, ST. (2016). Limonene and its ozone-initiated reaction products attenuate allergic lung inflammation in mice. J Immunotoxicol 13: 793-803. http://dx.doi.org/10.1080/1547691X.2016.1195462.

Harkema, JR; Hotchkiss, LA; Vetter, NA; Jackson-Humbles, DN; Lewandowski, RP; Wagner, JG. (2017). Strain differences in a murine model of air pollutant-induced nonatopic asthma and rhinitis. Toxicol Pathol 45: 161-171. http://dx.doi.org/10.1177/0192623316674274.

Hatch, GE; Crissman, K; Schmid, J; Richards, JE; Ward, WO; Schladweiler, MC; Ledbetter, AD; Kodavanti, UP. (2015). Strain differences in antioxidants in rat models of cardiovascular disease exposed to ozone. Inhal Toxicol 27: 54-62. http://dx.doi.org/10.3109/08958378.2014.954170.

Hatch, GE; Slade, R; Harris, LP; Mcdonnell, WF; Devlin, RB; Koren, HS; Costa, DL; Mckee, J. (1994). Ozone dose and effect in humans and rats: A comparison using oxygen-18 labeling and bronchoalveolar lavage. Am J Respir Crit Care Med 150: 676-683. http://dx.doi.org/10.1164/ajrccm.150.3.8087337.

Hazucha, MJ; Folinsbee, LJ; Bromberg, PA. (2003). Distribution and reproducibility of spirometric response to ozone by gender and age. J Appl Physiol (1985) 95: 1917-1925. http://dx.doi.org/10.1152/japplphysiol.00490.2003.

Hebbern, C; Cakmak, S. (2015). Synoptic weather types and aeroallergens modify the effect of air pollution on hospitalisations for asthma hospitalisations in Canadian cities. Environ Pollut 204: 9-16. http://dx.doi.org/10.1016/j.envpol.2015.04.010.

Heckbert, SR; Kooperberg, C; Safford, MM; Psaty, BM; Hsia, J; McTiernan, A; Gaziano, JM; Frishman, WH; Curb, JD. (2004). Comparison of self-report, hospital discharge codes, and adjudication of cardiovascular events in the Women's Health Initiative. Am J Epidemiol 160: 1152-1158. http://dx.doi.org/10.1093/aje/kwh314.

HEI (Health Effects Institute). (1997). Effects of ozone on normal and potentially sensitive human subjects.

Henriquez, AR; Snow, SJ; Schladweiler, MC; Miller, CN; Dye, JA; Ledbetter, AD; Richards, JE; Mauge-Lewis, K; Mcgee, MA; Kodavanti, UP. (2017). Adrenergic and glucocorticoid receptor antagonists reduce ozone-induced lung injury and inflammation. Toxicol Appl Pharmacol 339: 161-171. http://dx.doi.org/10.1016/j.taap.2017.12.006.

Hernandez, M; Brickey, WJ; Alexis, NE; Fry, RC; Rager, JE; Zhou, B; Ting, JP; Zhou, H; Peden, DB. (2012). Airway cells from atopic asthmatic patients exposed to ozone display an enhanced innate immune gene profile [Letter]. J Allergy Clin Immunol 129: 259-261.e251-252. http://dx.doi.org/10.1016/j.jaci.2011.11.007.

Hernandez, ML; Lay, JC; Harris, B; Esther, CR; Brickey, WJ; Bromberg, PA; Diaz-Sanchez, D; Devlin, RB; Kleeberger, SR; Alexis, NE; Peden, DB. (2010). Atopic asthmatic subjects but not atopic subjects without asthma have enhanced inflammatory response to ozone. J Allergy Clin Immunol 126: 537-544. http://dx.doi.org/10.1016/j.jaci.2010.06.043.

Herring, MJ; Putney, LF; St George, JA; Avdalovic, MV; Schelegle, ES; Miller, LA; Hyde, DM. (2015). Early life exposure to allergen and ozone results in altered development in adolescent rhesus macaque lungs. Toxicol Appl Pharmacol 283: 35-41. http://dx.doi.org/10.1016/j.taap.2014.12.006.

Higgins, ITT; D'Arcy, JB; Gibbons, DI; Avol, EL; Gross, KB. (1990). Effect of exposures to ambient ozone on ventilatory lung function in children. Am J Respir Crit Care Med 141: 1136-1146. http://dx.doi.org/10.1164/ajrccm/141.5_Pt_1.1136.

Hoffmeyer, F; Sucker, K; Monsé, C; Berresheim, H; Jettkant, B; Rosenkranz, N; Brüning, T; Bünger, J. (2015). Different patterns in changes of exhaled breath condensate pH and exhaled nitric oxide after ozone exposure. In M Pokorski (Ed.), Environment Exposure to Pollutants (pp. 39-47). Switzerland: Springer International Publishing.

Hoffmeyer, F; Sucker, K; Monsé, C; Berresheim, H; Rosenkranz, N; Jettkant, B; Beine, A; Brüning, T; Bünger, J. (2013). Relationship of pulmonary function response to ozone exposure and capsaicin cough sensitivity. Inhal Toxicol 25: 569-576. http://dx.doi.org/10.3109/08958378.2013.812699.

Holland, N; Davé, V; Venkat, S; Wong, H; Donde, A; Balmes, JR; Arjomandi, M. (2014). Ozone inhalation leads to a dose-dependent increase of cytogenetic damage in human lymphocytes. Environ Mol Mutagen 56: 378-387. http://dx.doi.org/10.1002/em.21921.

Holz, O; Biller, H; Mueller, M; Kane, K; Rosano, M; Hanrahan, J; Hava, DL; Hohlfeld, JM. (2015). Efficacy and safety of inhaled calcium lactate PUR118 in the ozone challenge model - a clinical trial. 16: 21. http://dx.doi.org/10.1186/s40360-015-0021-1.

Holze, C; Michaudel, C; Mackowiak, C; Haas, DA; Benda, C; Hubel, P; Pennemann, FL; Schnepf, D; Wettmarshausen, J; Braun, M; Leung, DW; Amarasinghe, GK; Perocchi, F; Staeheli, P; Ryffel, B; Pichlmair, A. (2018). Oxeiptosis, a ROS-induced caspase-independent apoptosis-like cell-death pathway. Nat Immunol 19: 130-140. http://dx.doi.org/10.1038/s41590-017-0013-y.

Horstman, DH; Ball, BA; Brown, J; Gerrity, T; Folinsbee, LJ. (1995). Comparison of pulmonary responses of asthmatic and nonasthmatic subjects performing light exercise while exposed to a low level of ozone. Toxicol Ind Health 11: 369-385. http://dx.doi.org/10.1177/074823379501100401.

Horstman, DH; Folinsbee, LJ; Ives, PJ; Abdul-Salaam, S; Mcdonnell, WF. (1990). Ozone concentration and pulmonary response relationships for 6.6-hour exposures with five hours of moderate exercise to 0.08, 0.10, and 0.12 ppm. Am J Respir Crit Care Med 142: 1158-1163. http://dx.doi.org/10.1164/ajrccm/142.5.1158.

Hsieh, N; Cheng, Y, iH; Liao, C. (2014). Changing variance and skewness as leading indicators for detecting ozone exposure-associated lung function decrement. Stoch Environ Res Risk Assess 28: 2205-2216. http://dx.doi.org/10.1007/s00477-014-0887-2.

Hulo, S; Tiesset, H; Lancel, S; Edmé, JL; Viollet, B; Sobaszek, A; Nevière, R. (2011). AMP-activated protein kinase deficiency reduces ozone-induced lung injury and oxidative stress in mice. Respir Res 12: 64. http://dx.doi.org/10.1186/1465-9921-12-64.

Hunter, DD; Carrell-Jacks, LA; Batchelor, TP; Dey, RD. (2011). Role of nerve growth factor in ozone-induced neural responses in early postnatal airway development. Am J Respir Cell Mol Biol 45: 359-365. http://dx.doi.org/10.1165/rcmb.2010-0345OC.

Hwang, BF; Jaakkola, JJK; Lee, YL; Lin, YC; Guo, YLL. (2006). Relation between air pollution and allergic rhinitis in Taiwananese schoolchildren.

Islam, T; Mcconnell, R; Gauderman, WJ; Avol, E; Peters, JM; Gilliland, FD. (2008). Ozone, oxidant defense genes and risk of asthma during adolescence. Am J Respir Crit Care Med 177: 388-395. http://dx.doi.org/10.1164/rccm.200706-863OC.

Ito, K; Thurston, GD; Silverman, RA. (2007). Characterization of PM2.5, gaseous pollutants, and meteorological interactions in the context of time-series health effects models. J Expo Sci Environ Epidemiol 17: S45-S60. http://dx.doi.org/10.1038/sj.jes.7500627.

Jerrett, M; Burnett, RT; Beckerman, BS; Turner, MC; Krewski, D; Thurston, G; Martin, RV; van Donkelaar, A; Hughes, E; Shi, Y; Gapstur, SM; Thun, MJ; Pope, CA, III. (2013). Spatial analysis of air pollution and mortality in California. Am J Respir Crit Care Med 188: 593-599. http://dx.doi.org/10.1164/rccm.201303-0609OC.

Jerrett, M; Burnett, RT; Pope, CA, III; Ito, K; Thurston, G; Krewski, D; Shi, Y; Calle, E; Thun, M. (2009). Long-term ozone exposure and mortality. N Engl J Med 360: 1085-1095. http://dx.doi.org/10.1056/NEJMoa0803894.

Jiu-Chiuan, C; Schwartz, J. (2008). Metabolic syndrome and inflammatory responses to long-term particulate air pollutants. Environ Health Perspect 116: 612-617.

Jones, SL; Kittelson, J; Cowan, JO; Flannery, EM; Hancox, RJ; McLachlan, CR; Taylor, DR. (2001). The predictive value of exhaled nitric oxide measurements in assessing changes in asthma control. Am J Respir Crit Care Med 164: 738-743. http://dx.doi.org/10.1164/ajrccm.164.5.2012125.

Just, J; Segala, C; Sahraoui, F; Priol, G; Grimfeld, A; Neukirch, F. (2002). Short-term health effects of particulate and photochemical air pollution in asthmatic children. Eur Respir J 20: 899-906. http://dx.doi.org/10.1183/09031936.02.00236902.

Kahle, JJ; Neas, LM; Devlin, RB; Case, MW; Schmitt, MT; Madden, MC; Diaz-Sanchez, D. (2015). Interaction effects of temperature and ozone on lung function and markers of systemic inflammation, coagulation, and fibrinolysis: a crossover study of healthy young volunteers. Environ Health Perspect 123: 310-316. http://dx.doi.org/10.1289/ehp.1307986.

Kasahara, DI; Kim, H; Mathews, JA; Verbout, NG; Williams, AS; Wurmbrand, AP; Ninin, FMC; Neto, FL; Benedito, LAP; Hug, C; Umetsu, DT; Shore, SA. (2014). Pivotal role of IL-6 in the hyperinflammatory responses to subacute ozone in adiponectin-deficient mice. Am J Physiol Lung Cell Mol Physiol 306: L508-L520. http://dx.doi.org/10.1152/ajplung.00235.2013.

Kasahara, DI; Kim, HY; Williams, AS; Verbout, NG; Tran, J; Si, H; Wurmbrand, AP; Jastrab, J; Hug, C; Umetsu, DT; Shore, SA. (2012). Pulmonary inflammation induced by subacute ozone is augmented in adiponectin-deficient mice: role of IL-17A. J Immunol 188: 4558-4567. http://dx.doi.org/10.4049/jimmunol.1102363.

Kasahara, DI; Mathews, JA; Park, CY; Cho, Y; Hunt, G; Wurmbrand, AP; Liao, JK; Shore, SA. (2015). ROCK insufficiency attenuates ozone-induced airway hyperresponsiveness in mice. Am J Physiol Lung Cell Mol Physiol 309: L736-L746. http://dx.doi.org/10.1152/ajplung.00372.2014.

Kasahara, DI; Williams, AS; Benedito, LA; Ranscht, B; Kobzik, L; Hug, C; Shore, SA. (2013). Role of the adiponectin binding protein, T-cadherin (cdh13), in pulmonary responses to subacute ozone. PLoS ONE 8: e65829. http://dx.doi.org/10.1371/journal.pone.0065829.

Katsouyanni, K; Samet, JM; Anderson, HR; Atkinson, R; Le Tertre, A; Medina, S; Samoli, E; Touloumi, G; Burnett, RT; Krewski, D; Ramsay, T; Dominici, F; Peng, RD; Schwartz, J; Zanobetti, A. (2009). Air pollution and health: A European and North American approach (APHENA) (pp. 5-90). (Research Report 142). Boston, MA: Health Effects Institute. http://pubs.healtheffects.org/view.php?id=327.

Kharitonov, SA; Barnes, PJ. (2000). Clinical aspects of exhaled nitric oxide [Review]. Eur Respir J 16: 781-792.

Kilkenny, C; Browne, WJ; Cuthill, IC; Emerson, M; Altman, DG. (2010). Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research [Review]. PLoS Biol 8: e1000412. http://dx.doi.org/10.1371/journal.pbio.1000412.

Kim, CS; Alexis, NE; Rappold, AG; Kehrl, H; Hazucha, MJ; Lay, JC; Schmitt, MT; Case, M; Devlin, RB; Peden, DB; Diaz-Sanchez, D. (2011). Lung function and inflammatory responses in healthy young adults exposed to 0.06 ppm ozone for 6.6 hours. Am J Respir Crit Care Med 183: 1215-1221. http://dx.doi.org/10.1164/rccm.201011-1813OC.

Kirsten, A; Watz, H; Kretschmar, G; Pedersen, F; Bock, D; Meyer-Sabellek, W; Magnussen, H. (2011). Efficacy of the pan-selectin antagonist Bimosiamose on ozone-induced airway inflammation in healthy subjects - A double blind, randomized, placebo-controlled, cross-over clinical trial. Pulm Pharmacol Ther 24: 555-558. http://dx.doi.org/10.1016/j.pupt.2011.04.029.

Klemm, RJ; Lipfert, FW; Wyzga, RE; Gust, C. (2004). Daily mortality and air pollution in Atlanta: two years of data from ARIES. Inhal Toxicol 16 Suppl 1: 131-141. http://dx.doi.org/10.1080/08958370490443213.

Klemm, RJ; Mason, RM, Jr. (2000). Aerosol Research and Inhalation Epidemiological Study (ARIES): air quality and daily mortality statistical modeling--interim results. J Air Waste Manag Assoc 50: 1433-1439.

Klemm, RJ; Thomas, EL; Wyzga, RE. (2011). The impact of frequency and duration of air quality monitoring: Atlanta, GA, data modeling of air pollution and mortality. J Air Waste Manag Assoc 61: 1281-1291. http://dx.doi.org/10.1080/10473289.2011.617648.

Klimisch, HJ; Andreae, M; Tillmann, U. (1997). A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regul Toxicol Pharmacol 25: 1-5. http://dx.doi.org/10.1006/rtph.1996.1076.

Kodavanti, UP; Ledbetter, AD; Thomas, RF; Richards, JE; Ward, WO; Schladweiler, MC; Costa, DL. (2015). Variability in ozone-induced pulmonary injury and inflammation in healthy and cardiovascular-compromised rat models. Inhal Toxicol 27: 39-53. http://dx.doi.org/10.3109/08958378.2014.954169.

Kousha, T; Castner, J. (2016). The air quality health index and emergency department visits for otitis media. J Nurs Scholarsh 48: 163-171. http://dx.doi.org/10.1111/jnu.12195.

Kousha, T; Rowe, BH. (2014). Ambient ozone and emergency department visits due to lower respiratory condition. Int J Occup Med Environ Health 27: 50-59. http://dx.doi.org/10.2478/s13382-014-0229-0.

Kreit, JW; Gross, KB; Moore, TB; Lorenzen, TJ; D'Arcy, J; Eschenbacher, WL. (1989). Ozone-induced changes in pulmonary function and bronchial responsiveness in asthmatics. J Appl Physiol (1985) 66: 217-222.

Kumagai, K; Lewandowski, R; Jackson-Humbles, DN; Li, N; Van Dyken, SJ; Wagner, JG; Harkema, JR. (2016). Ozone-induced nasal type 2 immunity in mice is dependent on innate lymphoid cells. Am J Respir Cell Mol Biol 54: 782-791. http://dx.doi.org/10.1165/rcmb.2015-0118OC.

Kumagai, K; Lewandowski, RP; Jackson-Humbles, DN; Buglak, N; Li, N; White, K; Van Dyken, SJ; Wagner, JG; Harkema, JR. (2017). Innate lymphoid cells mediate pulmonary eosinophilic inflammation, airway mucous cell metaplasia, and type 2 immunity in mice exposed to ozone. Toxicol Pathol 45: 692-704. http://dx.doi.org/10.1177/0192623317728135.

Kumarathasan, P; Blais, E; Saravanamuthu, A; Bielecki, A; Mukherjee, B; Bjarnason, S; Guénette, J; Goegan, P; Vincent, R. (2015). Nitrative stress, oxidative stress and plasma endothelin levels after inhalation of particulate matter and ozone. Part Fibre Toxicol 12: 28. http://dx.doi.org/10.1186/s12989-015-0103-7.

Kummarapurugu, AB; Fischer, BM; Zheng, S; Milne, GL; Ghio, AJ; Potts-Kant, EN; Foster, WM; Soderblom, EJ; Dubois, LG; Moseley, MA; Thompson, JW; Voynow, JA. (2013). NADPH: quinone oxidoreductase 1 regulates host susceptibility to ozone via isoprostane generation. J Biol Chem 288: 4681-4691. http://dx.doi.org/10.1074/jbc.M112.438440.

Kurhanewicz, N; Mcintosh-Kastrinsky, R; Tong, H; Walsh, L; Farraj, A; Hazari, MS. (2014). Ozone co-exposure modifies cardiac responses to fine and ultrafine ambient particulate matter in mice: Concordance of electrocardiogram and mechanical responses. Part Fibre Toxicol 11: 54. http://dx.doi.org/10.1186/s12989-014-0054-4.

Lazaar, AL; Sweeney, LE; Macdonald, AJ; Alexis, NE; Chen, C; Tal-Singer, R. (2011). SB-656933, a novel CXCR2 selective antagonist, inhibits ex-vivo neutrophil activation and ozone-induced airway inflammation in humans. Br J Clin Pharmacol 72: 282-293. http://dx.doi.org/10.1111/j.1365-2125.2011.03968.x.

Lee, D; Wallis, C; Van Winkle, LS; Wexler, AS. (2011). Disruption of tracheobronchial airway growth following postnatal exposure to ozone and ultrafine particles. Inhal Toxicol 23: 520-531. http://dx.doi.org/10.3109/08958378.2011.591447.

Lee, MS; Moon, KY; Bae, DJ; Park, MK; Jang, AS. (2013). The effects of pycnogenol on antioxidant enzymes in a mouse model of ozone exposure. 28: 216-223. http://dx.doi.org/10.3904/kjim.2013.28.2.216.

Lepeule, J; Bind, MA; Baccarelli, AA; Koutrakis, P; Tarantini, L; Litonjua, A; Sparrow, D; Vokonas, P; Schwartz, JD. (2014). Epigenetic influences on associations between air pollutants and lung function in elderly men: the normative aging study. Environ Health Perspect 122: 566-572. http://dx.doi.org/10.1289/ehp.1206458.

Leroy, P; Tham, A; Wong, H; Tenney, R; Chen, C; Stiner, R; Balmes, JR; Paquet, AC; Arjomandi, M. (2015). Inflammatory and repair pathways induced in human bronchoalveolar lavage cells with ozone inhalation. PLoS ONE 10: e0127283. http://dx.doi.org/10.1371/journal.pone.0127283.

Lewis, TC; Robins, TG; Mentz, GB; Zhang, X; Mukherjee, B; Lin, X; Keeler, GJ; Dvonch, JT; Yip, FY; O'Neill, MS; Parker, EA; Israel, BA; Max, PT; Reyes, A; Committee, CAAACS. (2013). Air pollution and respiratory symptoms among children with asthma: Vulnerability by corticosteroid use and residence area. Sci Total Environ 448: 48-55. http://dx.doi.org/10.1016/j.scitotenv.2012.11.070.

Liu, J; Mao, F; Mao, Z; Wang, F; Zhu, Y; Wang, H. (2016). Emodin inhibited ozone stress-induced airway hyperresponsiveness by regulating epithelial protection mechanisms against injury. International Journal of Clinical and Experimental Medicine 9: 17404-17411.

MacIntyre, EA; Karr, CJ; Koehoorn, M; Demers, PA; Tamburic, L; Lencar, C; Brauer, M. (2011). Residential air pollution and otitis media during the first two years of life. Epidemiology 22: 81-89. http://dx.doi.org/10.1097/EDE.0b013e3181fdb60f.

Madden, MC; Stevens, T; Case, M; Schmitt, M; Diaz-Sanchez, D; Bassett, M; Montilla, TS; Berntsen, J; Devlin, RB. (2014). Diesel exhaust modulates ozone-induced lung function decrements in healthy human volunteers. Part Fibre Toxicol 11: 37. http://dx.doi.org/10.1186/s12989-014-0037-5.

Magzamen, S; Oron, AP; Locke, ER; Fan, VS. (2018). Association of ambient pollution with inhaler use among patients with COPD: a panel study. Occup Environ Med. http://dx.doi.org/10.1136/oemed-2017-104808.

Malig, BJ; Pearson, DL; Chang, Y; Broadwin, R; Basu, R; Green, RS; Ostro, B. (2016). A time-stratified case-crossover study of ambient ozone exposure and emergency department visits for specific respiratory diagnoses in California (2005-2008). Environ Health Perspect 124: 745-753. http://dx.doi.org/10.1289/ehp.1409495.

Malik, F; Cromar, KR; Atkins, CL; Price, RE; Jackson, WT; Siddiqui, SR; Spencer, CY; Mitchell, NC; Haque, IU; Johnston, RA. (2017). Chemokine (C-C Motif) Receptor-Like 2 is not essential for lung injury, lung inflammation, or airway hyperresponsiveness induced by acute exposure to ozone. Physiol Rep 5: e13545. http://dx.doi.org/10.14814/phy2.13545.

Mathews, JA; Kasahara, DI; Cho, Y; Bell, LN; Gunst, PR; Karoly, ED; Shore, SA. (2017a). Effect of acute ozone exposure on the lung metabolomes of obese and lean mice. PLoS ONE 12: e0181017. http://dx.doi.org/10.1371/journal.pone.0181017.

Mathews, JA; Kasahara, DI; Ribeiro, L; Wurmbrand, AP; Ninin, FMC; Shore, SA. (2015). gamma delta T cells are required for M2 macrophage polarization and resolution of ozone-induced pulmonary inflammation in mice. PLoS ONE 10: e0131236. http://dx.doi.org/10.1371/journal.pone.0131236.

Mathews, JA; Krishnamoorthy, N; Kasahara, DI; Cho, Y; Wurmbrand, AP; Ribeiro, L; Smith, D; Umetsu, D; Levy, BD; Shore, SA. (2017b). IL-33 drives augmented responses to ozone in obese mice. Environ Health Perspect 125: 246-253. http://dx.doi.org/10.1289/EHP272.

Mathews, JA; Krishnamoorthy, N; Kasahara, DI; Hutchinson, J; Cho, Y; Brand, JD; Williams, AS; Wurmbrand, AP; Ribeiro, L; Cuttitta, F; Sunday, ME; Levy, BD; Shore, SA. (2018). Augmented responses to ozone in obese mice require IL-17A and gastrin-releasing peptide. Am J Respir Cell Mol Biol 58: 341-351. http://dx.doi.org/10.1165/rcmb.2017-0071OC.

McConnell, R; Berhane, K; Gilliland, F; London, SJ; Islam, T; Gauderman, WJ; Avol, E; Margolis, HG; Peters, JM. (2002). Asthma in exercising children exposed to ozone: A cohort study. Lancet 359: 386-391. http://dx.doi.org/10.1016/S0140-6736(02)07597-9.

McConnell, R; Berhane, K; Gilliland, F; Molitor, J; Thomas, D; Lurmann, F; Avol, E; Gauderman, WJ; Peters, JM. (2003). Prospective study of air pollution and bronchitic symptoms in children with asthma. Am J Respir Crit Care Med 168: 790-797. http://dx.doi.org/10.1164/rccm.200304-466OC.

McDonnell, WF; Abbey, DE; Nishino, N; Lebowitz, MD. (1999a). Long-term ambient ozone concentration and the incidence of asthma in nonsmoking adults: the Ahsmog study. Environ Res 80: 110-121. http://dx.doi.org/10.1006/enrs.1998.3894.

McDonnell, WF; Kehrl, HR; Abdul-Salaam, S; Ives, PJ; Folinsbee, LJ; Devlin, RB; O'Neil, JJ; Horstman, DH. (1991). Respiratory response of humans exposed to low levels of ozone for 6.6 hours. Arch Environ Occup Health 46: 145-150. http://dx.doi.org/10.1080/00039896.1991.9937441.

McDonnell, WF; Stewart, PW; Smith, MV. (2010). Prediction of ozone-induced lung function responses in humans. Inhal Toxicol 22: 160-168. http://dx.doi.org/10.3109/08958370903089557.

McDonnell, WF; Stewart, PW; Smith, MV. (2013). Ozone exposure-response model for lung function changes: An alternate variability structure. Inhal Toxicol 25: 348-353. http://dx.doi.org/10.3109/08958378.2013.790523.

McDonnell, WF; Stewart, PW; Smith, MV; Kim, CS; Schelegle, ES. (2012). Prediction of lung function response for populations exposed to a wide range of ozone conditions. Inhal Toxicol 24: 619-633. http://dx.doi.org/10.3109/08958378.2012.705919.

McDonnell, WF; Stewart, PW; Smith, MV; Pan, WK; Pan, J. (1999b). Ozone-induced respiratory symptoms: Exposure-response models and association with lung function. Eur Respir J 14: 845-853. http://dx.doi.org/10.1034/j.1399-3003.1999.14d21.x.

McIntosh-Kastrinsky, R; Diaz-Sanchez, D; Sexton, KG; Jania, CM; Zavala, J; Tilley, SL; Jaspers, I; Gilmour, MI; Devlin, RB; Cascio, WE; Tong, H. (2013). Photochemically altered air pollution mixtures and contractile parameters in isolated murine hearts before and after ischemia. Environ Health Perspect 121: 1344-1348. http://dx.doi.org/10.1289/ehp.1306609.

Medina-Ramon, M; Zanobetti, A; Schwartz, J. (2006). The effect of ozone and PM10 on hospital admissions for pneumonia and chronic obstructive pulmonary disease: A national multicity study. Am J Epidemiol 163: 579-588. http://dx.doi.org/10.1093/aje/kwj078.

Meng, YY; Rull, RP; Wilhelm, M; Lombardi, C; Balmes, J; Ritz, B. (2010). Outdoor air pollution and uncontrolled asthma in the San Joaquin Valley, California. J Epidemiol Community Health 64: 142-147. http://dx.doi.org/10.1136/jech.2008.083576.

Michaudel, C; Mackowiak, C; Maillet, I; Fauconnier, L; Akdis, CA; Sokolowska, M; Dreher, A; Tan, HT; Quesniaux, VF; Ryffel, B; Togbe, D. (2018). Ozone exposure induces respiratory barrier biphasic injury and inflammation controlled by IL-33. J Allergy Clin Immunol. http://dx.doi.org/10.1016/j.jaci.2017.11.044.

Mikerov, AN; Cooper, TK; Wang, G; Hu, S; Umstead, TM; Phelps, DS; Floros, J. (2011). Histopathologic evaluation of lung and extrapulmonary tissues show sex differences in Klebsiella pneumoniae - infected mice under different exposure conditions. International Journal of Physiology, Pathophysiology and Pharmacology 3: 176-190.

Miller, CN; Dye, JA; Ledbetter, AD; Schladweiler, MC; Richards, JH; Snow, SJ; Wood, CE; Henriquez, AR; Thompson, LC; Farraj, AK; Hazari, MS; Kodavanti, UP. (2017). Uterine artery flow and offspring growth in long-evans rats following maternal exposure to ozone during implantation. Environ Health Perspect 125: 127005. http://dx.doi.org/10.1289/EHP2019.

Miller, DB; Snow, SJ; Henriquez, A; Schladweiler, MC; Ledbetter, AD; Richards, JE; Andrews, DL; Kodavanti, UP. (2016a). Systemic metabolic derangement, pulmonary effects, and insulin insufficiency following subchronic ozone exposure in rats. Toxicol Appl Pharmacol 306: 47-57. http://dx.doi.org/10.1016/j.taap.2016.06.027.

Miller, DB; Snow, SJ; Schladweiler, MC; Richards, JE; Ghio, AJ; Ledbetter, AD; Kodavanti, UP. (2016b). Acute ozone-induced pulmonary and systemic metabolic effects are diminished in adrenalectomized rats. Toxicol Sci 150: 312-322. http://dx.doi.org/10.1093/toxsci/kfv331.

Mishra, V; Diangelo, SL; Silveyra, P. (2016). Sex-specific IL-6-associated signaling activation in ozone-induced lung inflammation. 7: 16. http://dx.doi.org/10.1186/s13293-016-0069-7.

Moore, BD; Hyde, D; Miller, L; Wong, E; Frelinger, J; Schelegle, ES. (2012). Allergen and ozone exacerbate serotonin-induced increases in airway smooth muscle contraction in a model of childhood asthma. Respiration 83: 529-542. http://dx.doi.org/10.1159/000336835.

Moore, K; Neugebauer, R; Lurmann, F; Hall, J; Brajer, V; Alcorn, S; Tager, I. (2008). Ambient ozone concentrations cause increased hospitalizations for asthma in children: An 18-year study in Southern California. Environ Health Perspect 116: 1063-1070. http://dx.doi.org/10.1289/ehp.10497.

Mortimer, KM; Neas, LM; Dockery, DW; Redline, S; Tager, IB. (2002). The effect of air pollution on inner-city children with asthma. Eur Respir J 19: 699-705. http://dx.doi.org/10.1183/09031936.02.00247102.

Mortimer, KM; Tager, IB; Dockery, DW; Neas, LM; Redline, S. (2000). The effect of ozone on inner-city children with asthma: Identification of susceptible subgroups. Am J Respir Crit Care Med 162: 1838-1845. http://dx.doi.org/10.1164/ajrccm.162.5.9908113.

Muhajarine, N; Mustard, C; Roos, LL; Young, TK; Gelskey, DE. (1997). Comparison of survey and physician claims data for detecting hypertension. J Clin Epidemiol 50: 711-718. http://dx.doi.org/10.1016/S0895-4356(97)00019-X.

Murgia, N; Brisman, J; Claesson, A; Muzi, G; Olin, AC; Torén, K. (2014). Validity of a questionnaire-based diagnosis of chronic obstructive pulmonary disease in a general population-based study. BMC Pulm Med 14: 49. http://dx.doi.org/10.1186/1471-2466-14-49.

Murphy, SR; Oslund, KL; Hyde, DM; Miller, LA; Van Winkle, LS; Schelegle, ES. (2014). Ozone-induced airway epithelial cell death, the neurokinin-1 receptor pathway, and the postnatal developing lung. Am J Physiol Lung Cell Mol Physiol 307: L471-L481. http://dx.doi.org/10.1152/ajplung.00324.2013.

Murphy, SR; Schelegle, ES; Edwards, PC; Miller, LA; Hyde, DM; Van Winkle, LS. (2012). Postnatal exposure history and airways oxidant stress responses in airway explants. Am J Respir Cell Mol Biol 47: 815-823. http://dx.doi.org/10.1165/rcmb.2012-0110OC.

Murphy, SR; Schelegle, ES; Miller, LA; Hyde, DM; Van Winkle, LS. (2013). Ozone exposure alters serotonin and serotonin receptor expression in the developing lung. Toxicol Sci 134: 168-179. http://dx.doi.org/10.1093/toxsci/kft090.

NAEPP (National Asthma Education and Prevention Program). (2008). Expert panel report 3 (EPR-3): Guidelines for the diagnosis and management of asthmasummary report 2007 - Updates [Erratum]. J Allergy Clin Immunol 120: 1330. http://dx.doi.org/10.1016/j.jaci.2008.04.033.

Neophytou, AM; White, MJ; Oh, SS; Thakur, N; Galanter, JM; Nishimura, KK; Pino-Yanes, M; Torgerson, DG; Gignoux, CR; Eng, C; Nguyen, EA; Hu, D; Mak, AC; Kumar, R; Seibold, MA; Davis, A; Farber, HJ; Meade, K; Avila, PC; Serebrisky, D; Lenoir, MA; Brigino-Buenaventura, E; Rodriguez-Cintron, W; Bibbins-Domingo, K; Thyne, SM; Williams, LK; Sen, S; Gilliland, FD; Gauderman, WJ; Rodriguez-Santana, JR; Lurmann, F; Balmes, JR; Eisen, EA; Burchard, EG. (2016). Air pollution and lung function in minority youth with asthma in the GALA II (Genesenvironments and admixture in Latino Americans) and SAGE II (Study of African Americans, asthma, genes, and environments) studies. Am J Respir Crit Care Med 193: 1271-1280. http://dx.doi.org/10.1164/rccm.201508-1706OC.

NHLBI (National Institutes of Health, National Heart Lung and Blood Institute). (2017). NHLBI fact book, fiscal year 2012: Disease statistics. Available online at https://www.nhlbi.nih.gov/about/documents/factbook/2012/chapter4 (accessed August 23, 2017).

Nishimura, KK; Galanter, JM; Roth, LA; Oh, SS; Thakur, N; Nguyen, EA; Thyne, S; Farber, HJ; Serebrisky, D; Kumar, R; Brigino-Buenaventura, E; Davis, A; Lenoir, MA; Meade, K; Rodriguez-Cintron, W; Avila, PC; Borrell, LN; Bibbins-Domingo, K; Rodriguez-Santana, J. R.; Sen, S; Lurmann, F; Balmes, J. R.; Burchard, EG. (2013). Early-life air pollution and asthma risk in minority children: The GALA II and SAGE II studies. Am J Respir Crit Care Med 188: 309-318. http://dx.doi.org/10.1164/rccm.201302-0264OC.

O' Lenick, CR; Chang, HH; Kramer, MR; Winquist, A; Mulholland, JA; Friberg, MD; Sarnat, SE. (2017). Ozone and childhood respiratory disease in three US cities: Evaluation of effect measure modification by neighborhood socioeconomic status using a Bayesian hierarchical approach. Environ Health 16: #36. http://dx.doi.org/10.1186/s12940-017-0244-2.

O'Connor, GT; Neas, L; Vaughn, B; Kattan, M; Mitchell, H; Crain, EF; Evans, R, III; Gruchalla, R; Morgan, W; Stout, J; Adams, GK; Lippmann, M. (2008). Acute respiratory health effects of air pollution on children with asthma in US inner cities. J Allergy Clin Immunol 121: 1133-1139.e1131. http://dx.doi.org/10.1016/j.jaci.2008.02.020.

O'Lenick, CR; Winquist, A; Mulholland, JA; Friberg, MD; Chang, HH; Kramer, MR; Darrow, LA; Sarnat, SE. (2017). Assessment of neighbourhood-level socioeconomic status as a modifier of air pollution-asthma associations among children in Atlanta. J Epidemiol Community Health 71: 129-136. http://dx.doi.org/10.1136/jech-2015-206530.

Ong, CB; Kumagai, K; Brooks, PT; Brandenberger, C; Lewandowski, RP; Jackson-Humbles, DN; Nault, R; Zacharewski, TR; Wagner, JG; Harkema, JR. (2016). Ozone-induced type 2 immunity in nasal airways. Development and lymphoid cell dependence in mice. Am J Respir Cell Mol Biol 54: 331-340. http://dx.doi.org/10.1165/rcmb.2015-0165OC.

Paffett, ML; Zychowski, KE; Sheppard, L; Robertson, S; Weaver, JM; Lucas, SN; Campen, MJ. (2015). Ozone inhalation impairs coronary artery dilation via intracellular oxidative stress: Evidence for serum-borne factors as drivers of systemic toxicity. Toxicol Sci 146: 244-253. http://dx.doi.org/10.1093/toxsci/kfv093.

Parker, JD; Akinbami, LJ; Woodruff, TJ. (2009). Air pollution and childhood respiratory allergies in the United States. Environ Health Perspect 117: 140-147. http://dx.doi.org/10.1289/ehp.11497.

Patel, MM; Chillrud, SN; Deepti, KC; Ross, JM; Kinney, PL. (2013). Traffic-related air pollutants and exhaled markers of airway inflammation and oxidative stress in New York City adolescents. Environ Res 121: 71-78. http://dx.doi.org/10.1016/j.envres.2012.10.012.

Peacock, JL; Anderson, HR; Bremner, SA; Marston, L; Seemungal, TA; Strachan, DP; Wedzicha, JA. (2011). Outdoor air pollution and respiratory health in patients with COPD. Thorax 66: 591-596. http://dx.doi.org/10.1136/thx.2010.155358.

Penard-Morand, C; Charpin, D; Raherison, C; Kopferschmitt, C; Caillaud, D; Lavaud, F; Annesi-Maesano, I. (2005). Long-term exposure to background air pollution related to respiratory and allergic health in schoolchildren. Clin Exp Allergy 35: 1279-1287. http://dx.doi.org/10.1111/j.1365-2222.2005.02336.x.

Peng, C; Luttmann-Gibson, H; Zanobetti, A; Cohen, A; De Souza, C; Coull, BA; Horton, ES; Schwartz, J; Koutrakis, P; Gold, DR. (2016). Air pollution influences on exhaled nitric oxide among people with type II diabetes. Air Qual Atmos Health 9: 265-273. http://dx.doi.org/10.1007/s11869-015-0336-5.

Qian, Z; Liao, D; Lin, HM; Whitsel, EA; Rose, KM; Duan, Y. (2005). Lung function and long-term exposure to air pollutants in middle-aged American adults. Arch Environ Occup Health 60: 156-163. http://dx.doi.org/10.3200/AEOH.60.3.156-163.

Rage, E; Jacquemin, B; Nadif, R; Oryszczyn, MP; Siroux, V; Aguilera, I; Kauffmann, F; Künzli, N. (2009). Total serum IgE levels are associated with ambient ozone concentration in asthmatic adults. Allergy 64: 40-46. http://dx.doi.org/10.1111/j.1398-9995.2008.01800.x.

Raizenne, ME; Burnett, RT; Stern, B; Franklin, CA; Spengler, JD. (1989). Acute lung function responses to ambient acid aerosol exposures in children. Environ Health Perspect 79: 179-185.

Ramadour, M; Burel, C; Lanteaume, A; Vervloet, D; Charpin, D; Brisse, F; Dutau, H; Charpin, D. (2000). Prevalence of asthma and rhinitis in relation to long-term exposure to gaseous air pollutants. Allergy 55: 1163-1169. http://dx.doi.org/10.1034/j.1398-9995.2000.00637.x.

Ramot, Y; Kodavanti, UP; Kissling, GE; Ledbetter, AD; Nyska, A. (2015). Clinical and pathological manifestations of cardiovascular disease in rat models: the influence of acute ozone exposure. Inhal Toxicol 27: 26-38. http://dx.doi.org/10.3109/08958378.2014.954168.

Razvi, SS; Richards, JB; Malik, F; Cromar, KR; Price, RE; Bell, CS; Weng, T; Atkins, CL; Spencer, CY; Cockerill, KJ; Alexander, AL; Blackburn, MR; Alcorn, JL; Haque, IU; Johnston, RA. (2015). Resistin deficiency in mice has no effect on pulmonary responses induced by acute ozone exposure. Am J Physiol Lung Cell Mol Physiol 309: L1174 L1185. http://dx.doi.org/10.1152/ajplung.00270.2015.

Rice, MB; Ljungman, PL; Wilker, EH; Gold, DR; Schwartz, JD; Koutrakis, P; Washko, GR; O'Connor, GT; Mittleman, MA. (2013). Short-term exposure to air pollution and lung function in the Framingham heart study. Am J Respir Crit Care Med 188: 1351-1357. http://dx.doi.org/10.1164/rccm.201308-1414OC.

Robertson, S; Colombo, ES; Lucas, SN; Hall, PR; Febbraio, M; Paffett, ML; Campen, MJ. (2013). CD36 mediates endothelial dysfunction downstream of circulating factors induced by O3 exposure. Toxicol Sci 134: 304-311. http://dx.doi.org/10.1093/toxsci/kft107.

Rodopoulou, S; Samoli, E; Chalbot, MG; Kavouras, IG. (2015). Air pollution and cardiovascular and respiratory emergency visits in Central Arkansas: A time-series analysis. Sci Total Environ 536: 872-879. http://dx.doi.org/10.1016/j.scitotenv.2015.06.056.

Rooney, AA; Boyles, AL; Wolfe, MS; Bucher, JR; Thayer, KA. (2014). Systematic review and evidence integration for literature-based environmental health science assessments. Environ Health Perspect 122: 711-718. http://dx.doi.org/10.1289/ehp.1307972.

Ross, MA; Persky, VW; Scheff, PA; Chung, J; Curtis, L; Ramakrishnan, V; Wadden, RA; Hryhorczuk, DO. (2002). Effect of ozone and aeroallergens on the respiratory health of asthmatics. Arch Environ Occup Health 57: 568-578. http://dx.doi.org/10.1080/00039890209602090.

Sacks, JD; Rappold, AG; Davis, JA, Jr; Richardson, DB; Waller, AE; Luben, TJ. (2014). Influence of urbanicity and county characteristics on the association between ozone and asthma emergency department visits in North Carolina. Environ Health Perspect 122: 506-512. http://dx.doi.org/10.1289/ehp.1306940.

Salam, MT; Byun, HM; Lurmann, F; Breton, CV; Wang, X; Eckel, SP; Gilliland, FD. (2012). Genetic and epigenetic variations in inducible nitric oxide synthase promoter, particulate pollution, and exhaled nitric oxide levels in children. J Allergy Clin Immunol 129: 232-239.e237. http://dx.doi.org/10.1016/j.jaci.2011.09.037.

Salam, MT; Islam, T; Gauderman, WJ; Gilliland, FD. (2009). Roles of arginase variants, atopy, and ozone in childhood asthma. J Allergy Clin Immunol 123: 596-602. http://dx.doi.org/10.1016/j.jaci.2008.12.020.

Sarnat, JA; Sarnat, SE; Flanders, WD; Chang, HH; Mulholland, J; Baxter, L; Isakov, V; Ozkaynak, H. (2013). Spatiotemporally resolved air exchange rate as a modifier of acute air pollution-related morbidity in Atlanta. J Expo Sci Environ Epidemiol 23: 606-615. http://dx.doi.org/10.1038/jes.2013.32.

Sarnat, SE; Winquist, A; Schauer, JJ; Turner, JR; Sarnat, JA. (2015). Fine particulate matter components and emergency department visits for cardiovascular and respiratory diseases in the St. Louis, Missouri-Illinois, metropolitan area. Environ Health Perspect 123: 437-444. http://dx.doi.org/10.1289/ehp.1307776.

Schelegle, ES; Adams, WC; Walby, WF; Marion, MS. (2012). Modelling of individual subject ozone exposure response kinetics. Inhal Toxicol 24: 401-415. http://dx.doi.org/10.3109/08958378.2012.683891.

Schelegle, ES; Morales, CA; Walby, WF; Marion, S; Allen, RP. (2009). 6.6-hour inhalation of ozone concentrations from 60 to 87 parts per billion in healthy humans. Am J Respir Crit Care Med 180: 265-272. http://dx.doi.org/10.1164/rccm.200809-1484OC.

Schelegle, ES; Walby, WF. (2012). Vagal afferents contribute to exacerbated airway responses following ozone and allergen challenge. Respir Physiol Neurobiol 181: 277-285. http://dx.doi.org/10.1016/j.resp.2012.04.003.

Schittny, JC. (2017). Development of the lung [Review]. Cell Tissue Res 367: 427-444. http://dx.doi.org/10.1007/s00441-016-2545-0.

Sheffield, PE; Zhou, J; Shmool, JL; Clougherty, JE. (2015). Ambient ozone exposure and children's acute asthma in New York City: a case-crossover analysis. Environ Health 14: 25. http://dx.doi.org/10.1186/s12940-015-0010-2.

Shmool, JLC; Kinnee, E; Sheffield, PE; Clougherty, JE. (2016). Spatio-temporal ozone variation in a case-crossover analysis of childhood asthma hospital visits in New York City. Environ Res 147: 108-114. http://dx.doi.org/10.1016/j.envres.2016.01.020.

Shore, SA; Williams, ES; Chen, L; Benedito, LA; Kasahara, DI; Zhu, M. (2011). Impact of aging on pulmonary responses to acute ozone exposure in mice: role of TNFR1. Inhal Toxicol 23: 878-888. http://dx.doi.org/10.3109/08958378.2011.622316.

Silverman, RA; Ito, K. (2010). Age-related association of fine particles and ozone with severe acute asthma in New York City. J Allergy Clin Immunol 125: 367-373. http://dx.doi.org/10.1016/j.jaci.2009.10.061.

Smith, GS; Van Den Eeden, SK; Garcia, C; Shan, J; Baxter, R; Herring, AH; Richardson, DB; Van Rie, A; Emch, M; Gammon, MD. (2016). Air pollution and pulmonary tuberculosis: A nested case-control study among members of a northern california health plan. Environ Health Perspect 124: 761-768. http://dx.doi.org/10.1289/ehp.1408166.

Snow, SJ; Cheng, WY; Henriquez, A; Hodge, M; Bass, V; Nelson, GM; Carswell, G; Richards, JE; Schladweiler, MC; Ledbetter, AD; Chorley, B; Gowdy, KM; Tong, H; Kodavanti, UP. (2018). Ozone-induced vascular contractility and pulmonary injury are differentially impacted by diets enriched with coconut oil, fish oil, and olive oil. Toxicol Sci 163: 5769. http://dx.doi.org/10.1093/toxsci/kfy003.

Snow, SJ; Gordon, CJ; Bass, VL; Schladweiler, MC; Ledbetter, AD; Jarema, KA; Phillips, PM; Johnstone, AF; Kodavanti, UP. (2016). Age-related differences in pulmonary effects of acute and subchronic episodic ozone exposures in Brown Norway rats. Inhal Toxicol 28: 313-323. http://dx.doi.org/10.3109/08958378.2016.1170910.

Speen, AM; Kim, HH; Bauer, RN; Meyer, M; Gowdy, KM; Fessler, MB; Duncan, KE; Liu, W; Porter, NA; Jaspers, I. (2016). Ozone-derived oxysterols affect liver X receptor (LXR) signaling: a potential role for lipid-protein adducts. J Biol Chem 291: 25192-25206. http://dx.doi.org/10.1074/jbc.M116.732362.

Spektor, DM; Lippmann, M. (1991). Health effects of ambient ozone on healthy children at a summer camp. In RL Berglund; DR Lawson; DJ McKee (Eds.), Tropospheric Ozone and the Environment: Papers from an International Conference; March 1990; Los Angeles, CA (pp. 83-89). Pittsburgh, PA: Air & Waste Management Association.

Spektor, DM; Lippmann, M; Lioy, PJ; Thurston, GD; Citak, K; James, DJ; Bock, N; Speizer, FE; Hayes, C. (1988). Effects of ambient ozone on respiratory function in active, normal children. Am Rev Respir Dis 137: 313-320. http://dx.doi.org/10.1164/ajrccm/137.2.313.

Stafoggia, M; Forastiere, F; Faustini, A; Biggeri, A; Bisanti, L; Cadum, E; Cernigliaro, A; Mallone, S; Pandolfi, P; Serinelli, M; Tessari, R; Vigotti, MA; Perucci, CA. (2010). Susceptibility factors to ozone-related mortality: A population-based case-crossover analysis. Am J Respir Crit Care Med 182: 376-384. http://dx.doi.org/10.1164/rccm.200908-1269OC.

Stieb, DM; Szyszkowicz, M; Rowe, BH; Leech, JA. (2009). Air pollution and emergency department visits for cardiac and respiratory conditions: A multi-city time-series analysis. Environ Health 8: 25. http://dx.doi.org/10.1186/1476-069X-8-25.

Stiegel, MA; Pleil, JD; Sobus, JR; Stevens, T; Madden, MC. (2017). Linking physiological parameters to perturbations in the human exposome: Environmental exposures modify blood pressure and lung function via inflammatory cytokine pathway. J Toxicol Environ Health A 80: 485-501. http://dx.doi.org/10.1080/15287394.2017.1330578.

Stober, VP; Johnson, CG; Majors, A; Lauer, ME; Cali, V; Midura, RJ; Wisniewski, HG; Aronica, MA; Garantziotis, S. (2017). TNF-stimulated gene 6 promotes formation of hyaluronan-inter-α-inhibitor heavy chain complexes necessary for ozone-induced airway hyperresponsiveness. J Biol Chem 292: 20845-20858. http://dx.doi.org/10.1074/jbc.M116.756627.

Strickland, MJ; Darrow, LA; Klein, M; Flanders, WD; Sarnat, JA; Waller, LA; Sarnat, SE; Mulholland, JA; Tolbert, PE. (2010). Short-term associations between ambient air pollutants and pediatric asthma emergency department visits. Am J Respir Crit Care Med 182: 307-316. http://dx.doi.org/10.1164/rccm.200908-1201OC.

Strickland, MJ; Klein, M; Flanders, WD; Chang, HH; Mulholland, JA; Tolbert, PE; Darrow, LA. (2014). Modification of the effect of ambient air pollution on pediatric asthma emergency visits: susceptible subpopulations. Epidemiology 25: 843-850. http://dx.doi.org/10.1097/EDE.0000000000000170.

Sunil, VR; Francis, M; Vayas, KN; Cervelli, JA; Choi, H; Laskin, JD; Laskin, DL. (2015). Regulation of ozone-induced lung inflammation and injury by the beta-galactoside-binding lectin galectin-3. Toxicol Appl Pharmacol 284: 236-245. http://dx.doi.org/10.1016/j.taap.2015.02.002.

Sunil, VR; Patel-Vayas, K; Shen, J; Laskin, JD; Laskin, DL. (2012). Classical and alternative macrophage activation in the lung following ozone-induced oxidative stress. Toxicol Appl Pharmacol 263: 195-202. http://dx.doi.org/10.1016/j.taap.2012.06.009.

Sunil, VR; Vayas, KJ; Massa, CB; Gow, AJ; Laskin, JD; Laskin, DL. (2013). Ozone-induced injury and oxidative stress in bronchiolar epithelium is associated with altered pulmonary mechanics. Toxicol Sci 133: 309-319. http://dx.doi.org/10.1093/toxsci/kft071.

Szyszkowicz, M; Kousha, T; Castner, J; Dales, R. (2018). Air pollution and emergency department visits for respiratory diseases: A multi-city case crossover study. Environ Res 163: 263-269. http://dx.doi.org/10.1016/j.envres.2018.01.043.

Tank, J; Biller, H; Heusser, K; Holz, O; Diedrich, A; Framke, T; Koch, A; Grosshennig, A; Koch, W; Krug, N; Jordan, J; Hohlfeld, JM. (2011). Effect of acute ozone induced airway inflammation on human sympathetic nerve traffic: a randomized, placebo controlled, crossover study. PLoS ONE 6: e18737. http://dx.doi.org/10.1371/journal.pone.0018737.

Tankersley, CG; Georgakopoulos, D; Tang, WY; Sborz, N. (2013). Effects of ozone and particulate matter on cardiac mechanics: role of the atrial natriuretic peptide gene. Toxicol Sci 131: 95-107. http://dx.doi.org/10.1093/toxsci/kfs273.

Tétreault, LF; Doucet, M; Gamache, P; Fournier, M; Brand, A; Kosatsky, T; Smargiassi, A. (2016a). Childhood exposure to ambient air pollutants and the onset of asthma: an administrative cohort study in Québec. Environ Health Perspect 124: 1276-1282. http://dx.doi.org/10.1289/ehp.1509838.

Tétreault, LF; Doucet, M; Gamache, P; Fournier, M; Brand, A; Kosatsky, T; Smargiassi, A. (2016b). Severe and moderate asthma exacerbations in asthmatic children and exposure to ambient air pollutants. Int J Environ Res Public Health 13: 771. http://dx.doi.org/10.3390/ijerph13080771.

Thomson, EM; Pal, S; Guénette, J; Wade, MG; Atlas, E; Holloway, AC; Williams, A; Vincent, R. (2016). Ozone inhalation provokes glucocorticoid-dependent and -independent effects on inflammatory and metabolic pathways. Toxicol Sci 152: 17-28. http://dx.doi.org/10.1093/toxsci/kfw061.

Thomson, EM; Vladisavljevic, D; Mohottalage, S; Kumarathasan, P; Vincent, R. (2013). Mapping acute systemic effects of inhaled particulate matter and ozone: multiorgan gene expression and glucocorticoid activity. Toxicol Sci 135: 169-181. http://dx.doi.org/10.1093/toxsci/kft137.

Tighe, RM; Birukova, A; Yeager, MJ; Reece, SW; Gowdy, KM. (2018). Euthanasia and lavage mediated effects on bronchoalveolar measures of lung injury and inflammation. Am J Respir Cell Mol Biol. http://dx.doi.org/10.1165/rcmb.2017-0357OC.

To, T; Zhu, J; Larsen, K; Simatovic, J; Feldman, L; Ryckman, K; Gershon, A; Lougheed, MD; Licskai, C; Chen, H; Villeneuve, PJ; Crighton, E; Su, Y; Sadatsafavi, M; Williams, D; Carlsten, C. (2016). Progression from asthma to chronic obstructive pulmonary disease (COPD): is air pollution a risk factor? Am J Respir Crit Care Med 194: 429-438. http://dx.doi.org/10.1164/rccm.201510-1932OC.

Tolbert, PE; Klein, M; Peel, JL; Sarnat, SE; Sarnat, JA. (2007). Multipollutant modeling issues in a study of ambient air quality and emergency department visits in Atlanta. J Expo Sci Environ Epidemiol 17: S29-S35. http://dx.doi.org/10.1038/sj.jes.7500625.

Toren, K; Brisman, J; Jarvholm, B. (1993). Asthma and asthma-like symptoms in adults assessed by questionnaires: A literature review [Review]. Chest 104: 600-608. http://dx.doi.org/10.1378/chest.104.2.600.

Turner, MC; Jerrett, M; Pope, A, III; Krewski, D; Gapstur, SM; Diver, WR; Beckerman, BS; Marshall, JD; Su, J; Crouse, DL; Burnett, RT. (2016). Long-term ozone exposure and mortality in a large prospective study. Am J Respir Crit Care Med 193: 1134-1142. http://dx.doi.org/10.1164/rccm.201508-1633OC.

U.S. EPA (U.S. Environmental Protection Agency). (1986). Air quality criteria for ozone and other photochemical oxidants; Volume V [EPA Report]. (EPA-600/8-84-020eF). Research Triangle Park, NC.

U.S. EPA (U.S. Environmental Protection Agency). (1991). Guidelines for developmental toxicity risk assessment (pp. 1-71). (EPA/600/FR-91/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=23162.

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

U.S. EPA (U.S. Environmental Protection Agency). (1996b). Guidelines for reproductive toxicity risk assessment (pp. 1-143). (EPA/630/R-96/009). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/sites/production/files/2014-11/documents/guidelines_repro_toxicity.pdf.

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

U.S. EPA (U.S. Environmental Protection Agency). (1998). Guidelines for neurotoxicity risk assessment [EPA Report] (pp. 1-89). (EPA/630/R-95/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/risk/guidelines-neurotoxicity-risk-assessment.

U.S. EPA (U.S. Environmental Protection Agency). (2005). Guidelines for carcinogen risk assessment [EPA Report] (pp. 1-166). (EPA/630/P-03/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/sites/production/files/2013-09/documents/cancer_guidelines_final_3-25-05.pdf.

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

U.S. EPA (U.S. Environmental Protection Agency). (2008). Integrated science assessment for sulfur oxides: Health criteria [EPA Report]. (EPA/600/R-08/047F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment-RTP. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=198843.

U.S. EPA (U.S. Environmental Protection Agency). (2013a). Integrated science assessment for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-10/076F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment-RTP Division. http://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=247492.

U.S. EPA (U.S. Environmental Protection Agency). (2013b). Toxicological review of trimethylbenzenes (CASRN 25551-13-7, 95-63-6, 526-73-8, and 108-67-8) in support of summary information on the Integrated Risk Information System (IRIS): revised external review draft [EPA Report]. (EPA/635/R13/171a). Washington, D.C.: U.S. Environmental Protection Agency, National Center for Environmental Assessment. http://yosemite.epa.gov/sab/SABPRODUCT.NSF/b5d8a1ce9b0729348525737500701 2b7/ee1e280e77586de985257b65005d37e7!OpenDocument.

U.S. EPA (U.S. Environmental Protection Agency). (2015). Preamble to the integrated science assessments [EPA Report]. (EPA/600/R-15/067). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, RTP Division. https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=310244.

U.S. EPA (U.S. Environmental Protection Agency). (2016). Integrated science assessment for oxides of nitrogen-Health Criteria (final report) [EPA Report]. (EPA/600/R-15/068). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment. http://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=526855.

Urman, R; McConnell, R; Islam, T; Avol, EL; Lurmann, FW; Vora, H; Linn, WS; Rappaport, EB; Gilliland, FD; Gauderman, WJ. (2014). Associations of children's lung function with ambient air pollution: Joint effects of regional and near-roadway pollutants. Thorax 69: 540-547. http://dx.doi.org/10.1136/thoraxjnl-2012-203159.

Vanos, JK; Hebbern, C; Cakmak, S. (2014). Risk assessment for cardiovascular and respiratory mortality due to air pollution and synoptic meteorology in 10 Canadian cities. Environ Pollut 185: 322-332. http://dx.doi.org/10.1016/j.envpol.2013.11.007.

Verhein, KC; Mccaw, Z; Gladwell, W; Trivedi, S; Bushel, PR; Kleeberger, SR. (2015). Novel roles for Notch3 and Notch4 receptors in gene expression and susceptibility to ozone-induced lung inflammation in mice. Environ Health Perspect 123: 799-805. http://dx.doi.org/10.1289/ehp.1408852.

Verhein, KC; Salituro, FG; Ledeboer, MW; Fryer, AD; Jacoby, DB. (2013). Dual p38/JNK mitogen activated protein kinase inhibitors prevent ozone-induced airway hyperreactivity in guinea pigs. PLoS ONE 8: e75351. http://dx.doi.org/10.1371/journal.pone.0075351.

Villeneuve, PJ; Chen, L; Rowe, BH; Coates, F. (2007). Outdoor air pollution and emergency department visits for asthma among children and adults: A case-crossover study in northern Alberta, Canada. Environ Health 6: 40. http://dx.doi.org/10.1186/1476-069X-6-40.

von Elm, E; Altman, DG; Egger, M; Pocock, SJ; Gøtzsche, PC; Vandenbroucke, JP. (2007). The strengthening the reporting of observational studies in epidemiology (strobe) statement: guidelines for reporting observational studies [Review]. PLoS Med 4: e296. http://dx.doi.org/10.1371/journal.pmed.0040296.

Wang, G; Zhao, J; Jiang, R; Song, W. (2013). Rat lung response to ozone and fine particulate matter (PM2.5) exposures. Environ Toxicol 30: 343-356. http://dx.doi.org/10.1002/tox.21912.

Wang, X; Dockery, DW; Wypij, D; Gold, DR; Speizer, FE; Ware, JH; Ferris, BG, Jr. (1993). Pulmonary function growth velocity in children 6 to 18 years of age. Am J Respir Crit Care Med 148: 1502-1508. http://dx.doi.org/10.1164/ajrccm/148.6_Pt_1.1502.

Ward, WO; Kodavanti, UP. (2015). Pulmonary transcriptional response to ozone in healthy and cardiovascular compromised rat models. Inhal Toxicol 27: 93-104. http://dx.doi.org/10.3109/08958378.2014.954173.

Ward, WO; Ledbetter, AD; Schladweiler, MC; Kodavanti, UP. (2015). Lung transcriptional profiling: insights into the mechanisms of ozone-induced pulmonary injury in Wistar Kyoto rats. Inhal Toxicol 27: 80-92. http://dx.doi.org/10.3109/08958378.2014.954172.

Weakley, J; Webber, MP; Ye, F; Zeig-Owens, R; Cohen, HW; Hall, CB; Kelly, K; Prezant, DJ. (2013). Agreement between obstructive airways disease diagnoses from self-report questionnaires and medical records. Prev Med 57: 38-42. http://dx.doi.org/10.1016/j.ypmed.2013.04.001.

Weichenthal, S; Pinault, LL; Burnett, RT. (2017). Impact of oxidant gases on the relationship between outdoor fine particulate air pollution and nonaccidental, cardiovascular, and respiratory mortality. Sci Rep 7: 16401. http://dx.doi.org/10.1038/s41598-017-16770-y.

Weir, CH; Yeatts, KB; Sarnat, JA; Vizuete, W; Salo, PM; Jaramillo, R; Cohn, RD; Chu, H; Zeldin, DC; London, SJ. (2013). Nitrogen dioxide and allergic sensitization in the 2005-2006 National Health and Nutrition Examination Survey. Respir Med 107: 1763-1772. http://dx.doi.org/10.1016/j.rmed.2013.08.010.

Wendt, JK; Symanski, E; Stock, TH; Chan, W; Du, XL. (2014). Association of short-term increases in ambient air pollution and timing of initial asthma diagnosis among Medicaid-enrolled children in a metropolitan area. Environ Res 131: 50-58. http://dx.doi.org/10.1016/j.envres.2014.02.013.

Williams, AS; Mathews, JA; Kasahara, DI; Wurmbrand, AP; Chen, L; Shore, S. (2015). Innate and ozone-induced airway hyperresponsiveness in obese mice: role of TNF-alpha. Am J Physiol Lung Cell Mol Physiol 308: L1168-L1177. http://dx.doi.org/10.1152/ajplung.00393.2014.

Winquist, A; Kirrane, E; Klein, M; Strickland, M; Darrow, LA; Sarnat, SE; Gass, K; Mulholland, J; Russell, A; Tolbert, P. (2014). Joint effects of ambient air pollutants on pediatric asthma emergency department visits in Atlanta, 1998-2004. Epidemiology 25: 666-673. http://dx.doi.org/10.1097/EDE.0000000000000146.

Winquist, A; Klein, M; Tolbert, P; Flanders, WD; Hess, J; Sarnat, SE. (2012). Comparison of emergency department and hospital admissions data for air pollution time-series studies. Environ Health 11: 70. http://dx.doi.org/10.1186/1476-069X-11-70.

Wolkoff, P; Clausen, PA; Larsen, ST; Hammer, M; Nielsen, GD. (2012). Airway effects of repeated exposures to ozone-initiated limonene oxidation products as model of indoor air mixtures. Toxicol Lett 209: 166-172. http://dx.doi.org/10.1016/j.toxlet.2011.12.008.

Wong, EM; Walby, WF; Wilson, DW; Tablin, F; Schelegle, ES. (2018). Ultrafine particulate matter combined with ozone exacerbates lung injury in mature adult rats with cardiovascular disease. Toxicol Sci 163: 140-151. http://dx.doi.org/10.1093/toxsci/kfy018.

Wood, AM; Harrison, RM; Semple, S; Ayres, JG; Stockley, RA. (2009). Outdoor air pollution is associated with disease severity in a1-antitrypsin deficiency. Eur Respir J 34: 346-353. http://dx.doi.org/10.1183/09031936.00087908.

Xiang, Y; Qin, XQ; Liu, HJ; Tan, YR; Liu, C; Liu, CX. (2012). Identification of transcription factors regulating CTNNAL1 expression in human bronchial epithelial cells. PLoS ONE 7: e31158. http://dx.doi.org/10.1371/journal.pone.0031158.

Xiao, Q; Liu, Y; Mulholland, JA; Russell, AG; Darrow, LA; Tolbert, PE; Strickland, MJ. (2016). Pediatric emergency department visits and ambient Air pollution in the U.S. State of Georgia: a case-crossover study. Environ Health 15: 115. http://dx.doi.org/10.1186/s12940-016-0196-y.

Yanagisawa, R; Warabi, E; Inoue, KI; Yanagawa, T; Koike, E; Ichinose, T; Takano, H; Ishii, T. (2012). Peroxiredoxin I null mice exhibits reduced acute lung inflammation following ozone exposure. J Biochem 152: 595-601. http://dx.doi.org/10.1093/jb/mvs113.

Yang, CL; To, T; Foty, RG; Stieb, DM; Dell, SD. (2011). Verifying a questionnaire diagnosis of asthma in children using health claims data. BMC Pulm Med 11. http://dx.doi.org/10.1186/1471-2466-11-52.

Ying, Z; Allen, K; Zhong, J; Chen, M; Williams, KM; Wagner, JG; Lewandowski, R; Sun, Q; Rajagopalan, S; Harkema, JR. (2016). Subacute inhalation exposure to ozone induces systemic inflammation but not insulin resistance in a diabetic mouse model. Inhal Toxicol 28: 155-163. http://dx.doi.org/10.3109/08958378.2016.1146808.

Yonchuk, JG; Foley, JP; Bolognese, BJ; Logan, G; Wixted, WE; Kou, JP; Chalupowicz, DG; Feldser, HG; Sanchez, Y; Nie, H; Callahan, JF; Kerns, JK; Podolin, PL. (2017). Characterization of the potent, selective Nrf2 activator, 3-(pyridin-3-ylsulfonyl)-5-(trifluoromethyl)-2h-chromen-2-one, in cellular and in vivo models of pulmonary oxidative stress. J Pharmacol Exp Ther 363: 114-125. http://dx.doi.org/10.1124/jpet.117.241794.

Zellner, LC; Brundage, KM; Hunter, DD; Dey, RD. (2011). Early postnatal ozone exposure alters rat nodose and jugular sensory neuron development. Toxicol Environ Chem 93: 2055-2071. http://dx.doi.org/10.1080/02772248.2011.610882.

Zhang, S; Li, J; Li, Y; Liu, Y; Guo, H; Xu, X. (2017). Nitric oxide synthase activity correlates with OGG1 in ozone-induced lung injury animal models. Front Physiol 8: 249. http://dx.doi.org/10.3389/fphys.2017.00249.

Zhong, J; Allen, K; Rao, X; Ying, Z; Braunstein, Z; Kankanala, SR; Xia, C; Wang, X; Bramble, LA; Wagner, JG; Lewandowski, R; Sun, Q; Harkema, JR; Rajagopalan, S. (2016). Repeated ozone exposure exacerbates insulin resistance and activates innate immune response in genetically susceptible mice. Inhal Toxicol 28: 383-392. http://dx.doi.org/10.1080/08958378.2016.1179373.

Zhu, Y; Li, J; Wu, Z; Lu, Y; You, H; Li, R; Li, B; Yang, X; Duan, L. (2016). Acute exposure of ozone induced pulmonary injury and the protective role of vitamin E through the Nrf2 pathway in Balb/c mice. Toxicology Research 5: 268-277. http://dx.doi.org/10.1039/c5tx00259a.

Zu, K; Liu, X; Shi, L; Tao, G; Loftus, CT; Lange, S; Goodman, JE. (2017). Concentration-response of short-term ozone exposure and hospital admissions for asthma in Texas. Environ Int 104: 139-145. http://dx.doi.org/10.1016/j.envint.2017.04.006.

Zychowski, KE; Lucas, SN; Sanchez, B; Herbert, G; Campen, MJ. (2016). Hypoxia-induced pulmonary arterial hypertension augments lung injury and airway reactivity caused by ozone exposure. Toxicol Appl Pharmacol 305: 40-45. http://dx.doi.org/10.1016/j.taap.2016.06.003.

APPENDIX 4 HEALTH EFFECTS—CARDIOVASCULAR

Summary of Causality Determinations for Short- and Long-Term Ozone Exposure and Cardiovascular Effects

This Appendix characterizes the scientific evidence that supports causality determinations for short- and long-term ozone exposure and cardiovascular effects. The types of studies evaluated within this Appendix are consistent with the overall scope of the ISA as detailed in the Preface. In assessing the overall evidence, the strengths and limitations of individual studies were evaluated based on scientific considerations detailed in the Annex for Appendix 4. More details on the causal framework used to reach these conclusions are included in the Preamble to the ISA (U.S. EPA, 2015).

Exposure Duration	Causality Determination
Short-term exposure	Suggestive of, but not sufficient to infer, a causal relationship
Long-term exposure	Suggestive of, but not sufficient to infer, a causal relationship

4.1 Short-Term Ozone Exposure and Cardiovascular Health Effects

4.1.1 Introduction, Summary from the 2013 Ozone ISA, and Scope for Current Review

The 2013 Ozone ISA concluded that "a likely causal relationship exists between short-term exposure to ozone and cardiovascular effects." This conclusion was based on multiple lines of evidence, including animal toxicological studies demonstrating ozone-induced impaired vascular and cardiac function, as well as changes in time domains of heart rate variability (HRV) (U.S. EPA, 2013a). There was also evidence from animal toxicological studies for changes in heart rate, although the ISA noted inconsistencies in that both bradycardia and tachycardia were reported. Controlled human exposure (CHE) studies also reported cardiovascular effects in response to short-term ozone exposure. More specifically, CHE studies reported both increases and decreases in measures in the high-frequency domain of HRV (U.S. EPA, 2013a). Changes in HRV observed in both animal and human studies provided putative evidence for ozone-induced modulation of the autonomic nervous system potentially through the

activation of neural reflexes in the lung. In addition, CHE studies from the last review demonstrated some evidence of ozone-induced effects on blood biomarkers of systemic inflammation and oxidative stress, as well as changes in biomarkers associated with increased coagulation and/or decreased fibrinolysis (U.S. EPA, 2013a). Taken together, this experimental evidence was coherent with the consistently positive associations reported in epidemiologic studies between short-term ozone exposure and cardiovascular mortality.

Key uncertainties from the last review included a lack of coherence between epidemiologic mortality and morbidity studies. Although multicity studies and a multicontinent study reported positive associations between short-term ozone exposure and cardiovascular mortality, with a few exceptions, the findings from epidemiologic studies on short-term ozone exposure and cardiovascular-related morbidity outcomes, specifically hospital admissions and emergency department (ED) visits, were generally null. In addition, given that relatively few epidemiologic studies in the 2013 Ozone ISA examined the potential for copollutant confounding, some uncertainty remains regarding the extent to which ozone is driving the positive associations reported in studies of mortality. However, the few studies that did examine the potential for copollutant confounding suggested an independent effect of ozone exposure (U.S. EPA, 2013a). The subsections below provide an evaluation of the most policy-relevant scientific evidence relating short-term ozone exposure to cardiovascular health effects. These sections focus on studies published since the 2013 Ozone ISA, and particular emphasis is placed on those studies that address uncertainties identified in that review. Importantly, when considered as a whole, these newer studies call into question that a likely causal relationship exists between short-term exposure to ozone and cardiovascular effects.

4.1.2 Population, Exposure, Comparison, Outcome, and Study Design (PECOS) Tool

The scope of this section is defined by a scoping tool that generally defines the relevant Population, Exposure, Comparison, Outcome, and Study Design (PECOS). The PECOS tool defines the parameters and provides a framework to help identify the relevant evidence in the literature to inform the ISA. Because the 2013 Ozone ISA concluded a likely to be a causal relationship between short-term ozone exposure and cardiovascular health effects, the epidemiologic studies evaluated are more limited in scope and targeted toward study locations, as reflected in the PECOS tool, that are most informative to address the policy-relevant considerations forming the basis of this section. The studies evaluated and subsequently discussed within this section were included because they satisfied all of the components of the following PECOS tool:

Experimental Studies:

- Population: Study populations of any controlled human exposure or animal toxicological study of mammals at any lifestage

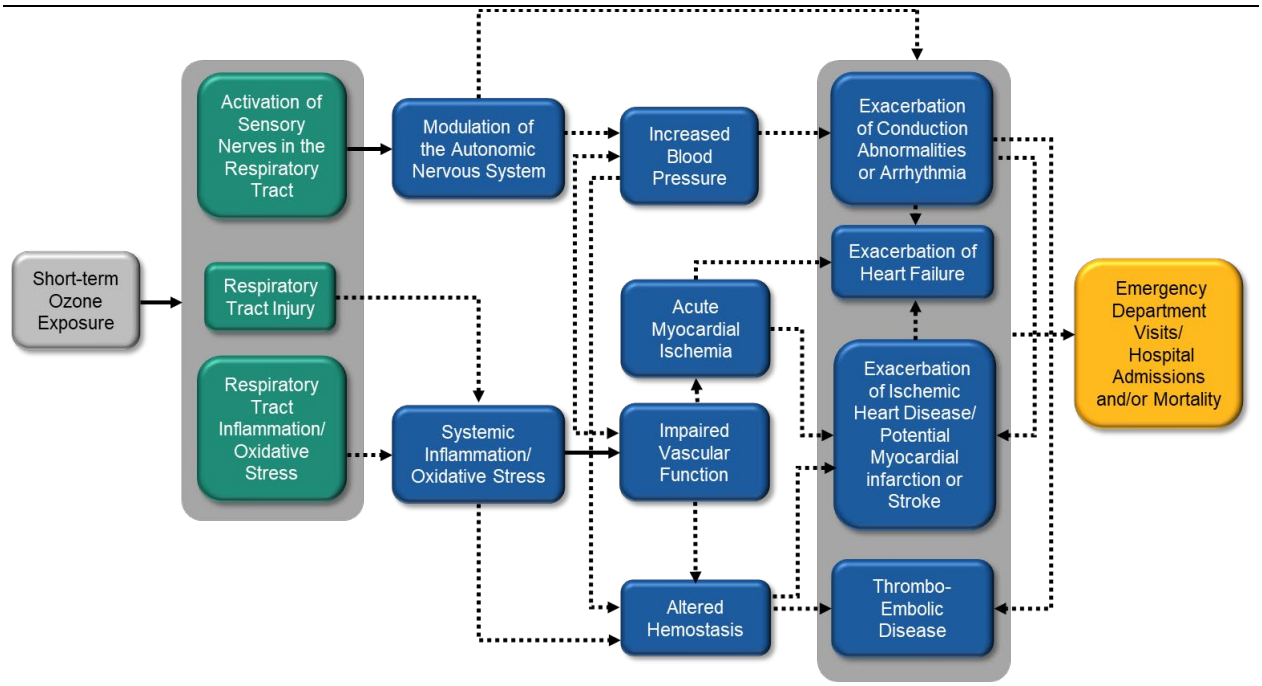
- Exposure: Short-term (on the order of minutes to weeks) inhalation exposure to relevant ozone concentrations (i.e., ≤0.4 ppm for humans; ≤2 ppm for other mammals)
- Comparison: Human subjects that serve as their own controls with an appropriate washout period or subjects compared to a reference population exposed to lower levels (when available), or, in toxicological studies of mammals, an appropriate comparison group that is exposed to a negative control (e.g., filtered air)
- Outcome: Cardiovascular effects
- Study Design: Controlled human exposure (i.e., chamber) studies; in vivo acute, subacute, or repeated-dose toxicity studies in mammals, immunotoxicity studies

Epidemiologic Studies:

- Population: Any U.S., Canadian, European, or Australian population, including populations or lifestages that might be at increased risk
- Exposure: Short-term ambient concentration of ozone
- Comparison: Per unit increase (in ppb)
- Outcome: Change in risk (incidence/prevalence) of cardiovascular effects
- Study Design: Epidemiologic studies consisting of panel, case-crossover, time-series studies, case-control studies, and cross-sectional studies with appropriate timing of exposure for the health endpoint of interest

4.1.3 Biological Plausibility

This subsection describes the biological pathways that potentially underlie cardiovascular health effects resulting from short-term inhalation exposure to ozone. Figure 4-1 graphically depicts these proposed pathways as a continuum of pathophysiological responses—connected by arrows—that may ultimately lead to the apical cardiovascular events associated with short-term exposures to ozone at concentrations observed in epidemiologic studies (e.g., ED visits and hospital admissions). This discussion of how short-term exposure to ozone may lead to these cardiovascular events also provides biological plausibility for the epidemiologic results reported later in this Appendix. In addition, most studies cited in this subsection are discussed in greater detail throughout this Appendix.



Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence related to ozone exposure, and the arrows indicate a proposed relationship between those effects. Solid arrows denote evidence of essentiality as provided, for example, by an inhibitor of the pathway or a genetic knockout model used in an experimental study involving ozone exposure. Shading around multiple boxes is used to denote a grouping of these effects. Arrows may connect individual boxes, groupings of boxes, and individual boxes within groupings of boxes. Progression of effects is generally depicted from left to right and color-coded (gray, exposure; green, initial effect; blue, intermediate effect; orange, effect at the population level or a key clinical effect). Here, population level effects generally reflect results of epidemiologic studies. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below.

Figure 4-1: Potential biological pathways for cardiovascular effects following short-term exposure to ozone.

When considering the available health evidence, plausible pathways connecting short-term exposure to ozone with the apical events reported in epidemiologic studies are proposed in Figure 4-1. The first pathway begins as respiratory tract inflammation leading to systemic inflammation. The second pathway involves activation of sensory nerve pathways in the respiratory tract that lead to modulation of the autonomic nervous system. Once these pathways are initiated, there is evidence from experimental and observational studies that short-term exposure to ozone may result in a series of pathophysiological responses that could lead to cardiovascular events such as ED visits and hospital admissions for ischemic heart disease (IHD), heart failure (HF), and possible mortality.

There are plausible pathways through which respiratory tract inflammation and oxidative stress could exacerbate existing IHD and HF and contribute to the development of a myocardial infarction or stroke. Inflammatory mediators, such as cytokines produced in the respiratory tract (Appendix 3), have the potential to enter into the circulatory system where they may amplify the initial inflammatory response and/or cause distal pathophysiological events that can contribute to overt cardiovascular disease.

Thus, it is important to note that there is evidence from epidemiologic panel studies for an increase in the cytokines IL-6 and TNF-α (Mirowsky et al., 2017), as well as for the TNF-α receptor (Li et al., 2016) following short-term exposure to ozone. Similarly, there is also evidence for increases in circulating inflammatory cells (e.g., monocytes, neutrophils) from CHE studies (Stiegel et al., 2016; Biller et al., 2011), an epidemiologic panel study (Mirowsky et al., 2017), and animal toxicological studies (Zhong et al., 2016; Paffett et al., 2015). Generally, increases in cytokines like interleukin 6 (IL-6) have been correlated with increases in liver-derived inflammatory markers such as C-reactive protein (CRP) and the promotion of hemostasis (i.e., the stopping of blood flow), which is characterized by increases in markers of coagulation (i.e., clot promoting factors) and/or decreases in markers of fibrinolysis (i.e., clot dissolving factors) (Tanaka et al., 2014). With respect to short-term ozone exposure, a CHE (Kahle et al., 2015) and an epidemiologic panel (Mirowsky et al., 2017) study reported changes in protein levels associated with fibrinolysis. In addition, CHE (Arjomandi et al., 2015; Biller et al., 2011) and an epidemiologic panel study (Bind et al., 2012) reported increases in serum levels of CRP following short-term exposure. In agreement with these studies, an animal toxicological study (Snow et al., 2018) demonstrated that in rats fed a normal, coconut oil, or fish oil supplemented diet, short-term exposure to ozone resulted in increases in platelet circulation. Platelets typically form a plug when the endothelium is damaged to prevent bleeding. However, platelets can also lead to clot formation when present in the endothelium in the absence of a wound. Taken together, enhanced inflammation, hemostasis, and increases in the circulation of platelets likely enhances the potential for thrombosis, which could exacerbate existing IHD and HF.

In addition to affecting hemostasis, systemic inflammation and/or oxidative stress may result in impaired vascular function. Impaired vascular function stems from impaired functioning of the endothelium, which maintains the normal balance of mediators that promote vasorelaxation (e.g., nitric oxide) and vasoconstriction (e.g., endothelin-1). In endothelial dysfunction, the balance is tipped towards greater production of vasoconstrictors causing increased vascular resistance which could lead to rupture of existing plaques (Halvorsen et al., 2008). Dislodged plaques might then obstruct blood flow to the heart or stimulate intra-vascular clotting (Karoly et al., 2007), both of which could result in acute myocardial ischemia, and set the stage for HF. If the dislodged plaque obstructs blood flow to the brain, there is potential for stroke. Impaired vascular function has been reported following short-term ozone exposure in epidemiologic panel studies (Mirowsky et al., 2017; Lanzinger et al., 2014) and animal toxicological studies (Paffett et al., 2015; Robertson et al., 2013; Chuang et al., 2009). With respect to impaired vascular function, Paffett et al. (2015) reported that following in-vivo ozone exposure, cotreatment of rat coronary artery segments with acetylcholine and an NAPDH oxidase inhibitor improved the vasodilatory response compared to acetylcholine and control, indicating that one likely mechanism of impaired vasodilation was through oxidative stress-related pathways. These results are in agreement with animal toxicological studies reporting increases in markers of oxidative stress following short-term exposure to ozone (Kumarathasan et al., 2015; Martinez-Campos et al., 2012). Moreover, Robertson et al. (2013) used knockout mice to determine that the presence of CD36 was required for ozone-induced impaired vascular function. We also note that clinical indicators of potential ischemia

(e.g., ST segment depression on an electrocardiogram) following short-term exposure to ozone have been shown in an animal toxicological study (Farraj et al., 2012), and increased odds of STEMI (ST-elevation myocardial infarction) have been reported in an epidemiologic panel study (Evans et al., 2016).

Impaired vascular function can also lead to increases in blood pressure (BP) through vasoconstriction. Increases in BP may then exacerbate IHD or HF through altered hemostasis and/or impaired vascular function. For example, in patients with high blood pressure, changes in arterial shear stress due to changes in blood flow (i.e., laminar vs. turbulent) are associated with impaired vascular function (Khder et al., 1998), which as noted above, could lead to a worsening of IHD or HF (Figure 4-1). Thus, it is notable that following short-term ozone exposure, there is evidence for increases in BP from epidemiologic panel (Cakmak et al., 2011) and animal toxicological studies (Farraj et al., 2016; Tankersley et al., 2013). Taken together, there are plausible pathways through which respiratory tract inflammation could exacerbate existing IHD and HF, contribute to the development of a myocardial infarction or stroke, and lead to ED visits and hospital admissions.

There is also evidence that exposure to ozone could lead to these outcomes potentially through activation of sensory nerves in the respiratory tract. Once activated, these nerves send sensory input to autonomic centers in the brain, which in turn relay reflex motor outputs that modulate autonomic tone (e.g., increased sympathetic tone) to the heart and vasculature. Shifts toward increased sympathetic nervous system tone may result in increases in BP and decreases in vascular function, which as mentioned above, could exacerbate IHD and/or HF. It is therefore important to note the evidence from CHE (Arjomandi et al., 2015), epidemiologic panel (Bartell et al., 2013; Cakmak et al., 2011) and animal toxicological (Wagner et al., 2014; McIntosh-Kastrinsky et al., 2013; Wang et al., 2013; Farraj et al., 2012) studies of autonomic nervous system modulation—including limited evidence for a shift toward increased sympathetic tone (as evidenced by changes in HRV)—following short-term ozone exposure. Similarly, there is evidence from epidemiologic panel (Cakmak et al., 2014; Bartell et al., 2013; Sarnat et al., 2006) and animal toxicological (Farraj et al., 2016; Farraj et al., 2012) studies that short-term exposure to ozone can result in conduction abnormalities or arrhythmia. Conduction abnormalities or arrhythmia could then potentially exacerbate IHD and/or HF. Taken together, there are multiple potential pathways by which activation of sensory nerves in the respiratory tract may lead to worsening of IHD or HF.

Overall, the evidence suggests plausible pathways through which short-term exposure to ozone may worsen IHD or HF as well as contribute to the development of MI or stroke (Figure 4-1). These proposed pathways also provide some biological plausibility for ED visits and hospital admissions following short-term ozone exposure. However, considerable uncertainties remain as the evidence supporting some of the individual events in these pathways is limited and not supported by CHE studies. This information will be used to inform causality, which is discussed later in the Appendix (Section 4.1.16).

4.1.4 Heart Failure, Impaired Heart Function, and Associated Cardiovascular Effects

Heart failure refers to a set of conditions in which the heart's pumping action is compromised. In congestive heart failure (CHF), the flow of blood from the heart slows and fails to meet the body's oxygen demand. Edema from heart failure frequently occurs from increased sodium reabsorption resulting in an increase in blood volume (hypervolemia) and fluid retention, which often causes swelling in the lungs or other tissues (typically in the legs and ankles). The effect of short-term ozone exposure on people with CHF, which is a chronic condition, is generally evaluated using ICD codes recorded when a patient is admitted or discharged from the hospital or ED. The relevant diagnostic codes for heart failure are ICD9 428 and ICD10 I50. These codes encompass left, systolic, diastolic, and combined heart failure. In experimental studies, indicators of heart failure include decreased contractility and/or relaxation in response to pharmacological challenge, reduced ejection fraction (i.e., the percentage of blood pumped from the ventricles during each contraction), reduced stroke volume (i.e., the volume of blood pumped per contraction) and reduced cardiac output (stroke volume multiplied by heart rate), as well as decreases in left ventricular developed pressure (LVDP). Of note, the most prevalent form of heart failure is diastolic heart failure (i.e., heart failure where cardiac filling is impaired, but ejection fraction is not).

4.1.4.1 Epidemiologic Studies of Emergency Department Visits and Hospital Admissions

The 2013 Ozone ISA reported the results of several studies in the U.S., Canada, and the U.K., all of which observed null results for the association between CHF-related emergency department or hospital visits and ozone exposure averaged over either 8 or 24 hours. A few additional studies have been conducted since the 2013 Ozone ISA with mixed results (Table 4-3). Specifically:

- While studies conducted in the U.K. and U.S. did not observe positive associations between CHF (alone or combined with hypertensive heart disease) and 8-hour max ozone concentrations (Rodopoulou et al., 2015; Milojevic et al., 2014), a study in St. Louis, MO reported a 5% increase in ED visits (95% CI: 1, 9%)[1] and hospital admissions (95% CI: 2, 9%) for CHF (Winquist et al., 2012) associated with 8-hour max ozone. Similarly, an additional study in St. Louis observed a 4% (95% CI: −1, 10%) increase in ED visits for CHF, which increased to 6% (95% CI: 0, 12%) when CO was included in the model (Sarnat et al., 2015). Copollutant models with either $PM_{2.5}$ or NO_2 did not change the predicted risk for ozone.
- Studies evaluating the role of lifestage in ozone's effects on heart failure reported no notable differences for older adults (≥65 or 70 years) compared with other adult age groups (19−64 or <70 years) (Milojevic et al., 2014; Winquist et al., 2012).

[1] All epidemiologic results standardized to a 15 ppb increase in 24 hour avg, 20 ppb increase in 8 hour daily max, 25 ppb increase in 1 hour daily max ozone concentrations, or a 10-ppb increase in seasonal/annual ozone concentrations to facilitate comparability across studies.

4.1.4.2 Controlled Human Exposure Studies

In the 2013 Ozone ISA, there were no CHE studies examining the relationship between short-term ozone exposure and impaired cardiac function. In a recent study in healthy subjects with or without deletion of GSTM1, Frampton et al. (2015) reported that short-term exposure (3 hours) to ozone (0.1, 0.2 ppm) did not result in statistically significant changes in stroke volume or left ventricular ejection time relative to FA. Results were independent of the GSTM1 phenotype. Additional information on this study can be found in Table 4-4.

4.1.4.3 Animal Toxicological Studies

In the 2013 Ozone ISA, an animal toxicological study demonstrated that ozone exposure resulted in decreased LVDP, rate of change of pressure development, and rate of change of pressure decay (Perepu et al., 2010). Another study demonstrated that ozone exposure resulted in an increase in left ventricular chamber dimensions at end-diastole in young and old mice, as well as a decrease in left ventricular posterior wall thickness at end-systole in older mice (Tankersley et al., 2010). Moreover, these authors also reported a decrease in fractional shortening—an indicator of impaired cardiac contraction characterized by the percent change in left ventricular diameter from end-diastole to end-systole following short-term ozone.

Since the publication of the 2013 Ozone ISA, there is additional evidence from animal toxicological studies that short-term exposure (3−4 hours, some studies with multiple day exposures) to ozone can result in impaired cardiac function. With respect to this evidence, we note the following key points:

- Tankersley et al. (2013) exposed wild-type mice to ozone (0.5 or 0.8 ppm) and then FA, and demonstrated that this short-term exposure resulted in a decrease in LVDP that was not statistically significant, as well as a decrease in left ventricular stroke volume ($p < 0.05$), and an increase in right ventricular pressure ($p < 0.05$) relative to an exposure of FA followed by a second FA exposure. Moreover, an approximately 33% decrease in left ventricular cardiac output relative to FA exposure was reported ($p < 0.05$). Tankersley et al. (2013) also demonstrated that short-term exposure to ozone resulted in a significant decrease in left ventricular minimum and maximum volumes, ($p < 0.05$) as well as an increase in total peripheral resistance. Finally, they also demonstrated through the use of knockout mice that many of these effects may be mediated by the atrial natriuretic peptide gene.
- In mice, Kurhanewicz et al. (2014) reported a decrease in LVDP and other measures of contractility 24-hours post-exposure (0.3 ppm) relative to FA. However, the authors did not denote these results as having statistical significance relative to FA in their figure.
- McIntosh-Kastrinsky et al. (2013) reported that short-term ozone exposure (0.245 ppm) reduced diastolic function (i.e., cardiac filling) as indicated by impaired cardiac relaxation rate (dP/dt_{minimum}) relative to FA exposure in isolated, perfused murine hearts ($p < 0.05$).

- Wang et al. (2013) reported at least some evidence of dissolved myofilaments (a potential indicator of cardiac damage) in right ventricles by microscopy following short-term ozone (0.8 ppm) exposure.

Although results from the studies mentioned above demonstrated an effect of short-term ozone exposure on changes in heart function, other results from these studies showed no effect. That is:

- McIntosh-Kastrinsky et al. (2013) reported that short-term ozone exposure (0.245 ppm) did not result in changes in LVDP, $dP/dt_{maximum}$, or coronary flow rate relative to FA exposure in isolated, perfused murine hearts prior to ischemia. Moreover, following ischemia/reperfusion there was no difference between ozone and FA exposure with respect to time to ischemic contracture, recovery of LVDP, or ischemia-induced infarct size. Similarly, Kurhanewicz et al. (2014) reported that in mice, there were no differences in time to ischemic contracture, or coronary flow rate prior or after ischemia with ozone exposure. They also reported no differences in the recovery of left ventricular developed pressure or pressure development over time post-ischemia.
- Tankersley et al. (2013) did not report changes in left ventricular pressure over time ($dP/dt_{minimum}$ or $dP/dt_{maximum}$) following short-term ozone (0.5, 0.8 ppm) exposure relative to FA in wild-type mice. Similarly, Zychowski et al. (2016) reported that short-term ozone (1.0 ppm) exposure did not result in appreciable right ventricular hypertrophy in mice kept in normal oxygen conditions, nor did ozone exacerbate right ventricular hypertrophy in hypoxia-induced mice.
- Ramot et al. (2015) reported no effect of ozone effect on heart pathology in rats (0.25, 0.5, 1.0 ppm).

Although not demonstrated by all studies, most of the studies presented above report some indicator of impaired cardiac function following short-term ozone exposure (Table 4-5). In addition, there is evidence suggesting that the atrial natriuretic peptide gene may mediate some of these ozone-induced cardiovascular effects.

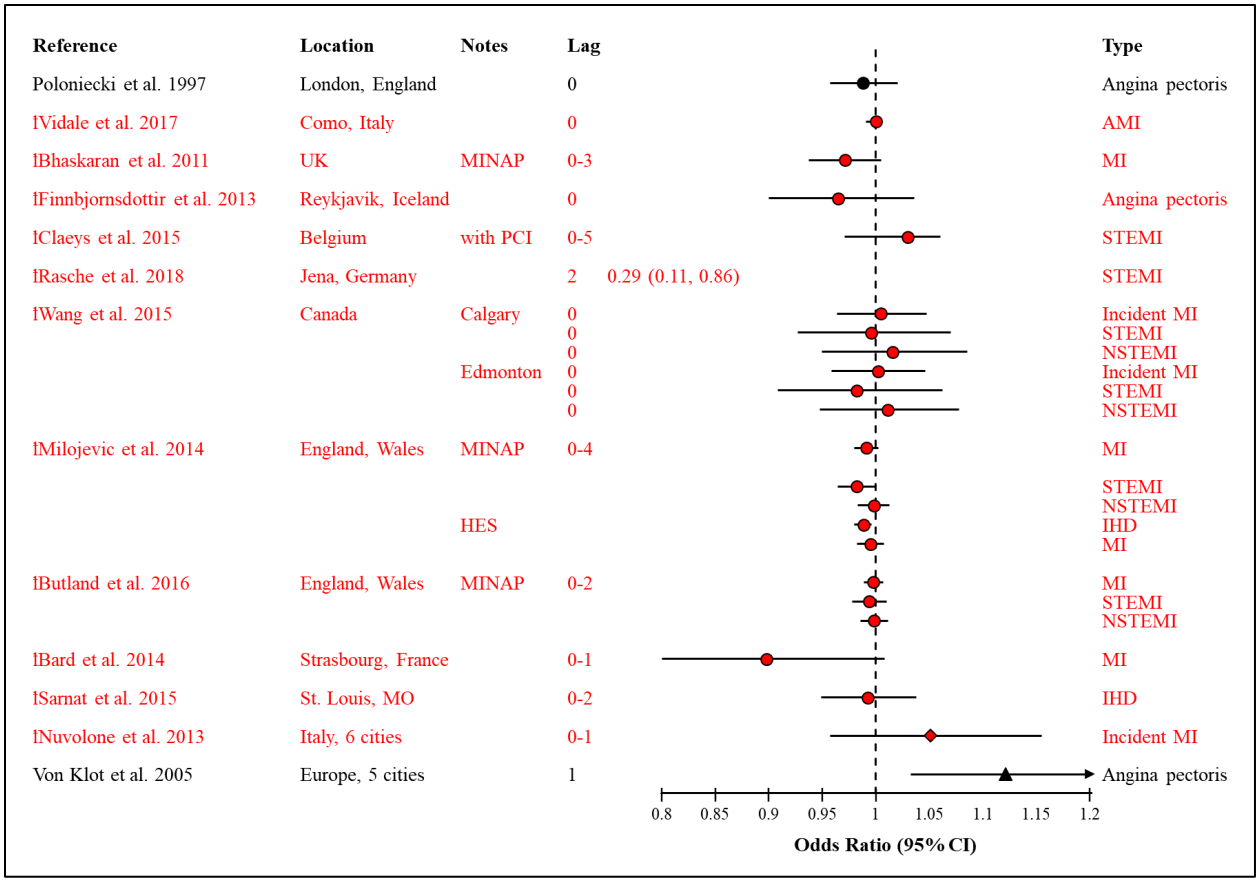
4.1.5 Ischemic Heart Disease and Associated Cardiovascular Effects

IHD is a chronic condition characterized by atherosclerosis and reduced blood flow to the heart. Myocardial infarction (MI), more commonly known as a heart attack, occurs when heart tissue death occurs secondary to prolonged ischemia due to occlusion of the coronary artery. The effect of short-term ozone exposure on acute MI, complications from recent MI, and other acute or chronic IHD are generally evaluated using ICD codes recorded when a patient is admitted or discharged from the hospital or emergency department (ICD9: 410−414 or ICD10: I20−I25). In experimental or epidemiologic panel studies, indicators of MI include ST segment depression as measured by an electrocardiograph (ECG). The ST segment of an electrocardiogram recorded by surface electrodes corresponds to the electrical activity of the heart registered between ventricular depolarization and repolarization, and is normally isoelectric.

4.1.5.1 Epidemiologic Studies of Emergency Department and Hospital Admission Studies

In the 2013 Ozone ISA, all of the studies involving U.S. or European populations reported null effect estimates for IHD and MI, but mixed findings for angina. A multicity study in Europe reported no association for MI, but the same study observed a positive association (OR: 1.19; 95% CI: 1.05, 1.35) for angina pectoris during the warm season (April−September) (von Klot et al., 2005). In contrast, a study in London, reported null results for angina (OR: 0.98; 95% CI: 0.94, 1.03) (Poloniecki et al., 1997).

- Recent studies from Europe, Canada, and the U.S. published since the 2013 ISA, several of which analyzed a large number of MI, IHD or angina events per day in multiple cities, confirm the pattern indicated by the earlier studies. These studies also consistently reported null or small positive effect estimates (i.e., OR ≤ 1.02) in analyses of MI, including ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI) (Table 4-6; Figure 4-2). A study of five urban areas in Tuscany, Italy reported a 5% increase in incident MI associated with an increase in ozone concentrations during the warm season using a 0−1-day distributed lag (95% CI: −4, 16%) (Nuvolone et al., 2013).
- A study in Iceland that analyzed associations with air pollutants, including ozone, and dispensing glyceryl trinitrate against angina pectoris did not observe increases in odds ratios in single pollutant models (Finnbjornsdottir et al., 2013).



AMI = acute myocardial infarction; HES = Hospital Episode Statistics; IHD = ischemic heart disease; MI = myocardial infarction; MINAP = Myocardial Ischaemia National Audit Project; NSTEMI = non-ST-elevation myocardial infarction; PCI = percutaneous coronary intervention; STEMI = ST-elevation myocardial infarction.

Note: †Studies published since the 2013 Ozone ISA. Studies are listed from the top in order of increasing mean or median ozone concentration reported in the publication. Associations are presented per 25 ppb increase in pollutant concentration for 1-h max averaging times, 20 ppb increase for 8-h avg times, and 15 ppb increase for 24-h avg times. Symbols represent point estimates, circles, triangles and squares represent the entire year, warm season and cold season, respectively; horizontal lines represent 95% confidence intervals for ozone. Black text and symbols represent evidence included in the 2013 Ozone ISA; red text and symbols represent recent evidence not considered in previous ISAs or AQCDs.

Figure 4-2 Associations between short-term exposure to ozone and ischemic heart disease (IHD)-related emergency department visits and hospital admissions.

4.1.5.2 Epidemiologic Panel Studies

The 2013 Ozone ISA reported inconsistent results with respect to an association between short-term ozone exposure and MI. One study reported elevated risks for recurrent MIs (Henrotin et al., 2010), while another observed no associations between short-term ozone exposure and ST-segment depression in elderly men with a history of coronary artery disease (Delfino et al., 2011).

Since the 2013 Ozone ISA, one additional study examined the potential for STEMI following short-term ozone exposure (Table 4-7). This study provides evidence of increased incidence of STEMI resulting from increased concentrations of short-term ozone exposure. Specifically, in a cohort of 362 subjects in Rochester, NY with acute coronary syndrome identified as STEMI, NSTEMI, or unstable angina Evans et al. (2016) reported a 35% (OR = 1.35; 95% CI: 1.00, 1.84) increase in the odds of STEMI for exposure over the previous hour; these associations were attenuated but remained positive at 12, 24, 48, and 72 hours prior to the event. Larger increases in the odds of STEMI were observed for increases in ozone concentrations measured over the previous hour for patients with previous MI (OR = 2.06; 95% CI: 0.96, 4.44), CVD (OR = 1.98; 95% CI: 1.02, 3.81), and hypertension (OR = 1.44; 95% CI: 1.00, 2.24). When evaluated by season, the authors observed elevated odds of STEMI in the cooler months (November to April), and decreased odds in the warmer months (May to October) for ozone exposure estimated over the 24, 48, and 72 hours preceding the event.

4.1.5.3 Controlled Human Exposure Studies

In the 2013 Ozone ISA, there were no controlled human exposure studies examining indicators of IHD. That said, a study from the previous AQCD indicated that exposure to ozone did not result in ST segment depression (Gong et al., 1998). Recently, Rich et al. (2018) reported no appreciable change in the ST segment as a result of ozone (0.07, 0.12 ppm) exposure (3 hours) in older adults (Table 4-8).

4.1.5.4 Animal Toxicological Studies

The 2013 Ozone ISA did not have any studies animal toxicological studies examining the relationship between short-term exposure to ozone and the ST segment (U.S. EPA, 2013a). Since the publication of that document, Farraj et al. (2012) reported that in spontaneously hypertensive (SH) rats, short-term (4 hours) exposure to 0.8 but not 0.2 ppm ozone resulted in ST-segment depression during exposure when compared to pre-exposure baseline conditions ($p < 0.05$). However, there were no statistically significant post-exposure effects when compared to baseline. Thus, evidence from animal toxicological studies that short-term exposure to ozone can result in potential indicators of ischemic heart disease is limited. Details from this study can be found in Table 4-9.

4.1.6 Endothelial Dysfunction

Endothelial dysfunction is the physiological impairment of the inner lining of blood vessels that is characterized by an imbalance between vasodilators such as nitric oxide and vasoconstrictors such as endothelin-1 (ET-1) that favors vasoconstrictors. Endothelial dysfunction is typically measured by flow-mediated dilation percentage (FMD%). It is a noninvasive technique involving measurement of the

percentage change in brachial artery diameter (BAD) after reactive hyperemia (increased blood flow following removal of an artery occluding blood pressure cuff) or pharmacological challenge. In addition to measuring FMD or BAD, experimental studies also examine arterial stiffness as indicated by pulse wave velocity and levels of biomarkers such as ET-1.

4.1.6.1 Epidemiologic Panel Studies

In the 2013 Ozone ISA, endothelial biomarkers indicated the potential for cardiovascular disease and injury. However, no epidemiologic studies had evaluated short-term ozone exposure and endothelial function. Recent panel studies have specifically evaluated short-term ozone exposure and the effects on endothelial function (e.g., FMD, BAD) and biomarkers. Considering a number of endpoints in epidemiologic panel studies, there is limited evidence of endothelial dysfunction following short-term ozone exposures (Table 4-10). However, this could be due to differences in study size, demographics, exposure, time lags, and the health endpoints examined across studies. With respect to this evidence, we note:

- Lanzinger et al. (2014) reported FMD decreases in 22 individuals between the ages of 48−78 years with type 2 diabetes at lag 0 (−29.2; 95% CI: −52.6, −5.80) and lag 1 (−27.0; 95% CI: −54.0, −0.08). However, Mirowsky et al. (2017) saw no change in FMD at any lag in 13 men with a previous diagnosis of coronary artery disease.
- In one study of 64 patients with type 2 diabetes, null associations were observed between short-term ozone effects and BAD (Zanobetti et al., 2014) (qualitative results only). However, Mirowsky et al. (2017) observed opposing effects in BAD in a small cohort of 13 men with coronary artery disease. They reported a decrease in BAD at lag 2 (−2.68; 95% CI: −5.36, 0.10) followed by an increase at lag 4 (3.75; 95% CI: 1.29, 6.32).
- Mirowsky et al. (2017) also evaluated several markers of endothelial dysfunction: I-CAM, V-CAM, LAEI, SAEI, and observed a decrease in V-CAM of 10.3% (95% CI: −18.43, −1.29) at a lag 2. Conversely, Bind et al. (2012) used the Normative Aging Study Cohort with 704 men in the greater Boston area who were free from chronic medical conditions and observed no change in either V-CAM or I-CAM (qualitative results only).

4.1.6.2 Controlled Human Exposure Studies

In the last review, Brook et al. (2009) found no effect of ozone exposure alone on clinical indicators of endothelial dysfunction, such as FMD. Recent CHE studies (1−3 hours in duration) have also shown no evidence of an ozone effect. Specifically:

- Barath et al. (2013) reported no decreases in measures of blood flow relative to FA in response to acetylcholine, sodium nitroprusside, verapamil, or bradykinin following ozone exposure (0.3 ppm) in healthy young men. In fact, the study authors reported an increase in blood flow with ozone relative to FA exposure following acetylcholine or nitroprusside challenge.

- Frampton et al. (2015) and Rich et al. (2018) also reported no changes in measures of vascular function (via peripheral arterial tonometry, FMD) due to short-term exposure to ozone (0.07, 0.1, 0.12, 0.2 ppm) in healthy subjects with or without a GSTM1 deletion or in older adults, respectively.

Thus, there is no evidence from CHE studies that short-term ozone exposure results in vasoconstriction. Additional information on these studies can be found in Table 4-11.

4.1.6.3 Animal Toxicological Studies

In the 2013 Ozone ISA, the Chuang et al. (2009) study reported that short-term ozone exposure inhibited acetylcholine-induced vasorelaxation. In addition, a few studies demonstrated that short-term exposure (4 hours, some studies with multiple day exposures) to ozone was associated with an increase in the vasoconstrictor ET-1. Since the publication of the 2013 ISA, additional studies have reported similar effects following short-term exposure to ozone (Table 4-12). With respect to this evidence, we note the following key points:

- In rats, Paffett et al. (2015) demonstrated that short-term ozone (1.0 ppm) exposure resulted in increased vasoconstriction and reduced vasodilation relative to control animals following ex-vivo treatment of coronary artery segments with serotonin and acetylcholine respectively ($p < 0.05$). The authors demonstrated that cotreatment of coronary artery segments with acetylcholine and an NAPDH oxidase inhibitor improved the vasodilatory response, suggesting one likely mechanism of impaired vasodilation was through oxidative-stress-related pathways (Paffett et al., 2015).
- Impaired vasodilation relative to control animals in response to acetylcholine was also reported in wild-type, but not CD 36 null, mouse abdominal and thoracic aortic segments following ozone (1.0 ppm) exposure (Robertson et al., 2013) ($p < 0.05$). These authors also provided some evidence that decreased vasodilation in wild type mice was due to impaired endothelial release of NO.
- In a dietary intervention study, relative to control rats, (Snow et al., 2018) reported significant phenylephrine-induced vasoconstriction in aortic rings from ozone-exposed rats fed either a normal diet ($p < 0.05$) or a diet supplemented with coconut oil, or olive oil ($p < 0.05$), but not in rats supplemented with fish oil prior to ozone exposure. However, neither ozone nor diet resulted in an impaired vasodilation response to acetylcholine or sodium nitroprusside (Snow et al., 2018).

With respect to blood markers of vasodilation, vasoconstriction, or vascular damage:

- Paffett et al. (2015) demonstrated decreased serum levels of NO_2/NO_3 in ozone (1.0 ppm)-exposed animals relative to FA ($p < 0.05$).
- In rats, Kumarathasan et al. (2015) found an increase in plasma ET-1 and BET-1 (i.e., the precursor to ET-1) following exposure to 0.8, but not 0.4 ppm ozone immediately after and 24-hours post-exposure ($p < 0.05$). Similarly, Thomson et al. (2013) reported increased ET-1 mRNA expression in rat heart tissue following short-term ozone (0.4, 0.8 ppm) exposure relative to control exposure.
- In contrast to these results, Wang et al. (2013) reported no difference in plasma levels of ET-1 or VEGF when comparing rats exposed to ozone (0.8 ppm) to animals exposed to FA.

Overall, the animal toxicological evidence is generally consistent. Some studies demonstrated increased vasoconstriction while others showed impaired vasodilation. This evidence is further supported by studies reporting increased blood markers associated with vasoconstriction and/or endothelial injury.

4.1.7 Cardiac Depolarization, Repolarization, Arrhythmia, and Arrest

In epidemiologic studies, the effect of short-term ozone exposure on arrhythmia is generally evaluated using ICD codes for ED visits, hospital admissions, and out-of-hospital cardiac arrests (OHCA). In addition, there is a body of epidemiologic studies that examine arrhythmias recorded on implantable cardio-defibrillators.

Experimental and epidemiologic panel studies typically use surface ECGs to measure electrical activity in the heart resulting from depolarization and repolarization of the atria and ventricles. The P wave of the ECG represents atrial depolarization, while the QRS represents ventricular depolarization and the T wave, ventricular repolarization. Because the ventricles account for the largest proportion of heart mass overall and thus are the primary determinants of the electrical activity recorded in the ECG, ECG changes indicating abnormal electrical activity in the ventricles are of greatest concern. Changes in QT, ST, as well as changes in T-wave shape, duration or amplitude may indicate abnormal impulse propagation in the ventricles. Cardiac arrhythmias can vary in severity from the benign to the potentially lethal, such as in cardiac arrest when an electrical disturbance disrupts the heart's pumping action causing loss of heart function.

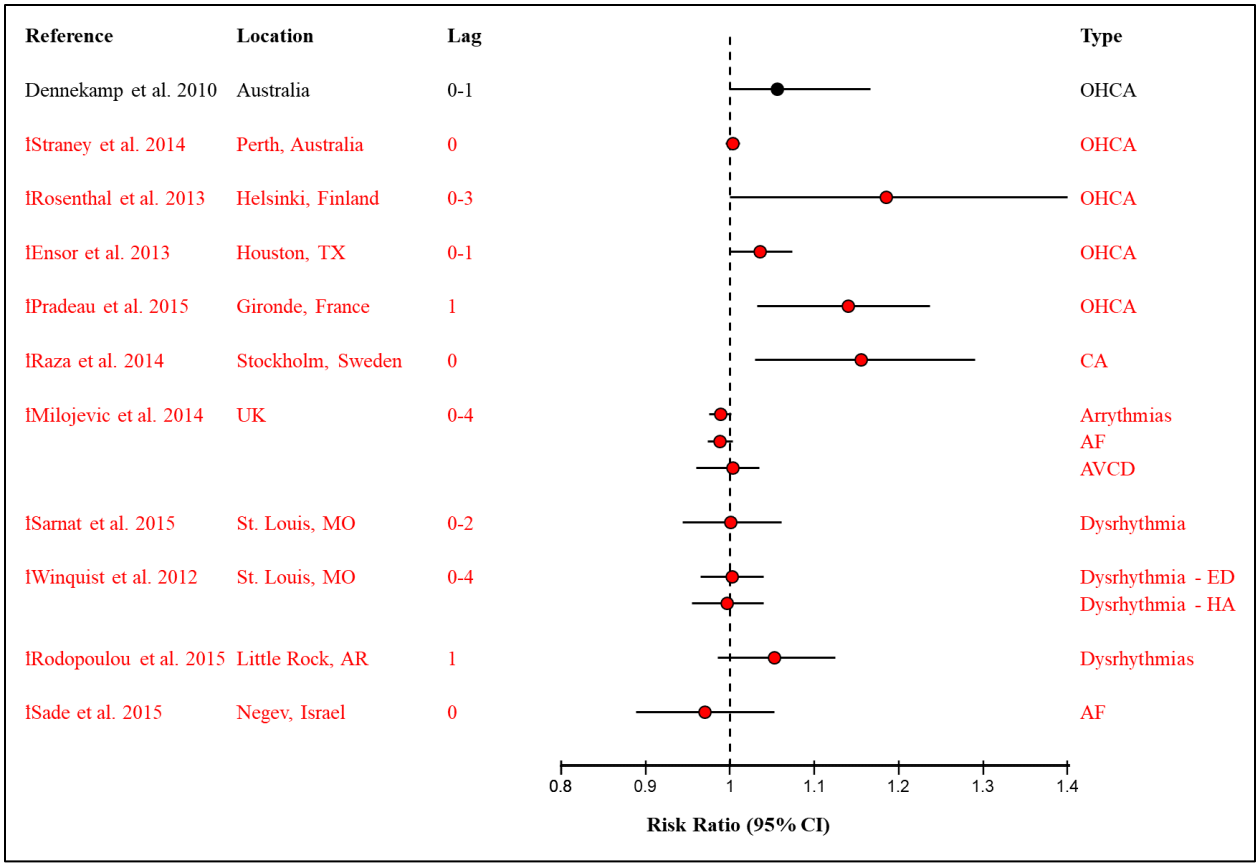
4.1.7.1 Epidemiologic Studies of Emergency Department Visits and Hospital Admissions

Few studies evaluating short-term ozone exposure and cardiac arrest, arrhythmias, or dysrhythmias were discussed in the 2013 Ozone ISA. Two studies in the U.S. and Australia reported no positive associations for out-of-hospital cardiac arrest (Dennekamp et al., 2010; Silverman et al., 2010). A modest elevation in risk of arrhythmia was associated with 8-hour max ozone concentrations during the warm season in Helsinki, Finland (OR: 1.04; 95% CI: 0.8, 1.35) (Halonen et al., 2009), but null results were reported in Atlanta, London, and a multicity study in Canada (Stieb et al., 2009; Peel et al., 2007; Poloniecki et al., 1997). Several recent studies in the U.S., Europe, and Australia have analyzed the association between ozone concentration and cardiac arrest, arrhythmias, or dysrhythmias. Findings from these studies indicate increases in out-of-hospital cardiac arrests associated with 8-hour max or 24-hour avg increases in ozone concentrations; however, null associations are reported for other endpoints (e.g., dysrhythmia, arrhythmia, or atrial fibrillation). (Table 4-13; Figure 4-3). Specifically:

- In Europe, odds ratios for out-of-hospital cardiac arrest associated with 24-hour average ozone concentration were 1.18 (95% CI: 1.00, 1.41) in Helsinki, Finland, 1.13 (95% CI: 1.03, 1.24) in

Gironde Department in France, and 1.16 (95% CI: 1.03, 1.29) in Stockholm County, Sweden (Pradeau et al., 2015; Raza et al., 2014; Rosenthal et al., 2013). These associations were observed in models using the average of the previous 3 days, a 1 day constrained lag, or the concentration on the same day as the hospitalization, respectively. Rosenthal et al. (2013) presented the results of models of cardiac arrest risk stratified by the underlying cause of the event, either acute myocardial infarction or other cardiac causes. The model results indicated that the elevated risk for cardiac arrest was primarily due to causes other than acute myocardial infarction. The odds ratios increased and remained statistically significant in copollutant models with $PM_{2.5}$, PM_{10}, other particulate size classes, NO, NO_2, SO_2, or CO. Raza et al. (2014) analyzed a high number of events per day and confirmed the independent effect of ozone on cardiac arrest in a copollutant model with NO_2. The authors also observed an exposure response pattern in a categorical analysis using 10.2 ppb increments from 11.7 ppb to >66 ppb.

- In contrast to the associations observed in Finland, France, and Sweden, a study in Perth, Australia analyzed hourly lags and cumulative hourly lags over a 48-hour period, and observed no association with out-of-hospital cardiac arrest and 1-hour max ozone concentrations (Straney et al., 2014).
- For a study in Houston, TX, an OR for cardiac arrest of 1.04 (95% CI:1.00, 1.07) was associated with an increase in 8-hour max ozone concentration, and the risk was higher during the warm season (Ensor et al., 2013).
- A few other studies assessed whether risk ratios varied by season, but no clear trend was apparent. In contrast to the findings by Ensor et al. (2013), no notable seasonal differences in the risk of either OHCA or onset of atrial fibrillation were observed by other studies (Pradeau et al., 2015; Sade et al., 2015; Rosenthal et al., 2013).
- A number of studies evaluating the onset of dysrhythmia or atrial fibrillation, identified via ED or hospital admission records, did not observe increased risk ratios associated with ozone concentrations using single- or multiple-day lags (Sade et al., 2015; Sarnat et al., 2015; Milojevic et al., 2014; Winquist et al., 2012). However, a study in Little Rock, AR observed a moderately increased risk ratio for conduction disorders and dysrhythmias associated with an 8-hour max ozone concentration using a 1-day lag (OR: 1.05; 95% CI: 0.99, 1.12) (Rodopoulou et al., 2015).
- Risk comparisons of OHCA by sex did not consistently indicate greater susceptibility for either men or women. Rosenthal et al. (2013) found the risk of out-of-hospital cardiac arrest from causes other than acute MI to be larger in women (OR = 1.76; 95% CI: 1.33−2.33, lag 1 day) than in men (*p*-value for difference by sex = 0.003). Another study reported increased odds ratios for OHCA with presumptive cardiac etiology for both women and men, although the odds ratios were higher among women (Pradeau et al., 2015). An opposite pattern was observed by Ensor et al. (2013) in their Texas study; the increased RR of OHCA associated with the average 1−3 hour lagged ozone concentration was statistically significant for men and higher than the RR for women.
- The risk of OHCA associated with 24-hour avg ozone concentrations were reported to be higher by two studies for individuals older than 64 years (Pradeau et al., 2015; Ensor et al., 2013).



AF = atrial fibrillation; AVCD = atrioventricular conduction disorders; CA = cardiac arrest; ED = emergency department; HA = hospital admissions; OHCA = out of hospital cardiac arrest.

Note: †Studies published since the 2013 Ozone ISA. Studies are listed from the top in order of increasing mean or median ozone concentration reported in the publication. Associations are presented per 25-ppb increase in pollutant concentration for 1-h max avg times, 20-ppb increase for 8-h avg times, and 15-ppb increase for 24-h avg times. Circles represent point estimates; horizontal lines represent 95% confidence intervals for ozone. Black text and circles represent evidence included in the 2013 Ozone ISA; red text and circles represent recent evidence not considered in previous ISAs or AQCDs.

Figure 4-3 Associations between short-term exposure to ozone and emergency department (ED) visits and hospital admissions related to cardiac arrest, arrhythmias, and dysrhythmias.

4.1.7.2 Epidemiologic Panel Studies

The 2013 Ozone ISA stated that many studies reported positive associations for arrhythmia-associated endpoints, yet collectively, results were inconsistent. In a population of subjects with an implantable cardioverter defibrillator (ICD), Metzger et al. (2007) observed no evidence of an association for tachyarrhythmic events with an increase in ozone concentrations. In contrast, in a study of nonsmoking adults, increased odds were observed for supraventricular ectopy (Sarnat et al., 2006). In the

few studies published since the 2013 Ozone ISA, none have the same endpoints so the results remain inconsistent (Table 4-14). Specifically:

- In a cohort of 50 elderly nonsmokers with previous coronary artery disease, Bartell et al. (2013) observed relatively strong associations between short-term ozone exposure and daily ventricular tachycardia (VT) events, specifically for the 24 hour avg lag period (RR = 1.50; 95% CI: 1.10, 2.05) and at the 3-day avg lag period (RR = 2.54; 95% CI: 1.25, 5.18). A secondary analysis, which adjusted for daytime and evening hours, found opposing associations for VT events: positive associations in the nighttime hourly exposure and negative associations for daytime hourly exposure. Specifically, at 24 hours after the increase in ozone exposure, the daytime odds ratio was 0.69 (95% CI: 0.40, 1.21) and the evening odds ratio was 2.34 (95% CI: 1.27, 4.32).
- In a study conducted in Boston, MA using 2,369 participants in the Framingham Heart Study Third Generation and Offspring Cohorts, a positive association between ozone and pulse amplitude was identified for the 2-day moving avg lag period (7.63%; 95% CI: 0.87, 14.40%). However, there was no change in pulse amplitude for the 1-,3-,5-, or 7-day moving avg lag periods (Ljungman et al., 2014).
- Cakmak et al. (2014) looked at eight endpoints of cardiac rhythm in 8,662 patients in Ottawa, Ontario and Gatineau, Quebec, Canada referred for 24 hour ambulatory cardiac monitoring. An increase in AV block (1.13%; 95% CI: 1.01, 1.26%) was observed for an increase of 15.67 ppb ozone calculated as a 3 hour max. When stratified by season, AV block was still elevated by 1.23% (95% CI: 1.07, 1.42%) in the warm season from April to September. Additionally, in the cold season, an increased number of supraventricular ectopic runs (8.15%; 95% CI: 0.34, 16.57%) was observed.

4.1.7.3 Controlled Human Exposure Studies

In the 2013 Ozone ISA Devlin et al. (2012) reported that the QTc interval significantly increased immediately after ozone exposure and that the QRS complex significantly decreased immediately after ozone exposure. However, an additional study from the previous review noted that ventricular repolarization was most affected by the combined pollutant exposure of ozone and PM rather than to ozone alone in healthy adults (Sivagangabalan et al., 2011). Similarly, a study from the 2006 AQCD noted that short-term ozone exposure alone did not result in ECG abnormalities (Gong et al., 1998). CHE studies published since the 2013 Ozone ISA provide limited evidence that short-term ozone exposure (2−3 hours) can appreciably effect cardiac electrophysiology. That is:

- In older adults, Rich et al. (2018) reported that short-term exposure to ozone (0.07, 0.12 ppm) did not result in changes in a variety of cardiac electrophysiological endpoints, including the QTc interval, QRS complexity, or T-wave amplitude. Moreover, there was no ozone effect on ventricular or supraventricular arrhythmia. However, the authors did report a trend toward an increase in the probability of ventricular but not supraventricular ectopy couplets or runs at 0.070 ppm (but not 0.12 ppm).
- In healthy adults, Kusha et al. (2012) reported a significant change in T-wave alternans during the first 5 minutes of exposure (0.12 ppm) ($p < 0.05$) relative to FA, but no change relative to FA later in the exposure. The authors speculated that the significant effect reported during the first

5 minutes of exposure was likely an artifact. Thus, they concluded that there was little evidence of an ozone-induced effect on t-wave alternans.

Altogether, there is very limited evidence from CHE studies indicating that ozone exposure may result in conduction abnormalities or arrhythmia. Additional information about these studies can be found in Table 4-15.

4.1.7.4 Animal Toxicological Studies

The 2013 Ozone ISA describes studies from the 2006 Ozone AQCD reporting an effect of short-term ozone exposure on cardiac electrophysiology and indicators of arrhythmia. For example, the 2013 ISA notes that short-term ozone exposure in rats induced premature atrial contraction, indicators of atrial block, and arrhythmia (U.S. EPA, 2013a). Recent studies demonstrate similar effects resulting from short-term exposure (3−4 hours, some studies with multiple day exposures) to ozone.

- Farraj et al. (2012) found that in spontaneously hypertensive (SH) rats, short-term exposure to a higher (0.8 ppm), but not a lower (0.2 ppm) ozone concentration resulted in a decrease in the QTc interval and an increase in the PR interval (indicative of atrial block, $p < 0.05$) during exposure. No post-exposure effects were reported. In addition, the authors demonstrated that during exposure, 0.8 ppm ozone resulted in an increase in atrial premature beats, atrioventricular block, and sinoatrial block. Importantly, this study also found that after 18-hours ozone exposure, both levels of ozone reduced increased sensitivity to aconitine-induced arrhythmia ($p < 0.05$). Similar results were also found in an another study by this group (Farraj et al., 2016).
- In contrast, Wang et al. (2013) noted that short-term ozone (0.8 ppm) exposure in normotensive rats resulted in ECGs that were similar to control exposures. Similarly, in normotensive mice, Kurhanewicz et al. (2014) reported no significant effect of ozone (0.3 ppm) exposure on ECG readings, including QRS, PR, and QTc, relative to FA exposure.

Overall, the results of these studies provide evidence that in SH rats, short-term exposure to ozone can result in conduction abnormalities and indicators of arrhythmia (Table 4-16). Importantly, these results also suggest that even at ozone exposure concentrations that do not result in overt symptoms, these ozone exposures could “prime” SH rats for arrhythmic responses to an arrhythmogenic agent (e.g., aconitine) at lower concentrations than would normally be expected. That said, results in normotensive rats indicated that short-term ozone exposure did not significantly alter ECG measures.

4.1.8 Blood Pressure Changes and Hypertension

High blood pressure is typically defined as a systolic blood pressure above 130 mm Hg or a diastolic blood pressure above 80 mm Hg. Hypertension, the clinically relevant consequence of chronically high blood pressure, typically develops over years. Systolic blood pressure (SBP) represents the pressure in the arteries as the heart contracts, while diastolic blood pressure (DBP) represents the pressure in the arteries as the heart is relaxed and is filling with blood. Prolonged high blood pressure is

known as hypertension and can lead to a thickening of the ventricular wall resulting in diminished filling during diastole. This can ultimately contribute to the development of arrythmia and heart failure. Pulse pressure (PP) or the difference between SBP and DBP, as well as mean arterial pressure (MAP), which is a function of cardiac output, systemic vascular resistance, and central venous pressure, are additional outcome metrics used in studies of air pollution on blood pressure. Moreover, hypertension is one of the array of conditions including high blood sugar, excess body fat around the waist, and abnormal triglyceride levels that comprise metabolic syndrome (see Appendix 5), which is a risk factor for heart disease, stroke, and diabetes.

4.1.8.1 Epidemiologic Studies of Emergency Department Visits and Hospital Admissions

No time-series or case-crossover studies analyzing ED visits or hospital admissions for hypertension were reported in the 2013 Ozone ISA. Recent evidence is limited in number and generally inconsistent.

- A study of ED visits for hypertension in two Canadian cities, Calgary and Edmonton, reported an increased OR of 1.15 among women (95% CI: 1, 1.31), but not men, during the warm season (Brook and Kousha, 2015). No association was observed for women or men during the cold season. A study in Lithuania analyzed emergency medical service records of emergency calls for exacerbations of essential hypertension with elevated arterial blood pressure and found associations with 8-hour max ozone concentrations primarily during the warm season (Vencloviene et al., 2017). While median ozone concentrations in the two study areas were similar (approximately 20 ppb), the maximum concentration in Kaunas, Lithuania (102 ppb) was twice that in the two Canadian cities (50 ppb). No association with ED visits for hypertension and 8-hour max ozone concentration was reported in a time-series study in Arkansas, an area with a higher median ozone concentration (39 ppb) compared with the two other studies that analyzed associations with hypertension (Rodopoulou et al., 2015) (Table 4-17).

4.1.8.2 Epidemiologic Panel Studies

Limited evidence was available in the 2013 Ozone ISA regarding the association between blood pressure endpoints and short-term ozone exposure. One study found a positive association between subjects with CVD and higher DBP associated with a 5-day avg; however, the effect estimate was not sustained when the model was adjusted for $PM_{2.5}$ (Zanobetti et al., 2004). The evidence from recent studies remains inconsistent and is characterized in Table 4-18. Specifically:

- Cakmak et al. (2011) observed positive associations between ozone concentration and resting SBP (1.17 mm Hg; 95% CI: 0.29, 2.05) and resting DBP (0.65 mm Hg; 95% CI: 0.06, 1.23) in a nationwide Canadian cohort with 5,011 subjects. However, in 70 subjects with pre-existing type 2 diabetes an opposing effect was observed over a 5-day mean of ozone exposure with decreases in MAP (−3.15; 95% CI: −5.86, −0.34), SBP (−4.51; 95% CI: −7.44, −1.58), and DBP (−2.26; 95% CI: −4.74, 0.02) (Hoffmann et al., 2012). Additionally, in a cohort of Canadian children ages

6−17 years of age, Dales and Cakmak (2016) observed increases in SBP (4.41; 95% CI: 1.91, 6.93) and DBP (3.55; 95% CI: 1.01,6.08) in children clinically diagnosed with a mood disorder and no change in SBP (−0.52; 95% CI: −1.18, 0.14) or DBP (−0.24; 95% CI: −0.85, 0.36) in children without a diagnosed mood disorder. Yet, several additional studies reported no changes in blood pressure measures (Cole-Hunter et al., 2018; Mirowsky et al., 2017; Cakmak et al., 2014).

4.1.8.3 Controlled Human Exposure Studies

In the 2013 Ozone ISA, CHE studies indicated that short-term ozone exposure alone did not have an effect on diastolic blood pressure (Sivagangabalan et al., 2011; Brook et al., 2009; Fakhri et al., 2009). Since the publication of the 2013 Ozone ISA, CHE studies continue to report little evidence of an effect of short-term (1−3 hours) ozone exposure on measures of blood pressure. Specifically:

- Frampton et al. (2015) reported a blunting of an exercise-induced increase in SBP ($p < 0.05$) (0.2 ppm), with no change in DBP following ozone exposure (0.1, 0.2 ppm) in healthy subjects with or without GSTM1 deletion. However, the authors were unclear of the clinical significance of the effect. Other CHE studies reported no effect of short-term ozone exposure (0.3, 0.7, 0.12 ppm) on SBP, DBP, or angiotensin converting enzyme (ACE) levels in healthy or older adults (Rich et al., 2018; M et al., 2015; Barath et al., 2013). Additional information with respect to these studies can be found in Table 4-19.
- Stiegel et al. (2017) reported a significant decrease in DBP but not SBP in response to ozone exposure (0.3 ppm) in healthy adults when comparing post vs pre-exposure.

4.1.8.4 Animal Toxicological Studies

The 2013 Ozone ISA cited a study by Chuang et al. (2009) that reported an increase in BP in mice following short-term ozone exposure when compared with control animals. Since the publication of the 2013 ISA, there is additional evidence from some, but not all studies to suggest that short-term exposure (3−8 hours, some studies with multiple day exposures) to ozone can result in changes in blood pressure in animals (Table 4-20). Moreover, some results also suggest that changes in diet may mediate these effects. With respect to this evidence we note the following key points:

- Farraj et al. (2016) reported that relative to FA exposed animals, SH rats exposed to ozone (0.3 ppm) alone experienced an increase in pulse pressure and a decrease in DBP ($p < 0.05$). No change in SBP was reported.
- In rats fed a high fructose diet, Wagner et al. (2014) reported a decrease in SBP, DBP, and MAP ($p < 0.05$) with ozone (0.5 ppm). In contrast, ozone-exposed rats fed a normal diet displayed an increase in DBP ($p < 0.05$). Furthermore, Tankersley et al. (2013) demonstrated an increase in right ventricular systolic pressure and total peripheral resistance in ozone (~0.5 ppm)-exposed mice compared to control mice ($p < 0.05$).
- No differences in SBP were found in studies of rats following short-term exposure to ozone (0.8 ppm) 24-hours after six exposures (Wang et al., 2013). Also, Ramot et al. (2015) reported no

change in ACE activity following short-term ozone exposure (0.25, 0.5, 1 ppm) in several different mouse strains.

4.1.9 Heart Rate (HR) and Heart Rate Variability (HRV)

Heart rate (HR), a key prognostic indicator, is modulated at the sinoatrial node of the heart by both parasympathetic and sympathetic branches of the autonomic nervous system and represents the number of times the heart beats in a given time frame (e.g., per minute). In general, increased sympathetic activation increases HR, while enhanced activation of parasympathetic, vagal tone, decreases HR (Lahiri et al., 2008). Heart rate variability (HRV) represents the degree of difference in the inter-beat intervals of successive heartbeats. Given that both arms of the autonomic nervous system contribute, changes in HRV are an indicator of the relative balance of sympathetic and parasympathetic tone to the heart and their interaction (Rowan III et al., 2007). Low HRV is associated with an increased risk of cardiac arrhythmia and an increased risk of mortality in patients with congestive heart failure awaiting a heart or lung transplant (Fauchier et al., 2004; Bigger et al., 1992). Low HRV has also been shown to be predictive of coronary artery disease (Kotecha et al., 2012). Notably, increases in HRV have also been associated with increases in mortality (Carll et al., 2018). In general, the two most common ways to measure HRV are time-domain measures of variability and frequency-domain analysis of the power spectrum. With respect to time-domain measures, the standard deviation of NN intervals (i.e., normal to normal or the interval between consecutive normal beats; SDNN) reflects overall heart rate variability, and root-mean-square of successive differences in NN intervals (rMSSD) reflect parasympathetic influence on the heart. In terms of frequency domain, high frequency (HF) domain is widely thought to reflect cardiac parasympathetic activity while the low frequency (LF) domain has been posited as an indicator of the interaction of the sympathetic and parasympathetic nervous systems (Billman, 2013), although its linkage with sympathetic tone is controversial and uncertain (Notarius et al., 1999).

4.1.9.1 Epidemiologic Panel Studies

The 2013 Ozone ISA noted inconsistent results in studies for HRV. It specifically noted that studies showing positive associations were in the same geographic area and that ozone may have been a proxy for other pollutants [U.S. EPA (2013a) pgs. 6-172 to 6-175]. Since the last ISA, studies evaluating heart rate and HRV have continued to have inconsistent results (Table 4-21). The inconclusive evidence may result from the variations in studies, including, but not limited to, sample size, demographics, exposure, and time lags evaluated for these endpoints. For example:

- Several studies that evaluated resting heart rate observed inconsistent results. One study of 5,011 subjects aged 6−79 years in the Canadian Health Measures Survey showed an increase of 0.90 BPM (95% CI: 0.18, 1.63) with short-term exposure to ozone (Cakmak et al., 2011). However, Cole-Hunter et al. (2018), who used 227 subjects from the TAPAS and EXPOsOMICS cohorts in Barcelona, Spain, did not observe changes in heart rate when assigning spatially

weighted ozone exposure according to residential address or when using a mixed model to assign exposure based on home and work address. Cakmak et al. (2014) used a population of 8,662 Ottawa and Gatineau patients referred for 24 hour ambulatory cardiac monitoring with exposure linked to the monitor closest to their home address and observed no differences in resting heart rate due to short-term exposure to ozone. Finally, in a cohort of Canadian children ages 6−17 years, Dales and Cakmak (2016) observed no change in heart rate (bpm) in children clinically diagnosed with a mood disorder (2.47; 95% CI: −1.52, 6.47) or in children without a diagnosed mood disorder (−0.42; 95% CI: −1.36, 0.52). However, the heart rate was higher in the clinically diagnosed population.

- Two studies evaluated the HRV measures SDNN and rMSSD in elderly populations with previously diagnosed coronary artery disease (CAD) (Mirowsky et al., 2017; Bartell et al., 2013). (Bartell et al., 2013) found decreases of −9.21% (95% CI: −15.80, −2.63%) for SDNN and −9.03% (95% CI: −19.23, 1.15%) for rMSSD in a pool of 50 elderly nonsmokers in the Los Angeles area (mean 24-hour avg ozone concentration 27.1 ppb). Conversely, Mirowsky et al. (2017) observed no change in these variables in 13 elderly men in the vicinity of Chapel Hill, NC (mean 24 hour avg ozone concentration 26.0 ppb).

4.1.9.2 Controlled Human Exposure Studies

In the 2013 Ozone ISA, a couple of controlled human exposure studies demonstrated limited evidence of changes in HRV following short-term ozone exposure. More specifically, both studies reported changes in HF following short-term ozone exposure. However, one study demonstrated an increase in HF, while the other reported a decrease (Devlin et al., 2012; Fakhri et al., 2009). In addition, there was some evidence of a trend for an increase in SDNN (Fakhri et al., 2009). One CHE study reported an increase in HR following ozone exposure in a combined group of hypertensive and healthy controls (Gong et al., 1998).

Since the publication of the 2013 Ozone ISA, additional CHE studies have examined the relationship between short-term exposure (1 to 4 hours) to ozone and HRV-related measures, but evidence of an ozone-mediated effect remains limited. There is also no evidence from more recent CHE studies for an ozone effect on HR. With respect to this evidence we note the following:

- In healthy men (Barath et al., 2013) and older adults (Rich et al., 2018), no changes in time and frequency measures of HRV following short-term ozone (0.07, 0.12, 0.3 ppm) exposure were reported.
- However, Arjomandi et al. (2015) reported that decreases in normalized HF and increases in normalized LF were statistically significantly associated with increasing ozone (0.1, 0.2 ppm) concentrations from 0 to 24 hours, but not from 0 to 4 hours in a group of asthmatics and nonasthmatics (measurements were taken at 0, 4, and 24 hours). However, no changes were reported in time-domain measures of HRV. Additional information about these studies can be found in Table 4-22.
- No CHE study reported a statistically significant effect of ozone (0.07, 0.1, 0.12, 0.2, 0.3 ppm) on changes in HR (Rich et al., 2018; Arjomandi et al., 2015; Frampton et al., 2015; Barath et al., 2013; Kusha et al., 2012).

4.1.9.3 Animal Toxicological Studies

The 2013 Ozone ISA presented some evidence that short-term exposure to ozone could result in changes in HR and HRV [U.S. EPA (2013a), pgs. 6-203 to 6-204]. With respect to HR, subsequent studies in animals have reported inconsistent results following short-term ozone exposure (3−8 hours, some studies with multiple-day exposures):

- McIntosh-Kastrinsky et al. (2013) reported a decrease in HR in mice following short-term ozone exposure (0.245 ppm) relative to FA, but not 40 minutes after reperfusion following ischemia. Note, however, that the results following reperfusion could have been due to the ex vivo nature of the experiment.
- Farraj et al. (2012) found that in rats, short-term exposure to a higher (0.8 ppm), but not a lower (0.2 ppm), ozone concentration resulted in a 22.1% decrease in HR relative to pre-exposure baseline levels ($p < 0.05$). In an additional study by this group (Farraj et al., 2016), no change in rat HR was reported following a FA exposure in the morning of Day 1 and a 0.3 ppm ozone exposure in the afternoon of Day 2 (relative to FA exposures on both days). Similarly,Wang et al. (2013) reported no change in HR following short-term exposure to ozone in rats.
- Kurhanewicz et al. (2014) reported, no change in HR following short-term exposure to ozone (0.3 ppm) in mice before ischemia. However, a significant decrease in HR was found relative to FA 20 minutes after reperfusion in the ozone group.
- Wagner et al. (2014) reported that rats fed either a normal or high fructose diet had a significantly decreased HR during a multiday ozone exposure (0.5 ppm) relative to FA. More information about these studies can be found in the Table 4-23.

With respect to HRV, there is some recent evidence in studies of rodents that short-term exposure (3−4 hours) to ozone can result in changes in HRV. It also appears from the limited available evidence that the direction of this change may be dependent upon the exposure concentration, duration, and time point examined. More specifically:

- In rats, Farraj et al. (2012) reported that exposure to a higher (0.8 ppm), but not a lower (0.2 ppm) ozone concentration resulted in an *increase* in both time and frequency measures of HRV during exposure, but not post-exposure relative to baseline. In an additional study by this group (Farraj et al., 2016), a *decrease* in time and frequency domains of HRV and no change in the LF:HF ratio were reported in rats 24-hours after a FA exposure in the morning of Day 1 and a 0.3 ppm ozone exposure in the afternoon of Day 2 (relative to FA exposures on both days). Together, these results suggest that ozone exposure may initially result in a parasympathetic response, but some hours later result in a transition to a more sympathetic response. The extent to which this phenomenon may apply to humans, however, remains unclear.
- In rats, Wang et al. (2013) reported an increase in LF after six but not three exposures to ozone (0.8 ppm) (see Table 4-23) and no change in HF or the LF:HF ratio at either time point.
- During a multiday ozone exposure (0.5 ppm) relative to FA, Wagner et al. (2014) reported a significant increase in SDNN and RMSDD in SD rats fed a normal diet and in RMSDD, but not SDNN in rats fed a high fructose diet.

- In addition, Kurhanewicz et al. (2014) reported no changes in time or frequency domains of HRV during or 1-hour post ozone exposure (0.3 ppm) in mice. More information about these studies can be found in the Table 4-23.

4.1.10 Coagulation and Thrombosis

Coagulation refers to the process by which blood changes from a liquid to a semisolid state to form a clot. Increases in coagulation factors (e.g., fibrinogen, thrombin) or decreases in factors that promote fibrinolysis like tissue plasminogen activator (tPA) can promote clot formation, and thus, increase the potential for MI.

4.1.10.1 Epidemiologic Studies of Emergency Department Visits and Hospital Admissions

In a case-crossover study of cases identified from discharge data in Spain from 2001−2013, an increased risk of pulmonary embolism was reported for ozone concentrations averaged over the 3 days around the time of diagnosis as compared to the average concentration for a similar period 3 weeks prior (de Miguel-Diez et al., 2016). No associations were observed when control periods closer to the time of diagnosis were analyzed. No associations with first diagnosis for pulmonary embolism and average monthly ozone concentration were reported by a case-control study in Italy (Spiezia et al., 2014) or in a case-crossover study in the U.K. that analyzed 8-hour max ozone concentrations and lags of 0−4 days (Milojevic et al., 2014) (Table 4-24).

4.1.10.2 Epidemiologic Panel Studies

Previously, short-term exposure to ozone showed inconsistent results for coagulation biomarkers such as PAI-1, fibrinogen, and vWF. These studies varied in location and study design, making conclusions difficult [U.S. EPA (2013a), pgs. 6-178 to 6-180]. Studies since the last ISA continued to be inconsistent with respect to changes in biomarkers of coagulation (Table 4-25). That is:

- A panel study conducted in six U.S. cities evaluated 2,086 women with an average age of 46.3 years reported no change in PAI-1 for lags of 1 or 30 days for short-term increases in ozone exposure (Green et al., 2015). Conversely, in a small sample size of men with pre-existing CAD (n = 13), Mirowsky et al. (2017) found positive associations of short-term ozone exposure and PAI of 21.43% (95% CI: 0.86, 45.86%) at lag 2 and 43.39% (95% CI: 9.32, 87.43%) for a 5-day moving avg.
- No studies observed changes in fibrinogen levels resulting from increases in short-term ozone exposure in large study populations (Li et al., 2017; Green et al., 2015; Bind et al., 2012).

4.1.10.3 Controlled Human Exposure Studies

In the 2013 Ozone ISA, a controlled human exposure study demonstrated changes in markers of coagulation following short-term ozone exposure. More specifically, Devlin et al. (2012) reported a statistically significant decrease in PAI-1 immediately following and 24 hours post-exposure, as well as a decrease in plasminogen levels and a trend toward an increase in tPA. Given these results, the authors suggested that ozone exposure may activate the fibrinolysis system [(U.S. EPA, 2013a) pg 6−166] Since the publication of the 2013 Ozone ISA, there is limited evidence from CHE studies that short-term ozone exposure (1−2 hours) can result in changes to markers of coagulation or fibrinolysis. Specifically:

- A study on the effect of temperature on ozone exposure (0.3 ppm) in healthy young volunteers reported a statistically significant decrease in PAI-1 and plasminogen levels 24-hours post-exposure ($p < 0.05$) when the experiment was carried out at 22°C, but a significant increase in these coagulation markers when the exposure was conducted at 32.5°C ($p < 0.05$) (Kahle et al., 2015). This study also reported no changes in D-dimer, tPA, or vWF at either temperature.
- However, other CHE studies (Arjomandi et al., 2015; Frampton et al., 2015; Barath et al., 2013) have reported that were no measurable changes in markers of coagulation or fibrinolysis (e.g., D-dimer, platelet activation, PAI-1, plasminogen) following short-term ozone (0.1, 0.2, 0.3 ppm) exposure. Additional information about these studies can be found in Table 4-26.

4.1.10.4 Animal Toxicological Studies

The 2013 Ozone ISA contained very limited animal toxicological evidence that short-term exposure (4 hours, some studies with multiple-day exposures) to ozone could result in changes to factors related to coagulation or fibrinolysis (U.S. EPA, 2013a). This remains the case in the current review (Table 4-27):

- Snow et al. (2018) demonstrated that in rats fed a normal or coconut oil or fish oil supplemented diet, short-term exposure to ozone resulted in an increase in circulating platelets relative to FA exposure given the same diet ($p < 0.05$).
- In a study comparing the susceptibility of six different strains of mice to ozone (0.25, 0.5, 1.0 ppm) (see Table 4-27), Ramot et al. (2015) reported that short-term ozone exposure did not increase blood D-dimer levels in any mouse strain and decreased fibrinogen levels in just one of these strains (FHH mice, which are characterized as developing hypertension and proteinuria at a young age).

4.1.11 Systemic Inflammation and Oxidative Stress

Systemic inflammation has been linked to a number of CVD-related outcomes. For example, circulating cytokines such as IL-6 can stimulate the liver to release inflammatory proteins (e.g., CRP) and coagulation factors that can ultimately increase the risk of thrombosis and embolism. Other indicators of systemic inflammation include an increase in inflammatory cells such as neutrophils and monocytes and

other cytokines such as TNF. Similarly, oxidative stress can result in damage to healthy cells and blood vessels and further increase the inflammatory response. Thus, this section discusses the evidence for changes in markers of systemic inflammation and oxidative stress following short-term ozone exposures.

4.1.11.1 Epidemiologic Panel Studies

Studies in the 2013 Ozone ISA showed inconsistent results for inflammatory and oxidative stress biomarkers. Specifically, a positive association was observed in IL-6 (Thompson et al., 2010), while CRP studies reported either no association (Rudez et al., 2009; Steinvil et al., 2008) or increases (Chuang et al., 2007) following short-term ozone exposure. In addition, oxidative stress markers had mixed results, with no studies evaluating the same biomarkers [U.S. EPA (2013a), pg. 6-180].

There are few studies that demonstrate short-term exposure to ozone results in changes in inflammatory biomarker levels. Studies reviewed for these endpoints are summarized in Table 4-28. Altogether, these epidemiologic panel studies provide evidence that short-term ozone exposure is associated with increased inflammatory responses.

- Most commonly, studies examined changes in C-reactive protein (CRP) as a biomarker to identify inflammation. Across these studies, a single study reported changes in CRP after short-term exposure to ozone. Bind et al. (2012) reported a 10.8% (95% CI: 2.2, 20.5%) increase in CRP in more than 700 elderly men free of chronic medical conditions, living in the greater Boston area at 24-hours post-exposure. Among the remaining studies, consisting of cohorts of middle-aged women, men with previously diagnosed CVD, and noncurrent smokers, there were no differences in CRP reported over several different lag times (Li et al., 2017; Mirowsky et al., 2017; Green et al., 2015).
- Mirowsky et al. (2017) found increases in IL-6 at lag 4 (17.04%; 95% CI: 3.86, 33.71%), neutrophils at lag 1 (9.32%; 95% CI: 1.61, 17.57%) and lag 2 (9.00%; 95% CI: 1.07, 17.46%), monocytes at lag 1 (10.92%; 95% CI: 1.07, 21.54%), and TNF-α at lag 2 (6.32; 95% CI: −0.96, 14.14) in 13 men with previously diagnosed CAD. However, these results occur at various time-lapses and have wide confidence intervals with a small sample size. These increases changed less than 10% when adjusted for $PM_{2.5}$, suggesting that they may be related to ozone exposure.
- TNFR2 increased in a cohort of over 3,000 subjects when evaluated over 1−7 day moving avg exposure to ozone. Additionally, when these results were stratified by age, CVD or no CVD, statin use, and season, the associations remained positive (Li et al., 2017).
- A single study in a cohort of over 3,000 subjects evaluated 1−7 day moving avg exposure to ozone reported no change in the oxidative stress biomarkers myeloperoxidase and indexed 8-epi-prostaglandin F2alpha (Li et al., 2016).

4.1.11.2 Controlled Human Exposure Studies

In the 2013 Ozone ISA, a controlled human exposure study reported significant increases in CRP, IL-1, and IL-8, but not TNF-α following exposure to ozone (Devlin et al., 2012). In addition, Brook et al. (2009) found a decrease in total white blood cell count, but not in TNF, or neutrophil percentage. Since the 2013 Ozone ISA, CHE studies have provided limited additional evidence for changes in inflammatory markers following short-term ozone exposure (0.5−4 hours). For example:

- Biller et al. (2011) reported an increase in percentage blood neutrophils ($p < 0.05$) relative to FA exposure at 5, 7, but not 24-hours post-exposure (0.25 ppm) in healthy volunteers. These authors also reported increased neutrophil activation at 5- and 7-, but not 24-hours post-exposure. With respect to total leukocytes, there was a significant increase at 5 and 7 hours, but not at 24-hours ($p < 0.05$).
- In a time course study, Bosson et al. (2013) reported a decrease in blood neutrophils ($p < 0.05$) in healthy volunteers at 1.5 hour post exposure (0.2 ppm) when compared to FA exposure. These levels rebounded above FA levels when measured at 6 hours ($p < 0.05$), and at 18 hours post exposure, there was no difference in neutrophil levels when compared to FA. Similar results were found with respect to total leukocytes. In addition, the authors also describe a correlation between neutrophil levels in the blood and the lung. No impact of ozone was found on blood monocytes or lymphocytes.
- In healthy volunteers, Stiegel et al. (2016) reported an increase in percentage neutrophils following ozone exposure (0.3 ppm) immediately after ($p > 0.05$), but not 24 hours post-exposure when compared to pre-exposure. However, similar results were reported following clean air exposure, calling into question the significance of the ozone exposure on these changes. These authors also reported a decrease in the total percentage of lymphocytes ($p < 0.05$), but no change in the percentage of monocytes. Again however, similar results were reported following clean air exposure. No appreciable changes in a number cytokines, including IL-8 and TNF-α were reported following ozone exposure.
- Arjomandi et al. (2015) reported a decline in eosinophil levels from 0−4 ($p < 0.05$), but not 0−24 hours associated with increasing ozone concentrations from 0.1 to 0.2 ppm in adults with or without asthma. However, asthma status of the volunteers had no impact on these changes. No significant changes in total leukocytes or monocytes, or neutrophils were reported. No significant changes in a number of cytokines were reported, including IL-1 and TNF-α.
- In addition, a study reported statistically significant increases in blood CRP levels across exposures ranging from 0 to 200 ppb, while another reported a significant increase in CRP when comparing post exposure to pre-exposure levels (Arjomandi et al., 2015; Biller et al., 2011).
- Ramanathan et al. (2016) also demonstrated that ozone exposure (0.12 ppm) did not alter HDL antioxidant or anti-inflammatory capacity in healthy adults.

Taken together, there is limited additional evidence that short-term exposure to ozone may result in changes to some inflammatory cells and cytokines in a manner that is concentration and timepoint dependent (Table 4-29). This may particularly the case with neutrophils. That is, exposure to ozone may first cause a decrease in neutrophils in the blood (perhaps as these cells migrate into the lung), followed by an increase later post-exposure.

4.1.11.3 Animal Toxicological Studies

In the 2013 Ozone ISA, animal toxicological studies demonstrated that short-term exposure to ozone resulted in an increase in inflammatory markers (U.S. EPA, 2013a). In addition, studies in mice and monkeys demonstrated that short-term exposure to ozone resulted in an increase in markers of oxidative stress. Although not entirely consistent within and across studies, more recent animal toxicological studies provide some evidence that short-term exposure (2−24 hours, some studies with multiple-day exposures) to ozone results in an increase in markers of inflammation and oxidative stress. With respect to this evidence, we note the following key points:

- Zhong et al. (2016) reported that in obese-prone mice, short-term exposure to ozone (0.5 ppm) resulted in an increase in inflammatory monocytes and CD4 T cells in blood ($p < 0.05$). Similarly, in rats Paffett et al. (2015) reported an increase in neutrophils and macrophages in blood as a result of short-term ozone exposure ($p < 0.05$).
- Studies also demonstrated that lymphocytes, T cells, or WBC counts decreased (Snow et al., 2018; Ramot et al., 2015; Thomson et al., 2013) following short-term ozone (0.25, 0.4, 0.5, 0.8, 1.0 ppm) exposure ($p < 0.05$). However, some of these studies also found no appreciable effect of short-term ozone exposure on other cell populations. For example, in rats Paffett et al. (2015) reported no change in lymphocytes or eosinophils in blood following short-term ozone (1 ppm) exposure.
- With respect to markers of inflammation, evidence was inconsistent across studies. For example, Thomson et al. (2013) reported a decrease in TNF-α mRNA and IL-1 ($p < 0.05$) in rat heart tissue following short-term ozone exposure of 0.8 ppm, but not 0.4 ppm. However, there was little effect of ozone (0.8 ppm) exposure on a panel of 24 cytokines in the blood of rats (Thomson et al., 2016).

With respect to markers of oxidative stress:

- Kumarathasan et al. (2015) reported that exposure to 0.8 but not 0.4 ppm ozone can increase *o*-tyrosine, but not *m*-tyrosine or the lipid peroxidation marker 8-isoPGF2α in rats.
- Martinez-Campos et al. (2012) also reported in rats that short-term ozone (0.5 ppm) exposure could lead to an increase in plasma levels of MDA and 8-IP. Moreover, Farraj et al. (2016) reported decreased SOD activity following short-term ozone (0.3 ppm) exposure in rats.
- However, Cestonaro et al. (2017) found that short-term exposure to ozone (0.05 ppm) resulted in no evidence of lipid peroxidation in rats. Similarly, Thomson et al. (2013) reported that in rats, short-term exposure to ozone (0.4, 0.8 ppm) did not cause an increase in MDA mRNA in heart tissue exposure. Furthermore, Wang et al. (2013) found that short-term exposure to ozone alone did not affect SOD l or MDA levels in rat hearts.

Although not demonstrated in all studies, the recent studies presented above provide some evidence that short-term exposure to ozone can result in changes in markers of inflammation and oxidative stress (Table 4-30).

4.1.12 Stroke and Associated Cardiovascular Effects

4.1.12.1 Epidemiologic Studies of Emergency Department Visits and Hospital Admissions

A few studies of cerebrovascular disease and stroke were discussed in the 2013 Ozone ISA, including one Finnish study, a multicity French study, and an analysis of stroke subtypes in Edmonton, Canada. The Canadian study reported a weak elevated risk of ischemic and hemorrhagic stroke for 24-hour avg ozone concentrations during the warm season, but not in other seasons; however, confidence intervals were wide (Villeneuve et al., 2006). In contrast, an inverse association with transient ischemic stroke during the warm season was observed. Several studies have been published since the 2013 Ozone ISA, and results have been inconsistent. Confidence intervals around the risk ratios tended to be wide, indicating the relative imprecision in the reported associations.

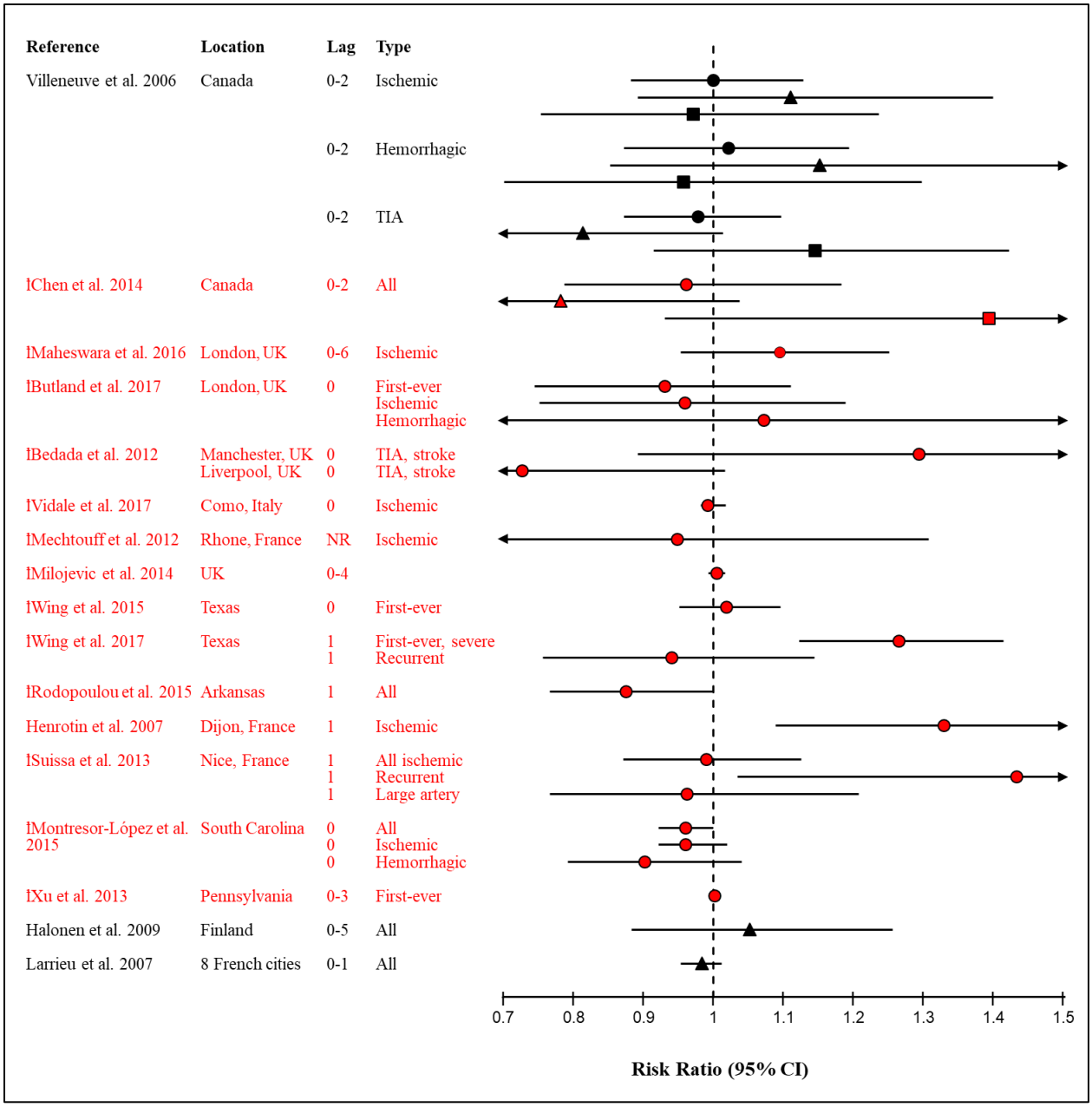
- A more recent study in Edmonton that evaluated acute ischemic stroke using lags of different time periods between 0 and 72 hours found an inverse association with 1-hour max ozone concentration during the warm season and a positive association during the cold season, although effect estimates were not precise (Chen et al., 2014). Several recent studies in Europe found no association with ischemic stroke (Table 4-31; Figure 4-4).
- While a study in Nice, France also found no associations with ischemic stroke overall with 8-hour max ozone concentrations in the preceding 3 days, the study reported a 43% increase in recurrent stroke (OR: 1.43; 95% CI: 1.03, 1.99) per 20 ppb 8-hour max ozone concentration at a lag of 1 day, and a 8% increase in large artery stroke risk (OR: 1.08; 95% CI: 1.01, 1.16) per 15 ppb 24-hour avg ozone concentration at lag Day 3 (Suissa et al., 2013). For recurrent stroke, larger odds ratios were observed with 8-hour max ozone concentrations concurrent with and the day prior to the event, and for large artery stroke, an elevated odds ratio was observed only for 24-hour avg ozone concentrations on lag Day 3. A dose-response trend was observed with increasing quartiles of ozone concentration for both stroke groups. The results of this study were consistent with those of a study in Dijon, (Henrotin et al., 2010; Henrotin et al., 2007). Two other studies that analyzed associations with ischemic stroke overall or for stroke subtypes primarily found null or weakly positive associations with 8-hour max or 24-hour avg ozone concentrations (Maheswaran et al., 2016; Corea et al., 2012).
- A comparison of risk of transient ischemic attacks (TIA) and minor stroke in the NORTHSTAR cohort in England found associations in opposite directions in two communities (Bedada et al., 2012). In Manchester, an increased TIA and stroke risk with increasing ozone concentration was found at lag Day 0 and an inverse association was found at lag Day 1. In Liverpool, an opposite pattern was observed: an inverse association at lag Day 0 and an increased OR at lag Day 1 (Table 4-31). The number of cases accrued over the 5 year study was low (N = 374 from Liverpool, N = 335 from Manchester) resulting in imprecise effect estimates.
- In the U.S., a small elevated risk was found for stroke hospitalizations in Allegheny County, PA, with 24-hour avg ozone concentrations on the day of hospitalization (Xu et al., 2013). One study in Nueces County, TX evaluated associations with incident stroke and stroke severity with cases identified in the Brain Attack Surveillance in Corpus Christi project between 2000 and 2012 (Wing et al., 2017b; Wing et al., 2015). The investigators reported a small elevated increase in

risk of incident stroke with a 20 ppb increase in 8-hour max ozone concentrations on the 4 days concurrent with and preceding the event record, with the highest increase on lag Day 2 (OR: 1.05; 95% CI: 0.97, 1.12). Effect measure estimates were not changed in a model that included $PM_{2.5}$. This study also reported an elevated risk among adults with severe incident stroke (OR: 1.27; 95% CI: 1.12, 1.41). Severe stroke was defined as the upper quartile (score ≥7) of the score obtained using the National Institutes of Health Stroke Scale (NIHSS). An analysis of first recurrent stroke also was conducted in the Texas population. A total of 317 recurrent ischemic strokes were identified between 2000 and 2012, and in contrast to the findings for incident stroke, no associations were observed with increases in 8-hour max ozone concentration (Wing et al., 2017a).

- Two other studies in the U.S. reported inverse associations with ED visits or hospital admissions for cerebrovascular disease (ICD-9 430−438) (Montresor-López et al., 2015; Rodopoulou et al., 2015). Rodopoulou et al. (2015) found an inverse association for both the cold and warm seasons in Little Rock, AR, which was not altered in a copollutant model with $PM_{2.5}$. Montresor-López et al. (2015) also conducted separate analyses for ischemic and hemorrhagic stroke in their study in South Carolina, and found no associations for these subgroups generally, other than a small increase at lag Day 2 (OR: 1.02; 95% CI: 0.9, 1.17).

- Few studies of cerebrovascular disease have examined differences by age, sex, or ethnicity. Studies conducted in the U.K. did not find notable differences between men and women or for individuals 75 years and older for ischemic stroke diagnoses (Maheswaran et al., 2016; Milojevic et al., 2014). In the U.S., an increase in risk of stroke hospitalization was strongest among men and individuals between the ages of 65 and 79 years compared with those 80 years or older (Xu et al., 2013). However, no difference in risk by sex was found in another study among hospitalized residents of South Carolina with a first diagnosis of stroke (Montresor-López et al., 2015). Wing et al. (2015) observed a higher risk among non-Hispanic whites compared to no elevated risk among Mexican-Americans associated with 8-hour max ozone concentrations at lag Days 2 and 3.

- The risk of ischemic stroke associated with a 0−6 day mean 24-hour avg ozone concentration was higher among stroke cases from the South London Stroke register with pre-existing hypertension or atrial fibrillation (Maheswaran et al., 2016).



TIA = transient ischemic attack.

Note: †Studies published since the 2013 Ozone ISA. Studies are listed from the top in order of increasing mean or median ozone concentration reported in the publication. Associations are presented per 25-ppb increase in pollutant concentration for 1-h max avg times, 20-ppb increase for 8-h avg times, and 15-ppb increase for 24-h avg times. Symbols represent point estimates, circles, triangles and squares represent the entire year, warm season and cold season, respectively horizontal lines represent 95% confidence intervals for ozone. Black text and symbols represent evidence included in the 2013 Ozone ISA; red text and symbols represent recent evidence not considered in previous ISAs or AQCDs.

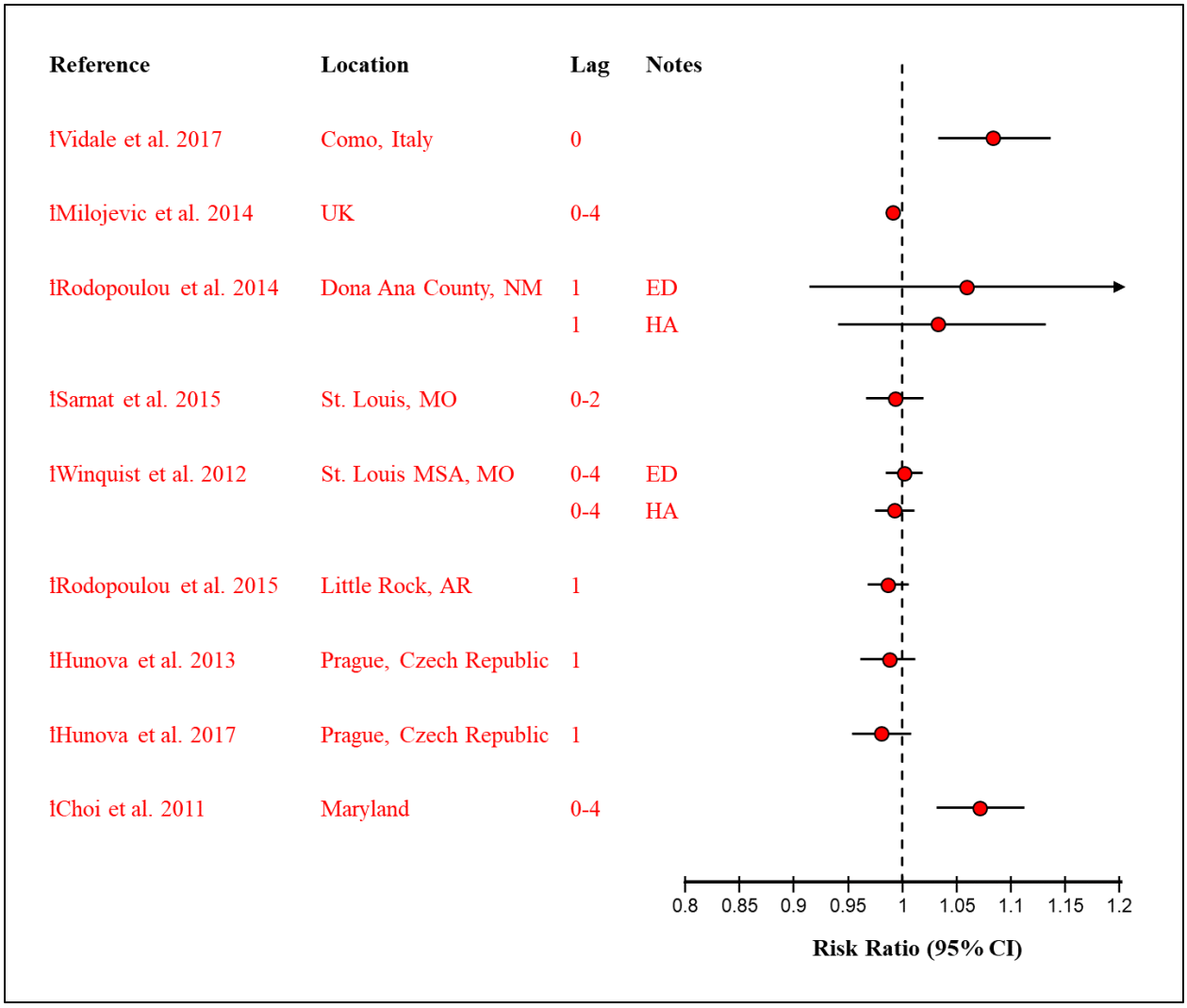
Figure 4-4 Associations between short-term exposure to ozone and cerebrovascular-related emergency department visits and hospital admissions.

4.1.13 Nonspecific Cardiovascular Effects

4.1.13.1 Epidemiologic Studies of Emergency Department Visits and Hospital Admissions

Several studies of ozone concentrations and cardiovascular hospital admissions and ED visits for all CVD diagnoses combined were discussed in the 2013 Ozone ISA. With few exceptions, these studies did not report an association between ozone concentrations and an increased risk of aggregated CVD in populations in the U.S., Canada, Europe, and Australia.

- Recent studies that reported a risk ratio for combined cardiovascular disease outcomes show a similar pattern to those studies included in the 2013 Ozone ISA (Table 4-32; Figure 4-5). Although changes were small (<1%), associations were positive during the cold season and negative during the warm season.
- Studies that evaluated effect modification by sex or age did not find notable differences (Milojevic et al., 2014; Rodopoulou et al., 2014). Winquist et al. (2012) observed a higher relative risk per 8-hour max ozone concentration among individuals residing in a poverty area.



ED = emergency department; HA = hospital admissions.

Note: †Studies published since the 2013 Ozone ISA. Studies are listed from the top in order of increasing mean or median ozone concentration reported in the publication. Associations are presented per 25-ppb increase in pollutant concentration for 1-h max avg times, 20-ppb increase for 8-h avg times, and 15-ppb increase for 24-h avg times. Circles represent point estimates; horizontal lines represent 95% confidence intervals for ozone. Black text and circles represent evidence included in the 2013 Ozone ISA; red text and circles represent recent evidence not considered in previous ISAs or AQCDs.

Figure 4-5 Associations between short-term exposure to ozone and nonspecific cardiovascular emergency department (ED) visits and hospital admissions.

4.1.14 Cardiovascular Mortality

No recent multicity study has extensively examined the relationship between short-term ozone exposure and cardiovascular mortality. The majority of evidence examining cardiovascular mortality consists of studies evaluated in the 2013 Ozone ISA, which reported positive associations for cardiovascular mortality in all-year and summer/warm season analyses. Of the recent multicity studies evaluated, only Vanos et al. (2014) examined cardiovascular mortality and reported positive associations in all-year and summer season analyses, which is consistent with the multicity studies previously evaluated. These studies are further characterized in Table 4-33. Additional single-city studies examined cardiovascular mortality and reported the following:

- In a study conducted in Philadelphia, PA, with the aim of examining the influence of model specification (i.e., control for seasonal/temporal trends, and weather covariates) on associations between air pollution and cardiovascular mortality using statistical models from recent multicity studies, Sacks et al. (2012) reported evidence of positive associations ranging from 1.30 to 2.20% for those studies that more aggressively controlled for temperature in the statistical model (i.e., either multiple temperature terms or a term for apparent temperature), while those studies that only included one temperature term did not, with associations ranging from −1.60 to 0.50% at lag 0−1 days for a 20-ppb increase in 8-hour max ozone concentrations.
- Klemm et al. (2011) conducted a study in Atlanta, GA that included 7.5 additional years of data than Klemm and Mason (2000) and Klemm et al. (2004). In analyses that examined cardiovascular mortality, the authors reported positive, but imprecise associations at lag 0−1 days in all-year analyses (0.69% [95% CI: −2.28, 3.75%] for a 20-ppb increase in 8-hour max ozone concentrations).

4.1.15 Potential Copollutant Confounding of the Ozone-Cardiovascular Disease (CVD) Relationship

Recent studies that examined potential copollutant confounding focused on either $PM_{2.5}$ or PM_{10}, or gaseous copollutants. The results of these studies extend the limited evidence from the 2013 Ozone ISA that demonstrated that ozone-cardiovascular health endpoint associations are relatively unchanged in copollutant models as detailed below:

- Associations between short-term ozone exposure and cardiovascular health endpoints are relatively unchanged in copollutant models that include PM. Specifically, Rosenthal et al. (2013) observed that the elevated risks for cardiac arrest were either unchanged or increased in copollutant models with $PM_{2.5}$, PM_{10}, or other particulate size classes. A study in St. Louis observed a 4% (95% CI: −1, 10%) elevation in ED visits for CHF, which was unchanged in copollutant models with $PM_{2.5}$ (Sarnat et al., 2015). Mirowsky et al. (2017) found increases in IL-6, neutrophils, monocytes, and TNF-α in men with previously diagnosed CVD. These increases were relatively unchanged when adjusted for $PM_{2.5}$, suggesting that these increases are directly related to ozone exposure alone.
- Associations between short-term ozone exposure and cardiovascular health endpoints are relatively unchanged in copollutant models that include other gaseous pollutants. Rosenthal et al.

(2013) observed that the elevated risks of cardiac arrest were either unchanged or increased in copollutant models with NO, NO_2, SO_2, or CO. Similar results were found for the effect of ozone on cardiac arrest (Raza et al., 2014) or CHF (Sarnat et al., 2015) after evaluating a copollutant model with NO_2. A study in St. Louis observed a 4% (95% CI: −1, 10%) elevation in ED visits for CHF, which was increased to a 6% increased risk (95% CI: 0−12%) when CO was included in the model (Sarnat et al., 2015).

4.1.16 Effect Modification of the Ozone-Cardiovascular Health Effects Relationship

4.1.16.1 Lifestage

The 1996 and 2006 Ozone AQCDs identified children, especially those with asthma, and older adults as at-risk populations (U.S. EPA, 2006, 1996a). The 2013 Ozone ISA confirmed these earlier findings and concluded that there was adequate evidence that children and older adults are at increased risk of ozone-related health effects (U.S. EPA, 2013a). Collectively, the majority of evidence for older adults has come from studies of short-term ozone exposure and mortality. No recent studies contribute evidence for whether children are at a greater risk of cardiovascular health effects due to short-term ozone exposure. A limited number of recent studies of short-term ozone exposure and cardiovascular health effects have compared associations between different age groups, but the studies do not report consistent evidence that older adults are at increased risk.

- Among the studies that evaluated the modification of the effect of exposure to ozone on heart failure by lifestage, no notable differences were reported for older adults compared with other adult age groups (Milojevic et al., 2014; Winquist et al., 2012).
- (Pradeau et al., 2015) and Ensor et al. (2013) observed higher risks among persons older than 64 years for out-of-hospital cardiac arrest with a cardiac etiology (Ensor et al., 2013). No differences for age were reported by other studies of out-of-hospital cardiac arrest (Milojevic et al., 2014; Raza et al., 2014).
- Cakmak et al. (2014) used a population of 8,662 Ottawa and Gatineau patients referred for 24-hour ambulatory cardiac monitoring with exposure linked to the monitor closest to their home address. In subjects over the age of 50 (n = 6,009) cardiac rhythm was disrupted by an increased presence of heart block (1.13; 95% CI: 1.01, 1.27).
- Increases in TNFR2 were associated with ozone exposure in a cohort of over 3,000 subjects. When stratified by above or below age of 53 years, the results persisted, however, there was no difference between the age groups (Li et al., 2017).

4.1.16.2 Pre-existing Disease

Individuals with certain pre-existing diseases may be considered at greater risk of an air pollution-related health effect because they are likely in a compromised biological state that can vary depending on the disease and severity. The 2013 Ozone ISA concluded that there was adequate evidence for increased ozone-related health effects among individuals with asthma (U.S. EPA, 2013a). The results of controlled human exposure studies, as well as epidemiologic and animal toxicological studies, contributed to this evidence. Specifically, the evidence from controlled human exposure studies provided support for increased decrements in FEV_1 and greater inflammatory responses to ozone in individuals with asthma than in healthy individuals without a history of asthma. Studies of short-term ozone exposure and mortality provided limited evidence for stronger associations among individuals with pre-existing cardiovascular disease or diabetes.

A limited number of recent studies provides some evidence that individuals with pre-existing diseases may be at greater risk of cardiovascular health effects associated with short-term ozone exposure. These studies focus on specific diseases of varying severity (e.g., previous CVD events, type 2 diabetes). Specifically:

- Larger increases in the odds of STEMI were observed for patients with previous MI (OR = 1.78; 95% CI: 0.97, 3.28), CVD (OR = 1.72; 95% CI: 1.02, 2.90), and hypertension (OR = 1.34; 95% CI: 1.00, 1.90) (Evans et al., 2016)
- Lanzinger et al. (2014) reported FMD decreases in individuals with type 2 diabetes. However, Mirowsky et al. (2017) saw no change in FMD in men with a previous diagnosis of coronary artery disease.
- Increases in TNFR2 were associated with short-term ozone exposure in a cohort of over 3,000 subjects. When these results were stratified by pre-existing CVD or no pre-existing CVD the associations remained positive and relatively unchanged (Li et al., 2017).
- A single study provided emerging evidence that children ages 6−17 years clinically diagnosed with mood disorders showed increases in SBP (mm Hg) (4.41; 95% CI: 1.91, 6.93), DBP (mm Hg) (3.55; 95% CI: 1.01, 6.08), and HR (bpm) (2.47; 95% CI: −1.52, 6.47) relative to children without a clinically diagnosed mood disorders for SBP (−0.52; 95% CI: −1.18, 0.14), DBP (−0.24; 95% CI: −0.85, 0.36), and HR (−0.42; 95% CI: −1.36, 0.52) (Dales and Cakmak, 2016).

4.1.16.3 The Role of Season on Ozone Associations with Cardiovascular Health Effects

As detailed in Appendix 1 Section 1.7, ozone concentrations are generally higher in the summer or warm months due to the atmospheric conditions that lead to ozone formation. Therefore, many locations, particularly within the U.S., only monitor ozone during the summer or warm months. Thus, many of the epidemiologic studies tend to focus on summer or warm season analyses. However, some studies conduct all-year analyses based on areas that monitor ozone year-round, with a subset of these

studies then examining whether the magnitude of the ozone-cardiovascular health association varies either across seasons or in the summer/warm season compared with the entire year. Studies evaluated in the 2013 Ozone ISA reported evidence of positive ozone-cardiovascular health associations in all-year analyses that tended to be larger in magnitude during the warm or summer months. A limited number of recent studies that conducted seasonal analyses reported associations that were similar for both warm season and cool season analyses, Specifically, recent studies indicate:

- Evans et al. (2016) observed increased odds of STEMI in the cooler months (November to April) for ozone exposure at 12-hours (OR = 1.43; 95% CI: 1.03, 1.98), 24-hours (OR = 1.45; 95% CI: 1.04, 2.03), and 72-hours (OR = 1.60; 95% CI: 1.05, 2.46), and no increased associations during the warmer months.
- Increases in TNFR2 were associated with short-term ozone exposure in a cohort of over 3,000 subjects. When stratified by warm and cool seasons, the associations remained positive in both season (Li et al., 2017).
- Seasonality altered cardiovascular electrophysiology in a population of 8,662 Ottawa and Gatineau patients referred for 24-hour ambulatory cardiac monitoring with exposure linked to the 3-hour max exposure for the 24-hours prior to the visit, based on the monitor closest to their home address. During the warm season (April−September), Cakmak et al. (2014) reported increases in the presence of heart block (1.23; 95% CI: 1.07, 1.42). However, in the cold season, the same study reported increases in the number of supraventricular ectopic runs (defined as more than three consecutive beats) (8.15; 95% CI: 0.34, 16.57) and the length of the longest ventricular ectopic runs (20.68; 95% CI: 5.3, 38.31).

4.1.17 Summary and Causality Determination

The 2013 Ozone ISA concluded that the strongest evidence for an effect of short-term ozone exposure on cardiovascular health was from animal toxicological studies demonstrating ozone-induced impaired vascular and cardiac function, as well as changes in HR and HRV (U.S. EPA, 2013a). This evidence was supported by a limited number of controlled human exposure studies in healthy adults demonstrating changes in HRV, as well as in blood markers associated with an increase in coagulation, systemic inflammation, and oxidative stress. Evidence of these effects in animals and humans was cited as providing biological plausibility for the evidence from epidemiologic studies reporting positive associations between short-term ozone exposure and cardiovascular-related mortality. However, there was limited or no evidence from controlled human exposure or epidemiologic studies for short-term ozone exposure and cardiovascular morbidity, such as effects related to HF, IHD and MI, arrhythmia and cardiac arrest, or thromboembolic disease. The lack of evidence connecting the effects observed on impaired vascular and cardiac function in animal toxicological studies and the association between short-term ozone exposure and cardiovascular mortality observed in epidemiologic studies was a major source of uncertainty in the 2013 Ozone ISA.

Animal toxicological studies published since the 2013 Ozone ISA provide generally consistent evidence for impaired cardiac function and endothelial dysfunction, but limited or inconsistent evidence

for endpoints including indicators of arrhythmia and markers of oxidative stress and inflammation. Additional controlled human exposure studies have been published in recent years, however the evidence for an ozone-induced effect on cardiovascular endpoints is inconsistent; no effect of ozone was reported from studies of cardiac function, indicators of IHD (i.e., ST segment), endothelial dysfunction, or HR, while other studies provide limited evidence that ozone exposure can result in changes in blood pressure, HRV, indicators of arrhythmia, markers of coagulation, and inflammatory markers. In addition, the number of epidemiologic studies evaluating short-term ozone exposure and cardiovascular health effects has grown somewhat, but overall remains limited and continues to provide little, if any, evidence for associations with HF, IHD and MI, arrhythmia and cardiac arrest, or thromboembolic disease. Recent epidemiologic evidence for associations between short-term ozone exposure and cardiovascular mortality is limited, and the studies included in the 2013 Ozone ISA continue to provide the strongest evidence for this association. Overall, many of the same limitations and uncertainties that existed in the body of evidence in the 2013 Ozone ISA continue to exist. However, the body of controlled human exposure studies evaluating short-term ozone exposure and cardiovascular endpoints has grown, and when evaluated in the context of the controlled human exposure studies available for the 2013 Ozone ISA, the evidence is less consistent and weaker, overall. Evidence published since the completion of the 2013 ISA and its effect on judgments regarding the extent to which short-term exposure to ozone causes cardiovascular effects is discussed in greater detail below.

Similar to the evidence in the 2013 Ozone ISA, there is evidence from some, but not all recent animal toxicological studies for an increase in markers associated with systemic inflammation and oxidative stress (Section 4.1.11.3) following short-term ozone exposure. The systemic inflammation results are coherent with generally consistent evidence from epidemiologic panel studies demonstrating increases in markers of systemic inflammation such as CRP following short-term ozone exposure (Section 4.1.11.2 and Section 4.1.11.1, respectively). However, there is limited evidence from controlled human exposure studies examining the potential for increased markers of inflammation and oxidative stress following short-term ozone exposure (Section 4.1.11.2). Additionally, the newly available epidemiologic panel study did not observe an association between short-term ozone concentrations and myeloperoxidase.

The 2013 Ozone ISA included evidence from animal toxicological studies for changes in cardiac and endothelial function following short-term exposure to ozone. There is generally consistent evidence from recent animal toxicological studies published since the last review demonstrating impaired cardiac and endothelial function in rodents following short-term ozone exposure (Section 4.1.4.3 and Section 4.1.6.3). However, coherence with studies in humans is lacking. A controlled human exposure study in healthy individuals did not report ozone-induced changes in stroke volume or left ventricular ejection time relative to FA. Moreover, multiple controlled human exposure studies in healthy subjects found no evidence of an ozone-induced effect on measures of endothelial function such as FMD following reactive hyperemia or pharmacological challenge (Section 4.1.6.2). In addition, results from

recent epidemiologic panel studies were inconsistent, with a limited number of studies reporting either positive, negative, or null associations with short-term ozone concentrations (Section 4.1.6.1).

In the last review, there was also a limited number of animal toxicological and controlled human exposure studies demonstrating changes in HR and HRV. In the current review, there is inconsistent evidence for changes in HR in animals (Section 4.1.9.3), and no additional evidence for changes in HR in healthy adults from multiple controlled human exposure studies (Section 4.1.9.2). With respect to HRV, there is inconsistent evidence in both animal toxicological and controlled human exposure studies of healthy adults (Section 4.1.9.2 and Section 4.1.9.3). Similarly, recent epidemiologic panel studies reported inconsistent associations between short-term exposure to ozone and both HR and HRV (Section 4.1.9.1). Moreover, although some but not all recent animal toxicological studies demonstrate ozone-induced changes in blood pressure (Section 4.1.8.4) and changes in indicators of conduction abnormalities in SH rats (Section 4.1.7.2), there is again a lack of coherence with evidence in humans. Multiple controlled human exposure studies reported little effect of short-term ozone exposure on conduction abnormalities, and little evidence of an ozone-induced effect on blood pressure. Few epidemiologic panel studies evaluated blood pressure, and the results were inconsistent.

In addition, a limited number of epidemiologic time-series and case-crossover studies published since the last review report inconsistent results. With respect to the limited number of recent studies of hospital admissions and ED visits that analyzed associations with heart failure, associations continued to be inconsistent. Studies conducted in the U.K. and Arkansas did not observe associations for CHF alone or combined with hypertensive heart disease and increases in ozone concentrations, but a pair of studies in St. Louis, MO reported a 5% increase in either ED visits or hospital admissions associated with short-term exposure to ozone (Section 4.1.4.1). Studies from Europe, Canada and the U.S., several of which analyzed a large number of events per day in multiple cities, consistently reported null or only small positive effect estimates (i.e., OR ≤ 1.02) in analyses of MI, including for STEMI and NSTEMI (Section 4.1.5.1). One multicity study in Italy reported a 5% increase in incident MI associated with an increase in 8-hour max ozone concentrations during the warm season using a 0−1 day distributed lag. Similarly, inconsistent results were observed in several studies that analyzed hospital admissions and ED visits for stroke and stroke subtypes in the U.S., Canada and Europe (Section 4.1.12.1). Increases in out-of-hospital cardiac arrests associated with 8-hour max or 24-hour avg increases in ozone concentrations were reported by a few case-crossover studies, however analyses of other endpoints (e.g., dysrhythmia, arrhythmia, or atrial fibrillation) generally reported null results (Section 4.1.7.1). In addition, increases in ED visits for hypertension of 11 to 15% were observed among females in a study conducted in two Canadian cities during the warm season, and in a study in Kaunas, Lithuania (Section 4.1.8.1). However, no association between ED visits for hypertension and ozone concentration was observed in a time-series study in Arkansas.

The 2013 Ozone ISA concluded that there was adequate evidence that children and older adults are at increased risk of ozone-related health effects (U.S. EPA, 2013a). No recent studies of short-term

ozone exposure and cardiovascular health effects contribute evidence to determine if children are at a greater risk compared to other lifestages. A limited number of recent studies of short-term ozone exposure and cardiovascular health effects have compared associations between different age groups, but do not report consistent evidence that older adults are at increased risk compared to other lifestages (Section 4.1.16.1). When considering pre-existing disease as a modifying factor, the 2013 Ozone ISA concluded that there was adequate evidence for increased ozone-related health effects among individuals with asthma (U.S. EPA, 2013a). A limited number of recent studies provides some evidence that individuals with pre-existing diseases may be at greater risk of cardiovascular health effects associated with short-term ozone exposure. These studies focus on specific cardiovascular and metabolic diseases (e.g., previous CVD events, type 2 diabetes) (Section 4.1.16.2).

Notably, there is a lack of coherence between cardiovascular effects when they are observed in animals and corresponding effects in humans, particularly when examining the results of controlled human exposure studies. This could be because a number of the animal toxicological studies were performed in rodent disease models, while controlled human exposure studies generally include healthy individuals. For example, evidence for changes in blood pressure were found in SH rats and in rats fed a high fructose diet, and in a panel study that included individuals with pre-existing type 2 diabetes (Hoffmann et al., 2012), but practically no evidence of an effect on blood pressure was reported in multiple controlled human exposure studies in generally healthy subjects. Thus, it is possible that if those with underlying cardiovascular or metabolic disease were included in controlled human exposure studies, results may have been different. That being said, controlled human exposure studies do not typically include unhealthy or diseased individuals for ethical reasons, and therefore this represents an important uncertainty to consider in interpreting the results of controlled human exposure studies.

In addition to underlying disease status, there are also substantial differences in exposure concentrations between animal toxicological and controlled human exposure studies. Animal toxicological studies generally expose rodents to 0.3 to 1 ppm, while CHE studies generally expose humans to 0.07 and 0.3 ppm. Thus, additional animal toxicological studies conducted at lower concentrations could help to reduce this uncertainty. In fact, there is evidence in SH rats that exposure to 0.2 ppm ozone results in no statistically significant effects on measures of cardiac electrophysiology, while exposure to 0.8 ppm exposure results in statistically significant effects on these endpoints (Farraj et al., 2012). A caveat to this study, however, is that both concentrations increased sensitivity to the arrhythmia-inducing drug aconitine (Farraj et al., 2012). Nevertheless, additional studies in wild-type and disease-model mice exposed to lower ozone concentrations would be greatly beneficial for future review. Finally, in addition to disease status and exposure concentration, the lack of coherence between some animal and human studies could be due to differences in physiology (e.g., rodents are obligate nose breathers), differences in the duration and timing of exposure (e.g., rodents are exposed during the day, during their resting cycle, while humans are exposed during the day when they are normally active), and the temperature at which the exposure was conducted (Kahle et al., 2015).

When considered as a whole the evidence is **suggestive of, but not sufficient to infer, a causal relationship between short-term exposure to ozone and cardiovascular effects**. This determination is different from the conclusion in the 2013 Ozone ISA. The evidence that supports this change in the causality determination includes: (1) a growing body of controlled human exposure studies providing less evidence for an effect of short-term ozone exposure and cardiovascular health endpoints; (2) a paucity of evidence for more severe cardiovascular morbidity endpoints (i.e., HF, IHD and MI, arrhythmia and cardiac arrest, and thromboembolic disease) to connect the evidence for impaired vascular and cardiac function from animal toxicological studies with the evidence from epidemiologic studies of cardiovascular mortality; and (3) uncertainties and limitations acknowledged in the 2013 Ozone ISA (e.g., lack of control for potential confounding by copollutants in epidemiologic studies) remain in recent evidence (Table 4-1). Although there exists some generally consistent evidence for a limited number of ozone-induced cardiovascular endpoints in animal toxicological studies, there is a general lack of coherence between these results and those in controlled human exposure and epidemiologic studies. Thus, while some consistent results in animals and limited positive results in humans provide biological plausibility for more serious endpoints such as mortality (Section 4.1.14), the underlying evidence supporting biological plausibility is limited and thus, important uncertainties remain. Additional animal toxicological studies at lower exposure concentrations in animal models of disease and epidemiologic studies in populations with underlying disease would be useful to address these uncertainties.

Table 4-1 Summary of evidence that is suggestive of, but not sufficient to infer, a causal relationship between short-term ozone exposure and cardiovascular effects.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Generally consistent evidence from animal toxicological studies at relevant ozone concentrations	Indicators of impaired heart function, endothelial dysfunction	Section 4.1.4.3, Section 4.1.6.3	~0.2 to 0.3 ppm
Limited or inconsistent evidence from animal toxicological studies at relevant ozone concentrations	ST-segment depression, changes in indicators of cardiac electrophysiology or potential arrhythmia in SH rats, changes in changes in BP and HR or HRV, markers of systemic inflammation and oxidative stress	Farraj et al. (2012), Section 4.1.7.4, Section 4.1.8.4, Section 4.1.9.3 Section 4.1.11.3	0.8 but not at 0.2 ppm

Table 4-1 (Continued): Summary of evidence that is suggestive of, but not sufficient to infer, a causal relationship between short-term ozone exposure and cardiovascular effects.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Limited and inconsistent evidence from controlled human exposure studies at relevant ozone concentrations	No changes in a number of electrophysiology measures by ECG, but there was increased probability of ventricular but not supraventricular ectopy couplets or runs,	Rich et al. (2018)	0.07 but not 0.12 ppm 0.12 ppm
	Change in T-wave alternans during the first 5 min of exposure, but no change relative to FA later in the exposure. The effect observed in first 5-minutes is likely not meaningful.	Kusha et al. (2012)	
	No meaningful changes in SBP and/or DBP	Frampton et al. (2015) Barath et al. (2013) Arjomandi et al. (2015) Rich et al. (2018)	
	Changes in HRV	Barath et al. (2013) Rich et al. (2018) Arjomandi et al. (2015)	
	Markers of coagulation, systemic inflammation and oxidative stress	Kahle et al. (2015) Barath et al. (2013) Arjomandi et al. (2015) Frampton et al. (2015) Section 4.1.11.2	
No evidence from controlled human exposure studies at relevant ozone concentrations	Changes in stroke volume or left ventricular ejection time	Frampton et al. (2015)	
	Changes in ST segment	Rich et al. (2018)	
	Clinical indicators of endothelial dysfunction	Section 4.1.6.2	
	Changes in HR	Frampton et al. (2015) Barath et al. (2013) Arjomandi et al. (2015) Rich et al. (2018) Kusha et al. (2012)	
Consistent evidence from high-quality, epidemiologic studies of cardiovascular mortality	A number of studies evaluated in the 2013 Ozone ISA reported positive associations for cardiovascular mortality in all-year and seasonal analyses. A more limited number of recent studies continue to report positive associations.	Section 4.1.14	

Table 4-1 (Continued): Summary of evidence that is suggestive of, but not sufficient to infer, a causal relationship between short-term ozone exposure and cardiovascular effects.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Limited epidemiologic evidence from multiple studies of CVD hospital admissions or ED visits	Generally null or inconsistent associations (both negative and positive direction) observed in studies of CVD hospital admissions or ED visits limited by low ozone concentrations (averaging <40 ppb), low number of daily events in many studies, and few multicity studies to allow for evaluation of geographic heterogeneity. Although there were a few exceptions, among studies averaging more events per day (>1), associations with heart failure, hypertension, stroke, ischemic heart disease, and dysrhythmia/atrial fibrillation were primarily null or in the negative direction. More consistent associations were reported by a few studies for out-of-hospital cardiac arrest.	Section 4.1.4, Section 4.1.5, Section 4.1.7, Section 4.1.8, Section 4.1.12	Mean: 20−40 ppb 75th : 27−50 ppb
Limited epidemiologic evidence from panel studies of CVD endpoints	Limited number of studies with generally positive associations (ventricular tachycardia, pulse amplitude, and myocardial infarction) observed among populations with or without pre-existing disease and without any repeated endpoints evaluated.	Section 4.1.5.2, Section 4.1.7.2	Mean: 23−40.56 ppb
Inconsistent epidemiologic evidence from multiple panel studies of CVD endpoints	Generally null or inconsistent associations (e.g., heart rate variability, endothelial outcomes, coagulation markers, BP) observed among populations with or without pre-existing disease; limited number of studies evaluating the same endpoint; limited number of subjects in some studies.	Section 4.1.6.1, Section 4.1.8.2, Section 4.1.9.1, Section 4.1.10.2	Mean: 22−41 ppb
Limited epidemiologic evidence from copollutant models provides some support for an independent ozone association	The magnitude of ozone associations remains relatively unchanged, but in some cases with wider confidence intervals in a limited number of studies evaluating copollutant models, including $PM_{2.5}$ and other gaseous pollutants. When reported, correlations with $PM_{2.5}$ or gaseous copollutants were primarily in the low to moderate range ($r < 0.7$).	Section 4.1.15	
Uncertainty due to limited coherence between CVD morbidity and CVD mortality	Consistent positive associations observed in studies of short-term ozone exposure and mortality, although limited evidence of a relationship between short-term ozone exposure and CVD morbidity (e.g., HF, IHD and MI, arrhythmia and cardiac arrest, and stroke) in epidemiologic and controlled human exposure studies		

Table 4-1 (Continued): Summary of evidence that is suggestive of, but not sufficient to infer, a causal relationship between short-term ozone exposure and cardiovascular effects.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Uncertainty regarding geographic heterogeneity in ozone associations	Multicity U.S. studies demonstrate city-to-city and regional heterogeneity in ozone-CVD ED visit and hospital admission associations. Evidence supports that a combination of factors, including composition and exposure factors may contribute to the observed heterogeneity.	Section 4.1.5.1, Section 4.1.7.1, Section 4.1.12.1, Section 4.1.13.1	

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble to the ISAs (U.S. EPA, 2015).

[b]Describes the key evidence and references, supporting or contradicting, contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where the full body of evidence is described.

[c]Describes the ozone concentrations with which the evidence is substantiated.

4.2 Long-Term Ozone Exposure and Cardiovascular Health Effects

4.2.1 Introduction, Summary from the 2013 Ozone ISA, and Scope for Current Review

The 2013 Ozone ISA concluded that evidence was suggestive of a causal relationship between long-term exposures to ozone and cardiovascular effects. In the last review, a small number of well-conducted animal toxicological studies provided evidence for ozone-enhanced atherosclerosis or ischemic/reperfusion injury. There was also evidence that long-term exposure to ozone resulted in systemic inflammation and oxidative stress. This evidence was in addition to a small number of epidemiologic studies reporting an association between long-term exposure to ozone and cardiovascular disease-related biomarkers. Of note, the only epidemiologic study to investigate the relationship between long-term ozone exposure and cardiovascular mortality did not observe a positive association. A key uncertainly from the last review was the mechanism by which ozone inhalation may result in systemic effects. However, there was limited evidence in the 2013 Ozone ISA that activation of LOX-1 by ozone-oxidized lipids and proteins could result in changes in genes involved in proteolysis, thrombosis, and vasoconstriction.

The subsections below provide an evaluation of the most policy-relevant scientific evidence relating long-term ozone exposure to cardiovascular health effects. These sections focus on studies

published since the completion of the 2013 Ozone ISA, and emphasis is placed on those studies that address uncertainties remaining from the last review. Overall, a limited number of animal toxicological and epidemiologic studies contribute some new evidence characterizing the relationship between long-term ozone exposure and cardiovascular health effects. There is some emerging epidemiologic evidence that long-term ozone exposure may be associated with blood pressure changes or hypertension among different lifestages or those with pre-existing disease. With respect to the toxicological evidence, there was some evidence for inflammation, oxidative stress, and impaired cardiac contractility in rodents following long-term ozone exposure. Overall, however, many of the uncertainties identified in the previous review remain.

4.2.1.1 Population, Exposure, Comparison, Outcome, and Study Design (PECOS) Tool

The scope of this section is defined by a scoping tool that generally defines the relevant Population, Exposure, Comparison, Outcome, and Study Design (PECOS). The PECOS tool defines the parameters and provides a framework to help identify the relevant evidence in the literature to inform the ISA. Because the 2013 Ozone ISA concluded that there is evidence to suggest a causal relationship between long-term ozone exposure and cardiovascular health effects, the epidemiologic studies evaluated are less limited in scope and not targeted towards specific study locations, as reflected in the PECOS tool. The studies evaluated and subsequently discussed within this section were identified using the following PECOS tool:

Experimental Studies:

- Population: Study population of any animal toxicological study of mammals at any lifestage
- Exposure: Long-term (on the order of months to years) inhalation exposure to relevant ozone concentrations (i.e., ≤2 ppm)
- Comparison: Appropriate comparison group exposed to a negative control (i.e., clean air or filtered air control)
- Outcome: Cardiovascular effects
- Study Design: In vivo chronic-duration, subchronic-duration, or repeated-dose toxicity studies in mammals or immunotoxicity studies

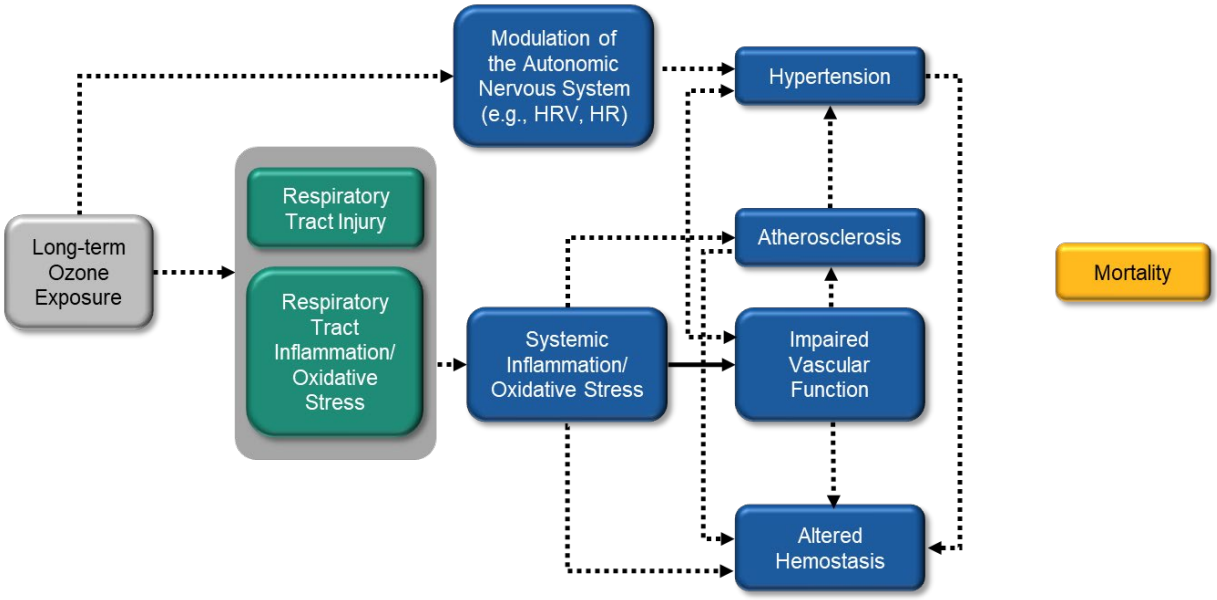
Epidemiologic Studies:

- Population: Any population, including populations or lifestages that might be at increased risk
- Exposure: Long-term ambient concentration of ozone
- Comparison: Per unit increase (in ppb)
- Outcome: Change in risk (incidence/prevalence) of a cardiovascular effect

- Study Design: Epidemiologic studies consisting of cohort, case-control studies, and cross-sectional studies with appropriate timing of exposure for the health endpoint of interest

4.2.2 Biological Plausibility

This subsection describes the biological pathways that potentially underlie cardiovascular health effects resulting from long-term inhalation exposure to ozone. Figure 4-6 graphically depicts these proposed pathways as a continuum of pathophysiological responses—connected by arrows—that may ultimately lead to the apical cardiovascular events observed in epidemiologic studies associated with long-term exposure. This discussion of "how" long-term exposure to ozone may lead to these cardiovascular events also provides biological plausibility for the epidemiologic results reported later in this Appendix. In addition, most studies cited in this subsection are discussed in greater detail throughout this Appendix.



Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence related to ozone exposure, and the arrows indicate a proposed relationship between those effects. Solid arrows denote evidence of essentiality as provided, for example, by an inhibitor of the pathway or a genetic knockout model used in an experimental study involving ozone exposure. Shading around multiple boxes is used to denote a grouping of these effects. Arrows may connect individual boxes, groupings of boxes, and individual boxes within groupings of boxes. Progression of effects is generally depicted from left to right and color-coded (gray, exposure; green, initial effect; blue, intermediate effect; orange, effect at the population level or a key clinical effect). Here, population level effects generally reflect results of epidemiologic studies. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below.

Figure 4-6: Potential biological pathways for cardiovascular effects following long-term exposure to ozone.

There is evidence from epidemiologic studies for cardiovascular-related mortality following long-term exposure to ozone. However, when attempting to construct a biologically plausible pathway that could result in cardiovascular-related mortality following long-term exposure to ozone, there are important gaps in the health evidence (Figure 4-6). Specifically, there is no evidence from epidemiologic studies of an association between long-term exposure to ozone and IHD or MI, HF, arrhythmia, or thromboembolic disease. More information on this pathway and the important gaps that exist are described below.

Long-term inhalation exposure to ozone may result in respiratory tract inflammation and oxidative stress (Appendix 3). In general, inflammatory mediators, such as cytokines produced in the respiratory tract, have the potential to enter into the circulatory system where they may cause distal pathophysiological responses that could lead to overt cardiovascular disease. In addition, release of inflammatory mediators into the circulation, such as monocyte chemoattractant protein-1 (MCP-1), can result in the recruitment of additional inflammatory cells, and thus amplify the initial inflammatory response. Thus, it is important to note that there is evidence from long-term experimental studies in animals (Miller et al., 2016; Perepu et al., 2012; Sethi et al., 2012) demonstrating an increase in cytokines, and/or oxidative stress markers in the circulatory system following long-term ozone exposure. The release of cytokines like IL-6 into the circulation can stimulate the liver to release inflammatory proteins and coagulation factors that can alter hemostasis and increase the potential for thrombosis (Tanaka et al., 2014). Thus, it is important to note animal toxicological studies demonstrating changes in these types of coagulation factors following long-term ozone exposure (Gordon et al., 2014; U.S. EPA, 2013a). These changes may alter the balance between pro- and anti-coagulation proteins, and therefore, increase the potential for thrombosis, which may then promote IHD, stroke, or thromboembolic disease elsewhere in the body. However, there is no epidemiologic evidence of an association between long-term exposure to ozone and IHD, stroke, or thromboembolic disease, and thus, considerable uncertainty in the potential pathway leading to mortality.

Systemic inflammation has the potential to result in impaired vascular function, a systemic pathological condition characterized by the altered production of vasoconstrictors and vasodilators, which over time promotes plaque formation leading to atherosclerosis. Specifically, vascular dysfunction is often accompanied by endothelial cell expression of adhesion molecules and release of chemo attractants that recruit inflammatory cells. Macrophages may then internalize circulating lipids, leading to the formation of foam cells: a hallmark of atherosclerotic lesions. Over time, these atherosclerotic lesions may become calcified, and this often leads to arteriole stiffening and promotion of IHD or stroke. Importantly, evidence for changes in molecular markers associated with impaired vascular function following ozone exposure are found in an experimental study in animals from the 2013 Ozone ISA [U.S. EPA (2013a), pg. 7−38]. This is in agreement with results from an epidemiologic study reporting a positive association between long-term exposure to ozone and increased CIMT (Breton et al., 2012), as well as with animal toxicological results indicating changes in caveolin 1 and caveolin 3 (Sethi et al., 2012), two molecular markers possibly associated with the development of atherosclerosis. Moreover,

one study in the 2013 Ozone ISA reported enhanced aortic atherosclerotic lesions in mice following long-term ozone exposure [U.S. EPA (2013a), pg. 7−38]. However, considerable uncertainty remains in how long-term ozone exposure may lead to mortality given that there is little epidemiologic evidence of an association between long-term exposure to ozone and other cardiovascular endpoints such as IHD, stroke, or thromboembolic disease. Thus, how these earlier events could lead to mortality remains unclear.

In addition to long-term ozone exposure leading to cardiovascular disease through inflammatory pathways, there is also evidence that long-term exposure to ozone could lead to cardiovascular disease through modulation of the autonomic nervous system. Studies in animals showed modulation of autonomic function (as evidenced by changes in HR) following long-term ozone exposure (Gordon et al., 2013). Moreover, there is epidemiologic evidence of positive associations between long-term exposure to ozone and increases in BP and hypertension (Cole-Hunter et al., 2018; Coogan et al., 2017; Yang et al., 2017; Dong et al., 2014).

When considering the available evidence, important uncertainties remain in potential pathways connecting long-term exposure to ozone to cardiovascular-related mortality. That is, while there is some evidence for a number of early and intermediate cardiovascular-related effects from animal toxicological and epidemiologic studies, there is no epidemiologic evidence of associations between long-term exposure to ozone and outcomes that could directly result in death, such as IHD, stroke, or arrhythmia.

4.2.3 Ischemic Heart Disease (IHD) and Associated Cardiovascular Effects

4.2.3.1 Epidemiologic Studies

No studies examining long-term ozone exposure and IHD were included in the 2013 Ozone ISA. A recent national cohort study conducted in England observed a null association between long-term ozone exposure and MIs (Atkinson et al., 2013) and a cohort study conducted in South Korea observed an inverse association (Kim et al., 2017) (Table 4-34).

4.2.4 Atherosclerosis

4.2.4.1 Epidemiologic Studies

No studies examining long-term ozone exposure and atherosclerosis were included in the 2013 Ozone ISA. A recent U.S. cohort study evaluated long-term ozone exposure, averaged in early life (ages 0−5 years), during elementary school (ages 6−12 years) and during the first 20 years of life (Breton et al., 2012). These authors observed positive associations between ozone averaged over all three exposure windows and increases in CIMT measured in southern California college students (Table 4-35). These results were robust to the inclusion of $PM_{2.5}$, PM_{10}, and NO_2 in copollutant models.

4.2.4.2 Animal Toxicological Studies

The 2013 Ozone ISA presented evidence that long-term exposure to ozone in ApoE−/− mice resulted in enhanced aortic atherosclerotic lesions when compared with filtered air exposure [U.S. EPA (2013a), pg. 7−38]. The last review also noted that activation of lectin-like oxidized-low density lipoprotein receptor-1 (LOX-1) could have a role in vascular pathology associated with atherosclerosis [U.S. EPA (2013a), pg. 7−38]. Since the 2013 Ozone ISA, there is inconsistent evidence of an effect of long-term ozone exposure (4−17 weeks) on potential markers of atherosclerosis (Table 4-36). Specifically:

- Sethi et al. (2012) reported that long-term exposure to ozone (0.8 ppm) decreased caveolin 1 and increased caveolin 3 expression at 28 days relative to control animals. At 56 days, caveolin 1 expression and caveolin 3 expression decreased, setting up the potential for a proapoptotic and atherosclerotic environment ($p < 0.05$). However, Gordon et al. (2013) reported that 17-week ozone exposure (0.8 ppm) had no effect on LOX-1, caveolin 1, or RAGE gene expression in the aortas of younger or older rats.

4.2.5 Heart Failure and Impaired Heart Function

4.2.5.1 Epidemiologic Studies

No studies examining long-term ozone exposure and heart failure were included in the 2013 Ozone ISA. A recent national cohort study conducted in England observed a negative association between long-term ozone exposure and heart failure (Atkinson et al., 2013), and a cohort study conducted in South Korea observed an inverse association (Kim et al., 2017) (Table 4-37).

4.2.5.2 Animal Toxicological Studies

In the 2013 Ozone ISA, there was evidence from an animal toxicological study that long-term ozone exposure decreased LVDP, rate of pressure development, and rate of change of pressure in isolated perfused rat hearts [U.S. EPA (2013a), pg. 7−39]. Similarly, two recent studies from the same laboratory that contributed evidence to the 2013 Ozone ISA reported a decrease in LVDP following long-term exposure (4−8 weeks) to ozone (0.8 ppm) in isolated perfused rat hearts ($p < 0.05$) (Perepu et al., 2012; Sethi et al., 2012) (Table 4-38). Moreover, Perepu et al. (2012) also reported a decrease in the rate of pressure development and a decrease in pressure decay in these hearts ($p < 0.05$). This decrease in pressure decay is consistent with impaired diastolic function (i.e., cardiac filling) and is consistent with additional results from this study indicating an increase in left ventricular end diastolic pressure. Thus, both studies demonstrate that long-term ozone exposure can result in abnormal cardiac function. However, these studies were all conducted by the same laboratory and therefore, there is uncertainty with respect to the broad applicability of the results.

4.2.6 Vascular Function

4.2.6.1 Animal Toxicological Studies

The 2013 Ozone ISA presented evidence from an animal toxicological study of an increase in ET-1, ET-1 receptor, and eNOS mRNA in rat aortas following long-term exposure to ozone [U.S. EPA (2013a), pg. 7−38]. However, a more recent study reported no change in eNOS/iNOS or ET-1 mRNA expression in adult or senescent rat aorta tissue following long-term ozone (0.8 ppm) exposure (17 weeks) [(Gordon et al., 2013), Table 4-39]. Thus, there remains limited evidence from animal toxicological studies that long-term exposure to ozone may result in an increase in markers that promote vasoconstriction.

4.2.7 Cardiac Depolarization, Repolarization, Arrhythmia, and Arrest

4.2.7.1 Epidemiologic Studies

No studies examining long-term ozone exposure and arrhythmia were included in the 2013 Ozone ISA. A recent national cohort study conducted in England observed a null association between long-term ozone exposure and arrhythmia (Atkinson et al., 2013).

4.2.8 Blood Pressure Changes and Hypertension

4.2.8.1 Epidemiologic Studies

At the time of the 2013 Ozone ISA, one study was available that investigated the relationship between long-term ozone exposure and blood pressure. Chuang et al. (2011) observed increases in both systolic and diastolic blood pressure associated with ozone concentrations among older adults in Taiwan, although these increases were attenuated in models that included copollutants. A number of recent studies, conducted mainly in Asia, observed inconsistent results between long-term ozone exposure and blood pressure or hypertension among healthy adults (Table 4-40). There is some emerging evidence that long-term ozone exposure may be associated with changes in blood pressure or hypertension among different lifestages or those with pre-existing disease. Specifically:

- A U.S. cohort study observed positive associations between long-term ozone exposure and incident hypertension among black women (Coogan et al., 2017). These associations were robust to copollutant adjustment with $PM_{2.5}$ and somewhat attenuated, though still positive, with adjustment for NO_2. Similarly, cross-sectional studies conducted in China observed positive associations between long-term ozone concentrations and prevalent prehypertension (Yang et al., 2017) and hypertension (Dong et al., 2015; Dong et al., 2014; Dong et al., 2013b; Zhao et al., 2013).
- A cohort study conducted in Spain (Cole-Hunter et al., 2018) observed positive associations between both systolic and diastolic blood pressure and long-term ozone exposure; the associations were robust to the inclusion of PM_{10} in a copollutant model. Similarly, cross-sectional studies conducted in China observed positive associations with both systolic and diastolic blood pressure (Yang et al., 2017; Liu et al., 2016; Dong et al., 2013b; Zhao et al., 2013; Chuang et al., 2011). In some instances, this effect was larger for systolic, compared to diastolic, blood pressure (Yang et al., 2017; Liu et al., 2016).
- A cross-sectional study conducted in China (Yang et al., 2017) observed stronger associations between long-term ozone exposure and prevalent prehypertension and blood pressure among women compared with the entire population. In contrast, a separate cross-sectional study conducted in China reported stronger associations between long-term ozone exposure and hypertension and blood pressure among men compared to women (Dong et al., 2013b).
- A cross-sectional study conducted in China (Yang et al., 2017) observed stronger associations between long-term ozone exposure and prevalent prehypertension among older adults (>55 years) compared with younger adults (<35 years). In an additional cross-sectional study conducted in China, (Dong et al., 2013b) observed stronger associations between long-term ozone exposure and hypertension in both older adults (>65 years) and younger adults (<55 years) compared to adults aged 55−64 years. Similarly, Yang et al. (2017) observed stronger associations between long-term ozone exposure and blood pressure in younger (<35 years) compared to older (>55 years) adults.
- Zhao et al. (2013) reported stronger associations between long-term ozone exposure and hypertension and blood pressure among overweight and obese adults, compared to normal weight adults. This trend was especially strong among men, and less apparent when in women. Similarly,

Dong et al. (2015) observed a higher magnitude of effect for both hypertension and blood pressure among overweight and obese children compared with normal-weight children, although no difference was observed between boys and girls.

- Dong et al. (2014) reported positive associations between long-term ozone exposure and hypertension and blood pressure in children and observed stronger associations among children that had never been breastfed. In a related analysis (Dong et al., 2015), they observed stronger associations in overweight and obese children compared to normal-weight children.

4.2.8.2 Animal Toxicological Studies

In the 2013 Ozone ISA, no studies examined the relationship between long-term exposure to ozone and changes in BP. Recently, Gordon et al. (2013) reported that long-term exposure (17 weeks) to ozone (0.8 ppm) did not result in changes in SBP or DBP in adult or senescent rats (Table 4-41). Thus, there continues to be no evidence from animal toxicological studies that long-term exposure to ozone can result in changes in BP.

4.2.9 Heart Rate and Heart Rate Variability

4.2.9.1 Epidemiologic Studies

No studies examining long-term ozone exposure and heart rate were included in the 2013 Ozone ISA. A recent cohort study observed positive associations between annual average ozone concentrations and increases in heart rate in a Spanish population (Cole-Hunter et al., 2018). These associations were robust to the inclusion of PM_{10} in a copollutant model.

4.2.9.2 Animal Toxicological Studies

No animal toxicological studies examining the relationship between long-term exposure to ozone and HR or HRV were included in the 2013 Ozone ISA. Recently, Gordon et al. (2013) reported that long-term exposure (17 weeks) to ozone (0.8 ppm) did not result in changes in HR in adult or senescent rats. However, in an additional study using a different exposure protocol (Table 4-42), this group did find an increase in HR following long-term episodic exposure (13 weeks) to ozone (1.0 ppm) ($p < 0.05$) in adult or senescent rats (Gordon et al., 2014). Overall, the evidence for an effect of long-term exposure to ozone on HR remains limited. There were no studies examining the relationship between long-term exposure to ozone and HRV.

4.2.10 Coagulation

4.2.10.1 Animal Toxicological Studies

The 2013 Ozone ISA presented some evidence that long-term exposure to ozone resulted in changes in factors involved in coagulation, such as tissue plasminogen activator, plasminogen activator inhibitor-1, and von Willebrand factor [U.S. EPA (2013a), pg 7−38]. Since the 2013 Ozone ISA was published, Gordon et al. (2013) has reported that long-term exposure (17 weeks) to ozone (0.8 ppm) results in small changes in aortic mRNA levels of TF ($p < 0.05$), but not tPA, vWF, thrombomodulin, and other mRNA markers of coagulation in adult or senescent rats. These authors also report no effect of long-term ozone exposure on platelet levels in blood in adult or senescent rats. Overall, there is limited evidence from animal toxicological studies that long-term exposure to ozone can result in changes in mRNA levels of coagulation factors (Table 4-43).

4.2.11 Systemic Inflammation and Oxidative Stress

4.2.11.1 Epidemiologic Studies

The majority of studies evaluating long-term ozone exposure and cardiovascular outcomes included in the 2013 Ozone ISA assessed cardiovascular disease-related biomarkers. The studies used annual or multiyear averages of air monitoring data for exposure assessment and reported generally null effects with common biomarkers, including CRP, fibrinogen, and IL-6. A limited number of recent studies provide evidence that is generally consistent with the evidence included in the 2013 Ozone ISA. Specifically:

- A cohort study of midlife, multiethnic women conducted in the U.S. (Green et al., 2015) observed positive associations with factor VIIc and hs-CRP.
- Cross-sectional studies conducted in Germany (Pilz et al., 2018) and Taiwan (Chuang et al., 2011) reported null or negative associations with CRP and IL-6, respectively. Chuang et al. (2011) observed positive associations between long-term ozone exposure and increases in neutrophils and small changes in hemoglobin A1c.

4.2.11.2 Animal Toxicological Studies

In the 2013 Ozone ISA, there was evidence that long-term exposure to ozone resulted in increased levels of TNF-α while decreasing the anti-inflammatory cytokine IL-10 [U.S. EPA (2013a),

pg 7−39]. In addition, there was evidence that long-term exposure to ozone decreased SOD enzyme activity and increased levels of malondialdehyde. Recent studies provide some evidence that long-term exposure (4−17 weeks) to ozone can result in an increase in markers of inflammation and oxidative stress (Table 4-44). Specifically:

- The same laboratory cited in the 2013 Ozone ISA reported that in rats, long-term exposure to ozone resulted in an increase in myocardial production of TNF-α ($p < 0.05$) (Perepu et al., 2012; Sethi et al., 2012). This laboratory (Perepu et al., 2012) also reported a decrease in the anti-inflammatory cytokine IL-10 following long-term exposure to ozone (0.8 ppm).
- In rats, Miller et al. (2016) also reported an increase in serum levels of IL-4, IL-10, and IFN-γ, but no change in IL-1 or TNF-α following long-term ozone (1 ppm) exposure.
- Notably, some studies also found that long-term exposure to ozone (0.8, 1.0 ppm) resulted in no appreciable changes in other inflammatory markers, including TNF-α, IL-1 (Miller et al., 2016), and total lymphocytes (Gordon et al., 2013).

With respect to markers of oxidative stress, there is limited evidence that long-term exposure to ozone can result in markers of oxidative stress. That is:

- In rats, Sethi et al. (2012) and Perepu et al. (2012) reported a decrease in SOD activity ($p < 0.05$) following long-term ozone (0.8 ppm) exposure. Perepu et al. (2012) also reported an increase in lipid peroxidation.
- However, Gordon et al. (2013) reported no appreciable change in HO-1 levels following long-term ozone (0.8 ppm) exposure in rats.

4.2.12 Stroke and Associated Cardiovascular Effects

4.2.12.1 Epidemiologic Studies

No studies examining long-term ozone exposure and stroke or other cerebrovascular outcomes were included in the 2013 Ozone ISA. A recent national cohort study conducted in England observed null associations between long-term ozone exposure and both stroke and cerebrovascular disease (Atkinson et al., 2013). In addition, several recent publications report results from a cross-sectional study conducted in 33 Chinese communities, noting positive associations between long-term ozone exposure and stroke (Dong et al., 2013a). When stratified by obesity status, positive associations were observed between long-term ozone exposure and stroke for adults that were overweight or obese, and null associations for adults with normal weight (Qin et al., 2015). These studies are characterized in Table 4-45.

4.2.13 Other Cardiovascular Endpoints

4.2.13.1 Pulmonary Embolism

No studies examining long-term ozone exposure and heart rate were included in the 2013 Ozone ISA. A recent case-control study conducted in Italy (Spiezia et al., 2014) reported negative associations between monthly average ozone concentrations and unprovoked acute isolated pulmonary embolism.

4.2.13.2 Erectile Dysfunction Incidence

No studies examining long-term ozone exposure and erectile dysfunction were included in the 2013 Ozone ISA. A recent U.S. nationwide study in a cohort of older men (Tallon et al., 2017) observed positive (though imprecise) associations between self-reported incident erectile dysfunction and long-term warm-season ozone exposure averaged over 1 to 7 years.

4.2.14 Aggregate Cardiovascular Disease

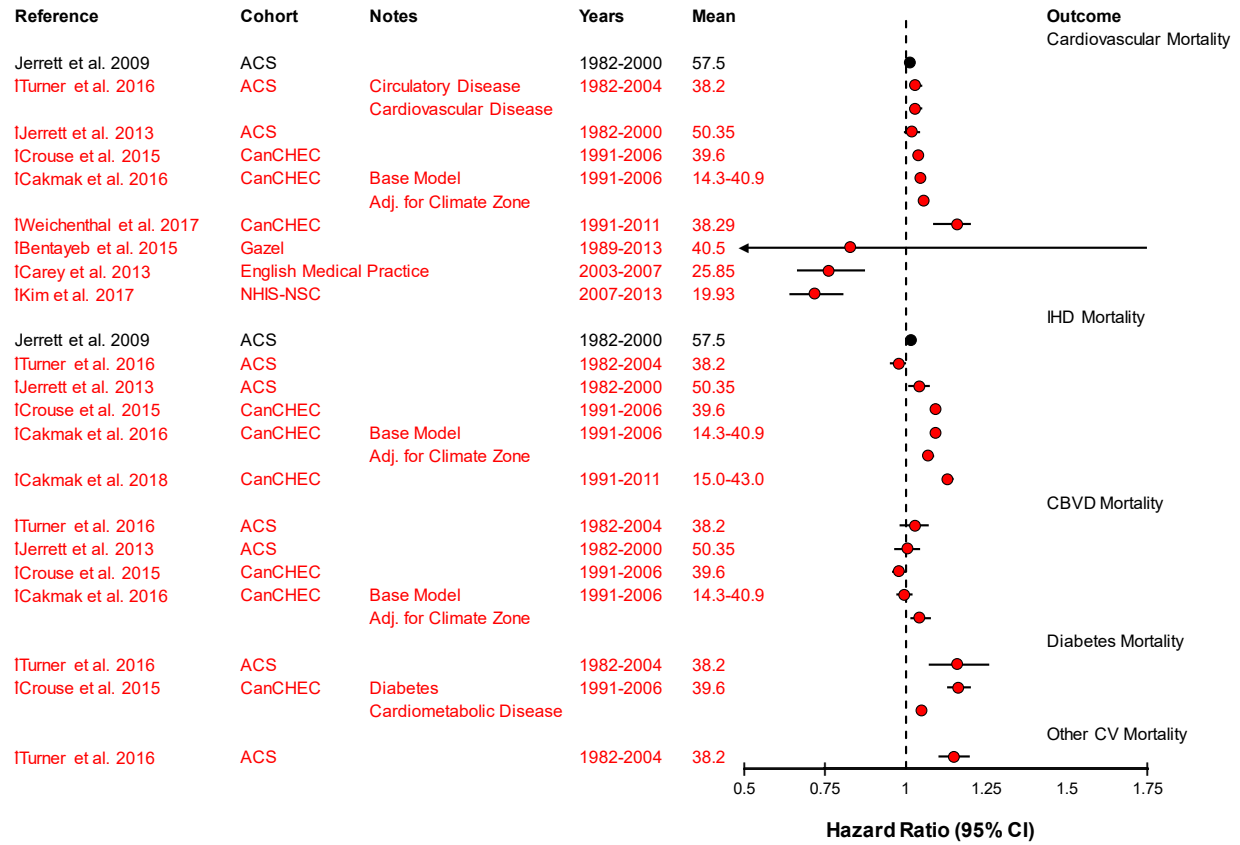
4.2.14.1 Epidemiologic Studies

No studies examining long-term ozone exposure and aggregate endpoints related to cardiovascular disease were included in the 2013 Ozone ISA. A recent national cohort study conducted in England observed null associations between long-term ozone exposure and cardiovascular disease (Atkinson et al., 2013). In addition, several recent publications report results from a cross-sectional study conducted in 33 Chinese communities, noting positive associations between long-term ozone exposure and cardiovascular disease, although when stratified by sex, a positive association was only observed for males (Dong et al., 2013a). When stratified by obesity status, positive associations were observed between long-term ozone exposure and cardiovascular disease for adults that were obese, and null associations were observed for normal-weight or overweight adults (Qin et al., 2015). In females, the association was positive among those with higher BMIs (i.e., >25 kg/m^2) and negative among those with lower BMIs. These studies are characterized in Table 4-46.

4.2.15 Cardiovascular Mortality

Recent cohort studies extend the body of evidence for the relationship between long-term ozone exposure and cardiovascular-related mortality. The 2013 Ozone ISA noted inconsistent evidence for cardiopulmonary mortality, and there was limited evidence from an analysis of the ACS cohort for the association between long-term ozone exposure and cardiovascular mortality (Jerrett et al., 2009). Recent analyses from the ACS cohort in the U.S. and the CanCHEC cohort in Canada provide consistent evidence for positive associations between long-term ozone exposure and cardiovascular and IHD mortality, as well as mortality due to diabetes or cardiometabolic diseases. Associations with mortality due to cerebrovascular disease (e.g., stroke) were less consistent, and generally yielded closer-to-the-null values. Other recent studies conducted in Europe and Asia report null or negative associations. Recent studies used a variety of fixed-site (i.e., monitors), models (e.g., CMAQ, dispersion models) and hybrid methods (combining fixed-site and model techniques) to measure or estimate ozone concentrations for use in assigning long-term ozone exposure in epidemiologic studies (Appendix 2, Section 2.3). The differences in the way exposure to ozone was assessed do not explain the heterogeneity in the observed associations. The results from studies evaluating long-term ozone exposure and cardiovascular mortality are presented in Figure 4-7. Overall, there is increased evidence that long-term ozone exposure is associated with cardiovascular mortality compared to the evidence included in the 2013 Ozone ISA. Specifically:

- The strongest evidence for an association between long-term ozone exposure and cardiovascular mortality comes from nationwide analyses of the ACS cohort, demonstrating positive associations with cardiovascular mortality (Turner et al., 2016; Jerrett et al., 2013; Jerrett et al., 2009), IHD mortality (Jerrett et al., 2013), cerebrovascular disease mortality (Turner et al., 2016), and mortality due to dysrhythmia and heart failure (Turner et al., 2016).
- Several recent analyses of the CanCHEC cohort in Canada provide consistent evidence for a positive association between long-term ozone exposure and cardiovascular and IHD mortality (Cakmak et al., 2017; Cakmak et al., 2016; Crouse et al., 2015).
- Cohort studies conducted in France (Bentayeb et al., 2015), the U.K. (Carey et al., 2013), and South Korea (Kim et al., 2017) report negative associations between long-term ozone exposure and cardiovascular mortality.
- Several recent studies conducted in the U.S. and Canada provide limited and inconsistent evidence for an association between long-term ozone exposure and mortality due to cerebrovascular disease (Figure 4-7).
- A limited body of evidence demonstrates positive associations between long-term ozone exposure and mortality from diabetes and cardiometabolic diseases (Turner et al., 2016; Crouse et al., 2015).



ACS = American Cancer Society; CanCHEC = Canadian Census Health and Environment Cohort; CBVD = cerebrovascular disease; CV = cardiovascular; IHD = ischemic heart disease; NHIS-NSC = National Health Insurance Service—National Sample Cohort.

Note: †Studies published since the 2013 Ozone ISA. Associations are presented per 10-ppb increase in pollutant concentration. Circles represent point estimates; horizontal lines represent 95% confidence intervals for ozone. Black text and circles represent evidence included in the 2013 Ozone ISA; red text and circles represent recent evidence not considered in previous ISAs or AQCDs.

Corresponding quantitative results are reported in Supplemental Table A5-C (HERO).

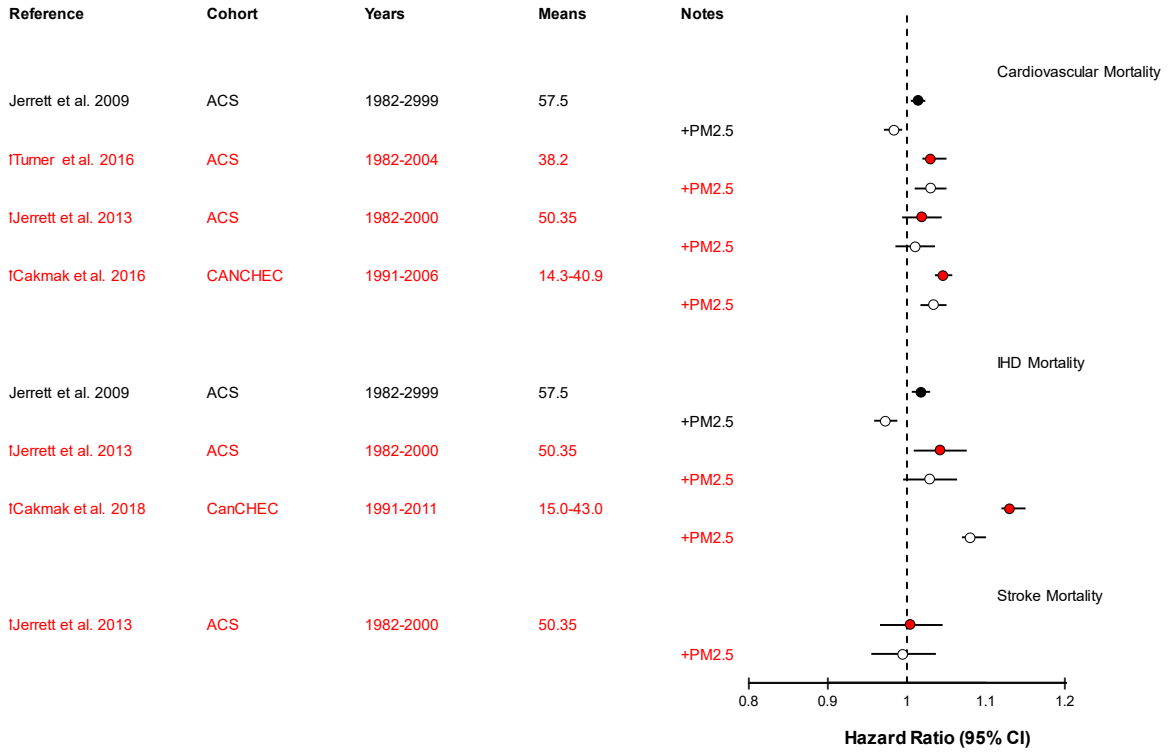
Figure 4-7 Associations between long-term exposure to ozone and cardiovascular mortality in recent cohort studies.

4.2.16 Potential Copollutant Confounding of the Ozone-Cardiovascular Disease (CVD) Relationship

The evaluation of potential confounding effects of copollutants on the relationship between long-term ozone exposure and cardiovascular effects allows for examination of whether ozone risks are changed in copollutant models. In the 2013 Ozone ISA, Jerrett et al. (2009) reported associations with cardiovascular mortality that were attenuated, changing from positive to negative, after adjustment for $PM_{2.5}$ concentrations. Recent studies examined the potential for copollutant confounding by evaluating

copollutant models that included $PM_{2.5}$, PM_{10}, and NO_2. These recent studies address a previously identified data gap by informing the extent to which effects associated with long-term ozone exposure are independent of coexposure to correlated copollutants in long-term analyses.

- Several recent studies of cardiovascular mortality observe that the association between long-term ozone exposure and cardiovascular mortality is attenuated in models that also include $PM_{2.5}$ (Figure 4-8), consistent with the results presented in (Jerrett et al., 2009). Whereas the associations were attenuated and changed from positive to negative in Jerrett et al. (2009), the associations between long-term ozone exposure and cardiovascular mortality are attenuated but remain positive after adjusting for $PM_{2.5}$ in several recent studies. Similarly, the inclusion of $PM_{2.5}$ in copollutant models had little effect on the association between long-term ozone exposure and markers of inflammation in a cohort of multiethnic women (Green et al., 2015).
- When examining other cardiovascular endpoints, several recent studies report that the associations with long-term ozone exposure were robust to the inclusion of $PM_{2.5}$ or PM_{10} in copollutant models. The association between long-term ozone exposure and incident hypertension in a cohort of black women was relatively unchanged when $PM_{2.5}$ was included in copollutant models (Coogan et al., 2017). Adding $PM_{2.5}$ to the model had little impact on the association with cardiovascular health effects across studies. When PM_{10} was included in copollutant models, it had little effect on the association between long-term ozone exposure and MI, stroke, arrhythmia or heart failure (Atkinson et al., 2013), measures of blood pressure or heart rate (Cole-Hunter et al., 2018), or changes in CIMT (Breton et al., 2012).
- When NO_2 was included in copollutant models, it had little effect on the association between long-term ozone exposure and MI, stroke, arrhythmia or heart failure (Atkinson et al., 2013), or changes in CIMT (Breton et al., 2012). The association between long-term ozone exposure and incident hypertension in a cohort of black women was attenuated, but remained positive, when NO_2 was included in copollutant models (Coogan et al., 2017).



ACS = American Cancer Society; CanCHEC = Canadian Census Health and Environment Cohort; IHD = ischemic heart disease.
Note: Studies published since the 2013 Ozone ISA. Associations are presented per 10-ppb increase in pollutant concentration. Circles represent point estimates; horizontal lines represent 95% confidence intervals for ozone. Black text and circles represent evidence included in the 2013 Ozone ISA; red text and circles represent recent evidence not considered in previous ISAs or AQCDs. Closed circles represent effect of ozone in single pollutant models, open circles represent effect of ozone adjusted for $PM_{2.5}$.

Figure 4-8 Associations between long-term exposure to ozone and cardiovascular mortality with and without adjustment for $PM_{2.5}$ concentrations in recent cohort studies.

4.2.17 Effect Modification of the Ozone-Cardiovascular Relationship

4.2.17.1 Pre-existing Disease

Individuals with certain pre-existing diseases may be considered at greater risk of an air pollution-related health effect because they are likely in a compromised biological state that can vary depending on the disease and severity. The 2013 Ozone ISA concluded that there was adequate evidence for increased ozone-related health effects among individuals with asthma (U.S. EPA, 2013a). The results

of controlled human exposure studies, as well as epidemiologic and animal toxicological studies, contributed to this evidence. No studies evaluated in the 2013 Ozone ISA evaluated the potential of pre-existing disease to modify the relationship between long-term ozone exposure and cardiovascular health effects. Several recent studies conducted in China have evaluated the potential for pre-exiting disease (e.g., hypertension, obesity) to modify the associations between long-term ozone exposure and stroke, hypertension or measures of blood pressure.

- Yang et al. (2017) observed increases in SBP and DBP associated with long-term ozone exposure; these associations were stronger among those with prehypertension compared to "normotensive" adults, although the associations were attenuated to near-null when hypertensive adults are compared with normotensive adults, regardless of medication use.
- Positive associations were observed between long-term ozone exposure and stroke among overweight and obese adults but not for normal-weight adults (Qin et al., 2015). Zhao et al. (2013) reported positive associations between long-term ozone exposure and hypertension, SBP, and DBP among adults, which increased in magnitude when restricted to overweight and obese adults. This trend was especially strong among men, and was less apparent in analyses restricted to women. In evaluations of children, the associations between long-term ozone exposure and hypertension, SBP, and DBP were stronger among overweight children compared to normal-weight children (Dong et al., 2015).
- Qin et al. (2015) provided evidence of an interaction between sex and obesity status on the effect of long-term ozone of cardiovascular health effects. Among all adults, there were positive associations between ozone exposure and cardiovascular health effects, and these associations were positive and higher in magnitude for those with higher BMI (i.e., >25 kg/m^2). When stratifying by BMI and sex, positive associations were observed between long-term ozone exposure and cardiovascular health effects in men, with stronger associations in men with higher BMIs. In females, the association was positive among those with higher BMIs (i.e., >25 kg/m^2) and negative among those with lower BMIs. Positive associations were observed between long-term ozone exposure and CVD effects among obese adults; these associations remained positive but were attenuated and near null for normal-weight and overweight adults. Among all adults, there were positive associations between ozone exposure and CVD effects, and these associations were similar after stratifying by BMI among all adults and males. In females, the association was positive among those with higher BMIs (i.e., >25 kg/m^2) and negative among those with lower BMIs.

4.2.17.2 Lifestage

The 1996 and the 2006 Ozone AQCDs identified children, especially those with asthma, and older adults as at-risk populations (U.S. EPA, 2006, 1996a). In addition, the 2013 Ozone ISA concluded that there was adequate evidence to conclude that children and older adults are at increased risk of ozone-related health effects (U.S. EPA, 2013a). Collectively, the majority of evidence for older adults has come from studies of short-term ozone exposure and mortality, with little evidence contributed by studies of long-term ozone exposure. No recent studies contribute evidence to determine whether children are at a greater risk of cardiovascular health effects due to long-term ozone exposure compared to adults. A limited number of recent studies of long-term ozone exposure and cardiovascular effects have compared

associations between different age groups, but do not report consistent evidence that older adults are at increased risk.

- In an English cohort, Atkinson et al. (2013) observed no difference in the association between long-term ozone exposure and heart failure for participants aged 40−64 years compared with those aged 65−89 years.
- In a cross-sectional study of 33 Chinese communities (Dong et al., 2013a), the association between long-term ozone exposure and prevalent prehypertension was stronger among older women (>55 years) compared with younger women (<35 years), whereas the association for increases in blood pressure were stronger among younger adults (<35 years) compared with older adults (>55 years). In an additional cross-sectional analysis of a Chinese population, stronger associations were observed between long-term ozone exposure and hypertensions in both younger (<55 years) and older (>65 years) adults, compared with adults that were between 55 and 64 years old.

4.2.18 Summary and Causality Determination

This section evaluates evidence for cardiovascular health effects, with respect to the causality determination for long-term exposures to ozone using the framework described in the Preamble to the ISA (U.S. EPA, 2015). The key evidence, as it relates to the causal framework, is summarized in Table 4-2. A small number of toxicological studies reviewed in the 2013 Ozone ISA provided some evidence for enhanced atherosclerosis and impaired cardiac contraction in isolated perfused rat hearts following long-term ozone exposure [U.S. EPA (2013a). see pg 7−40]. In addition, an animal toxicological study demonstrated increases in markers associated with inflammation, oxidative stress, thrombosis, and vasoconstriction following long-term exposure [U.S. EPA (2013a), see pg 7-40]. The limited body of epidemiologic evidence included in the 2013 Ozone ISA included studies of long-term ozone exposure and circulating biomarkers, as well as a study evaluating cardiovascular mortality. Recent epidemiologic evidence remains limited, although several recent studies provide some evidence for changes in measures of blood pressure or increases in hypertension outcomes. Further, the number of studies of cardiovascular mortality has increased, and these studies generally report positive associations. Overall, the limited number of recent studies are consistent with, and in some cases extend, the conclusions in the 2013 Ozone ISA. This evidence is discussed in greater detail below.

Overall, the evidence base describing the relationship between long-term ozone exposure and cardiovascular effects remains limited. A couple of recent animal toxicological studies continue to demonstrate impaired cardiac function following long-term ozone exposure. Note that these studies were conducted by the same laboratory and show similar effects to those studies included in the 2013 Ozone ISA (Section 4.2.5.2). In addition, a limited number of recent animal toxicological studies show inconsistent evidence with respect to increases in markers of inflammation, oxidative stress, and a proatherosclerotic environment.

There continues to be a limited number of epidemiologic studies evaluating the association between long-term ozone exposure and cardiovascular effects. In the 2013 Ozone ISA, a number of studies considered the relationship between long-term ozone exposure and circulating biomarkers in the blood, observing generally null associations. Few recent studies evaluated circulating biomarkers, but instead focused on changes in blood pressure or hypertension, with relatively few studies evaluating outcomes such as IHD or MI, HF, or stroke. In addition, a number of recent epidemiologic studies of cardiovascular mortality provide evidence of positive associations with long-term ozone exposure. Compared to the 2013 Ozone ISA, a greater number of recent epidemiologic studies of cardiovascular morbidity and mortality evaluate the potential for copollutant confounding, especially with PM_{10} and NO_2 (Section 4.2.16). One study (Coogan et al., 2017) evaluated $PM_{2.5}$ in copollutant models. Generally, these studies report that the ozone association is relatively unchanged or slightly attenuated in copollutant models (Section 4.2.16; Figure 4-8). Potential copollutant confounding continues to be a source of uncertainty when characterizing the relationship between long-term ozone exposure and cardiovascular health effects.

Consistent with previous evidence, recent studies continue to demonstrate associations between long-term ozone exposure and cardiovascular health effects among older adults, although the limited number of studies that evaluated effect modification by age do not provide evidence that older adults are at increased risk of cardiovascular health effects related to long-term ozone exposure compared with other adults. Similarly, there is some emerging evidence that long-term ozone exposure may be associated with changes in blood pressure among children, but there are no studies that evaluate whether children are at increased risk of ozone-related cardiovascular health effects compared with adults. With regard to pre-existing disease, there is limited recent evidence that BMI or obesity status may modify the risk of long-term ozone exposure on changes in blood pressure, but this evidence base is small and not entirely consistent.

Overall, recent animal toxicological and epidemiologic studies add to the body of evidence that formed the basis of the conclusions in the 2013 Ozone ISA for cardiovascular health effects. This body of evidence is limited, however, with some experimental and observational evidence for subclinical cardiovascular health effects and little evidence for associations with outcomes such as IHD or MI, HF, or stroke. The strongest evidence for the association between long-term ozone exposure and cardiovascular health outcomes continues to come from animal toxicological studies of impaired cardiac contractility and epidemiologic studies of blood pressure changes and hypertension and cardiovascular mortality. Recent epidemiologic studies observed positive associations with changes in blood pressure or hypertension, but animal toxicological studies do not report effects of ozone on blood pressure changes. In conclusion, the results observed across both recent and older experimental and observational studies conducted in various locations provide limited evidence for an association between long-term ozone exposure and cardiovascular health effects. Collectively, **the body of evidence for long-term ozone exposure and cardiovascular effects is suggestive of, but not sufficient to infer, a causal relationship.**

Table 4-2 Summary of evidence that is suggestive of, but not sufficient to infer, a causal relationship between long-term ozone exposure and cardiovascular effects.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Limited or inconsistent evidence from animal toxicological studies at relevant ozone concentrations	Impaired cardiac contractility, increased markers associated with systemic inflammation/oxidative stress, and a proatherosclerotic environment	Section 4.2.4.2 Section 4.2.11	
Consistent evidence from epidemiologic studies of cardiovascular mortality at relevant ozone concentrations	Nationwide analyses of the ACS cohort, demonstrating positive associations with cardiovascular mortality; CanCHEC cohort in Canada provides consistent evidence for a positive association with IHD mortality	Section 4.2.15	14.3−57.5 ppb
Generally null evidence from epidemiologic cohort studies of IHD, HF, and stroke	A limited number of studies evaluated these cardiovascular morbidity endpoints and generally report null or inverse associations with ozone exposure	Section 4.2.3.1 Section 4.2.5.1 Section 4.2.12.1	19.9−24.7 ppb
No evidence from a limited number of animal toxicological studies	Changes in blood pressure	Section 4.2.8.2	

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble to the ISAs (U.S. EPA, 2015).

[b]Describes the key evidence and references, supporting or contradicting, contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where full body of evidence is described.

[c]Describes the ozone concentrations with which the evidence is substantiated.

4.3 Evidence Inventories—Data Tables to Summarize Study Details

4.3.1 Short-Term Ozone Exposure

Table 4-3 Epidemiologic studies of short-term exposure to ozone and heart failure.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Winquist et al. (2012) St. Louis MSA, U.S. Ozone: January 1, 2001−June 27, 2007 Follow-up: January 1, 2001−June 27, 2007 Time-series study	n = 22.4 Counts of daily ED visits and HA for CHF among people residing in the St. Louis MSA	Concentrations from U.S. EPA AQS at Tudor Street stationary monitor; data missing 1.9% of days 8-h max	Mean: 36.3 Maximum: 111.8	Correlation (*r*):$PM_{2.5}$: 0.25 Copollutant models with: NR	ED visits, lag 0−4: 1.05 (1.01, 1.09) HA, lag 0−4: 1.05 (1.02, 1.09)
↑Milojevic et al. (2014) England and Wales, U.K. Ozone: 2003−2009 Follow-up: 2003−2009 Study	HES n = 312,332 Emergency hospital admissions for heart failure to NHS hospitals, 2003−2008, in HES database using centroid of census ward; median age (IQR) 73 yr (60−82), 54% male HES	Data from nearest monitoring station to residence on event day. Control exposure days defined using time-stratified design using other days of the month when case occurred 8-h max	Mean: NR Median: 30.96 75th: 38.58	Correlation (*r*): $PM_{2.5}$: −0.096; NO_2: −0.3489; SO_2: −0.0849; Other: PM_{10} 0.0302, CO −0.2973 Copollutant models with: NA	Heart failure, lag 0−4: 0.99 (0.98, 1.01)

Table 4-3 (Continued): Epidemiologic studies of short-term exposure to ozone and heart failure.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Sarnat et al. (2015) St. Louis, MO, U.S. Ozone: June 1, 2001–May 30, 2003 Follow-up: June 1, 2001–May 30, 2003 Time-series study	n = 69,679 ED visit records of patients with CHF residing in St. Louis MSA (eight counties each in Missouri and Illinois) from 36 out of 43 acute care hospitals	Averaged hourly concentrations in St. Louis from U.S. EPA AQS 8-h max	Mean: 36.2	Correlation (*r*): $PM_{2.5}$: 0.23; NO_2: 0.37; SO_2: −0.04; Other: CO −0.01 Copollutant models with: NR	0−2 day distributed lag: 1.04 (0.99, 1.10) Copollutant model with NO_2, 2 day distributed lag: 1.02 (0.96, 1.08) Copollutant model with $PM_{2.5}$, 2 day distributed lag: 1.02 (0.97, 1.08) Copollutant model with CO, 2 day distributed lag: 1.06 (1.00, 1.12)
↑Rodopoulou et al. (2015) Little Rock, AR, U.S. Ozone: 2002−2012 Follow-up: 2002−2012 Time-series study	n = 84,269 Daily emergency room visits among persons 15 yr and older, 19% 65 yr and older, 42.5% male	U.S. AQS data from stationary monitor in Little Rock 8-h max	Mean: 40 Median: 39 75th: 50	Correlation (*r*): NR Copollutant models with: NR	Hypertensive heart disease and heart failure, lag 1: 0.97 (0.91, 1.05)
↑Winquist et al. (2012) St. Louis MSA, U.S. Ozone: January 1, 2001−June 27, 2007 Follow-up: January 1, 2001−June 27, 2007 Time-series study	n = 22.4 Counts of daily ED visits and HA for CHF among people residing in the St. Louis MSA	Concentrations from U.S. EPA AQS at Tudor Street stationary monitor; data missing 1.9% of days 8-h max	Mean: 36.3 Maximum: 111.8	Correlation (*r*): $PM_{2.5}$: 0.25; Copollutant models with: NR	ED visits, lag 0−4: 1.05 (1.01, 1.09) HA, lag 0−4: 1.05 (1.02, 1.09)

Table 4-3 (Continued): Epidemiologic studies of short-term exposure to ozone and heart failure.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Milojevic et al. (2014) England and Wales, U.K. Ozone: 2003−2009 Follow-up: 2003−2009	HES n = 312,332 Emergency hospital admissions for heart failure to NHS hospitals, 2003−2008, in HES database using centroid of census ward; median age (IQR) 73 yr (60−82), 54% male HES	Data from nearest monitoring station to residence on event day. Control exposure days defined using time-stratified design using other days of the month when case occurred 8-h max	Mean: NR Median: 30.96 75th: 38.58	Correlation (*r*): $PM_{2.5}$: −0.096; NO_2: −0.3489; SO_2: −0.0849; Other: PM_{10} 0.0302, CO −0.2973 Copollutant models with: NA	Heart failure, lag 0−4: 0.99 (0.98, 1.01)
↑Sarnat et al. (2015) St. Louis, MO, U.S. Ozone: June 1, 2001–May 30, 2003 Follow-up: June 1, 2001–May 30, 2003 Time-series study	n = 69,679 ED visit records of patients with CHF residing in St. Louis MSA (eight counties each in Missouri and Illinois) from 36 out of 43 acute care hospitals	Averaged hourly concentrations in St. Louis from U.S. EPA AQS 8-h max	Mean: 36.2	Correlation (*r*): $PM_{2.5}$: 0.23; NO_2: 0.37; SO_2: −0.04; Other: CO −0.01 Copollutant models with: NR	0−2 day distributed lag: 1.04 (0.99, 1.10) Copollutant model with NO_2, 0−2 day distributed lag: 1.02 (0.96, 1.08) Copollutant model with $PM_{2.5}$, lag 1: 0.97 (0.90, 1.05) Copollutant model with $PM_{2.5}$, 0−2 day distributed lag: 1.02 (0.97, 1.08) Copollutant model with CO, 0−2 day distributed lag: 1.06 (1.00, 1.12)

Table 4-3 (Continued): Epidemiologic studies of short-term exposure to ozone and heart failure.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
†Rodopoulou et al. (2015) Little Rock, AR, U.S. Ozone: 2002−2012 Follow-up: 2002−2012 Time-series study	n = 84,269 Daily emergency room visits among persons 15 yr and older, 19% 65 yr and older, 42.5% male	U.S. AQS data from stationary monitor in Little Rock 8-h max	Mean: 40 Median: 39 75th: 50	Correlation (*r*): NR Copollutant models with: NR	Hypertensive heart disease and heart failure, lag 1: 0.97 (0.91, 1.05)

Table 4-4 Study-specific details from controlled human exposure studies of impaired heart function.

Study	Population n, Sex, Age (Range or Mean ± SD)	Exposure Details (Concentration, Duration)	Endpoints Examined
Frampton et al. (2015)	Healthy adults (GSTM WT, GSTM null) n = GSTM WT 8, GSTM null seven males, GSTM WT 4, GSTM null five females Age: GSTM null: 27.3 ± 4.2 yr, GSTM WT: 25.4 ± 2.8 yr	0.1, 0.2 ppm, 3 h (alternating 15 min periods of rest and exercise)	LVDP and LV ejection time 1.5 h the day before and 2.5 h post-exposure

GSTM = glutathione S-transferase M1, LV = left ventricular; LVDP = left ventricular developed pressure; WT = wild type.

Table 4-5 Study-specific details from short-term animal toxicological studies of impaired heart function.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Tankersley et al. (2013)	Mice (C57BL/6J) n = 10−16/group males, 0 females Age: NR Nppa null mice n = 10−16/group males, 0 females Age: NR	Approximately 0.5 ppm, 3 h of ozone followed by 3 h of FA	Measures of cardiac function (8−10 h PE)
McIntosh-Kastrinsky et al. (2013)	Mice (C57BL/6) n = 0 males, 14−15/group females Age: NR	0.245 ppm, 4 h (aged, FA, or ozone) on 3 separate days outdoors	LVDP, *dP/dt,* coronary flow in isolated perfused hearts (8−11 h PE hearts were isolated and post-induced ischemia)
Kurhanewicz et al. (2014)	Mice (C57BL/6) n = 5−8/group males, 0 females Age: 10−12 weeks	0.3 ppm, 4 h	LVDP, contractility (24 h PE)

Table 4-5 (Continued): Study-specific details from short-term animal toxicological studies of impaired heart function.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Ramot et al. (2015)	Rats (FHH) n = NR males, NR females Age: 10−12 weeks Rats (S-D) n = NR males, NR females Age: 10−12 weeks Rats (SH) n = NR males, NR females Age: 10−12 weeks Rats (SHHF) n = NR males, NR females Age: 10−12 weeks Rats (SHSP) n = NR males, NR females Age: 10−12 weeks Rats (WKY) n = NR males, NR females Age: 10−12 weeks Rats (Wistar) n = NR males, NR females Age: 10−12 weeks	0.25 ppm, 4 h 0.5 ppm, 4 h 1 ppm, 4 h	Cardiac pathology (immediately after and 24 h PE)
Wang et al. (2013)	Rats (Wistar) n = 6/group males, 0 females Age: NR	0.8 ppm, 4 h of ozone followed by intra-tracheal instillation of saline or $PM_{2.5}$ twice/week for 3 weeks	Cardiac microscopy after 6th exposure sacrifice

Table 4-5 (Continued): Study-specific details from short-term animal toxicological studies of impaired heart function.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Zychowski et al. (2016)	Mice (C57BL/6J) n = 4−8/group males, 0 females Age: 6−8 weeks	1 ppm, 4 h of ozone (acute hypoxia [10.0% O_2] or normoxia [20.9% O_2] 24 h/day for 3 weeks prior to exposure)	RV hypertrophy (18−20 h PE)

FA = filtered air; FHH = fawn-hooded hypertensive; LVDP = left ventricular developed pressure; PE = post-exposure; SH = spontaneously hypertensive; SHHF = spontaneously hypertensive heart failure; S-D = Sprague-Dawley, WKY = Wistar Kyoto.

Table 4-6 Epidemiologic studies of short-term exposure to ozone and ischemic heart disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Finnbjornsdottir et al. (2013) Reykjavik, Iceland Ozone: January 1, 2005−December 31, 2009 Follow-up: January 1, 2005−December 31, 2009 Case-crossover study	Icelandic Medicines Registry n = 5,246 Adults 18 yr or older living in Reykjavik capital area to whom glyceryl trinitrates were dispensed at least once, mean age 74 yr, 57.9% male	Averaged hourly concentrations at busy intersection; calculated 24-h avg and running avg of 3-day means including the day of dispensing and 2 days prior. Control exposure days selected using symmetric bidirectional design, 7 days before and after the index day of event 24-h avg	Mean: 20.66 Maximum: 144.5 μg/m^3	Correlation (*r*): NO_2: −0.62; Other: PM_{10} 0.13 Copollutant models with: NA	24-h avg, lag 0: 0.96 (0.90, 1.04) 24-h avg, lag 1: 1.05 (0.81, 1.13) 24-h avg, lag 2: 1.11 (0.99, 1.26) 24-h avg, lag 3: 1.06 (0.94, 1.20) Multipollutant model with NO_2 and PM_{10} 24-h avg, lag 0: 1.11 (0.99, 1.25) 24-h avg, lag 1: 1.28 (1.14, 1.37) 3-day mean, lag 0: 1.29 (1.11, 1.50)
↑Nuvolone et al. (2013) Tuscany region, five urban areas, Italy Ozone: January 2002−December 2005 Follow-up: January 2002−December 2005 Time-series study	Cardiovascular Risk and Air Pollution in Tuscany (RISCAT) study n = 4,555 All hospitalized MI cases in the study region and period; 49.1 <75 yr, mean age 72.5 yr, 60.2% male	Daily 8-h max moving average concentrations for each of 29 sites were combined into 5 areas with homogenous concentration levels 8-h max	Mean: 47.51	Correlation (*r*): NO_2: −0.08; Other: CO −0.15, PM_{10} 0.21 Copollutant models with: NR	0−1 distributed lag: 1.05 (0.96, 1.16)

Table 4-6 (Continued): Epidemiologic studies of short-term exposure to ozone and ischemic heart disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Milojevic et al. (2014) England and Wales, U.K. Ozone: 2003−2009 Follow-up: 2003−2009 Case-crossover study	MINAP register, HES n = 410 341 MI All MI events, 2003−2009, in MINAP registry located in enumeration district of residence (100-m resolution) and emergency hospital admissions to NHS hospitals, 2003−2008, in HES database using centroid of census ward; median age (IQR) 71 yr (60−81) MINAP, 73 yr (60−82), 65% male MINAP, 54% male HES	Data from nearest monitoring station to residence on event day. Control exposure days defined using time-stratified design using other days of the month when case occurred 8-h max	Mean: NR Median: 30.96 75th: 38.58	Correlation (*r*): $PM_{2.5}$: −0.096; NO_2: −0.3489; SO_2: −0.0849; Other: PM_{10} 0.0302, CO −0.2973 Copollutant models with: NA	All MI, MINAP, lag 0−4: 0.99 (0.98, 1.00) STEMI, MINAP, lag 0−4: 0.98 (0.96, 1.00) nonSTEMI, MINAP, lag 0−4: 1.00 (0.98, 1.01) IHD, HES, lag 0−4: 0.99 (0.98, 1.00) MI, HES, lag 0−4: 0.99 (0.98, 1.01)
↑Bard et al. (2014) Strasbourg metropolitan area, France Ozone: 2000−2007 Follow-up: 2000−2007 Case-crossover study	Bas-Rhin Coronary Heart Disease Register, a WHO MONICA center n = 2,134 Fatal and nonfatal MI cases, aged 35−74 yr, 76.9% male	Modeled hourly concentrations at census block level using ADMS-Urban air dispersion model. Control days selected using a monthly time-stratified design 8-h avg	Mean: 32.13 Median: 30.16 75th: 43.15 Maximum: 228.3 μg/m^3	Correlation (*r*): NO_2: −0.34; Other: PM_{10} −0.16, CO −0.34, benzene −0.51 Copollutant models with: NA	Lag 0: 0.95 (0.86, 1.05) Lag 1: 0.88 (0.79, 0.98) Lag 0−1: 0.90 (0.80, 1.01)
↑Sarnat et al. (2015) St. Louis, MO, U.S. Ozone: June 1, 2001–May 30, 2003 Follow-up: June 1, 2001–May 30, 2003 Time-series study	n = 69,679 ED visit records of patients residing in St. Louis MSA (eight counties each in Missouri and Illinois) from 36 out of 43 acute care hospitals	Averaged hourly concentrations in St. Louis from U.S. EPA AQS 8-h max	Mean: 36.2	Correlation (*r*): $PM_{2.5}$: 0.23; NO_2: 0.37; SO_2: −0.04; Other: CO −0.01 Copollutant models with: NR	Ischemic heart disease, 0−2 day distributed lag: 0.99 (0.95, 1.04)

Table 4-6 (Continued): Epidemiologic studies of short-term exposure to ozone and ischemic heart disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
†Wang et al. (2015a) Calgary, Canada Ozone: April 1, 1999−March 31, 2010 Follow-up: April 1, 1999−March 31, 2010 Case-crossover study	n = 25,894 Cases who were residents of Alberta, 20 yr or older, 67.5% male, living within 15 km to closest stationary pollution monitor and 50 km to closest meteorological monitor	Hourly concentrations from 41 monitor locations used to calculate 24-h avg, 6-h avg for morning and afternoon, 12-h avg, daily 1-h max and daily 1-h min. Cases linked to pollution data by postal code, missing records were imputed using linear interpolation. Control exposure days selected using time-stratified design matching on weekday stratified on month and year	Mean: NR	Correlation (*r*): NR Copollutant models with: NR	Analytical results were not reported for main effects for ozone, only statistically significant results reported

Table 4-6 (Continued): Epidemiologic studies of short-term exposure to ozone and ischemic heart disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
†Wang et al. (2015b) Calgary, Edmonton, Canada Ozone: April 1, 1999−March 31, 2010 Follow-up: April 1, 1999−March 31, 2010 Case-crossover study	n = 12,066 AMI cases aged 20 or older living in urban Calgary and Edmonton	Averaged hourly concentrations from four monitor locations in each city. Control exposure days selected using time-stratified design matching by weekday of event stratified on month and year 24-h avg	Mean: NR	Correlation (*r*): NR Copollutant models with: NR	Calgary-whole, lag 0: 1.00 (0.96, 1.05) Calgary-whole, lag 1: 0.97 (0.93, 1.01) Calgary-whole, lag 2: 0.98 (0.94, 1.02) Calgary-STEMI, lag 0: 1.00 (0.93, 1.07) Calgary-STEMI, lag 1: 0.94 (0.87, 1.01) Calgary-STEMI, lag 2: 0.97 (0.90, 1.04) Calgary-NSTEMI, lag 0: 1.02 (0.95, 1.09) Calgary-NSTEMI, lag 1: 0.98 (0.92, 1.05) Calgary-NSTEMI, lag 2: 1.00 (0.93, 1.06) Edmonton-whole, lag 0: 1.00 (0.96, 1.05) Edmonton-whole, lag 1: 1.00 (0.95, 1.04) Edmonton-whole, lag 2: 1.01 (0.97, 1.06) Edmonton-STEMI, lag 0: 0.98 (0.91, 1.06) Edmonton-STEMI, lag 1: 0.98 (0.91, 1.06) Edmonton-STEMI, lag 2: 0.99 (0.92, 1.07) Edmonton-NSTEMI, lag 0: 1.01 (0.95, 1.08) Edmonton-NSTEMI, lag 1: 1.01 (0.95, 1.08) Edmonton-NSTEMI, lag 2: 1.02 (0.96, 1.09)

Table 4-6 (Continued): Epidemiologic studies of short-term exposure to ozone and ischemic heart disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Claeys et al. (2015) National, Belgium Ozone: 2006−2009 Follow-up: 2006−2009 Time-series study	National percutaneous coronary intervention (PCI) database n = 15,964 All cases receiving PCI procedures within 24 h of symptom onset, 2006−2009, at 32 PCI centers in Belgium, mean age 63 yr, 75% male	Averaged hourly concentrations measured across all 73 monitors in Belgium, daily average and 5-day avg	Mean: 21.68 Maximum: 65.5	Correlation (*r*): $PM_{2.5}$: −0.35; Other: PM_{10} −0.24 Copollutant models with: NR	Lag 5: 1.03 (0.97, 1.06)
↑Butland et al. (2016) National, U.K. Ozone: 2003−2010 Follow-up: 2003−2010 Case-crossover study	MINAP n = 626,239 Acute coronary cases from the MINAP registry covering National Health Service hospitals in England and Wales excluding missing geocodes, missing data on date of event, discharge diagnosis or not residing in England and Wales and missing exposure or covariate data, median age 70.6 yr, 65% male	Daily concentrations (using hourly data) with 5- × 5-km resolution from EMEP4 U.K. atmospheric chemistry transport model (ACTM); calculated daily max 8-h running mean for ozone. MI events linked to concentrations in closest 5-km grid. Control concentrations selected using time-stratified analysis using event day stratified on month 8-h max	Mean: NR	Correlation (*r*): NR Copollutant models with: NR	All MI, lag 0−2: 1.00 (0.99, 1.01) STEMI, lag 0−2: 0.99 (0.98, 1.01) nonSTEMI, lag 0−2: 1.00 (0.99, 1.01)

Table 4-6 (Continued): Epidemiologic studies of short-term exposure to ozone and ischemic heart disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
†Argacha et al. (2016) National, Belgium Ozone: 2009−2013 Follow-up: 2009−2013 Case-crossover study	Belgian STEMI Registry n = 11,420 STEMI cases included in registry, 2009−2013, mean age 62.8 yr and 75.4% male	National daily average estimated using measurements from 41 monitors, interpolation, and adjustment for population density. Control exposure days selected using a time-stratified design, stratifying by month and year with a 4-day exclusion period around the event day 24-h avg	Mean: 5.38 Median: 21.32 75th: 27.51 95th: 36.19	Correlation (*r*): $PM_{2.5}$: −0.388; NO_2: −0.6; Other: PM_{10} −0.287 Copollutant models with: NR	Not statistically significant, results in figure
†Collart et al. (2017) Wallonia, Belgium Ozone: January 1, 2008–December 31, 2011 Follow-up: January 1, 2008–December 31, 2011 Time-series study	n = 21,491 Daily counts of hospital admissions at 42 hospitals in study region, ages 25 yr and older, mean age 66.9 yr, 66.9% male	Averaged daily concentrations from 6−16 stationary monitors 24-h avg		Correlation (*r*): NR Copollutant models with: NR	Analytic results displayed in Figure 4. No associations using any lag
†Vidale et al. (2017) Como, Italy Ozone: January 2005−December 2014 Follow-up: January 2005−December 2014 Time-series study	n = 4,110 All residents of Como with hospital admission for acute MI between January 2005 and December 2014, mean age 71 yr, 65% male	Average daily concentrations from two stationary monitors 24-h avg		Correlation (*r*): NR Copollutant models with: NR	Lag 0: 1.00 (0.99, 1.00) Lag 1: 0.98 (0.97, 1.01)
†Rasche et al. (2018) Jena, Germany Ozone: January 1, 2003−December 31, 2010 Follow-up: January 1, 2003−December 31, 2010 Case-crossover study	n = 693 STEMI cases admitted to university hospital within 72 h of symptom onset and residing within 10 km around the hospital, median age 69 yr, 67.2% male	Daily average concentration from monitor. Control exposure days selected using bidirectional design, previous and following week 24-h avg	Median: 22.71 Maximum: 117.29 μg/m^3	Correlation (*r*): NR Copollutant models with: NR	Lag 2: 0.29 (0.11, 0.86)

Table 4-6 (Continued): Epidemiologic studies of short-term exposure to ozone and ischemic heart disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Hanna et al. (2011) North Carolina, five cities, U.S. Ozone: January 1, 1996−December 31, 2004 Follow-up: January 1, 1996−December 31, 2005 Time-series study	All hospital admissions in North Carolina	Daily concentrations from U.S. EPA AQS in five cities in North Carolina 1-h max		Correlation (*r*): NR Copollutant models with: NR	Data in figures by air mass type and city; only one statistically significant association for extreme moist tropical air mass at 5 day lag
↑Bhaskaran et al. (2011) National, U.K. Ozone: 2003−2006 Follow-up: 2003−2006 Case-crossover study	MINAP n = 79,288 MI cases, 64% male, aged 59−80 yr, with time of event recorded in MINAP within 15 conurbations during 2003−2006	Averaged hourly concentrations for each conurbation from stationary monitors, average concentration for the hour of the event. Referent exposures selected using time-stratified approach using day of week within each month	Median: 19.29 75th: 28.43	Correlation (*r*): NO_2: −0.58; SO_2: −0.14; Other: CO −0.24 Copollutant models with: NA	1-h avg, lag 1−6 h: 0.99 (0.96, 1.02) 1-h avg, lag 7−12 h: 1.02 (0.99, 1.06) 1-h avg, lag 13−18 h: 0.97 (0.94, 1.00) 1-h avg, lag 19−24 h: 1.00 (0.97, 1.02) 1-h avg, lag 1−72 h: 0.97 (0.94, 1.00) 1-h avg, lag 25−72 h: 0.99 (0.96, 1.02)

Table 4-7 Epidemiologic panel studies of short-term exposure to ozone and ischemic heart disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
↑Evans et al. (2016) Rochester, NY, U.S. Ozone: 2007−2012 Panel study	n = 362 Treated for STEMI, NSTEMI, or unstable angina	Mean concentrations from NYDEC monitor 1-h max, 12, 24, 48, and 72-h avg	Mean: 27.4 Median: 27 75th: 36.9 Maximum: 104	Correlation (*r*): NR Copollutant models with: NR	Increased odds of STEMI 1 h prior to event: 1.35 (1.00, 1.85) 12 h prior to event: 1.26 (0.94, 1.69) 24 h prior to event: 1.16 (0.90, 1.50) 48 h prior to event: 1.11 (0.81, 1.51) 72 h prior to event: 1.21 (0.84, 1.74)

Table 4-8 Study-specific details from controlled human exposure studies of ST segment depression.

Study	Population n, Sex, Age (Range or Mean ± SD)	Exposure Details (Concentration, Duration)	Endpoints Examined
Rich et al. (2018)	Older adults n = 35 males, 52 females Age: 55−70 yr	0, 0.07,120 ppm, 3 h (alternating 15-min periods of rest and exercise)	ST-segment depression 15 min, 4 and 24 h PE

MI = myocardial infarction; NSTEMI = non-ST elevation myocardial infarction; PE = post-exposure; STEMI = ST elevation myocardial infarction; NYDEC = New York State Department of Environmental Conservation.

Table 4-9 Study-specific details from short-term animal toxicological studies of ST-segment depression.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Farraj et al. (2012)	Rats (SH) n = 6/group males, 0 females Age: 12 weeks	0.2 ppm, 4 h 0.8 ppm, 4 h	ST-segment depression during exposure

SH = spontaneously hypertensive; ST = beginning of the S wave to the end of the T wave.

Table 4-10 Epidemiologic panel studies of short-term exposure to ozone and endothelial function.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
↑Zanobetti et al. (2014) Boston, MA, U.S. Ozone: 2006−2009 Panel study	n = 64 T2D	Averaged hourly concentrations from local sites 24-h avg	Mean: 10 Median: 28 75th: 33 Maximum: 47	Correlation (*r*): NR Copollutant models with: NR	No change in BAD at 5-day avg exposure to ozone—qualitative result
↑Ljungman et al. (2014) Boston, MA, U.S. Ozone: 2003−2008 Panel study Lags examined: 1−7 day moving avg	Framingham Offspring/Third Generation n = 2,369	Hourly concentrations from Boston area monitors were averaged to create moving averages 24-h avg	Mean: 23 Maximum: 64	Correlation (*r*): NR Copollutant models with: NR	Percentage increase PAT ratio: 1-day moving avg: −3.43 (−6.33, −0.53) 2-day moving avg: −4.42 (−7.90, −0.93) 5-day moving avg: −0.32 (−5.01, 4.37)
↑Lanzinger et al. (2014) Chapel Hill, NC, U.S. Ozone: 2004−2005 Panel study	n = 22 Subjects with T2D aged 48−78 yr	Monitor data 8-h max	Mean: 41 Median: 39 75th: 52 Maximum: 82	Correlation (*r*): NR Copollutant models with: NR	Percentage increase FMD Lag 0: −29.2 (−52.6, −5.80) Lag 1: −27.0 (−54.0, −0.08)
↑Mirowsky et al. (2017) Chapel Hill, NC, U.S. Ozone: 2012−2014 Panel study Lags examined: 0−4, 5-day avg Additional endpoints reported: LAEI, SAEI	CATHGEN n = 13 Have undergone cardiac catheterization Age 53−68	AQS monitor 24-h avg	Mean: 26 Median: 25 75th: 33 Maximum: 63	Correlation (*r*): NR Copollutant models with: $PM_{2.5}$	Percentage increase FMD Lag 0: −17.14 (−40.82, 15.11) Lag 1: 4.82 (−27.21, 49.82) 5-day avg: −19.93 (−53.46, 34.39) Percentage increase BAD Lag 0: −2.25 (−5.46, 1.07) Lag 1: −2.04 (−5.25, 1.29) 5-day avg: 1.82 (−3.11, 7.07)

BAD = brachial artery diameter; PAT = pulse amplitude tonometry; FMD = flow-mediated dilation; LAEI = large artery elasticity index; SAEI = small artery elasticity index; T2D = type 2 diabetes.

Table 4-11 Study-specific details from controlled human exposure studies of vascular function.

Study	Population n, Sex, Age (Range or Mean ± SD)	Exposure Details (Concentration, Duration)	Endpoints Examined
Rich et al. (2018)	Older adults n = 35 males, 52 females Age: 55−70 yr	0, 0.07, 120 ppm, 3 h (alternating 15-min periods of rest and exercise)	BAD, FMD, VTI (day before exposure and at the end of each of the three exposures)
Barath et al. (2013)	Healthy adults n = 36 males, 0 females Age: 26 ± 1 yr	0.3 ppm, 75 min (alternating 15-min periods of exercise and rest)	Forearm blood flow in response to acetylcholine, sodium nitroprusside, verapamil, or bradykinin. 2 and 6 h post-exposure
Frampton et al. (2015)	Healthy adults (GSTM WT, GSTM null) n = GSTM WT 8, GSTM null seven males, GSTM WT 4, GSTM null five females Age: GSTM null: 27.3 ± 4.2 yr, GSTM WT: 25.4 ± 2.8 yr	0.1, 0.2 ppm, 3 h (alternating 15-min periods of rest and exercise)	Indicators of endothelial dysfunction including flow in response to reactive hyperemia measured by arterial tonometry 1.5 h the day before and 2.5 h post-exposure

BAD = brachial artery diameter; FMD = flow-mediated dilation; GSTM = glutathione S-transferase M1; VTI = velocity-time interval; WT = wild type.

Table 4-12 Study-specific details from short-term animal toxicological studies of vascular function.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Wang et al. (2013)	Rats (Wistar) n = 6/group males, 0 females Age: NR	0.8 ppm, 4 h of ozone followed by intra-tracheal instillation of saline or $PM_{2.5}$ twice/week for 3 weeks	Markers of endothelial dysfunction in blood (after 6th exposure animals sacrificed blood drawn)
Robertson et al. (2013)	Mice (C57BL/6) n = NR males, NR females Age: 8−10 weeks Mice (CD 36-/-) n = NR males, NR females Age: 8−10 weeks	1 ppm, 4 h	Relaxation of aortic rings in response to acetylcholine (aortic rings isolated 24 h PE)
Paffett et al. (2015)	Rats (S-D) n = 65 males, 0 females Age: 8−12 weeks	1 ppm, 4 h	Serum-induced vascular dysfunction (serum collected immediately before sacrifice) Vascular function (24 h PE)
Kumarathasan et al. (2015)	Rats (F344) n = 8/exposure group, 17/control group males; 0 females Age: NA	0.8 ppm, 4 h	Markers of endothelial dysfunction in blood immediately and 24 h PE) Markers of oxidative stress in blood (immediately and 24 h PE)
Snow et al. (2018)	Rats (WKY) n = 6−8/group males, 0 females Age: ~12 weeks	0.8 ppm, 4 h/day for 2 consecutive days (diets enriched with coconut, olive, or fish oil for 8 weeks prior)	Endothelial function (2 h PE)
Thomson et al. (2013)	Rats (Fischer) n = 4−6/group males, 0 females Age: NR	0.4 ppm, 4 h 0.8 ppm, 4 h	mRNA markers of vascular function (tissue collected immediately PE)

PE = post-exposure, S-D = Sprague-Dawley, WKY = Wistar Kyoto.

Table 4-13 Epidemiologic studies of short-term exposure to ozone and emergency department visits or hospital admissions for electrophysiological changes, arrhythmia, and cardiac arrest.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Ensor et al. (2013) Houston, TX, U.S. Ozone: 2004−2011 Follow-up: 2004−2011 Case-crossover study	n = 11,677 All adults in EMS database, aged 18 yr and over, mean 64 yr, 59% male, 46% black	TCEQ monitoring data, hourly concentration from 47 monitors, calculated daily max 8-h running mean. Control days selected using time-stratified design matching on day (or hour) of event for the same month. 8-h max	Mean: NR	Correlation (*r*): $PM_{2.5}$: 0.4; NO_2: −0.33; SO_2: 0.11; Other: CO −0.32 Copollutant models with: NA	8-h max, lag 0: 1.04 (1.00, 1.07) 8-h max, lag 1: 1.02 (0.99, 1.05) 8-h max, lag 2: 1.03 (0.99, 1.06) 8-h max, lag 0−1: 1.04 (1.00, 1.07) 8-h max, lag 1−2: 1.03 (0.99, 1.07) 1-h max, lag 0: 1.05 (1.00, 1.10) 1-h max, lag 1: 1.05 (1.01, 1.10) 1-h max, lag 2: 1.06 (1.01, 1.11) 1-h max, lag 3: 1.05 (1.00, 1.10) 1-h max, 1−3 h distributed lag: 1.06 (1.01, 1.11)
↑Rosenthal et al. (2013) Helsinki, Finland Ozone: 1998−2006 Follow-up: 1998−2006 Case-crossover study	n = 2,134 Out-of-hospital cardiac arrests due to cardiac, mean age 67.7 yr, 66.2% male	Hourly concentrations from four stationary monitors. Control exposure days selected using time-stratified design matching on day of week stratified on month and year 24-h avg	Mean: 23.76	Correlation (*r*): NR Copollutant models with: PM coarse, $PM_{2.5}$, PM_{10}, UFP, CO, NO, NO_2, SO_2	Lag 0−7 h: 1.04 (0.95, 1.16) Lag 0−24 h: 1.08 (0.96, 1.21) Lag 24−48 h: 1.11 (0.99, 1.26) Lag 48−72 h: 1.16 (1.03, 1.31) Lag 0−3 days: 1.18 (1.00, 1.41)

Table 4 13 (Continued): Epidemiologic studies of short term exposure to ozone and emergency department visits or hospital admissions for electrophysiological changes, arrhythmia, and cardiac arrest.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Winquist et al. (2012) St. Louis MSA, U.S. Ozone: January 1, 2001−June 27, 2007 Follow-up: January 1, 2001−June 27, 2007 Time-series study	n = 18.3 Counts of daily ED visits and HA for dysrhythmia among people residing in the St. Louis MSA	Concentrations from U.S. EPA AQS at Tudor Street stationary monitor, data missing 1.9% of days 8-h max	Mean: 36.3 Maximum: 111.8	Correlation (*r*): $PM_{2.5}$: 0.2; Copollutant models with: NR	ED visits, lag 0−4: 1.00 (0.97, 1.04) HA, lag 0−4: 1.00 (0.95, 1.04)
↑Raza et al. (2014) Stockholm County, Sweden Ozone: 2000−2010 Follow-up: 2000−2010 Case-crossover study	Swedish Cardiac Arrest Register n = 55,973 All cases that occurred in Stockholm County between 2000 and 2010, excluding those classified as noncardiac, dead on arrival of EMS or missing time data, mean age 74 yr in women and 70 yr in men, 67% male	Hourly concentrations from central monitors in Stockholm and one monitor in a rural location. Control exposure days selected using time-stratified design matching on week day stratified on month and year 24-h avg	Mean: 31.57 Maximum: 143.4 μg/m^3	Correlation (*r*): $PM_{2.5}$: 0.22; NO_2: −0.32 Copollutant models with: NR	OR remained elevated (not significant) in two-pollutant model with NO_2 (3-day mean). Independent association observed for lag 0 and nonsignificant associations for lag 1, lag 2 and lag 4 using 24-h distributed lags up to 168 h Lag 0: 1.16 (1.03, 1.29)

Table 4 13 (Continued): Epidemiologic studies of short term exposure to ozone and emergency department visits or hospital admissions for electrophysiological changes, arrhythmia, and cardiac arrest.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Straney et al. (2014) Perth, WA, Australia Ozone: 2000−2010 Follow-up: 2000−2010 Case-crossover study	n = 8,551 Adult cases over 35 yr old attended by a paramedic. Time of event defined by date and time of emergency call. Referent exposures selected using time-stratified approach using day of the week within each month	Averaged hourly concentrations from monitor with closest distance to case based on postal code for the day and hour of cardiac arrest 1-h max	Median: 20 75th: 27.3 95th: 35	Correlation (*r*): $PM_{2.5}$: −0.1346; NO_2: −0.5612; SO_2: 0.1301; Other: PM_{10} 0.0067, CO −0.405 Copollutant models with: NR	Lag 0−1 h: 1.00 (0.99, 1.01) Lag 0−2 h: 1.00 (0.99, 1.01) Lag 0−3 h: 1.00 (0.99, 1.01) Lag 0−4 h: 1.00 (0.99, 1.01) Lag 0−8 h: 1.00 (0.99, 1.01) Lag 0−12 h: 1.00 (0.99, 1.01) Lag 0−24 h: 1.00 (0.99, 1.01) Lag 0−48 h.: 1.00 (0.99, 1.01) No associations observed in multipollutant models, or effect modification by sex or age category 35−65, >65, >75 yr
↑Milojevic et al. (2014) England and Wales, U.K. Ozone: 2003−2008 Follow-up: 2003−2009	HES n = 352,775 Emergency hospital admissions for arrythmias, atrial fibrillation and conduction disorders to NHS hospitals, in HES database using centroid of census ward; median age (IQR) 73 yr (60−82 yr), 54% male HES	Data from nearest monitoring station to residence on event day. Control exposure days defined using time-stratified design using other days of the month when case occurred 8-h max	Mean: NR Median: 30.96 75th: 38.58	Correlation (*r*): $PM_{2.5}$: −0.096; NO_2: −0.3489; SO_2: −0.0849; Other: PM_{10} 0.0302, CO −0.2973 Copollutant models with: NA	Arrythmias, lag 0−4: 0.99 (0.98, 1.00) Atrial fibrillation, lag 0−4: 0.99 (0.97, 1.00) AVCD, lag 0−4: 1.00 (0.96, 1.03)
↑Sarnat et al. (2015) St. Louis, MO, U.S. Ozone: June 1, 2001–May 30, 2003 Follow-up: June 1, 2001–May 30, 2003 Time-series study	n = 69,679 ED visit records of patients with dysrhythmia residing in St. Louis MSA (eight counties each in Missouri and Illinois) from 36 out of 43 acute care hospitals	Averaged hourly concentrations in St. Louis from U.S. EPA AQS 8-h max	Mean: 36.2	Correlation (*r*): $PM_{2.5}$: 0.23; NO_2: 0.37; SO_2: −0.04; Other: CO −0.01 Copollutant models with: NR	0−2 day distributed lag: 1.00 (0.94, 1.06)

Table 4 13 (Continued): Epidemiologic studies of short term exposure to ozone and emergency department visits or hospital admissions for electrophysiological changes, arrhythmia, and cardiac arrest.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Rodopoulou et al. (2015) Little Rock, AR, U.S. Ozone: 2002−2012 Follow-up: 2002−2012 Time-series study	n = 84,269 Daily emergency room visits among persons 15 yr and older, 19% were 65 yr and older, 42.5% male	U.S. AQS data from stationary monitor in Little Rock 8-h max	Mean: 40 Median: 39 75th: 50	Correlation (*r*): NR Copollutant models with: NR	Conduction disorders and cardiac dysrhythmias, lag 1: 1.05 (0.99, 1.12) Copollutant model with $PM_{2.5}$, lag 1: 1.06 (0.99, 1.13)
↑Sade et al. (2015) Negev, Israel Ozone: 2006−2010 Follow-up: 2006−2010 Case-crossover study	n = 1,458 All medical center patients with first episode of atrial fibrillation, living within 20 km of the monitoring site, mean age 69 yr, 45.5% male	Averaged concentrations over 24 h. Control exposure days selected using time-stratified design matching on day of week stratifying on month and year 24-h avg	Mean: 60.6−85.2	Correlation (*r*): NR Copollutant models with: NA	Lag 0: 0.97 (0.89, 1.05) Similar results for analyses stratified by season
↑Pradeau et al. (2015) Gironde Department, France Ozone: 2007−2012 Follow-up: 2007−2012 Case-crossover study	n = 4,558 OHCA events among adults aged 18 yr or older recorded in the EMS database, mean age 70 yr, 64% male	Averaged hourly concentrations from eight stationary monitors located in Gironde. Control exposure days were selected using time-stratified design matching by day of week stratifying on month 24-h avg	Mean: 27.26 Median: 27.51 Maximum: 114 μg/m^3	Correlation (*r*): NR Copollutant models with: NR	Lag 1: 1.14 (1.03, 1.24)

Table 4-14 Epidemiologic panel studies of short-term exposure to ozone and electrophysiology, arrhythmia, and cardiac arrest.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
↑Bartell et al. (2013) Los Angeles, CA, U.S. Ozone: 2005−2007 Panel study Lags reported: 4-, 8-, 24-h, or 3-, and 5-day avg	n = 55 Elderly nonsmokers	Hourly monitor values 24-h avg	Mean: 27.1 Maximum: 60.7	Correlation (*r*): NR Copollutant models with: NR	Increased risk for SVT, 24-h: 1.13 (0.83, 1.53) 3-day avg: 0.51 (0.27, 0.96) 5-day avg: 0.80 (0.28, 2.31) Increased risk for VT 24-h: 1.50 (1.10, 2.05) 3-day avg: 2.54 (1.25, 5.18) 5-day avg: 0.94 (0.14, 6.11)
↑Ljungman et al. (2014) Boston, MA, U.S. Ozone: 2003−2008 Panel study Lags reported: 1−7-day moving avg	Framingham Offspring/Third Generation n = 2,369	Hourly concentrations from Boston area monitors were averaged to create moving averages 24-h avg	Mean: 23 Maximum: 64	Correlation (*r*): NR Copollutant models with: NR	Percentage increase pulse amplitude 1-day avg: 4.45 (−1.18, 10.08) 2-day avg: 7.64 (0.87, 14.40) 5-day avg: 3.87 (−5.22, 12.96)
↑Cakmak et al. (2014) Ottawa and Gatineau, Canada Ozone: 2004−2009 Panel study Additional endpoints: SVT ectopic runs, VT ectopic runs	n = 8,595 Referred for cardiac monitoring ages 12−99 yr	Gatineau residents were assigned levels at single monitor serving the area; Ottawa residents had three monitors averaged to create exposure 3-h max concentration for preceding 24-h period	Mean: 34.89	Correlation (*r*):NR Copollutant models with: NR	Nonstandardized data due to unique exposure assessment Percentage increase atrial fibrillation 1.58 (−0.95, 4.17) Percentage increase heart block 1.13 (1.01, 1.26)

SVT = supraventricular tachycardia; VT = ventricular tachycardia.

Table 4-15 Study-specific details from controlled human exposure studies of electrophysiology, arrhythmia, cardiac arrest.

Study	Population n, Sex, Age (Range or Mean ± SD)	Exposure Details (Concentration, Duration)	Endpoints Examined
Kusha et al. (2012)	Healthy adults n = 8 males, 9 females Age: 18−38 yr	0.12 ppm, 2 h at rest	ECG endpoints, e.g., T-wave alternans (continuously during exposure)
Rich et al. (2018)	Older adults n = 35 males, 52 females Age: 55−70 yr	0, 0.07, 120 ppm, 3 h (alternating 15-min periods of rest and exercise)	Arrhythmia (over 24-h recording period including during exposure 3 h after exposure ECG recordings made) ECG endpoints (over 24-h recording period including during exposure)

ECG = electrocardiography.

Table 4-16 Study-specific details from short-term animal toxicological studies of electrophysiology, arrhythmia, cardiac arrest.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Farraj et al. (2012)	Rats (SH) n = 6/group males, 0 females Age: 12 weeks	0.2 ppm, 4 h 0.8 ppm, 4 h	Arrhythmia induced by aconitine (PE) QRS QT PR ST intervals and R-amplitude T-wave amplitude (before, during, and after exposure)
Wang et al. (2013)	Rats (Wistar) n = 6/group males, 0 females Age: NR	0.8 ppm, 4 h of ozone followed by intra-tracheal instillation of saline or $PM_{2.5}$ twice/week for 3 weeks	ECG measures (24 h after 3rd and 6th exposure)
Kurhanewicz et al. (2014)	Mice (C57BL/6) n = 5−8/group males, 0 females Age: 10−12 weeks	0.3 ppm, 4 h	ECG (before, during and after exposure)
Farraj et al. (2016)	Rats (SH) n = 6/group males, 0 females Age: 12 weeks	0.3 ppm Day 1: 3 h of FA in the morning, 3 h of FA in the afternoon; Day 2: 3 h 0.5 ppm NO_2 or FA exposure in the morning, 0.3 ppm ozone or FA in the afternoon	Cardiac sensitivity to aconitine challenge (24 h after Day 2 exposure animals sacrificed) PR interval (during exposure) QT interval (during exposure)

ECG = electrocardiography; FA = filtered air; PE = post-exposure; PR = time interval between the beginning of the P wave to the peak of the R wave; QRS = time interval between the beginning of the Q wave and the peak of the S wave; QT = time interval between the beginning of the Q wave to end of the T wave; SH = spontaneously hypertensive.

Table 4-17 Epidemiologic studies of short-term exposure to ozone and blood pressure.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
↑Brook and Kousha (2015) Edmonton and Calgary, Alberta, Canada Ozone: January 2010−December 2011 Follow-up: January 2010−December 2011 Case-crossover study	NACRS n = males 2,688, females 3,844 All ED visits for hypertension in the NACRS with residence within 35 km from an air monitor; included all ages (97% >30 yr), 41% male. Controls days were selected using time-stratified design matching on day of week for case and stratifying on month and year	Averaged hourly concentrations from monitors within 35 km of residential postal code centroid 24-h avg	Mean: NR Median: 22 Maximum: 50.1	Correlation (*r*): NR Copollutant models with: NA	Females, cold season, lag 0; pooled results for two cities: 0.98 (0.84, 1.12) Females, cold season, lag 1; pooled results for two cities: 0.98 (0.84, 1.12) Females, cold season, lag 2; pooled results for two cities: 0.96 (0.82, 1.10) Females, cold season, lag 3; pooled results for two cities: 0.98 (0.84, 1.12) Females, warm season, lag 3, pooled results for two cities: 1.15 (1.00, 1.31)
↑Rodopoulou et al. (2015) Little Rock, AR, U.S. Ozone: 2002−2012 Follow-up: 2002−2012 Time-series study	n = 84,269 Daily emergency room visits for hypertension among persons 15 yr and older, 19% 65 yr and older, 42.5% male	U.S. AQS data from stationary monitor in Little Rock 8-h max	Mean: 40 Median: 39 75th: 50	Correlation (*r*): NR Copollutant models with: NR	Lag 1: 0.98 (0.96, 1.00)

Table 4-17 (Continued): Epidemiologic studies of short-term exposure to ozone and blood pressure.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Vencloviene et al. (2017) Kaunas, Lithuania Ozone: January 1, 2009−June 30, 2011 Follow-Up: January 1, 2009−June 30, 2011 Time-series study	n = 17,114 calls Individuals residing in Kaunas and recorded in the emergency calls database, ages 17−104, 60.2% >65 yr, 21.6% male	Averaged hourly concentrations from one stationary monitor 8-h max	Mean: 21.17 Median: 20.91 75th: 27.36 Maximum: 101.76	Correlation (*r*): Other: PM_{10} −0.028, CO −0.298 Copollutant models with: NR	All year, lag 0: 0.94 (0.88, 0.98) All year, lag 2−4: 1.06 (0.98, 1.14) Autumn−winter, lag 0: 0.94 (0.87, 1.03) Autumn−winter, lag 2−4: 0.96 (0.84, 1.08) Spring−summer, lag 0: 0.93 (0.85, 1.00) All year, lag 0, low ozone: 1.08 (1.00, 1.23) All year, lag 0, high ozone: 0.93 (0.85, 1.03) All year, lag 2−4, high ozone: 1.08 (0.97, 1.22) Autumn−winter, lag 0, low ozone: 1.08 (0.97, 1.23) Autumn−winter, lag 0, high ozone: 0.97 (0.81, 1.16) Autumn−winter, lag 2−4, high ozone: 0.93 (0.70, 1.25) Spring−summer, lag 2−4: 1.11 (1.00, 1.23) Spring−summer, lag 0, low ozone: 1.17 (1.00, 1.43) Spring−summer, lag 0, high ozone: 0.93 (0.83, 1.03) Spring−summer, lag 2−4, high ozone: 1.16 (1.03, 1.34)

Table 4-18 Epidemiologic panel studies of short-term exposure to ozone and blood pressure.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Cakmak et al. (2011) Canada Ozone: 2007−2009 Panel study	Canadian Health Measures Survey n = 5,604	Monitor 1-h max	Mean: 34.1 95th: 59.6	Correlation (*r*): NR Copollutant models with: none	Absolute change DBP (mm Hg), lag 0: 0.65 (0.06, 1.23) Absolute change SBP (mm Hg), lag 0: 1.17 (0.29, 2.05)
↑Hoffmann et al. (2012) Boston, MA, U.S. Ozone: 2006−2010 Panel study	n = 70 T2D; 40−85 yr	Monitor 24-h avg	Mean: 25 Median: 24 75th: 32	Correlation (*r*): $PM_{2.5}$: 0.09; Copollutant models with: $PM_{2.5}$	Percentage Increase CMP 2-day mean: −0.33 (−2.30, 1.64) 5-day mean: −3.16 (−5.86, −0.34) Percentage Increase SBP 2-day mean: −0.66 (−2.74, 1.53) 5-day mean: −4.51 (−7.44, −1.58) Percentage Increase DBP 2-day mean: 0.11 (−1.64, 1.86) 5-day mean: −2.26 (−4.74, 0.02)

Table 4-18 (Continued): Epidemiologic panel studies of short-term exposure to ozone and blood pressure.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Dales and Cakmak (2016) National, Canada Ozone: 2007−2009 Follow-up: 2007−2009 Cross-sectional study	Canada Health Measures Survey n = 1,883 (n = 1,693 absence of mood disorder, n = 190 presence of mood disorder) Population-based national sample, aged 6−17 yr, stratified by the presence or absence of clinically diagnosed mood disorder	Concentration on the day of testing from monitors located closest to clinic site 8-h max	Mean: 29.5 Maximum: 83	Correlation (*r*): $PM_{2.5}$: NR; NO_2: NR; SO_2: NR; Other: NR Copollutant models with: NA	Percentage increase SBP Absence of mood disorder: −0.52 (−1.18, 0.14) Presence of mood disorder 4.41(1.91, 6.93) Percentage Increase DBP Absence of mood disorder −0.24(−0.85, 0.36) Presence of mood disorder 3.55(1.01, 6.08)
↑Mirowsky et al. (2017) Chapel Hill, NC, U.S. Ozone: 2012−2014 Panel study Lags reported 0−4, 5 day avg	CATHGEN n = 13 Have undergone cardiac catheterization	AQS Monitor 24-h avg	Mean: 26 Median: 25 75th: 33 Maximum: 63	Correlation (*r*): NR Copollutant models with: $PM_{2.5}$	Percentage increase SBP Lag 0: 2.46 (−2.14, 7.18) Lag 1: −0.11 (−3.54, 3.64) 5-day avg: 1.50 (−3.75, 6.96) Percentage increase DBP Lag 0: 2.46 (−2.25, 7.39) Lag 1: −1.93 (−5.57, 1.82) 5-day avg: −0.43 (−5.89, 5.36)
↑Cole-Hunter et al. (2018) Barcelona, Spain Ozone : 2011−2014 Panel study	TAPAS/EXPOsOMICS n = 57 Healthy, nonsmokers	Monitored values used to model daily time weighted based on location (home/work) 24-h avg	Mean: 22 Maximum: 32.9	Correlation (*r*): NR Copollutant models with: NR	Percentage increase SBP home, exposure 3-days prior: −0.52 (−1.75, 0.71) Percentage increase home DBP, exposure 3-days prior: −0.20 (−1.04, 0.64)

BP = blood pressure, DBP = diastolic blood pressure, SBP = systolic blood pressure, T2D = type 2 diabetes.

Table 4-19 Study-specific details from controlled human exposure studies of blood pressure.

Study	Population n, Sex, Age (Mean ± SD)	Exposure Details (Concentration, Duration)	Endpoints Examined
Barath et al. (2013)	Healthy adults n = 36 males, 0 females Age: 26 ± 1 yr	0.3 ppm, 75 min (alternating 15 min periods of exercise and rest)	SBP, DBP, ACE levels (2 and 6 h PE)
Frampton et al. (2015)	Healthy adults n = GSTM WT eight males, GSTM null seven males; GSTM WT four females, GSTM null five females Age: GSTM null: 27.3 ± 4.2 yr, GSTM WT: 25.4 ± 2.8 yr	0.1, 0.2 ppm; 3 h (alternating 15 min periods of rest and exercise)	SBP, DBP (during exposure and immediately and 2.5 h PE)
Rich et al. (2018)	Older adults n = 35 males, 52 females Age: 55−70 yr	0, 0.07, 0.120 ppm, 3 h (alternating 15 min periods of rest and exercise)	DBP 15 min, 4 and 22 h PE
Stiegel et al. (2017)	Healthy adults n = 11 males, four females Age: 23 to 31 yr	0.3 ppm, 2 h (four 15 min periods of exercise)	SBP (pre- and immediately post-exposure)
Arjomandi et al. (2015)	Adults with asthma (n = 10) and adults without asthma (n = 16) n = 13 males, 13 females Age: asthma: 33.5 ± 8.8 yr, healthy: 30.8 ± 6.9 yr	0.1, 0.2 ppm, 4 h (alternating 30 min periods of exercise and rest)	SBP, DBP, (before, immediately after and 20 h PE) ACE activity (before, immediately after and 20 h PE)

ACE = angiotensin-converting enzyme; DBP = diastolic blood pressure; GSTM = glutathione S-transferase M1; PE = post-exposure; SBP = systolic blood pressure; WT = wild type.

Table 4-20 Study-specific details from short-term animal toxicological studies of blood pressure.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Wang et al. (2013)	Rats (Wistar) n = 6/group males, 0 females Age: NR	0.8 ppm, 4 h of ozone followed by intra-tracheal instillation of saline or $PM_{2.5}$ twice/week for 3 weeks	Blood pressure (24 h after 3rd and 6th exposure)
Wagner et al. (2014)	Rats (S-D), fed high fructose or normal diet n = 4/group males, 0 females Age: 8 weeks	0.5 ppm, 8 h/day for 9 consecutive weekdays	Blood pressure (during 9-day exposure)
Farraj et al. (2016)	Rats (SH) n = 6/group males, 0 females Age: 12 weeks	0.3 ppm Day 1: 3 h of FA in the morning, 3 h of FA in the afternoon Day 2: 3 h 0.5 ppm NO_2 or FA exposure in the morning, 0.3 ppm ozone or FA in the afternoon	Blood pressure (during exposure) Pulse pressure (during exposure)
Tankersley et al. (2013)	Mice (C57BL/6J) n = 10−16/group males, 0 females Age: NR Nppa null mice n = 10−16/group males, 0 females Age: NR	Approximately 0.5 ppm, 3 h of ozone followed by 3 h of FA	Right ventricular systolic pressure and total peripheral resistance (8−10 h PE)

Table 4-20 (Continued): Study-specific details from short-term animal toxicological studies of blood pressure.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Ramot et al. (2015)	Rats (FHH) n = NR males, NR females Age: 10−12 weeks Rats (S-D) n = NR males, NR females Age: 10−12 weeks Rats (SH) n = NR males, NR females Age: 10−12 weeks Rats (SHHF) n = NR males, NR females Age: 10−12 weeks Rats (SHSP) n = NR males, NR females Age: 10−12 weeks Rats (WKY) n = NR males, NR females Age: 10−12 weeks Rats (Wistar) n = NR males, NR females Age: 10−12 weeks	0.25 ppm, 4 h 0.5 ppm, 4 h 1 ppm, 4 h	ACE levels in blood (immediately after and 24 h PE)

ACE = angiotensin-converting enzyme; FHH = fawn-hooded hypertensive; PE = post-exposure; S-D = Sprague-Dawley; SH = spontaneously hypertensive; SHHF = spontaneously hypertensive heart failure; WKY = Wistar Kyoto.

Table 4-21 Epidemiologic panel studies of short-term exposure to ozone and heart rate variability (HRV), and heart rate (HR).

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
†Cakmak et al. (2011) Canada Ozone: 2007−2009 Panel study	Canadian Health Measures Survey n = 5,604	Monitor 1-h max	Mean: 34.1 95th: 59.6	Correlation (*r*): NR Copollutant models with: None	Absolute change resting heart rate (bpm), lag 0: 0.90 (0.18, 1.63)
†Bartell et al. (2013) Los Angeles, CA, U.S. Ozone: 2005−2007 Panel study Lags reported: 4, 8, 24 h, or 3-, and 5-day avg Additional endpoints reported: pNN50	n = 55 Elderly nonsmokers	Hourly monitor values 24-h avg	Mean: 27.1 Maximum: 60.7	Correlation (*r*): NR Copollutant models with: NR	Percentage increase rMSSD, 24-h: 0.54 (−3.04, 4.13) 3-day avg: −1.68 (−7.71, 4.34) 5-day avg: −9.03 (−19.23, 1.16) Percentage increase SDNN, 24-h: 2.09 (−0.28, 4.45) 3-day avg: −0.04 (−3.91, 3.84) 5-day avg: −9.21 (−15.79, −2.63)
†Cakmak et al. (2014) Ottawa and Gatineau, Canada Ozone: 2004−2009 Panel study	n = 8,595 Referred for cardiac monitoring ages 12−99 yr	Gatineau residents were assigned levels at single monitor serving the area, Ottawa residents had three monitors averaged to create exposure 3-h max concentration for preceding 24-h period	Mean: 34.89	Correlation (*r*): NR Copollutant models with: NR	Nonstandardized data due to unique exposure metric Percentage increase maximum HR 0.54 (−0.09, 1.16) Percentage increase average HR 0.11 (−0.46, 0.67)

Table 4-21 (Continued): Epidemiologic panel studies of short-term exposure to ozone and heart rate variability (HRV), heart rate (HR).

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Dales and Cakmak (2016) Canada Ozone: 2007−2009 Cross-sectional study	Canada Health Measures Survey n = 1,883 (n = 1,693 absence of mood disorder, n = 190 presence of mood disorder) Population-based national sample, aged 6−17 yr, stratified by the presence or absence of clinically diagnosed mood disorder	Concentration on the day of testing from monitors located closest to clinic site 8-h max	Mean: 29.5 Maximum: 83	Correlation (*r*): $PM_{2.5}$: NR; NO_2: NR; SO_2: NR; Other: NR Copollutant models with: NA	Percentage increase HR Absence of mood disorder: −0.42 (−1.36, 0.52) Presence of mood disorder 2.47 (−1.52, 6.47)
↑Mirowsky et al. (2017) Chapel Hill, NC, U.S. Ozone: 2012−2014 Panel Study Lags reported: 0−4, 5-day avg	CATHGEN n = 13 Have undergone cardiac catheterization	AQS monitor 24-h avg	Mean: 26 Median: 25 75th: 33 Maximum: 63	Correlation (*r*): NR Copollutant models with: $PM_{2.5}$	Percentage increase SDNN, lag 0: 0.21 (−11.79, 13.71) Lag 1: −2.89 (−12.96, 8.25) Lag 2: 1.07 (−9.21, 12.32) 5-day avg: −6.64 (−20.25, 9.11) Percentage increase rMSSD, lag 0 : 6.11 (−13.18, 29.25) Lag 1: 2.14 (−13.61, 20.46) Lag 2: 4.29 (−11.14, 22.29) 5-day avg: −5.25 (−25.29, 19.71)
↑Cole-Hunter et al. (2018) Barcelona, Spain Ozone: 2011−2014 Panel study	TAPAS/EXPOsOMICS n = 62 Healthy nonsmokers	Monitored values used to model daily time weighted based on location (home/work) 24-h avg	Mean: 22 Maximum: 32.9	Correlation (*r*): NR Copollutant models with: NR	Percentage increase HR, 3-days prior: 0.41 (−0.66, 1.49)

CATHGEN = catheterization genetics; HR = heart rate; pNN50 = the proportion of NN50 divided by the total number of NN (R-R) intervals, rMSSD = root-mean-square of the successive differences between adjacent NNs, SDNN = standard deviation of NN intervals.

Table 4-22 Study-specific details from controlled human exposure studies of heart rate variability (HRV), heart rate (HR).

Study	Population n, Sex, Age (Range or Mean ± SD)	Exposure Details (Concentration, Duration)	Endpoints Examined
Kusha et al. (2012)	Healthy adults n = eight males, nine females Age: 18−38 yr	0.12 ppm, 2 h at rest	Heart rate (first and last 5 min of exposure)
Barath et al. (2013)	Healthy adults n = 36 males, 0 females Age: 26 ± 1 yr	0.3 ppm, 75 min (alternating 15-min periods of exercise and rest)	HRV time and frequency parameters (2 and 6 h PE) Heart rate (2 and 6 h PE)
Frampton et al. (2015)	Healthy adults (GSTM WT, GSTM null) n = GSTM WT 8, GSTM null seven males, GSTM WT 4, GSTM null five females Age: GSTM null: 27.3 ± 4.2 yr, GSTM WT: 25.4 ± 2.8 yr	0.1, 0.2 ppm, 3 h (alternating 15-min periods of rest and exercise)	Heart rate (during exposure and immediately and 2.5 h PE)
Arjomandi et al. (2015)	Adults with asthma (n = 10) and adults without asthma (n = 16) n = 13 males, 13 females Age: asthma: 33.5 ± 8.8 yr, healthy: 30.8 ± 6.9 yr	0.1, 0.2 ppm, 4 h (alternating 30-min periods of exercise and rest)	HRV time and frequency parameters (before, immediately after and 20 h PE)
Rich et al. (2018)	Older adults n = 35 males, 52 females Age: 55−70 yr	0, 0.07, 0.120 ppm, 3 h (alternating 15-min periods of rest and exercise)	HR (over 24-h recording period including during exposure) HRV time and frequency parameters (over 24-h recording period including during exposure)

GSTM = glutathione S-transferase M1, HR = heart rate; HRV = heart rate variability; PE = post-exposure; WT = wild type.

Table 4-23 Study-specific details from short-term animal toxicological studies of heart rate variability (HRV), heart rate (HR).

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Farraj et al. (2012)	Rats (SH) n = 6/group males, 0 females Age: 12 weeks	0.2 ppm, 4 h 0.8 ppm, 4 h	HRV (before, during, and after exposure) HRV: time and frequency domains (before, during, and after exposure) Heart rate (before, during, and after exposure)
Wang et al. (2013)	Rats (Wistar) n = 6/group males, 0 females Age: NR	0.8 ppm, 4 h of ozone followed by intra-tracheal instillation of saline or $PM_{2.5}$ twice/week for 3 weeks	Heart rate (24 h after 3rd and 6th exposure) Measures of HRV (24 h after 3rd and 6th exposure)
McIntosh-Kastrinsky et al. (2013)	Mice (C57BL/6) n = 0 males, 14−15/group females Age: NR	0.245 ppm, 4 h (aged, FA, or ozone) on 3 separate days outdoors	Heart rate in isolated perfused hearts (8−11 h PE hearts were isolated and post-induced ischemia)
Wagner et al. (2014)	Rats (S-D), fed high-fructose or normal diet n = 4/group males, 0 females Age: 8 weeks	0.5 ppm, 8 h/day for 9 consecutive weekdays	HR (during 9-day exposure) Time domains of HRV (during 9-day exposure)
Kurhanewicz et al. (2014)	Mice (C57BL/6) n = 5−8/group males, 0 females Age: 10−12 weeks	0.3 ppm, 4 h	HR (before, during, and after exposure)

Table 4-23 (Continued): Study-specific details from short-term animal toxicological studies of heart rate variability (HRV), heart rate (HR).

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Farraj et al. (2016)	Rats (SH) n = 6/group males, 0 females Age: 12 weeks	0.3 ppm Day 1: 3 h of FA in the morning, 3 h of FA in the afternoon; Day 2: 3 h 0.5 ppm NO_2 or FA exposure in the morning, 0.3 ppm ozone or FA in the afternoon	Heart rate (during exposure) Time and frequency domains of HRV (during exposure)

FA = filtered air; HR = heart rate; HRV = heart rate variability; S-D = Sprague-Dawley; SH = spontaneously hypertensive.

Table 4-24 Epidemiologic studies of short-term exposure to ozone and pulmonary vascular disease (PVD), thrombosis.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
†Spiezia et al. (2014) Padua, Italy Ozone: January 2008 and October 2012 Follow-up: January 2008 and October 2012 Case-control study	n = 33 cases and 72 controls All consecutive hospital admissions to thrombosis unit at university hospital with objective identification of acute first episode of isolated pulmonary embolism between January 2008 and October 2012. Cases defined as having no predisposition and controls defined as having permanent or transient risk factors; mean age of cases and controls, 67 yr and 68 yr, respectively. Patients excluded if under 18 yr, being treated with anticoagulants, had previous episode of pulmonary embolism, or did not reside in Padua	Averaged concentration using two stationary monitors in the city; data from the closest monitor to the patient's address was used. Mean concentration over month preceding date of diagnosis		Correlation (*r*): NR Copollutant models with: NR	No associations with monthly average ozone >37 ppb

Table 4-24 (Continued): Epidemiologic studies of short-term exposure to ozone and pulmonary vascular disease (PVD), thrombosis.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Milojevic et al. (2014) England and Wales, U.K. Ozone: 2003−2009 Follow-up: 2003−2009 Case-crossover study	HES n = 82,231 Emergency hospital admissions for pulmonary embolism to NHS hospitals, 2003−2008, in HES database using centroid of census ward; median age (IQR) 73 (60−82), 54% male HES	Data from the nearest monitoring station to residence on event day. Control exposure days defined using time-stratified design using other days of the month when case occurred 8-h max	Mean: NR Median: 30.96 75th: 38.58	Correlation (*r*): $PM_{2.5}$: −0.096; NO_2: −0.3489; SO_2: −0.0849; Other: PM_{10} 0.0302, CO −0.2973 Copollutant models with: NA	Lag 0−4: 0.99 (0.96, 1.02)
↑de Miguel-Diez et al. (2016) National, Spain Ozone: January 1, 2000−December 31, 2013 Follow-up: January 1, 2001−December 31, 2013 Case-crossover study	Spanish Minimum Basic Data Set, covers 97.7% of all admissions to public hospitals n = 105,117 Cases recorded in SMBD database during the study period, mean age 70.73 yr, 45.8% male	Concentration from stationary monitor nearest to postal code, calculated 3-day avg including day of embolism and Days 1 and 2 prior. Control exposures were 3-day avg at 1 week, 1.5 weeks, 2 weeks, and 3 weeks before the event	Mean: NR	Correlation (*r*): NR Copollutant models with: NR	Control period 3 weeks prior to event: 1.03 (1.01, 1.06); effect estimate not standardized, increment not reported

PVD = peripheral vascular disease.

Table 4-25 Epidemiologic panel studies of short-term exposure to ozone and coagulation.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
↑Green et al. (2015) Multicity; Chicago, Detroit, Los Angeles, Newark, Oakland, and Pittsburgh, U.S. Ozone: 1999−2004 Cohort study	SWAN n = 2,086 Midlife women (42−52 yr)	Monthly averages of AQS data from one monitor within 20 km of residence 8-h max	Mean: 35.2 Maximum: 122	Correlation (*r*):NR Copollutant models with: NR	Percentage increase factor IIc, 1-day: −0.80 (−2.00, 0.20) Percentage increase fibrinogen, 1-day: −0.80 (−3.20, 1.60) Percentage increase PAI-1, 1-day: −2.20 (−5.00, 0.80) Percentage increase tPA, 1-day: 0.20 (−1.20, 1.80)
↑Bind et al. (2012) Boston, MA, U.S. Ozone: 2000−2009 Panel study Lags reported: 4 and 24 h, or 3-, 7-, 14-, 21- or 28-day avg	Normative Aging Study n = 704	Monitors in the Boston area 24-h avg	Mean: 24 95th: 49	Correlation (*r*):NR Copollutant models with: NR	No change in fibrinogen, qualitative results only
↑Mirowsky et al. (2017) Chapel Hill, NC, U.S. Ozone: 2012−2014 Panel study Lags reported: 0−4, 5-day avg	CATHGEN n = 13 Have undergone cardiac catheterization	AQS monitor 24-h avg	Mean: 26 Median: 25 75th: 33 Maximum: 63	Correlation (*r*):NR Copollutant models with: NR	Percentage increase tPA Lag 0: 5.79 (−3.32, 15.75) Lag 1: −0.96 (−7.71, 6.32) Lag 2: 2.89 (−4.29, 10.71) 5-day avg: 9.43 (−2.14, 22.18) Percentage increase PAI-1 Lag 0: 8.79 (−13.71, 36.75) Lag 1: 11.36 (−7.82, 34.29) Lag 2: 21.43 (0.86, 45.86) 5-day avg: 43.39 (9.32, 87.43)

CATHGEN = catheterization genetics; PAI-1 = plasminogen activator inhibitor 1; SWAN = study of women's health across nations; tPA = tissue plasminogen activator.

Table 4-26 Study-specific details from controlled human exposure studies of coagulation.

Study	Population n, Sex, Age (Range or Mean ± SD)	Exposure Details (Concentration, Duration)	Endpoints Examined
Barath et al. (2013)	Healthy adults n = 36 males, 0 females Age: 26 ± 1 yr	0.3 ppm, 75 min (alternating 15-min periods of exercise and rest)	Markers of coagulation in blood (2 and 6 h PE)
Frampton et al. (2015)	Healthy adults (GSTM WT, GSTM null) n = GSTM WT 8, GSTM null seven males, GSTM WT 4, GSTM null five females Age: GSTM null: 27.3 ± 4.2 yr, GSTM WT: 25.4 ± 2.8 yr	0.1, 0.2 ppm; 3 h (alternating 15-min periods of rest and exercise)	Platelet activation and microparticle circulation 1.5 h the day before and 2.5 h PE
Kahle et al. (2015)	Healthy adults n = 14 males, 2 females Age: 20−36	0.3 ppm, 2 h, 15 min of exercise alternating with 15 min of rest one exposure at 22°C other at 32.5°C	Markers of coagulation (24 h PE)
Arjomandi et al. (2015)	Adults with asthma (n = 10) and adults without asthma (n = 16) n = 13 males, 13 females Age: Asthma: 33.5 ± 8.8 yr, healthy: 30.8 ± 6.9 yr	0.1, 0.2 ppm, 4 h (alternating 30-min periods of exercise and rest)	Markers of coagulation in blood (before, immediately after and 20 h PE)

GSTM = glutathione S-transferase M1; PE = post-exposure; WT = wild type.

Table 4-27 Study-specific details from short-term animal toxicological studies of coagulation.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Snow et al. (2018)	Rats (WKY) n = 6−8/group males, 0 females Age: ~12 weeks	0.8 ppm, 4 h/day for 2 consecutive days (diets enriched with coconut, olive, or fish oil for 8 weeks prior)	Circulating platelets

WKY = Wistar Kyoto

Table 4-28 Epidemiologic panel studies of short-term exposure to ozone and inflammation.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
†Bind et al. (2012) Boston, MA, U.S. Ozone: 2000−2009 Panel study	Normative aging study n = 704	Monitors in the Boston area 24-h avg	Mean: 24 95th: 49	Correlation (*r*): NR Copollutant models with: NR	Percentage increase CRP 24-h: 0.87 (0.81, 0.95)
†Gandhi et al. (2014) Rutgers, NJ, U.S. Ozone: 2006−2009 Panel study Lags reported: 0−6	n = 49 Healthy, nonsmoking young adults	Hourly concentrations from East Brunswick from AQS 24-h avg	Mean: 25.3 Median: 24.8 75th: 33.2 Maximum: 67.7	Correlation (*r*): $PM_{2.5}$: −0.05, SO_4:−0.05, NO_X:−0.52 Copollutant models with: NR	Percentage increase plasma nitrite Lag 0: −5.61 (−20.61, 9.47) Lag 1: −4.91 (−18.33, 8.42)
†Green et al. (2015) Multicity; Chicago, Detroit, Los Angeles, Newark, Oakland, Pittsburgh, U.S. Ozone: 1999−2004 Cohort study	SWAN n = 2,086 Midlife women (42−52 yr)	Monthly averages of AQS data from one monitor within 20 km of residence 8-h max	Mean: 35.2 Maximum: 122	Correlation (*r*): NR Copollutant models with: NR	Percentage increase CRP 1-day : 0.80 (−2.00, 3.60)
†Li et al. (2016) Boston, MA, U.S. Ozone: 1998−2008 Panel study	Framingham Offspring n = 2,035 Nonsmokers	Mean concentrations from Harvard supersite 24-h avg	Mean: 20	Correlation (*r*): NR Copollutant models with: NR	Qualitative results for myeloperoxidase and indexed 8-epi-prostaglandin F2alpha show no change

Table 4-28 (Continued): Epidemiologic panel studies of short-term exposure to ozone and inflammation.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Mirowsky et al. (2017) Chapel Hill, NC, U.S. Ozone: 2012−2014 Panel study Lags reported: 0−4, 5-day avg Additional endpoints reported: VCAM, monocytes, neutrophils	CATHGEN n = 13 Have undergone cardiac catheterization	AQS monitor 24-h avg	Mean: 26 Median: 25 75th: 33 Maximum: 63	Correlation (*r*): NR Copollutant models with: NR	Percentage increase CRP Lag 0: −1.61 (−45.32, 73.07) Lag 1: 3.43 (−34.93, 62.36) 5-day avg: 2.68 (−52.50, 113.57) Percentage increase ICAM Lag 0 : 4.39 (−6.21, 15.96) Lag 1: 0.32 (−7.71, 8.89) 5-day avg: 4.82 (−8.04, 19.39) Percentage increase IL−6, Lag 0: 14.46 (−3.36, 35.46) Lag 1: 7.5 (−6.00, 22.82) 5-day avg: 18.86 (−3.64, 46.18) Percentage increase TNF-α Lag 0: 6.75 (−2.25, 16.50) Lag 1: 2.25 (−4.82, 9.64) 5-day avg: 4.61 (−6.11, 16.50)
↑Li et al. (2017) Boston, MA, U.S. Ozone: 2005−2008 Panel study Lags reported: 1−7 day moving avg	Framingham Offspring Cohort n = 3,396	Averaged ozone monitors in the area and made moving averages per lag 24-h avg	Mean: 23.7	Correlation (*r*): NR Copollutant models with: NR	Percentage increase TNFR2 1-day moving avg: 1.69 (0.45, 2.93) 2-day moving avg: 2.34 (0.84, 3.83) 7-day avg: 5.40 (2.99, 7.81)

CATHGEN = catheterization genetics; CRP = high sensitivity c-reactive protein; CVD = cardiovascular disease; ICAM = inter-cellular adhesion model; IL6 = interleukin 6; MA = moving average; SWAN = study of women's health across the nation; TNF-α= tumor necrosis factor alpha; TNFR2 = tumor necrosis factor receptor 2; VCAM = vascular cell adhesion model.

Table 4-29 Study-specific details from controlled human exposure studies of systemic inflammation and oxidative stress.

Study	Population n, Sex, Age (Range or Mean ± SD)	Exposure Details (Concentration, Duration)	Endpoints Examined
Biller et al. (2011)	Healthy adults n = 11 males, 3 females Age: 33.1 ± 9.5	0.25 ppm, 15 min of exercise alternating with 15 min of rest	Markers of systemic inflammation (before exposure and 5, 7, and 24 h PE)
Barath et al. (2013)	Healthy adults n = 36 males, 0 females Age: 26 ± 1 yr	0.3 ppm, 75 min (alternating 15-min periods of exercise and rest)	Markers of systemic inflammation in blood (2 and 6 h PE)
Kahle et al. (2015)	Healthy adults n = 14 males, 2 females Age: 20−36	0.3 ppm, 15 min of exercise alternating with 15 min of rest one exposure at 22°C other at 32.5°C	Markers of systemic inflammation (24 h PE)
Arjomandi et al. (2015)	Adults with asthma (n = 10) and adults without asthma (n = 16) n = 13 males, 13 females Age: asthma: 33.5 ± 8.8 yr, healthy: 30.8 ± 6.9 yr	0.1, 0.2 ppm, 4 h (alternating 30 min periods of exercise and rest)	Markers of systemic inflammation in blood (before, immediately after and 20 h PE)
Stiegel et al. (2016)	Healthy adults n = 11 males, 4 females Age: 23–31 yr	0.3 ppm, 2 h (four 15-min periods of exercise)	Markers of systemic inflammation in blood (before, immediately after, and next day)
Ramanathan et al. (2016)	Healthy adults n = 13 males, 17 females Age: 23 ± 4 yr	0.12 ppm, 2 h	HDL antioxidant and anti-inflammatory capacity (before exposure and 1 and 20 h PE)

Table 4-29 (Continued): Study-specific details from controlled human exposure studies of systemic inflammation and oxidative stress.

Study	Population n, Sex, Age (Range or Mean ± SD)	Exposure Details (Concentration, Duration)	Endpoints Examined
Stiegel et al. (2017)	Healthy adults n = 11 males, 4 females Age: 23–31 yr	0.3 ppm, 2 h (four 15-min periods of exercise)	Markers of systemic inflammation in blood (before, immediately after, and next day)
Bosson et al. (2013)	Healthy adults N = 24 males, 19 females Age: 19-32 yr	0.2 ppm, 2 h (moderate exercise and rest)	Markers of systemic inflammation in blood (before, 1.5, 6, and 18 h PE)

HDL = high-density lipoproteins; PE = post-exposure.

Table 4-30 Study-specific details from short-term animal toxicological studies of systemic inflammation and oxidative stress.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Farraj et al. (2012)	Rats (SH) n = 6/group males, 0 females Age: 12 weeks	0.2 ppm, 4 h 0.8 ppm, 4 h	Markers of systemic inflammation in blood (animals sacrificed 1 h PE)
Martinez-Campos et al. (2012)	Rats (Wistar) n = 6/group males, 0 females Age: 10 weeks	0.5 ppm, 4 h/day for 2 weeks (with or without exercise)	Markers of oxidative stress in blood (at the end of 2-week exposure)
Wang et al. (2013)	Rats (Wistar) n = 6/group males, 0 females Age: NR	0.8 ppm, 4 h of ozone followed by intra-tracheal instillation of saline or $PM_{2.5}$ twice/week for 3 weeks	Markers of antioxidants in heart tissue (heart tissue collected after 6th exposure) Markers of systemic inflammation in blood (blood drawn after 6th exposure)
Thomson et al. (2013)	Rats (Fischer) n = 4−6/group males, 0 females Age: NR	0.4 ppm, 4 h 0.8 ppm, 4 h	mRNA markers of oxidative stress (tissue collected immediately PE) mRNA markers of systemic inflammation in tissue (tissue collected immediately PE)
McIntosh-Kastrinsky et al. (2013)	Mice (C57BL/6) n = 0 males, 14−15/group females Age: NR	0.245 ppm, 4 h (aged, FA, or ozone) on 3 separate days outdoors	Heart rate in isolated perfused hearts (8−11 h PE hearts were isolated and post-induced ischemia)
Kurhanewicz et al. (2014)	Mice (C57BL/6) n = 5−8/group males, 0 females Age: 10−12 weeks	0.3 ppm, 4 h	Markers of systemic inflammation in blood (24 h PE)

Table 4-30 (Continued): Study-specific details from short-term animal toxicological studies of systemic inflammation and oxidative stress.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Paffett et al. (2015)	Rats (S-D) n = 65 males, 0 females Age: 8−12 weeks	1 ppm, 4 h	Markers of systemic inflammation in blood (serum collected immediately before sacrifice)
Kumarathasan et al. (2015)	Rats (Fischer) n = 8/exposure group 17/control group males, 0 females Age: NA	0.4 ppm, 4 h 0.8 ppm, 4 h	Markers of oxidative stress in blood (immediately and 24 h PE) Markers of systemic inflammation in blood (immediately and 24 h PE)
Ramot et al. (2015)	Rats (FHH) n = NR males, NR females Age: 10−12 weeks Rats (S-D) n = NR males, NR females Age: 10−12 weeks Rats (SH) n = NR males, NR females Age: 10−12 weeks Rats (SHHF) n = NR males, NR females Age: 10−12 weeks Rats (SHSP) n = NR males, NR females Age: 10−12 weeks Rats (WKY) n = NR males, NR females Age: 10−12 weeks Rats (Wistar) n = NR males, NR females Age: 10−12 weeks	0.25 ppm, 4 h 0.5 ppm, 4 h 1 ppm, 4 h	Markers of systemic inflammation in blood (immediately after and 24 h PE)
Hatch et al. (2015)	Rats (multiple strains) n = NR males, NR females Age: 10−12 weeks	0.25, 0.5, or 1 ppm, 4 h	Markers of oxidative stress in blood (24 h after Day 2 exposure animals sacrificed)

Table 4-30 (Continued): Study-specific details from short-term animal toxicological studies of systemic inflammation and oxidative stress.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Ying et al. (2016)	Mice (KKAy) n = NR males, 0 females Age: 7 weeks	0.8 ppm, 4 h/day for 13 consecutive weekdays	Markers of systemic inflammation in blood (1 or 3 days PE)
Zhong et al. (2016)	Mice (Japanese KK) n = 8/group males, 0 females Age: NA	0.5 ppm, 4 h/day for 13 consecutive weekdays	Inflammatory cell populations in blood (24 h PE) Markers of systemic inflammation in blood (24 h PE)
Martinez-Campos et al. (2012)	Rats 6−10 per group	0.5 ppm 4 h/day for 2 weeks then exercise 4 h/day for 2 weeks no exercise	Markers of oxidative stress in blood (at the end of 2-week exposure)
Thomson et al. (2016)	Rats (F344) n = 5/group males, 0 females Age: NR	0.8 ppm, 4 h of ozone exposure (treated with metyrapone, corticosterone, or vehicle for 1-h prior)	Markers of systemic inflammation in blood PE
Henriquez et al. (2017)	Rats (WKY) n = 8/group males, 0 females Age: 10 weeks	0.8 ppm, 1−2 days of ozone (with or without pretreatment with propranolol, mifepristine, or propranolol followed by mifepristine)	Markers of systemic inflammation in blood (immediately PE Day 1 or Day 2)
Cestonaro et al. (2017)	Rats (Wistar) n = 12/group males, 0 females Age: 9−10 weeks	0.05 ppm, 24 h/day for 14 or 28 days 0.05 ppm, 3 h/day for 14 and 28 days	Markers of oxidative stress (at the end of a given exposure)
Snow et al. (2018)	Rats (WKY) n = 6−8/group males, 0 females Age: ~12 weeks	0.8 ppm, 4 h/day for 2 consecutive days (diets enriched with coconut, olive, or fish oil for 8 weeks prior)	Markers of oxidative stress in blood (2 h PE) Markers of systemic inflammation in blood (2 h PE)

Table 4-30 (Continued): Study-specific details from short-term animal toxicological studies of systemic inflammation and oxidative stress.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Francis et al. (2017)	Mice (C57BL/6J WT and CCR2 null) n = 0 males, 3−4/group females Age: 8−11 weeks	0.8 ppm, 3 h	Markers of systemic inflammation in blood (24−72 h PE)

CCR2 = C-C chemokine receptor type 2; FHH = fawn-hooded hypertensive; PE = post-exposure; S-D = Sprague-Dawley; SH = spontaneously hypertensive; SHHF = spontaneously hypertensive heart failure; SHSP = spontaneously hypertensive stroke-prone; WKY = Wistar Kyoto; WT = wild type.

Table 4-31 Epidemiologic studies of short-term exposure to ozone and cerebrovascular disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Mechtouff et al. (2012) Rhone Department, France Ozone: November 6, 2006−June 6, 2007 Follow-up: November 6, 2006−June 6, 2007 Case-crossover study	AVC69 study n = 376 Consecutive patients 18 yr or older enrolled in AVC69 study living within study area, excluding nonstroke, TIA, ICH and undetermined stroke, mean age 76.6 yr, 46.3% male	Averaged hourly concentrations from two to five stationary monitors, calculated max of 8 h moving avg. Control exposure days selected using time-stratified design matching on day of week stratifying by month 8-h max	Mean: 28.02 Median: 29.44 75th: 39.09 Maximum: 65.99	Correlation (*r*): $PM_{2.5}$: −0.2 to 0.53; NO_2: −0.2 to 0.53; SO_2: −0.2 to 0.53; Copollutant models with: NA	Lag NR: 0.95 (0.68, 1.31)
↑Bedada et al. (2012) Manchester and Liverpool, U.K. Ozone: 2003−2007 Follow-up: 2003−2007 Case-crossover study	NORTHSTAR n = 335 Manchester 709 patients with incident TIA or minor stroke confirmed by stroke physician or neurologist with symptom onset within preceding 6 weeks, recruited from TIA clinics, ER or hospital stroke units in Northwest England, age >18 yr with no comorbidity or disability, mean age 66.8 yr, 58.7% male.	Averaged hourly concentrations from eight monitors; separate estimates for Manchester and Liverpool. Control exposure days selected using time-stratified design matched on day of week for the event date in the same month. 8-h avg	Mean: 18.98 Median: 19.29 75th: 24.37	Correlation (*r*): NO_2: −0.68; SO_2: −0.38; Other: CO −0.54, PM_{10} −0.23 Copollutant models with: NA	Liverpool, lag 0: 0.73 (0.52, 1.02) Liverpool, lag 1: 1.10 (0.79, 1.57) Liverpool, lag 2: 1.16 (0.82, 1.63) Liverpool, lag 3: 1.31 (0.92, 1.87) Manchester, lag 0: 1.29 (0.89, 1.86) Manchester, lag 1: 0.82 (0.57, 1.19) Manchester, lag 2: 1.11 (0.77, 1.62) Manchester, lag 3: 0.77 (0.53, 1.13)

Table 4-31 (Continued): Epidemiologic studies of short-term exposure to ozone and cerebrovascular disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Corea et al. (2012) Mantua County, Italy Ozone: 2006−2008 Follow-up: 2006−2008 Case-crossover study	Lombardia Stroke Unit Network registry n = 781 781 of 1,680 consecutive cases admitted to stroke unit over 3 yr between 2006−2008; lived in urban area within 10 km from a stationary monitor, mean age 71.2 yr, 46.8% male	Averaged hourly concentrations from seven stationary monitors. Control days were selected using a bidirectional symmetric design, Days 1, 3, 5, 7 before and after the admission for stroke. 8-h avg	Mean: NR	Correlation (*r*): $PM_{2.5}$: NA Copollutant models with: PM_{10}, SO_2, NO_2, NO, CO, benzene	No association at lag 0 for any CV event, cardioembolic disease or ischemic stroke or stroke subtypes; increment per 8-h avg ozone not reported
↑Xu et al. (2013) Allegheny County, PA, U.S. Ozone: September 1994−December 2000 Follow-up: 1994−2000 Case-crossover study	n = 26,210 Stroke cases aged 65 yr and older who lived in Allegheny County between 1994 and 2000; 41.2% male	Daily concentrations from U.S. EPA AQS. Control exposure days selected using a time-stratified design matching on day of week stratified on month and year. 24-h avg		Correlation (*r*): NR Copollutant models with: NR	Lag 0: 1.00 (1.00, 1.00) Lag 1: 1.00 (1.00, 1.00) Lag 2: 1.00 (1.00, 1.00) Lag 3: 1.00 (1.00, 1.00) Lag 0−3: 1.00 (1.00, 1.01)

Table 4-31 (Continued): Epidemiologic studies of short-term exposure to ozone and cerebrovascular disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
Suissa et al. (2013) Nice, France Ozone: 2007−2011 Follow-up: 2007−2011 Case-crossover study	n = 1,729 2,067 consecutive patients admitted to university hospital for stroke, 1,729 with diagnosis confirmed by neurologists using medical records, residents of Nice, mean age 76 yr, 46.7% male. Referent exposures selected using time-stratified approach using day of week within each month.	Averaged hourly concentrations from urban stationary monitor 8-h avg	Mean: 40.98 Median: 42.83 75th: 53.49 Maximum: 79.83	Correlation (*r*): NO_2: −0.54; Other: minimal temperature 0.67 Copollutant models with: NR	Recurrent stroke, 8-h avg, lag 1: 1.43 (1.03, 1.99) Large artery stroke, 8-h avg, lag 1: 0.96 (0.77, 1.21) All ischemic stroke, 8-h avg, lag 0: 0.97 (0.85, 1.11) All ischemic stroke, 8-h avg, lag 1: 0.99 (0.87, 1.13) All ischemic stroke, 8-h avg, lag 2: 0.99 (0.88, 1.13) All ischemic stroke, 8-h avg, lag 3: 1.03 (0.91, 1.16) All ischemic stroke, 1-h avg, lag 0: 0.98 (0.84, 1.15) All ischemic stroke, 1-h avg, lag 1: 1.02 (0.88, 1.19) All ischemic stroke, 1-h avg, lag 2: 0.98 (0.85, 1.14) All ischemic stroke, 1-h avg, lag 3: 0.99 (0.85, 1.15) All ischemic stroke, 24-h avg, lag 0: 1.01 (0.93, 1.16) All ischemic stroke, 24-h avg, lag 1: 1.00 (0.89, 1.14) All ischemic stroke, 24-h avg, lag 2: 1.01 (0.90, 1.14) All ischemic stroke, 24-h avg, lag 3: 1.03 (0.92, 1.17)

Table 4-31 (Continued): Epidemiologic studies of short-term exposure to ozone and cerebrovascular disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Chen et al. (2014) Edmonton, Alberta, Canada Ozone: April 17, 1998−March 31, 2002 Follow-up: April 17, 1998−March 31, 2002 Case-crossover study	n = 5,229 Acute ischemic stroke cases aged 25 yr or older presenting to EDs, 50.7% male	Average of hourly mean concentrations from three stationary monitors in National Air Pollution Surveillance network. Control exposure days selected using time-stratified design matching on day of week stratified on month and year 1-h max	Mean: 17.22 Median: 15 95th: 41.17	Correlation (*r*): $PM_{2.5}$: −0.15; NO_2: −0.59; SO_2: −0.02; Other: CO −0.47 Copollutant models with: NR	All seasons, lag 1−8 h: 0.97 (0.86, 1.10) All seasons, lag 9−16 h: 0.96 (0.86, 1.08) All seasons, lag 1−24 h: 0.96 (0.84, 1.12) All seasons, lag 25−48 h: 0.92 (0.80, 1.06) All seasons, 1−72 h: 0.96 (0.79, 1.18) Warm season, lag 1−8 h: 0.87 (0.72, 1.02) Warm season, lag 9−16 h: 0.85 (0.73, 1.01) Warm season, lag 1−24 h: 0.82 (0.67, 1.01) Warm season, lag 25−48 h: 0.82 (0.67, 1.00) Warm season, 1−72 h: 0.78 (0.59, 1.04) Cold season, lag 1−8 h: 1.16 (0.91, 1.48) Cold season, lag 9−16 h: 1.14 (0.91, 1.41) Cold season, lag 1−24 h: 1.22 (0.91, 1.60) Cold season, lag 25−48 h: 1.09 (0.83, 1.43) Cold season, 1−72 h: 1.39 (0.93, 2.08)

Table 4-31 (Continued): Epidemiologic studies of short-term exposure to ozone and cerebrovascular disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
†Milojevic et al. (2014) England and Wales, U.K. Ozone: 2003−2009 Follow-up: 2003−2009 Study	HES n = 426,940 Emergency hospital admissions for stroke to NHS hospitals, 2003−2008, in HES database using centroid of census ward; median age (IQR) 73 yr (60−82 yr), 54% male HES	Data from nearest monitoring station to residence on event day. Control exposure days defined using time-stratified design using other days of the month when case occurred 8-h max	Mean: NR Median: 30.96 75th: 38.58	Correlation (*r*): $PM_{2.5}$: −0.096; NO_2: −0.3489; SO_2: −0.0849; Other: PM_{10} 0.0302, CO −0.2973 Copollutant models with: NA	All stroke, lag 0−4: 1.01 (0.99, 1.02) <70 yr, lag 0−4: 0.99 (0.97, 1.01) 70+ yr, lag 0−4: 1.01 (1.00, 1.03) Females, lag 0−4: 1.00 (0.98, 1.01) Males, lag 0−4: 1.01 (1.00, 1.03)
†Rodopoulou et al. (2015) Little Rock, AR, U.S. Ozone: 2002−2012 Follow-up: 2002−2012 Time-series study	n = 84,269 Daily emergency room visits among persons 15 yr and older, 19% 65 yr and older, 42.5% male	U.S. AQS data from stationary monitor in Little Rock 8-h max	Mean: 40 Median: 39 75th: 50	Correlation (*r*): NR Copollutant models with: NR	Cerebrovascular disease, lag 1: 0.88 (0.77, 1.00)
†Wing et al. (2015) Nueces County, TX, U.S. Ozone: January 1, 2000−June 30, 2012 Follow-up: January 1, 2000−June 30, 2012 Case-crossover study	Brain Attack Surveillance in Corpus Christi register; active and passive surveillance n = 2,948 Incident ischemic stroke cases with exposure data over 45 yr old living in Nueces County, median age 71 yr, 56% Mexican American, 48.7% male	Daily maximal 8-h concentration from one central monitor in TCEQ TAMIS. Control exposure days selected using time-stratified design matching on week day stratifying on month and year 8-h max	Mean: NR Median: 35.7 75th: 46.3	Correlation (*r*): NR Copollutant models with: NA	Lag 0: 1.02 (0.95, 1.10) Lag 1: 1.04 (0.97, 1.12) Lag 2: 1.05 (0.97, 1.12) Lag 3: 1.02 (0.95, 1.09) Copollutant model $PM_{2.5}$, lag 0: 1.02 (0.95, 1.10) Copollutant model $PM_{2.5}$, lag 1: 1.05 (0.98, 1.13) Copollutant model $PM_{2.5}$, lag 2: 1.05 (0.98, 1.12) Copollutant model $PM_{2.5}$, lag 3: 1.02 (0.95, 1.10)

Table 4-31 (Continued): Epidemiologic studies of short-term exposure to ozone and cerebrovascular disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Montresor-López et al. (2015) South Carolina, U.S. Ozone: 2002−2006 Follow-up: 2002−2006 Case-crossover study	n = 21,301 Hospitalized cases with no prior stroke in the last 24 mo, 18 yr old or older and residents of South Carolina, mean age 68.7 yr, 47.4% male	Hourly concentrations modeled using U.S. EPA's Hierarchical Bayesian Model combining measurements and CMAQ outputs at 12-km grid cell resolution 8-h max	Mean: 46 Median: 46.2	Correlation (*r*): NR Copollutant models with: NR	All stroke, lag 0: 0.96 (0.92, 1.00) All stroke, lag 1: 0.94 (0.90, 1.00) All stroke, lag 2: 0.96 (0.90, 1.00) Ischemic stroke, lag 0: 0.96 (0.92, 1.02) Ischemic stroke, lag 1: 0.94 (0.90, 1.02) Ischemic stroke, lag 2: 0.94 (0.90, 1.00) Hemorrhagic stroke, lag 0: 0.90 (0.79, 1.04) Hemorrhagic stroke, lag 1: 0.96 (0.85, 1.08) Hemorrhagic stroke, lag 2: 1.02 (0.90, 1.17)
↑Maheswaran et al. (2016) London, U.K. Ozone: 1995−2006 Follow-up: 1995−2006 Case-crossover study	South London Stroke Register n = 2,590 First-ever ischemic stroke cases recorded on stroke register between 1995 and 2006, mean age 71.7 yr, 50.3% male	Averaged hourly concentrations from monitors nearest to residential postal code centroid. Control exposure days selected using time-stratified design matching on week day stratified by season 24-h avg	Mean: 15.3	Correlation (*r*): NR Copollutant models with: NR	Lag 0: 0.99 (0.89, 1.07) Lag 1: 1.00 (0.92, 1.09) Lag 2: 1.04 (0.94, 1.13) Lag 3: 1.05 (0.96, 1.15) Lag 0−6: 1.09 (0.95, 1.25)

Table 4-31 (Continued): Epidemiologic studies of short-term exposure to ozone and cerebrovascular disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
†Wing et al. (2017a) Nueces County, TX, U.S. Ozone: 2000−2012 Follow-up: 2000−2012 Case-crossover study	Brain Attack Surveillance in Corpus Christi register n = 317 First recurrent stroke on a different day after incident event recorded in BASIC, cases were 45 yr or older and lived in Nueces County, and had air pollution data available, mean age 71 yr, 47% male, 64% Mexican American	Daily maximal 8-h concentration from one central monitor in TCEQ TAMIS. Control exposure days selected using time-stratified design matching on day of week stratifying on month and year 8-h max	Median: 35.2 75th: 46.1	Correlation (*r*): NR Copollutant models with: NR	Lag 1: recurrent stroke: 0.94 (0.76, 1.14) Lag 1: severe incident stroke: 1.27 (1.12, 1.41)
†Butland et al. (2017) London, U.K. Ozone: 2005−2012 Follow-up: 2005−2012 Case-crossover study	South London Stroke Register n = 1,799 Stroke cases (and subtypes) included in the register; 63% with ages over 64 yr and 52.4% male	Annual mean concentration with a 20 m by 20 m spatial resolution modeled using measurements, emissions data, and dispersion modeling, linked at postal-code level and year, and then modified to daily mean concentrations using time-series scaling factors for the Years 2005−2012. Control exposure days selected using time-stratified design matching on week day and stratifying on month 24-h avg	Mean: 18.68 Median: 18.48 75th: 25.03	Correlation (*r*): $PM_{2.5}$: −0.4; NO_2: −0.59; Other: NO_X −0.72, PM_{10} −0.33 Copollutant models with: NR	All stroke, 8-h avg, lag 0: 0.93 (0.74, 1.11) All stroke, 24-h avg, lag 0: 0.96 (0.85, 1.09) Ischemic stroke, 8-h avg, lag 0: 0.96 (0.75, 1.19) Ischemic stroke, 24-h avg, lag 0: 0.98 (0.85, 1.13) Hemorrhagic stroke, 8-h avg, lag 0: 1.07 (0.65, 1.85) Hemorrhagic stroke, 24-h avg, lag 0: 1.09 (0.78, 1.52)

Table 4-31 (Continued): Epidemiologic studies of short-term exposure to ozone and cerebrovascular disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
†Vidale et al. (2017) Como, Italy Ozone: January 2005−December 2014 Follow-up: January 2005−December 2014 Time-series study	n = 4,110 All residents of Como with hospital admission for acute MI or ischemic stroke between January 2005 and December 2014, mean age 71 yr, 65% male	Average daily concentrations from 2 stationary monitors 24-h avg		Correlation (*r*): NR Copollutant models with: NR	Ischemic stroke, lag 0: 0.99 (0.98, 1.02) Ischemic stroke, lag 1: 1.00 (0.99, 1.01)
†Wing et al. (2017b) Nueces County, TX, U.S. Ozone: 2000−2012 Follow-up: 2000−2012 Time-series study	Brain Attack Surveillance in Corpus Christi register n = 3,035 Cases recorded in registry in Nueces County, TX, mean age 70 yr, 48.7% male, 53% Mexican American	Daily maximal 8-h concentration from one central monitor in TCEQ TAMIS 24-h avg		Correlation (*r*): NR Copollutant models with: NR	Severe incident stroke risk, lag 1: 1.27 (1.12, 1.41) Severe incident stroke risk, lag 1, with neighborhood disadvantage: 1.27 (1.12, 1.41)

Table 4-32 Epidemiologic studies of short-term exposure to ozone and aggregate cardiovascular disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
†Kalantzi et al. (2011) Magnesia Prefecture, Greece Ozone: January 1, 2001−December 31, 2007 Follow-up: January 1, 2001−December 31, 2007 Time-series study	n = 4.88/day Emergency hospital admissions, counts over 3 days, during the study period among patients over 14 yr of age with a respiratory or cardiovascular disease diagnosis (ICD-10)	Averaged concentrations measured continuously from three stationary monitors within 5 km of the hospital 24-h avg	Mean: 25.53	Correlation (*r*): NR Copollutant models with: NR	Lag 0: 1.02 (1.01, 1.03) Lag 1: 1.02 (1.01, 1.03)
†Hůnová et al. (2013) Prague, Czech Republic Ozone: April−September 2002−2006 Follow-up: April−September 2002−2006 Time-series study	Daily counts of hospital admissions among all permanent residents in Prague	Averaged hourly concentrations from three stationary monitors; 24-h mean and max daily running 8-h mean 8-h avg	Mean: 47.463 Median: 45.84 75th: 56.24 Maximum: 83.45	Correlation (*r*): NR Copollutant models with: PM_{10}	24-h mean, lag 1: 0.97 (0.95, 1.00) 24-h mean, lag 2: 0.99 (0.97, 1.01) 8-h max, lag 1: 0.99 (0.96, 1.01) 8-h max, lag 2: 1.00 (0.97, 1.02)
†Winquist et al. (2012) St. Louis MSA, U.S. Ozone: January 1, 2001−June 27, 2007 Follow-up: January 1, 2001−June 27, 2007 Time-series study	n = 88.8 Counts of daily ED visits and HA among people residing in the St. Louis MSA	Concentrations from U.S. EPA AQS at Tudor Street stationary monitor, data missing 1.9% of days 8-h max	Mean: 36.3 Maximum: 111.8	Correlation (*r*): $PM_{2.5}$: 0.25; Copollutant models with: NR	HA, lag 0−4: 0.99 (0.95, 1.00) ED, lag 0−4: 1.00 (0.98, 1.02)

Table 4-32 (Continued): Epidemiologic studies of short-term exposure to ozone and aggregate cardiovascular disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Rodopoulou et al. (2014) Dona Ana County, NM, U.S. Ozone: 2007−2010 Follow-up: 2007−2010 Time-series study	n = ED visits 2,031, HA 5,161 Daily ED visits and hospital admissions for the adult population (18 yr and older)	Averaged hourly concentrations from three sites in the county, U.S. EPA AQS data 8-h max	Mean: 43.2 Median: 43 75th: 51 Maximum: 70	Correlation (*r*): $PM_{2.5}$: −0.05; Other: PM_{10} 0.18 Copollutant models with: NR	HA, lag 1: 1.03 (0.94, 1.13) ED visits, lag 1: 1.06 (0.91, 1.23)
↑Milojevic et al. (2014) England and Wales, U.K. Ozone: 2003−2008 Follow-up: 2003−2008 Case-crossover study	HES n = 2,663,067 Emergency hospital admissions to NHS hospitals, in HES database using centroid of census ward; median age (IQR) 73 (60−82), 54% male HES	Data from nearest monitoring station to residence on event day. Control exposure days defined using time-stratified design using other days of the month when case occurred 8-h max	Mean: NR Median: 30.96 75th: 38.58	Correlation (*r*): $PM_{2.5}$: −0.096; NO_2: −0.3489; SO_2: −0.0849; Other: PM_{10} 0.0302, CO −0.2973 Copollutant models with: NA	All CVD, lag 0−4: 0.99 (0.99, 1.00)
↑Sarnat et al. (2015) St. Louis, MO, U.S. Ozone: June 1, 2001–May 30, 2003 Follow-up: June 1, 2001–May 30, 2003 Time-series study	n = 69,679 ED visit records of patients residing in St. Louis MSA (eight counties each in Missouri and Illinois) from 36 out of 43 acute care hospitals	Averaged hourly concentrations in St. Louis from U.S. EPA AQS 8-h max	Mean: 36.2	Correlation (*r*): $PM_{2.5}$: 0.23; NO_2: 0.37; SO_2: −0.04; Other: CO −0.01 Copollutant models with: NR	Lag 0−2: 0.99 (0.97, 1.02)
↑Rodopoulou et al. (2015) Little Rock, AR, U.S. Ozone: 2002−2012 Follow-up: 2002−2012 Time-series study	n = 84,269 Daily emergency room visits among persons 15 yr and older, 19% 65 yr and older, 42.5% male	U.S. AQS data from stationary monitor in Little Rock 8-h max	Mean: 40 Median: 39 75th: 50	Correlation (*r*): NR Copollutant models with: NR	All, lag 1: 0.99 (0.97, 1.01)

Table 4-32 (Continued): Epidemiologic studies of short-term exposure to ozone and aggregate cardiovascular disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Vidale et al. (2017) Como, Italy Ozone: January 2005−December 2014 Follow-up: January 2005−December 2014 Time-series study	n = 4,110 All residents of Como with hospital admission for acute MI or ischemic stroke between January 2005 and December 2014, mean age 71 yr, 65% male	Average daily concentrations from two stationary monitors 24-h avg		Correlation (*r*): NR Copollutant models with: NR	CVD, lag 0: 1.08 (1.03, 1.14) CVD, lag 1: 1.07 (1.03, 1.12)
↑Hunova et al. (2017) Prague, Czech Republic Ozone: April–September 2002−2006 Follow-up: April–September 2002−2006 Time-series study	Daily counts of hospital admissions among all permanent residents in Prague	Averaged hourly mean concentration from up to three stationary monitors in Prague 8-h max		Correlation (*r*): Other: PM_{10} lag-1 0.457 Copollutant models with: NR	0.98 (0.95, 1.01)
↑Choi et al. (2011) Maryland, U.S. Ozone: June−August 2002 Follow-up: June−August 2002 Time-series study	n = 19,752 total, 214.7 visits/day All ED visits for CVD in Maryland	Daily mean concentrations during June−August, 2002 for each zip code tabulation area using block kriging and monitoring data in Maryland (16 sites) and sites near border zip codes in adjoining states 8-h max	Mean: 76.68 Maximum: 119.42	Correlation (*r*): NR Copollutant models with: NR	Lag 0−4: 1.07 (1.03, 1.11)

Table 4-33 Epidemiologic studies of short-term exposure to ozone and cardiovascular mortality.

Study	Study Population	Exposure Assessment	Mean (μg/m³)	Copollutant Examination	Effect Estimates HR (95% CI)
†Klemm et al. (2011) Atlanta, GA, U.S. August 1998−December 2007 Time-series study	65+	Data from several monitors 8-h max	Mean: 35.54 75th: 47.82 Maximum: 109.07	Correlation (*r*): NR Copollutant models with: NR	Lag 0−1: 0.69 (−2.28, 3.75)
†Sacks et al. (2012) Philadelphia, PA, U.S. May 12, 1992−September 30, 1995 Time-series study	All ages	Single monitor ~6 km west/southwest of City Hall 8-h max	Mean: 36 Median: 33 Maximum: 110	Correlation (*r*): $PM_{2.5}$: 0.43; NO_2: 0.18; SO_2: −0.19; Other: CO: −0.35 Copollutant models with: NR	Harvard (lag 0−1): −1.60 (−5.10, 2.10) California (lag 0−1): 0.20 (−3.40, 3.90) Canada (lag 0−1): 0.50 (−3.10, 4.30) Harvard AT (lag 0−1): 1.30 (−2.10, 4.90) APHEA2 (lag 0−1): 1.70 (−1.80, 5.30) NMMAPS (lag 0−1): 2.20 (−1.80, 6.40)
†Vanos et al. (2014) 10 Canadian cities 1981−1999 Time-series study	All ages	Monitor located downtown or at city airports within 27 km of downtown in each city 24-h avg	Mean: 19.3	Correlation (*r*): NR Copollutant models with: NR	All-year (lag 0): 4.65 (1.86, 7.43) Spring (lag 0): 3.16 (0.25, 6.08) Summer (lag 0): 5.58 (1.94, 9.21) Fall (lag 0): 1.96 (0.13, 3.78) Winter (lag 0): 4.46 (1.55, 7.37)

4.3.2 Long-Term Ozone Exposure

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Table 4-34 Epidemiologic studies of long-term exposure to ozone and ischemic heart disease (IHD).

Study	Study Population	Exposure Assessment	Mean (μg/m^3)	Copollutant Examination	Effect Estimates HR 95% CI
†Atkinson et al. (2013) Nationwide, U.K. Ozone: 2002−2007 Follow-up: 2003−2007 Cohort study	English cohort n = 836,557 Age: 40−89 yr	Annual average from emission-based model with 1- × 1-km resolution		Correlation (*r*): $PM_{2.5}$: −0.43 Copollutant models with: NR	MI; 2003−2007 exposure period; NO_2 copollutant: 0.71 (0.57, 0.87) MI; 2003−2007 exposure period; PM_{10} copollutant: 0.71 (0.57, 0.87) MI; 2002 exposure period: 0.76 (0.62, 1.00) MI; 2003−2007 exposure period: 0.77 (0.62, 0.96) MI; 2003−2007 exposure period; SO_2 copollutant: 0.87 (0.71, 1.14)
Kim et al. (2017) Seoul, South Korea Ozone: NR Follow-up: 2007−2013 Cohort study	NHIS-NSC n = 136,094 Healthy adults	Average from monitors linked to participants' zip codes	Mean: 19.93 Median: 18.75 75th: 27.08 Maximum: 71.12	Correlation (*r*): $PM_{2.5}$: 0.67; NO_2: 0.68; SO_2: 0.84; Other: CO: 0.55; PM_{10}−2.5: 0.37 Copollutant models with: NR	HR for Acute MI: 0.81 (0.75, 0.88)

Table 4-35 Epidemiologic studies of long-term exposure to ozone and atherosclerosis.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
†Breton et al. (2012) Southern California, U.S. Ozone: 1980−2009 Follow-up: 2007−2009 Cohort study	TROY n = 768 College students	Monthly AQS data from up to four monitors within 50 km spatially interpolated to residence using IDW averaged for ages 6−12	Mean: 23.2 Maximum: 41.8	Correlation (*r*): $PM_{2.5}$: −0.15; NO_2: 0.35; Other: PM_{10}: −0.05 Copollutant models with: NO_2, PM_{10}, $PM_{2.5}$	Change in CIMT for exposure averaged during ages 0−5; NO_2 copollutant: 10.00 (1.40, 18.60) Change in CIMT for exposure averaged over lifetime; PM_{10} copollutant: 10.13 (−0.51, 20.63) Change in CIMT for exposure averaged during ages 6−12; $PM_{2.5}$ copollutant: 10.22 (1.18, 19.35) Change in CIMT for exposure averaged during ages 6−12: 10.86 (1.94, 19.89) Change in CIMT for exposure averaged during ages 6−12; PM_{10} copollutant: 10.86 (1.83, 19.89) Change in CIMT for exposure averaged during ages 0−5: 7.80 (−0.30, 15.90) Change in CIMT for exposure averaged during ages 0−5; PM_{10} copollutant: 8.50 (0.20, 16.90) Change in CIMT for exposure averaged over lifetime; NO_2 copollutant: 8.86 (−2.03, 19.75) Change in CIMT for exposure averaged over lifetime; $PM_{2.5}$ copollutant: 8.86 (−1.65, 19.37) Change in CIMT for exposure averaged during ages 0−5; $PM_{2.5}$ copollutant: 9.10 (0.90, 17.40) Change in CIMT for exposure averaged during ages 6−12; NO_2 copollutant: 9.46 (−0.11, 19.03) Change in CIMT for exposure averaged over lifetime: 9.49 (−1.01, 20.00)

Table 4-35 (Continued): Epidemiologic studies of long-term exposure to ozone and atherosclerosis.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
†Breton et al. (2016) Southern California, U.S. Ozone: NR Follow-up: 2002−2003 Case-control study	Children's Health Study n = 459 Public school children enrolled in kindergarten or first grade; CV measures at age 11	IDW from up to four monitors averaged over prenatal trimesters; based on residential history at birth and age 6−7 yr	Mean: about 40, presented in box plot only	Correlation (*r*): $PM_{2.5}$: 0.21−0.41; NO_2: −0.63; Other: PM_{10}: 0.21−0.66 Copollutant models with: NR	Left CIMT (mm); first trimester: −0.00 (−0.00, 0.00) Left CIMT (mm); third trimester: −0.00 (−0.00, 0.00) Right CIMT (mm); third trimester: −0.00 (−0.00, 0.00) Right CIMT (mm); first trimester: −0.00 (−0.00, 0.00) Left CIMT (mm); second trimester: 0.00 (−0.00, 0.00) Right CIMT (mm); second trimester: 0.00 (−0.00, 0.00)

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Table 4-36 Study-specific details from animal toxicological studies of atherosclerosis.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Gordon et al. (2013)	Rats (BN) n = 12/treatment group males, 0 females Age: 4 and 20 mo	0.8 ppm, 6hr/day, 1 day/week for 17 weeks	Potential markers of atherosclerosis at the end of the given exposure (28 or 56 days)
Sethi et al. (2012)	Rats (S-D) n = 6/treatment group males, 0 females Age: adult	0.8 ppm, 8 h/day for 28 or 56 days	Potential markers of atherosclerosis at the end of the given exposure (28 or 56 days)

BN = brown Norway; S-D = Sprague-Dawley.

Table 4-37 Epidemiologic studies of long-term exposure to ozone and heart failure.

Study	Study Population	Exposure Assessment	Mean (μg/m^3)	Copollutant Examination	Effect Estimates HR 95% CI
↑Atkinson et al. (2013) Nationwide, U.K. Ozone: 2002−2007 Follow-up: 2003−2007 Cohort study	English cohort n = 836,557 Age: 40−89 yr	Annual average from emission-based model with 1- × 1-km resolution		Correlation (*r*): $PM_{2.5}$: −0.43 Copollutant models with: NR	Heart failure; 2002 exposure period: 0.66 (0.50, 0.87) Heart failure; 2003−2007 exposure period: 0.66 (0.49, 0.85) Heart failure; 2003−2007 exposure period; NO_2 copollutant: 0.71 (0.53, 0.94) Heart failure; 2003−2007 exposure period; PM_{10} copollutant: 0.71 (0.53, 0.94) Heart failure; 2003−2007 exposure period; SO_2 copollutant: 0.71 (0.53, 0.94)
↑Kim et al. (2017) Seoul, South Korea Ozone: NR Follow-up: 2007−2013 Cohort study	NHIS-NSC n = 136,094 Healthy adults	Average from monitors linked to participants' zip codes	Mean: 19.93 Median: 18.75 75th: 27.08 Maximum: 71.12	Correlation (*r*): $PM_{2.5}$: 0.67; NO_2: 0.68; SO_2: 0.84; Other: CO: 0.55; $PM_{10-2.5}$: 0.37 Copollutant models with: NR	HR for CHF: 0.76 (0.71, 0.81)

Table 4-38 Study-specific details from animal toxicological studies of impaired heart function.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Perepu et al. (2012)	Rats (S-D) n = 6/treatment group males, 0 females Age: adult	0.8 ppm, 8 h/day for 28 or 56 days	LVDP (28 and 56 days PE)
Sethi et al. (2012)	Rats (S-D) n = 6/treatment group males, 0 females Age: adult	0.8 ppm, 8 h/day for 28 or 56 days	LVDP (28 and 56 days PE)

LVDP = left ventricular developed pressure; PE = post-exposure; S-D = Sprague-Dawley.

Table 4-39 Study-specific details from animal toxicological studies of vascular function.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Gordon et al. (2013)	Rats (BN) n = 12/treatment group males, 0 females Age: 4 and 20 mo	0.8 ppm, 6 h/day, 1 day/week for 17 weeks	Markers of endothelial function blood drawn day after final exposure

BN = brown Norway.

Table 4-40 Epidemiologic studies of long-term exposure to ozone and blood pressure.

Study	Study Population	Exposure Assessment	Mean (μg/m^3)	Copollutant Examination	Effect Estimates HR (95% CI)
†Dong et al. (2013b) Three northeastern cities, China Ozone: 2006−2008 Follow-up: 2009−2010 Cross-sectional study	33 Communities Chinese Health Study n = 24,845 Age: 18−74 yr	3-yr avg concentration from single monitor within 1 mile of residence 8-h avg	Mean: 24.7 Median: 25 Maximum: 35.5	Correlation (*r*): NR Copollutant models with: NR	Absolute increase in DBP; women: 0.02 (−0.26, 0.31) Absolute increase in SBP; women: 0.04 (−0.45, 0.53) Absolute increase in DBP; all: 0.34 (0.13, 0.55) Absolute increase in DBPl; men: 0.53 (0.22, 0.83) Absolute increase in SBP; all: 0.66 (0.32, 1.01) Absolute increase in SBP; men: 0.95 (0.47, 1.44) OR for hypertension; 55−64 yr: 1.02 (0.93, 1.13) OR for hypertension; women: 1.06 (0.92, 1.16) OR for hypertension; <55 yr: 1.12 (1.06, 1.18) OR for hypertension; all: 1.12 (1.05, 1.18) OR for hypertension; 65+ yr: 1.14 (0.96, 1.35) OR for hypertension; men: 1.19 (1.04, 1.34)

Table 4-40 (Continued): Epidemiologic studies of long-term exposure to ozone and blood pressure.

Study	Study Population	Exposure Assessment	Mean (μg/m^3)	Copollutant Examination	Effect Estimates HR (95% CI)
↑Zhao et al. (2013) Three northeastern cities, China Ozone: 2006−2008 Follow-up: 2009−2010 Cross-sectional study	33 Communities Chinese Health Study n = 24,845 Age: 18−74 yr	3-yr avg concentration from single monitor within 1 mile of residence 8-h avg	Mean: 24.7 Median: 25 Maximum: 35.5	Correlation (*r*): NR Copollutant models with: NR	Absolute difference for SBP; normal weight—female: −0.12 (−0.66, 0.44) Absolute difference for DBP; normal weight—female: −0.13 (−0.45, 0.20) Absolute difference for DBP; overweight—female: −0.15 (−0.66, 0.35) Absolute difference for SBP; overweight—female: 0.14 (−0.75, 1.04) Absolute difference for DBP; obese—female: 0.21 (−0.95, 1.37) Absolute difference for DBP; normal weight—all: 0.26 (0.02, 0.51) Absolute difference for SBP; normal weight—all: 0.31 (−0.10, 0.72) Absolute difference for DBP; overweight—all: 0.32 (−0.02, 0.65) Absolute difference for SBP; normal weight—male: 0.39 (−0.20, 0.99) Absolute difference for SBP; obese—female: 0.50 (−1.74, 2.74) Absolute difference for DBP; overweight—male: 0.59 (0.14, 1.04) Absolute difference for DBP; normal weight—male: 0.61 (0.25, 0.97) OR for hypertension; obese—female: 0.90 (0.69, 1.16) OR for hypertension; normal weight—female: 0.95 (0.86, 1.04) OR for hypertension; normal weight—all: 1.05 (0.99, 1.12) OR for hypertension; overweight—female: 1.06 (0.95, 1.19) OR for hypertension; normal weight—male: 1.09 (1.01, 1.19) OR for hypertension; overweight—all: 1.17 (1.09, 1.25) Absolute difference for DBP; obese—all: 1.19 (0.33, 2.06) OR for hypertension; obese—all: 1.22 (1.03, 1.44) OR for hypertension; overweight—male: 1.22 (1.12, 1.33) OR for hypertension; obese—male: 1.44 (1.14, 1.82) Absolute difference for SBP; overweight—all: 1.56 (0.98, 2.12) Absolute difference for DBP; obese—male: 1.76 (0.58, 2.93) Absolute difference for SBP; overweight—male: 2.36 (1.65, 3.08) Absolute difference for SBP; obese—all: 3.15 (1.61, 4.67) Absolute difference for SBP; Obese—male: 4.04 (2.20, 5.86)

Table 4-40 (Continued): Epidemiologic studies of long-term exposure to ozone and blood pressure.

Study	Study Population	Exposure Assessment	Mean (μg/m^3)	Copollutant Examination	Effect Estimates HR (95% CI)
†Dong et al. (2014) Seven northeastern cities, China Ozone: 2009−2012 Follow-up: 2012−2013 Cross-sectional study	n = 9,354 School-aged children, 5−17 yr	4-yr avg concentration from monitor within 1 km of school 8-h avg	Mean: 54 Median: 21.9	Correlation (*r*): NR Copollutant models with: NR	Absolute increase in SBP; girls: 0.20 (0.16, 0.24) Absolute Increase in SBP; breastfeeding only: 0.22 (0.18, 0.25) Absolute increase in SBP; all: 0.22 (0.19, 0.25) Absolute increase in SBP; no breastfeeding: 0.22 (0.16, 0.28) Absolute Increase in DBP; breastfeeding only: 0.23 (0.21, 0.27) Absolute increase in SBP; boys: 0.23 (0.19, 0.28) Absolute increase in DBP; all: 0.25 (0.22, 0.27) Absolute increase in DBP; girls: 0.25 (0.22, 0.29) Absolute increase in DBP; boys: 0.25 (0.21, 0.28) Absolute increase in DBP; no breastfeeding: 0.27 (0.22, 0.32) OR for hypertension; breastfeeding only: 1.04 (1.03, 1.05) OR for hypertension; boys: 1.05 (1.04, 1.06) OR for hypertension; girls: 1.05 (1.04, 1.06) OR for hypertension; no breastfeeding: 1.07 (1.05, 1.08)
†van Rossem et al. (2015) Boston, MA, U.S. Ozone: 1999−2002 Follow-up: 1999−2002 Cohort study	Project Viva n = 1,131 Newborn infants	Area-wide average of AQS monitors (n = 4) averaged over trimesters 24-h avg	Median: 23.4 75th: 29.2	Correlation (*r*): $PM_{2.5}$: −0.13; NO_2: −0.69; Other: BC: −0.35; NO_X: −0.92 Copollutant models with: NR	Increase in SBP for 3rd trimester exposure: −1.84 (−3.31, −0.29) Increase in SBP for 1st trimester exposure: 0.92 (−0.77, 2.69) Increase in SBP for 2nd trimester exposure: 1.33 (0.23, 2.34)

Table 4-40 (Continued): Epidemiologic studies of long-term exposure to ozone and blood pressure.

Study	Study Population	Exposure Assessment	Mean (μg/m^3)	Copollutant Examination	Effect Estimates HR (95% CI)
↑Dong et al. (2015) Seven northeastern cities, China Ozone: 2009−2012 Follow-up: 2012−2013 Cross-sectional study	n = 9,354 School-aged children, 5−17 yr	4-yr avg concentration from monitor within 1 km of school 8-h avg	Mean: 54 Median: 21.9 Maximum: 287	Correlation (*r*): NO_2: 0.33; SO_2: 0.6; Other: PM_{10}: 0.85 Copollutant models with: NR	Absolute increase in SBP; normal weight children: 0.13 (0.10, 0.17) Absolute increase in SBP; normal weight girls: 0.13 (0.09, 0.18) Absolute increase in SBP; normal weight boys: 0.14 (0.08, 0.19) Absolute increase in SBP; overweight boys: 0.14 (0.03, 0.25) Absolute increase in DBP; normal weight boys: 0.17 (0.13, 0.22) Absolute increase in SBP; overweight children: 0.17 (0.09, 0.25) Absolute increase in DBP; normal weight children: 0.19 (0.16, 0.22) Absolute increase in DBP; normal weight girls: 0.19 (0.16, 0.23) Absolute increase in SBP; overweight girls: 0.20 (0.08, 0.32) Absolute increase in DBP; overweight girls: 0.24 (0.13, 0.35) Absolute increase in DBP; overweight children: 0.25 (0.18, 0.33) Absolute increase in SBP; obese boys: 0.25 (0.13, 0.36) Absolute increase in SBP; obese children: 0.25 (0.16, 0.34) Absolute increase in DBP; obese boys: 0.26 (0.17, 0.35) Absolute increase in SBP; obese girls: 0.26 (0.10, 0.42) Absolute increase in DBP; obese children: 0.27 (0.20, 0.35) Absolute increase in DBP; overweight boys: 0.29 (0.19, 0.38) Absolute increase in DBP; obese girls: 0.30 (0.17, 0.44) OR for hypertension; normal weight boys: 1.03 (1.02, 1.04) OR for hypertension; normal weight children: 1.03 (1.03, 1.04) OR for hypertension; normal weight girls: 1.03 (1.02, 1.05) OR for hypertension; overweight boys: 1.05 (1.03, 1.08) OR for hypertension; overweight children: 1.05 (1.03, 1.07) OR for hypertension; overweight girls: 1.05 (1.03, 1.07) OR for hypertension; obese boys: 1.06 (1.04, 1.08) OR for hypertension; obese children: 1.07 (1.05, 1.08) OR for hypertension; obese girls: 1.07 (1.04, 1.10)

Table 4-40 (Continued): Epidemiologic studies of long-term exposure to ozone and blood pressure.

Study	Study Population	Exposure Assessment	Mean (μg/m^3)	Copollutant Examination	Effect Estimates HR (95% CI)
↑Liu et al. (2016) Taipei, Taiwan Ozone: 2005−2012 Follow-up: 2005−2012 Cohort study	n = 3,762 Age: 20−80 yr	Annual average of nearest monitor	Mean: 27 Maximum: 28.7	Correlation (*r*): NR Copollutant models with: NR	Absolute difference for DBP; AHI 0−4: −0.10 (−0.85, 0.65) Absolute difference for SBP; AHI 5−29: −1.20 (−2.12, −0.27) Absolute difference for SBP; AHI 30+: −1.54 (−2.48, −0.61) Absolute difference for SBP; all: −1.54 (−2.11, −0.98) Absolute difference for SBP; AHI 0−4: −1.92 (−2.96, −0.87) Absolute difference for DBP; AHI 30+: 0.19 (−0.46, 0.84) Absolute difference for DBP; all: 0.27 (−0.12, 0.66) Absolute difference for DBP; AHI 5−29: 0.70 (0.10, 1.30)
↑Breton et al. (2016) Southern CA, U.S. Ozone: NR Follow-up: 2002−2003 Case-control study	Children's Health Study n = 459 Public school children enrolled in kindergarten or first grade; CV measures at age 11 yr	IDW from up to four monitors averaged over prenatal trimesters; based on residential history at birth and age 6−7 yr	Mean: about 40, presented in box plot only	Correlation (*r*): $PM_{2.5}$: 0.21−0.41; NO_2: −0.63; Other: PM_{10}: 0.21−0.66 Copollutant models with: NR	DBP (mm Hg); second trimester: −0.04 (−0.32, 0.24) SBP (mm Hg); first trimester: −0.14 (−0.53, 0.25) DBP (mm Hg); first trimester: −0.15 (−0.43, 0.13) SBP (mm Hg); second trimester: 0.05 (−0.33, 0.43) SBP (mm Hg); third trimester: 0.05 (−0.39, 0.48) DBP (mm Hg); third trimester: 0.07 (−0.25, 0.39)
↑Coogan et al. (2017) Nationwide, U.S. Ozone: 2007−2008 Follow-up: 1995−2011 Cohort study	BWHS n = 33,771 African American women	CMAQ downscaler 8-h max	Mean: 37.4 Maximum: 56.4	Correlation (*r*): $PM_{2.5}$: 0.14; NO_2: −0.54; Copollutant models with: NO_2; $PM_{2.5}$	Hypertension incidence copollutant—NO_2: 1.06 (0.91, 1.23) Hypertension incidence copollutant—$PM_{2.5}$: 1.12 (0.99, 1.30) Hypertension incidence: 1.14 (1.00, 1.28)

Table 4-40 (Continued): Epidemiologic studies of long-term exposure to ozone and blood pressure.

Study	Study Population	Exposure Assessment	Mean (μg/m^3)	Copollutant Examination	Effect Estimates HR (95% CI)
†Yang et al. (2017) Three northeastern cities, China Ozone: 2006−2008 Follow-up: 2009 Cross-sectional study	33 Communities Chinese Health Study n = 24,845 Age: 18−74 yr	Data from monitoring stations 8-h avg	Mean: 24.7 Maximum: 35.5	Correlation (*r*): NR Copollutant models with: NR	Increase in DBP (mm Hg), hypertensive: −0.11 (−0.42, 0.20) Increase in SBP (mm Hg), hypertensive: 0.03 (−0.49, 0.55) Increase in SBP (mm Hg), hypertensive without medication: 0.04 (−0.55, 0.61) Increase in DBP (mm Hg), 55+ yr: 0.15 (−0.23, 0.55) Increase in DBP (mm Hg), hypertensive without medication: 0.15 (−0.20, 0.49) Increase in DBP (mm Hg), prehypertensive: 0.18 (0.03, 0.33) Increase in DBP (mm Hg), men: 0.35 (0.12, 0.49) Increase in DBP (mm Hg), normotensive: 0.35 (0.16, 0.55) Increase in DBP (mm Hg), <35 yr: 0.45 (0.16, 0.73) Increase in DBP (mm Hg), all: 0.45 (0.29, 0.60) Increase in SBP (mm Hg), normotensive: 0.48 (0.22, 0.75) Increase in DBP (mm Hg), women: 0.49 (0.28, 0.71) Increase in DBP (mm Hg), 35−55 yr: 0.50 (0.29, 0.71) Increase in SBP (mm Hg), men: 0.61 (0.29, 1.22) Increase in SBP (mm Hg), 55+ yr: 0.85 (0.25, 1.46) Increase in SBP (mm Hg), prehypertensive: 0.87 (0.64, 1.10) Increase in SBP (mm Hg), men 35−55 yr: 0.96 (0.65, 1.28) Increase in SBP (mm Hg), <35 yr: 1.03 (0.64, 1.41) Increase in SBP (mm Hg), all: 1.03 (0.79, 1.25) Prehypertension, men: 1.03 (0.91, 1.17) Prehypertension, <35 yr: 1.08 (1.03, 1.15) Prehypertension, 35−55 yr: 1.10 (1.04, 1.14) Prehypertension, all: 1.12 (0.99, 1.25) Prehypertension, women: 1.18 (1.05, 1.33) Increase in SBP (mm Hg), women: 1.22 (0.89, 1.55) Prehypertension, 55+ yr: 1.24 (1.14, 1.33)

Table 4-40 (Continued): Epidemiologic studies of long-term exposure to ozone and blood pressure.

Study	Study Population	Exposure Assessment	Mean (μg/m^3)	Copollutant Examination	Effect Estimates HR (95% CI)
↑Cole-Hunter et al. (2018) Barcelona, Spain Ozone: 2011−2014 Follow-up: 2011−2014 Cohort study	TAPAS/EXPOsOMICS n = 57 Healthy adults Age: 18−60 yr	Annual average assigned to participant address from closest reference station	Mean: 22 Maximum: 32.9	Correlation (*r*): $PM_{2.5}$: −0.4; NO_2: −0.21; Other: PM_{10}: −0.56; NO_X: −0.37 Copollutant models with: PM_{10}	Increase in SBP (mm Hg): 4.13 (−1.13, 9.38) Increase in SBP (mm Hg) copollutant PM_{10}: 4.87 (−1.36, 11.10) Increase in DBP (mm Hg): 6.42 (2.15, 10.69) Increase in DBP (mm Hg) copollutant PM_{10}: 7.60 (2.64, 12.55)
↑Chuang et al. (2011) Taiwan, Taiwan Ozone: 2000 Follow-up: 2000 Case-crossover study	SEBAS n = 1,023 Age: 54+ yr	City- or countywide annual average from monitoring stations	Mean: 22.95 Maximum: 42.3	Correlation (*r*): NR Copollutant models with: NR	Change in DBP (mm Hg): 22.97 (20.27, 25.66) Change in SBP (mm Hg): 24.03 (18.88, 29.20)

Table 4-41 Study-specific details from animal toxicological studies of blood pressure.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Gordon et al. (2013)	Rats (BN) n = 12/treatment group males, 0 females Age: 4 and 20 mo	0.8 ppm, 6 h/day, 1 day/week for 17 weeks	Blood pressure (biweekly through Week 15)

BN = brown Norway.

Table 4-42 Study-specific details from animal toxicological studies of heart rate variability (HRV), heart rate (HR).

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Gordon et al. (2014)	Rats (BN) n = 12/treatment group males, 0 females Age: 4 and 20 mo	1 ppm, 6 h/day, 2 day/week for 13 weeks	Heart rate (rats implanted with telemeter)
Gordon et al. (2013)	Rats (BN) n = 12/treatment group males, 0 females Age: 4 and 20 mo	0.8 ppm, 6 h/day, 1 day/week for 17 weeks	Heart rate (biweekly through Week 15)

BN = brown Norway; HR = heart rate; HRV = heart rate variability.

Table 4-43 Study-specific details from animal toxicological studies of coagulation.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Gordon et al. (2013)	Rats (BN) n = 12/treatment group males, 0 females Age: 4 and 20 mo	0.8 ppm, 6 h/day, 1 day/week for 17 weeks	mRNA levels of coagulation factors in aorta tissue collected a day after final exposure

BN = brown Norway.

Table 4-44 Study-specific details from animal toxicological studies of inflammation.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Perepu et al. (2012)	Rats (S-D) n = 6/treatment group males, 0 females Age: adult	0.8 ppm, 8 h/day for 28 or 56 days	Markers of oxidative stress (28 and 56 days PE) Markers of systemic inflammation in heart tissue (28 and 56 days PE)
Sethi et al. (2012)	Rats (S-D) n = 6/treatment group males, 0 females Age: adult	0.8 ppm, 8 h/day for 28 or 56 days	Markers of oxidative stress (28 and 56 days PE) Markers of systemic inflammation in heart tissue (28 and 56 days PE)
Gordon et al. (2013)	Rats (BN) n = 12/treatment group males, 0 females Age: 4 and 20 mo	0.8 ppm, 6 h/day, 1 day/week for 17 weeks	Histology (17 weeks PE) Markers of oxidative stress (17 weeks PE) Markers of systemic inflammation (17 weeks PE)
Miller et al. (2016)	Rats (WKY) n = four to five/treatment group males, 0 females Age: 10 weeks	1 ppm, 5 h/day, 3 consecutive days/week for 13 weeks	Markers of systemic inflammation (13 weeks PE)

BN = brown Norway; PE = post-exposure; S-D = Sprague-Dawley; WKY = Wistar Kyoto.

Table 4-45 Epidemiologic studies of long-term exposure to ozone and cerebrovascular disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Atkinson et al. (2013) Nationwide, U.K. Ozone: 2002−2007 Follow-up: 2003−2007 Cohort study	English Cohort n = 836,557 Ages 40−89	Annual average from emission-based model with 1- × 1-km resolution		Correlation (*r*): $PM_{2.5}$: −0.43 Copollutant models with: NR	Stroke; 2003−2007 exposure period; PM_{10} copollutant: 0.94 (0.76, 1.22) CBVD; 2003−2007 exposure period: 0.96 (0.90, 1.02) Stroke; 2002 exposure period: 1.00 (0.82, 1.30) Stroke; 2003−2007 exposure period; NO_2 copollutant: 1.00 (0.76, 1.22) Stroke; 2003−2007 exposure period: 1.02 (0.81, 1.28) Stroke; 2003−2007 exposure period; SO_2 copollutant: 1.07 (0.87, 1.38)
↑Dong et al. (2013a) Three northeastern cities, China Ozone: 2006−2008 Follow-up: 2009−2010 Cross-sectional study	33 Communities Chinese Health Study n = 24,845 Age: 18−74 yr	3-yr avg concentration from single monitor within 1 mile of residence 8-h avg	Mean: 24.7 Median: 25 Maximum: 35.5	Correlation (*r*): NO_2: 0.45 ;SO_2: 0.87; Other: PM_{10}: 0.80 Copollutant models with: NR	OR for stroke; female: 1.13 (0.92, 1.39) OR for stroke; all: 1.14 (0.99, 1.30) OR for stroke; male: 1.14 (0.95, 1.37)
↑Spiezia et al. (2014) Padua, Italy Ozone: NR Follow-up: 2008−2012 Case-control study	n = 105 (33 cases) Patients with “high probability” of a PE	Average monthly mean concentrations from nearest monitoring site	75th: 37	Correlation (*r*): NR Copollutant models with: NR	OR compares exposures >37 ppb to those lower than 37 ppb: 0.83 (0.26, 2.70)

Table 4-45 (Continued): Epidemiologic studies of long-term exposure to ozone and cerebrovascular disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Qin et al. (2015) Three northeastern cities, China Ozone: 2006−2008 Follow-up: 2009−2010 Cross-sectional study	33 Communities Chinese Health Study n = 24,845 Age: 18−74 yr	3-yr avg concentration from single monitor within 1 mile of residence 8-h avg	Mean: 24.7 Median: 25 Maximum: 35.5	Correlation (*r*): NR Copollutant models with: NR	OR for stroke; BMI <25 kg/m^2—female: 0.89 (0.66, 1.19) OR for stroke; normal weight: 0.98 (0.83, 1.16) OR for stroke; BMI <25 kg/m^2—all: 1.03 (0.87, 1.21) OR for stroke; BMI <25 kg/m^2—male: 1.14 (0.94, 1.39) OR for stroke; overweight: 1.26 (1.05, 1.52) OR for stroke; BMI >25 kg/m^2—male: 1.27 (0.99, 1.63) OR for stroke; BMI >25 kg/m^2—all: 1.29 (1.08, 1.54) OR for stroke; BMI >25 kg/m^2—female: 1.32 (1.02, 1.71) OR for stroke; obese: 1.42 (0.84, 2.38)
↑Kim et al. (2017) Seoul, South Korea Ozone: NR Follow-up: 2007−2013 Cohort study	NHIS-NSC n = 136,094 Healthy adults	Average from monitors linked to participants' zip codes	Mean: 19.93 Median: 18.75 75th: 27.08 Maximum: 71.12	Correlation (*r*): $PM_{2.5}$: 0.67; NO_2: 0.68; SO_2: 0.84; Other: CO: 0.55; $PM_{10-2.5}$: 0.37 Copollutant models with: NR	HR for ischemic stroke: 0.73 (0.68, 0.77) HR for stroke: 0.73 (0.69, 0.76) HR for hemorrhagic stroke: 0.74 (0.67, 0.81)

Table 4-46 Epidemiologic studies of long-term exposure to ozone and aggregate cardiovascular disease.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR 95% CI
↑Dong et al. (2013a) Three northeastern cities, China Ozone: 2006−2008 Follow-up: 2009−2010 Cross-sectional study	33 Communities Chinese Health Study n =24,845 Age: 18−74 yr	3-yr avg concentration from single monitor within 1 mile of residence 8-h avg	Mean: 24.7 Median: 25 Maximum: 35.5	Correlation (*r*): NO_2: 0.45; SO_2: 0.87; Other: PM_{10}: 0.80 Copollutant models with: NR	OR for CVDs; female: 0.98 (0.64, 1.43) OR for CVDs; all: 1.08 (0.85, 1.37) OR for CVD; male: 1.10 (0.80, 1.52)
↑Qin et al. (2015) Three northeastern cities, China Ozone: 2006−2008 Follow-up: 2009−2010 Cross-sectional study	33 Communities Chinese Health Study n = 24,845 Age: 18−74 yr	3-yr avg concentration from single monitor within 1 mile of residence 8-h avg	Mean: 24.7 Median: 25 Maximum: 35.5	Correlation (*r*): NR Copollutant models with: NR	OR for CVDs; BMI <25 kg/m^2—female: 0.71 (0.42, 1.22) OR for CVDs; normal weight: 1.07 (0.88, 1.31) OR for CVDs; overweight: 1.07 (0.87, 1.31) OR for CVDs; BMI <25 kg/m^2—all: 1.09 (0.89, 1.33) OR for CVDs; BMI >25 kg/m^2—male: 1.15 (0.94, 1.41) OR for CVDs; BMI >25 kg/m^2—all: 1.16 (0.96, 1.39) OR for CVDs; BMI <25 kg/m^2—male: 1.17 (0.94, 1.45) OR for CVDs; BMI >25 kg/m^2—female: 1.23 (0.82, 1.86) OR for CVDs; obese: 1.50 (1.02, 2.21)

Annex for Appendix 4: Evaluation of Studies on Health Effects of Ozone

This annex describes the approach used in the Integrated Science Assessment (ISA) for Ozone and Related Photochemical Oxidants to evaluate study quality in the available health effects literature. As described in the Preamble to the ISA (U.S. EPA, 2015), causality determinations were informed by the integration of evidence across scientific disciplines (e.g., exposure, animal toxicology, epidemiology) and related outcomes and by judgments of the strength of inference in individual studies. Table Annex 4-1 describes aspects considered in evaluating study quality of controlled human exposure, animal toxicological, and epidemiologic studies. The aspects found in Table Annex 4-1 are consistent with current best practices for reporting or evaluating health science data.1 Additionally, the aspects are compatible with published U.S. EPA guidelines related to cancer, neurotoxicity, reproductive toxicity, and developmental toxicity (U.S. EPA, 2005, 1998, 1996b, 1991).

These aspects were not used as a checklist, and judgments were made without considering the results of a study. The presence or absence of particular features in a study did not necessarily lead to the conclusion that a study was less informative or to exclude it from consideration in the ISA. Further, these aspects were not used as criteria for determining causality in the five-level hierarchy. As described in the Preamble, causality determinations were based on judgments of the overall strengths and limitations of the collective body of available studies and the coherence of evidence across scientific disciplines and related outcomes. Table Annex 4-1 is not intended to be a complete list of aspects that define a study's ability to inform the relationship between ozone and health effects, but it describes the major aspects considered in this ISA to evaluate studies. Where possible, study elements, such as exposure assessment and confounding (i.e., bias due to a relationship with the outcome and correlation with exposures to ozone), are considered specifically for ozone. Thus, judgments on the ability of a study to inform the relationship between an air pollutant and health can vary depending on the specific pollutant being assessed.

[1] For example, NTP OHAT approach (Rooney et al., 2014), IRIS Preamble (U.S. EPA, 2013b), ToxRTool (Klimisch et al., 1997), STROBE guidelines (von Elm et al., 2007), and ARRIVE guidelines (Kilkenny et al., 2010).

Table Annex 4-1 Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Study Design
Controlled Human Exposure:
Studies should clearly describe the primary and any secondary objectives of the study or specific hypotheses being tested. Study subjects should be randomly exposed without knowledge of the exposure condition. Preference is given to balanced crossover (repeated measures) or parallel design studies which include controlled exposures (e.g., to clean filtered air). In crossover studies, a sufficient and specified time between exposure days should be provided to avoid carry over effects from prior exposure days. In parallel design studies, all arms should be matched for individual characteristics such as age, sex, race, anthropometric properties, and health status. In studies evaluating effects of disease, appropriately matched healthy controls are desired for interpretative purposes.
Animal Toxicology:
Studies should clearly describe the primary and any secondary objectives of the study or specific hypotheses being tested. Studies should include appropriately matched controlled exposures (e.g., to clean filtered air, time matched) and use methods to limit differences in baseline characteristics of control and exposure groups. Studies should randomize assignment to exposure groups and where possible conceal allocation to research personnel. Groups should be subjected to identical experimental procedures and conditions; animal care including housing, husbandry, etc. should be identical between groups. Blinding of research personnel to study group may not be possible due to animal welfare and experimental considerations; however, differences in the monitoring or handling of animals in all groups by research personnel should be minimized.
Epidemiology:
Inference is stronger for studies that clearly describe the primary and any secondary aims of the study or specific hypotheses being tested. For short-term exposure, time-series, case-crossover, and panel studies are emphasized over cross-sectional studies because they examine temporal correlations and are less prone to confounding by factors that differ between individuals (e.g., SES, age). Panel studies with scripted exposures, in particular, can contribute to inference because they have consistent, well-defined exposure durations across subjects, measure personal ambient pollutant exposures, and measure outcomes at consistent, well-defined lags after exposures. Studies with large sample sizes and those conducted over multiple years are considered to produce more reliable results. Additionally, multicity studies are preferred over single-city studies because they examine associations for large diverse geographic areas using a consistent statistical methodology, avoiding the publication bias often associated with single-city studies.[a] If other quality parameters are equal, multicity studies carry more weight than single-city studies because they tend to have larger sample sizes and lower potential for publication bias. For long-term exposure, inference is considered to be stronger for prospective cohort studies and case-control studies nested within a cohort (e.g., for rare diseases) than cross-sectional, other case-control, or ecological studies. Cohort studies can better inform the temporality of exposure and effect. Other designs can have uncertainty related to the appropriateness of the control group or validity of inference about individuals from group-level data. Study design limitations can bias health effect associations in either direction.

Table Annex 4-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Study Population/Test Model
Controlled Human Exposure:
In general, the subjects recruited into study groups should be similarly matched for age, sex, race, anthropometric properties, and health status. In studies evaluating effects of specific subject characteristics (e.g., disease, genetic polymorphism, etc.), appropriately matched healthy controls are preferred. Relevant characteristics and health status should be reported for each experimental group. Criteria for including and excluding subjects should be clearly indicated. For the examination of populations with an underlying health condition (e.g., asthma), independent, clinical assessment of the health condition is ideal, but self-report of physician diagnosis generally is considered to be reliable for respiratory and cardiovascular disease outcomes.[b] The loss or withdrawal of recruited subjects during the course of a study should be reported. Specific rationale for excluding subject(s) from any portion of a protocol should be explained.
Animal Toxicology:
Ideally, studies should report species, strain, substrain, genetic background, age, sex, and weight. Unless data indicate otherwise, all animal species and strains are considered appropriate for evaluating effects of ozone exposure. It is preferred that the authors test for effects in both sexes and multiple lifestages and report the result for each group separately. All animals used in a study should be accounted for, and rationale for exclusion of animals or data should be specified.
Epidemiology:
There is greater confidence in results for study populations that are recruited from and representative of the target population. Studies that have high participation, have low drop-out over time, and are not dependent on exposure or health status are considered to have low potential for selection bias. Clearly specified criteria for including and excluding subjects can aid assessment of selection bias. For populations with an underlying health condition, independent, clinical assessment of the health condition is valuable, but self-report of physician diagnosis generally is considered to be reliable for respiratory and cardiovascular diseases.[b] Comparisons of groups with and without an underlying health condition are more informative if groups are from the same source population. Selection bias can influence results in either direction or may not affect the validity of results but rather reduce the generalizability of findings to the target population.
Pollutant
Controlled Human Exposure:
The focus is on studies testing ozone exposure.
Animal Toxicology:
The focus is on studies testing ozone exposure.
Epidemiology:
The focus is on studies testing ozone exposure.

Table Annex 4-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Exposure Assessment or Assignment
Controlled Human Exposure:
For this assessment, the focus is on studies that use ozone concentrations <0.4 ppm. Studies that use higher exposure concentrations may provide information relevant to biological plausibility, dosimetry, or inter-species variation. Studies should have well-characterized pollutant concentration, temperature, and relative humidity and/or have measures in place to adequately control the exposure conditions. Preference is given to balanced crossover or parallel design studies which include control exposures (e.g., to clean filtered air). Study subjects should be randomly exposed without knowledge of the exposure condition. Method of exposure (e.g., chamber, facemask, etc.) should be specified and activity level of subjects during exposures should be well characterized.
Animal Toxicology:
For this assessment, the focus is on studies that use ozone concentrations <2 ppm. Studies that use higher exposure concentrations may provide information relevant to biological plausibility, dosimetry, or inter-species variation. Studies should characterize pollutant concentration, temperature, and relative humidity and/or have measures in place to adequately control the exposure conditions. The focus is on inhalation exposure. Noninhalation exposure experiments (i.e., intra-tracheal instillation [IT]) are informative for size fractions that cannot penetrate the airway of a study animal and may provide information relevant to biological plausibility and dosimetry. In vitro studies may be included if they provide mechanistic insight or examine similar effects as in vivo studies but are generally not included. All studies should include exposure control groups (e.g., clean filtered air).
Epidemiology:
Of primary relevance are relationships of health effects with the ambient component of ozone exposure. However, information about ambient exposure rarely is available for individual subjects; most often, inference is based on ambient concentrations. Studies that compare exposure assessment methods are considered to be particularly informative. Inference is stronger when the duration or lag of the exposure metric corresponds with the time course for physiological changes in the outcome (e.g., up to a few days for symptoms) or latency of disease (e.g., several years for cancer). Ambient ozone concentration tends to have low spatial heterogeneity at the urban scale, except near roads where ozone concentration is lower because ozone reacts with emitted nitric oxide. For studies involving individuals with near-road or on-road exposures to ozone in which ambient ozone concentrations are more spatially heterogeneous and relationships between personal exposures and ambient concentrations are potentially more variable, validated methods that capture the extent of variability for the epidemiologic study design (temporal vs. spatial contrasts) and location carry greater weight. Fixed-site measurements, whether averaged across multiple monitors or assigned from the nearest or single available monitor, typically have smaller biases and smaller reductions in precision compared with spatially heterogeneous air pollutants. Concentrations reported from fixed-site measurements can be informative if correlated with personal exposures, closely located to study subjects, highly correlated across monitors within a location, or combined with time-activity information. Atmospheric models may be used for exposure assessment in place of or to supplement ozone measurements in epidemiologic analyses. For example, grid-scale models (e.g., CMAQ) that represent ozone exposure over relatively large spatial scales (e.g., typically greater than 4- × 4-km grid size) often do provide adequate spatial resolution to capture acute ozone peaks that influence short-term health outcomes. Uncertainty in exposure predictions from these models is largely influenced by model formulations and the quality of model input data pertaining to precursor emissions or meteorology, which tends to vary on a study-by-study basis. In studies of short-term exposure, temporal variability of the exposure metric is of primary interest. For long-term exposures, models that capture within-community spatial variation in individual exposure may be given more weight for spatially variable ambient ozone. Given the low spatial variability of ozone at the urban scale, exposure measurement error typically causes health effect estimates to be underestimated for studies of either short-term or long-term exposure. Biases and decreases in the precision of the association (i.e., wider 95% CIs) tend to be small. Even when spatial variability is higher near roads, the reduction in ozone exposure would cause the exposure to be overestimated at a monitor distant from the road or when averaged across a model grid cell, so that health effects would likely be underestimated.

Table Annex 4-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Outcome Assessment/Evaluation
Controlled Human Exposure:
Endpoints should be assessed in the same manner for control and exposure groups (e.g., time after exposure, methods, endpoint evaluator) using valid, reliable methods. Blinding of endpoint evaluators is ideal, especially for qualitative endpoints (e.g., histopathology). For each experiment and each experimental group, including controls, precise details of all procedures carried out should be provided including how, when, and where. Time of the endpoint evaluations is a key consideration that will vary depending on endpoint evaluated. Endpoints should be assessed at time points that are appropriate for the research questions.
Animal Toxicology:
Endpoints should be assessed in the same manner for control and exposure groups (e.g., time after exposure, methods, endpoint evaluator) using valid, reliable methods. Blinding of endpoint evaluators is ideal, especially for qualitative endpoints (e.g., histopathology). For each experiment and each experimental group, including controls, precise details of all procedures carried out should be provided including how, when, and where. Time of the endpoint evaluations is a key consideration that will vary depending on endpoint evaluated. Endpoints should be assessed at time points that are appropriate for the research questions.
Epidemiology:
Inference is stronger when outcomes are assessed or reported without knowledge of exposure status. Knowledge of exposure status could produce artefactual associations. Confidence is greater when outcomes assessed by interview, self-report, clinical examination, or analysis of biological indicators are defined by consistent criteria and collected by validated, reliable methods. Independent, clinical assessment is valuable for outcomes such as lung function or incidence of disease, but report of physician diagnosis has shown good reliability.[b] When examining short-term exposures, evaluation of the evidence focuses on specific lags based on the evidence presented in individual studies. Specifically, the following hierarchy is used in the process of selecting results from individual studies to assess in the context of results across all studies for a specific health effect or outcome: • Distributed lag models; • Multiple days (e.g., 0−2) are averaged; • Effect estimates are presented for lag days selected a priori by the study authors; or • If a study focuses on only a series of individual lag days, expert judgment is applied to select the appropriate result to focus on considering the time course for physiologic changes for the health effect or outcome being evaluated. When health effects of long-term exposure are assessed by acute events such as symptoms or hospital admissions, inference is strengthened when results are adjusted for short-term exposure. Validated questionnaires for subjective outcomes such as symptoms are regarded to be reliable,[c] particularly when collected frequently and not subject to long recall. For biological samples, the stability of the compound of interest and the sensitivity and precision of the analytical method is considered. If not based on knowledge of exposure status, errors in outcome assessment tend to bias results toward the null.
Potential Copollutant Confounding
Controlled Human Exposure:
Exposure should be well characterized to evaluate independent effects of ozone.
Animal Toxicology:
Exposure should be well characterized to evaluate independent effects of ozone.

Table Annex 4-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Epidemiology:
Not accounting for potential copollutant confounding can produce artifactual associations; thus, studies that examine copollutant confounding carry greater weight. The predominant method is copollutant modeling (i.e., two-pollutant models), which is especially informative when correlations are not high. However, when correlations are high ($r > 0.7$), such as those often encountered for UFP and other traffic-related copollutants, copollutant modeling is less informative. Although the use of single-pollutant models to examine the association between ozone and a health effect or outcome are informative, ideally studies should also include copollutant analyses. Copollutant confounding is evaluated on an individual study basis considering the extent of correlations observed between the copollutant and ozone, and relationships observed with ozone and health effects in copollutant models.
Other Potential Confounding Factors[d]
Controlled Human Exposure:
Preference is given to studies using experimental and control groups that are matched for individual level characteristics (e.g., race/ethnicity, sex, body weight, smoking history, age) and time varying factors (e.g., seasonal and diurnal patterns).
Animal Toxicology:
Preference is given to studies using experimental and control groups that are matched for individual level characteristics (e.g., strain, sex, body weight, litter size, food and water consumption) and time varying factors (e.g., seasonal and diurnal patterns).
Epidemiology:
Factors are considered to be potential confounders if demonstrated in the scientific literature to be related to health effects and correlated with ozone. Not accounting for confounders can produce artifactual associations; thus, studies that statistically adjust for multiple factors or control for them in the study design are emphasized. Less weight is placed on studies that adjust for factors that mediate the relationship between ozone and health effects, which can bias results toward the null. Confounders vary according to study design, exposure duration, and health effect and may include, but are not limited to the following: • Short-term exposure studies: Meteorology, day of week, season, medication use, allergen exposure, and long-term temporal trends. • Long-term exposure studies: Socioeconomic status, race, age, medication use, smoking status, stress, noise, and occupational exposures.
Statistical Methodology
Controlled Human Exposure:
Statistical methods should be clearly described and appropriate for the study design and research question (e.g., correction for multiple comparisons). Generally, statistical significance is used to evaluate the findings of controlled human exposure studies. However, consistent trends are also informative. Detection of statistical significance is influenced by a variety of factors including, but not limited to, the size of the study, exposure and outcome measurement error, and statistical model specifications. Sample size is not a criterion for exclusion; ideally, the sample size should provide adequate power to detect hypothesized effects (e.g., sample sizes less than three are considered less informative). Because statistical tests have limitations, consideration is given to both trends in data and reproducibility of results.

Table Annex 4-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Animal Toxicology:
Statistical methods should be clearly described and appropriate for the study design and research question (e.g., correction for multiple comparisons). Generally, statistical significance is used to evaluate the findings of animal toxicological studies. However, consistent trends are also informative. Detection of statistical significance is influenced by a variety of factors including, but not limited to, the size of the study, exposure and outcome measurement error, and statistical model specifications. Sample size is not a criterion for exclusion; ideally, the sample size should provide adequate power to detect hypothesized effects (e.g., sample sizes less than three are considered less informative). Because statistical tests have limitations, consideration is given to both trends in data and reproducibility of results.
Epidemiology:
Multivariable regression models that include potential confounding factors are emphasized. However, multipollutant models (more than two pollutants) are considered to produce too much uncertainty due to copollutant collinearity to be informative. Models with interaction terms aid in the evaluation of potential confounding as well as effect modification. Sensitivity analyses with alternate specifications for potential confounding inform the stability of findings and aid in judgments of the strength of inference from results. In the case of multiple comparisons, consistency in the pattern of association can increase confidence that associations were not found by chance alone. Statistical methods that are appropriate for the power of the study carry greater weight. For example, categorical analyses with small sample sizes can be prone to bias results toward or away from the null. Statistical tests such as *t*-tests and chi-squared tests are not considered sensitive enough for adequate inferences regarding ozone-health effect associations. For all methods, the effect estimate and precision of the estimate (i.e., width of 95% CI) are important considerations rather than statistical significance.

[a]U.S. EPA (2008).

[b]Murgia et al. (2014); Weakley et al. (2013); Yang et al. (2011); Heckbert et al. (2004); Barr et al. (2002); Muhajarine et al. (1997); Toren et al. (1993).

[c]Burney et al. (1989).

4.4 References

Argacha, JF; Collart, P; Wauters, A; Kayaert, P; Lochy, S; Schoors, D; Sonck, J; de Vos, T; Forton, M; Brasseur, O; Beauloye, C; Gevaert, S; Evrard, P; Coppieters, Y; Sinnaeve, P; Claeys, MJ. (2016). Air pollution and ST-elevation myocardial infarction: A case-crossover study of the Belgian STEMI registry 2009-2013. Int J Cardiol 223: 300-305. http://dx.doi.org/10.1016/j.ijcard.2016.07.191.

Arjomandi, M; Wong, H; Donde, A; Frelinger, J; Dalton, S; Ching, W; Power, K; Balmes, JR. (2015). Exposure to medium and high ambient levels of ozone causes adverse systemic inflammatory and cardiac autonomic effects. Am J Physiol Heart Circ Physiol 308: H1499-1509. http://dx.doi.org/10.1152/ajpheart.00849.2014.

Atkinson, RW; Carey, IM; Kent, AJ; van Staa, TP; Anderson, HR; Cook, DG. (2013). Long-term exposure to outdoor air pollution and incidence of cardiovascular diseases. Epidemiology 24: 44-53. http://dx.doi.org/10.1097/EDE.0b013e318276ccb8.

Barath, S; Langrish, JP; Lundbäck, M; Bosson, JA; Goudie, C; Newby, DE; Sandström, T; Mills, NL; Blomberg, A. (2013). Short-term exposure to ozone does not impair vascular function or affect heart rate variability in healthy young men. Toxicol Sci 135: 292-299. http://dx.doi.org/10.1093/toxsci/kft157.

Bard, D; Kihal, W; Schillinger, C; Fermanian, C; Ségala, C; Glorion, S; Arveiler, D; Weber, C. (2014). Traffic-related air pollution and the onset of myocardial infarction: disclosing benzene as a trigger? A small-area case-crossover study. PLoS ONE 9: e100307. http://dx.doi.org/10.1371/journal.pone.0100307.

Barr, RG; Herbstman, J; Speizer, FE; Camargo, CA, Jr. (2002). Validation of self-reported chronic obstructive pulmonary disease in a cohort study of nurses. Am J Epidemiol 155: 965-971. http://dx.doi.org/10.1093/aje/155.10.965.

Bartell, SM; Longhurst, J; Tjoa, T; Sioutas, C; Delfino, RJ. (2013). Particulate air pollution, ambulatory heart rate variability, and cardiac arrhythmia in retirement community residents with coronary artery disease. Environ Health Perspect 121: 1135-1141. http://dx.doi.org/10.1289/ehp.1205914.

Bedada, GB; Smith, CJ; Tyrrell, PJ; Hirst, AA; Agius, R. (2012). Short-term effects of ambient particulates and gaseous pollutants on the incidence of transient ischaemic attack and minor stroke: a case-crossover study. Environ Health 11: 77. http://dx.doi.org/10.1186/1476-069X-11-77.

Bentayeb, M; Wagner, V; Stempfelet, M; Zins, M; Goldberg, M; Pascal, M; Larrieu, S; Beaudeau, P; Cassadou, S; Eilstein, D; Filleul, L; Le Tertre, A; Medina, S; Pascal, L; Prouvost, H; Quénel, P; Zeghnoun, A; Lefranc, A. (2015). Association between long-term exposure to air pollution and mortality in France: A 25-year follow-up study. Environ Int 85: 5-14. http://dx.doi.org/10.1016/j.envint.2015.08.006.

Bhaskaran, K; Hajat, S; Armstrong, B; Haines, A; Herrett, E; Wilkinson, P; Smeeth, L. (2011). The effects of hourly differences in air pollution on the risk of myocardial infarction: Case crossover analysis of the MINAP database. BMJ 343: d5531. http://dx.doi.org/10.1136/bmj.d5531.

Bigger, JT, Jr; Fleiss, JL; Steinman, RC; Rolnitzky, LM; Kleiger, RE; Rottman, JN. (1992). Frequency domain measures of heart period variability and mortality after myocardial infarction. Circulation 85: 164-171.

Biller, H; Holz, O; Windt, H; Koch, W; Müller, M; Jörres, RA; Krug, N; Hohlfeld, JM. (2011). Breath profiles by electronic nose correlate with systemic markers but not ozone response. Respir Med 105: 1352-1363. http://dx.doi.org/10.1016/j.rmed.2011.03.002.

Billman, GE. (2013). The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. Front Physiol 4: 26. http://dx.doi.org/10.3389/fphys.2013.00026.

Bind, MA; Baccarelli, A; Zanobetti, A; Tarantini, L; Suh, H; Vokonas, P; Schwartz, J. (2012). Air pollution and markers of coagulation, inflammation, and endothelial function: Associations and epigene-environment interactions in an elderly cohort. Epidemiology 23: 332-340. http://dx.doi.org/10.1097/EDE.0b013e31824523f0.

Bosson, JA; Blomberg, A; Stenfors, N; Helleday, R; Kelly, FJ; Behndig, AF; Mudway, I. (2013). Peripheral blood neutrophilia as a biomarker of ozone-induced pulmonary inflammation. PLoS ONE 8: e81816. http://dx.doi.org/10.1371/journal.pone.0081816.

Breton, CV; Wang, X; Mack, WJ; Berhane, K; Lopez, M; Islam, TS; Feng, M; Lurmann, F; McConnell, R; Hodis, HN; Künzli, N; Avol, E. (2012). Childhood air pollutant exposure and carotid artery intima-media thickness in young adults. Circulation 126: 1614-1620. http://dx.doi.org/10.1161/CIRCULATIONAHA.112.096164.

Breton, CV; Yao, J; Millstein, J; Gao, L; Siegmund, KD; Mack, W; Whitfield-Maxwell, L; Lurmann, F; Hodis, H; Avol, E; Gilliland, FD. (2016). Prenatal Air Pollution Exposures, DNA Methyl Transferase Genotypes, and Associations with Newborn LINE1 and Alu Methylation and Childhood Blood Pressure and Carotid Intima-Media Thickness in the Children's Health Study. Environ Health Perspect 124: 1905-1912. http://dx.doi.org/10.1289/EHP181.

Brook, RD; Kousha, T. (2015). Air pollution and emergency department visits for hypertension in Edmonton and Calgary, Canada: A case-crossover study. Am J Hypertens 28: 1121-1126. http://dx.doi.org/10.1093/ajh/hpu302.

Brook, RD; Urch, B; Dvonch, JT; Bard, RL; Speck, M; Keeler, G; Morishita, M; Marsik, FJ; Kamal, AS; Kaciroti, N; Harkema, J; Corey, P; Silverman, F; Gold, DR; Wellenius, G; Mittleman, MA; Rajagopalan, S; Brook, JR. (2009). Insights into the mechanisms and mediators of the effects of air pollution exposure on blood pressure and vascular function in healthy humans. Hypertension 54: 659-667. http://dx.doi.org/10.1161/hypertensionaha.109.130237.

Burney, PG; Laitinen, LA; Perdrizet, S; Huckauf, H; Tattersfield, AE; Chinn, S; Poisson, N; Heeren, A; Britton, JR; Jones, T. (1989). Validity and repeatability of the IUATLD (1984) Bronchial Symptoms Questionnaire: an international comparison. Eur Respir J 2: 940-945.

Butland, BK; Atkinson, RW; Crichton, S; Barratt, B; Beevers, S; Spiridou, A; Hoang, U; Kelly, FJ; Wolfe, CD. (2017). Air pollution and the incidence of ischaemic and haemorrhagic stroke in the South London Stroke Register: a case-cross-over analysis. J Epidemiol Community Health 71: 707-712. http://dx.doi.org/10.1136/jech-2016-208025.

Butland, BK; Atkinson, RW; Milojevic, A; Heal, MR; Doherty, RM; Armstrong, BG; Mackenzie, IA; Vieno, M; Lin, C; Wilkinson, P. (2016). Myocardial infarction, ST-elevation and non-ST-elevation myocardial infarction and modelled daily pollution concentrations: a case-crossover analysis of MINAP data. 3: e000429. http://dx.doi.org/10.1136/openhrt-2016-000429.

Cakmak, S; Dales, R; Leech, J; Liu, L. (2011). The influence of air pollution on cardiovascular and pulmonary function and exercise capacity: Canadian Health Measures Survey (CHMS). Environ Res 111: 1309-1312. http://dx.doi.org/10.1016/j.envres.2011.09.016.

Cakmak, S; Hebbern, C; Pinault, L; Lavigne, E; Vanos, J; Crouse, DL; Tjepkema, M. (2017). Associations between long-term PM2.5 and ozone exposure and mortality in the Canadian Census Health and Environment Cohort (CANCHEC), by spatial synoptic classification zone. Environ Int 111: 200-211. http://dx.doi.org/10.1016/j.envint.2017.11.030.

Cakmak, S; Hebbern, C; Vanos, J; Crouse, DL; Burnett, R. (2016). Ozone exposure and cardiovascular-related mortality in the Canadian Census Health and Environment Cohort (CANCHEC) by spatial synoptic classification zone. Environ Pollut 214: 589-599. http://dx.doi.org/10.1016/j.envpol.2016.04.067.

Cakmak, S; Kauri, L; Shutt, R; Liu, L; Green, MS; Mulholland, M; Stieb, D; Dales, R. (2014). The association between ambient air quality and cardiac rate and rhythm in ambulatory subjects. Environ Int 73: 365-371. http://dx.doi.org/10.1016/j.envint.2014.08.015.

Carey, IM; Atkinson, RW; Kent, AJ; van Staa, T; Cook, DG; Anderson, HR. (2013). Mortality associations with long-term exposure to outdoor air pollution in a national English cohort. Am J Respir Crit Care Med 187: 1226-1233. http://dx.doi.org/10.1164/rccm.201210-1758OC.

Carll, AP; Farraj, AK; Roberts, AM. (2018). Role of the autonomic nervous system in cardiovascular toxicity. In C McQueen (Ed.), Comprehensive toxicology (3rd ed., pp. 61-114). Amsterdam, Netherlands: Elsevier. http://dx.doi.org/10.1016/B978-0-12-801238-3.64259-9.

Cestonaro, LV; Marcolan, AM; Rossato-Grando, LG; Anzolin, AP; Goethel, G; Vilani, A; Garcia, SC; Bertol, CD. (2017). Ozone generated by air purifier in low concentrations: friend or foe? Environ Sci Pollut Res Int 24: 22673-22678. http://dx.doi.org/10.1007/s11356-017-9887-3.

Chen, L; Villeneuve, PJ; Rowe, BH; Liu, L; Stieb, DM. (2014). The Air Quality Health Index as a predictor of emergency department visits for ischemic stroke in Edmonton, Canada. J Expo Sci Environ Epidemiol 24: 358-364. http://dx.doi.org/10.1038/jes.2013.82.

Choi, M; Curriero, FC; Johantgen, M; Mills, ME; Sattler, B; Lipscomb, J. (2011). Association between ozone and emergency department visits: An ecological study. Int J Environ Health Res 21: 201-221. http://dx.doi.org/10.1080/09603123.2010.533366.

Chuang, GC; Yang, Z; Westbrook, DG; Pompilius, M; Ballinger, CA; White, RC; Krzywanski, DM; Postlethwait, EM; Ballinger, SW. (2009). Pulmonary ozone exposure induces vascular dysfunction, mitochondrial damage, and atherogenesis. Am J Physiol Lung Cell Mol Physiol 297: L209-L216. http://dx.doi.org/10.1152/ajplung.00102.2009.

Chuang, KJ; Chan, CC; Su, TC; Lee, CT; Tang, CS. (2007). The effect of urban air pollution on inflammation, oxidative stress, coagulation, and autonomic dysfunction in young adults. Am J Respir Crit Care Med 176: 370-376. http://dx.doi.org/10.1164/rccm.200611-1627OC.

Chuang, KJ; Yan, YH; Chiu, SY; Cheng, TJ. (2011). Long-term air pollution exposure and risk factors for cardiovascular diseases among the elderly in Taiwan. Occup Environ Med 68: 64-68. http://dx.doi.org/10.1136/oem.2009.052704.

Claeys, MJ; Coenen, S; Colpaert, C; Bilcke, J; Beutels, P; Wouters, K; Legrand, V; Van Damme, P; Vrints, C. (2015). Environmental triggers of acute myocardial infarction: Results of a nationwide multiple-factorial population study. Acta Cardiol 70: 693-701. http://dx.doi.org/10.2143/AC.70.6.3120182.

Cole-Hunter, T; de Nazelle, A; Donaire-Gonzalez, D; Kubesch, N; Carrasco-Turigas, G; Matt, F; Foraster, M; Martínez, T; Ambros, A; Cirach, M; Martinez, D; Belmonte, J; Nieuwenhuijsen, M. (2018). Estimated effects of air pollution and space-time-activity on cardiopulmonary outcomes in healthy adults: A repeated measures study. Environ Int 111: 247-259. http://dx.doi.org/10.1016/j.envint.2017.11.024.

Collart, P; Dramaix, M; Levêque, A; Coppieters, Y. (2017). Short-term effects of air pollution on hospitalization for acute myocardial infarction: Age effect on lag pattern. Int J Environ Health Res 27: 68-81. http://dx.doi.org/10.1080/09603123.2016.1268678.

Coogan, PF; White, LF; Yu, J; Brook, RD; Burnett, RT; Marshall, JD; Bethea, TN; Rosenberg, L; Jerrett, M. (2017). Long-term exposure to NO2 and ozone and hypertension incidence in the black women's health study. Am J Hypertens 30: 367-372. http://dx.doi.org/10.1093/ajh/hpw168.

Corea, F; Silvestrelli, G; Baccarelli, A; Giua, A; Previdi, P; Siliprandi, G; Murgia, N. (2012). Airborne pollutants and lacunar stroke: a case cross-over analysis on stroke unit admissions. Neurology International 4: e11. http://dx.doi.org/10.4081/ni.2012.e11.

Crouse, DL; Peters, PA; Hystad, P; Brook, JR; van Donkelaar, A; Martin, RV; Villeneuve, PJ; Jerrett, M; Goldberg, MS; Pope, CA; Brauer, M; Brook, RD; Robichaud, A; Menard, R; Burnett, RT. (2015). Ambient PM 2.5, O 3, and NO 2 exposures and associations with mortality over 16 years of follow-up in the Canadian Census Health and Environment Cohort (CanCHEC). Environ Health Perspect 123: 1180-1186. http://dx.doi.org/10.1289/ehp.1409276.

Dales, RE; Cakmak, S. (2016). Does mental health status influence susceptibility to the physiologic effects of air pollution? A population based study of Canadian children. PLoS ONE 11: e0168931. http://dx.doi.org/10.1371/journal.pone.0168931.

de Miguel-Diez, J; Jimenez-Garcia, R; de Andres, A; Hernandez-Barrera, V; Carrasco-Garrido, P; Monreal, M; Jimenez, D; Jara-Palomares, L; Alvaro-Meca, A. (2016). Analysis of environmental risk factors for pulmonary embolism: A case-crossover study (2001-2013). Eur J Intern Med 31: 55-61. http://dx.doi.org/10.1016/j.ejim.2016.03.001.

Delfino, RJ; Gillen, DL; Tjoa, T; Staimer, N; Polidori, A; Arhami, M; Sioutas, C; Longhurst, J. (2011). Electrocardiographic ST-segment depression and exposure to trafficrelated aerosols in elderly subjects with coronary artery disease. Environ Health Perspect 119: 196-202. http://dx.doi.org/10.1289/ehp.1002372.

Dennekamp, M; Akram, M; Abramson, MJ; Tonkin, A; Sim, MR; Fridman, M; Erbas, B. (2010). Outdoor air pollution as a trigger for out-of-hospital cardiac arrests. Epidemiology 21: 494-500. http://dx.doi.org/10.1097/EDE.0b013e3181e093db.

Devlin, RB; Duncan, KE; Jardim, M; Schmitt, MT; Rappold, AG; Diaz-Sanchez, D. (2012). Controlled exposure of healthy young volunteers to ozone causes cardiovascular effects. Circulation 126: 104-111. http://dx.doi.org/10.1161/CIRCULATIONAHA.112.094359.

Dong, G; Qian, Z; Wang, J; Chen, W; Ma, W; Trevathan, E; Xaverius, PK; DeClue, R; Wiese, A; Langston, M; Liu, MM; Wang, D; Ren, W. (2013a). Associations between ambient air pollution and prevalence of stroke and cardiovascular diseases in 33 Chinese communities. Atmos Environ 77: 968-973. http://dx.doi.org/10.1016/j.atmosenv.2013.06.034.

Dong, GH; Qian, ZM; Trevathan, E; Zeng, XW; Vaughn, MG; Wang, J; Zhao, Y; Liu, YQ; Ren, WH; Qin, XD. (2014). Air pollution associated hypertension and increased blood pressure may be reduced by breastfeeding in Chinese children: the Seven Northeastern Cities Chinese Children's Study. Int J Cardiol 176: 956-961. http://dx.doi.org/10.1016/j.ijcard.2014.08.099.

Dong, GH; Qian, ZM; Xaverius, PK; Trevathan, E; Maalouf, S; Parker, J; Yang, L; Liu, MM; Wang, D; Ren, WH; Ma, W; Wang, J; Zelicoff, A; Fu, Q; Simckes, M. (2013b). Association between long-term air pollution and increased blood pressure and hypertension in China. Hypertension 61: 578-584. http://dx.doi.org/10.1161/HYPERTENSIONAHA.111.00003.

Dong, GH; Wang, J; Zeng, XW; Chen, L; Qin, XD; Zhou, Y; Li, M; Yang, M; Zhao, Y; Ren, WH; Hu, QS. (2015). Interactions between air pollution and obesity on blood pressure and hypertension in Chinese children. Epidemiology 26: 740-747. http://dx.doi.org/10.1097/EDE.0000000000000336.

Ensor, KB; Raun, LH; Persse, D. (2013). A case-crossover analysis of out-of-hospital cardiac arrest and air pollution. Circulation 127: 1192-1199. http://dx.doi.org/10.1161/CIRCULATIONAHA.113.000027.

Evans, KA; Hopke, PK; Utell, MJ; Kane, C; Thurston, SW; Ling, FS; Chalupa, D; Rich, DQ. (2016). Triggering of ST-elevation myocardial infarction by ambient wood smoke and other particulate and gaseous pollutants. J Expo Sci Environ Epidemiol 27: 198-206. http://dx.doi.org/10.1038/jes.2016.15.

Fakhri, AA; Ilic, LM; Wellenius, GA; Urch, B; Silverman, F; Gold, DR; Mittleman, MA. (2009). Autonomic effects of controlled fine particulate exposure in young healthy adults: Effect modification by ozone. Environ Health Perspect 117: 1287-1292. http://dx.doi.org/10.1289/ehp.0900541.

Farraj, AK; Hazari, MS; Winsett, DW; Kulukulualani, A; Carll, AP; Haykal-Coates, N; Lamb, CM; Lappi, E; Terrell, D; Cascio, WE; Costa, DL. (2012). Overt and latent cardiac effects of ozone inhalation in rats: evidence for autonomic modulation and increased myocardial vulnerability. Environ Health Perspect 120: 348-354. http://dx.doi.org/10.1289/ehp.1104244.

Farraj, AK; Malik, F; Haykal-Coates, N; Walsh, L; Winsett, D; Terrell, D; Thompson, LC; Cascio, WE; Hazari, MS. (2016). Morning NO2 exposure sensitizes hypertensive rats to the cardiovascular effects of same day O3 exposure in the afternoon. Inhal Toxicol 28: 170-179. http://dx.doi.org/10.3109/08958378.2016.1148088.

Fauchier, L; Babuty, D; Melin, A; Bonnet, P; Cosnay, P; Paul Fauchier, J. (2004). Heart rate variability in severe right or left heart failure: the role of pulmonary hypertension and resistances. Eur J Heart Fail 6: 181-185. http://dx.doi.org/10.1016/j.ejheart.2003.09.007.

Finnbjornsdottir, RG; Zoëga, H; Olafsson, O; Thorsteinsson, T; Rafnsson, V. (2013). Association of air pollution and use of glyceryl trinitrate against angina pectoris: a population-based case-crossover study. Environ Health 12: 38. http://dx.doi.org/10.1186/1476-069X-12-38.

Frampton, MW; Pietropaoli, A; Dentler, M; Chalupa, D; Little, EL; Stewart, J; Frasier, L; Oakes, D; Wiltshire, J; Vora, R; Utell, MJ. (2015). Cardiovascular effects of ozone in healthy subjects with and without deletion of glutathione-S-transferase M1. Inhal Toxicol 27: 113-119. http://dx.doi.org/10.3109/08958378.2014.996272.

Francis, M; Groves, AM; Sun, R; Cervelli, JA; Choi, H; Laskin, JD; Laskin, DL. (2017). Editor's highlight: CCR2 regulates inflammatory cell accumulation in the lung and tissue injury following ozone exposure. Toxicol Sci 155: 474-484. http://dx.doi.org/10.1093/toxsci/kfw226.

Gandhi, SK; Rich, DQ; Ohman-Strickland, PA; Kipen, HM; Gow, A. (2014). Plasma nitrite is an indicator of acute changes in ambient air pollutant concentrations. Inhal Toxicol 26: 426-434. http://dx.doi.org/10.3109/08958378.2014.913216.

Gong, H, Jr; Wong, R; Sarma, RJ; Linn, WS; Sullivan, ED; Shamoo, DA; Anderson, KR; Prasad, SB. (1998). Cardiovascular effects of ozone exposure in human volunteers. Am J Respir Crit Care Med 158: 538-546. http://dx.doi.org/10.1164/ajrccm.158.2.9709034.

Gordon, CJ; Jarema, KA; Lehmann, J. R.; Ledbetter, AD; Schladweiler, MC; Schmid, JE; Ward, WO; Kodavanti, UP; Nyska, A; Macphail, RC. (2013). Susceptibility of adult and senescent Brown Norway rats to repeated ozone exposure: an assessment of behavior, serum biochemistry and cardiopulmonary function. Inhal Toxicol 25: 141-159. http://dx.doi.org/10.3109/08958378.2013.764946.

Gordon, CJ; Johnstone, AF; Aydin, C; Phillips, PM; Macphail, RC; Kodavanti, UP; Ledbetter, AD; Jarema, KA. (2014). Episodic ozone exposure in adult and senescent Brown Norway rats: acute and delayed effect on heart rate, core temperature and motor activity. Inhal Toxicol 26: 380-390. http://dx.doi.org/10.3109/08958378.2014.905659.

Green, R; Broadwin, R; Malig, B; Basu, R; Gold, EB; Qi, L; Sternfeld, B; Bromberger, JT; Greendale, GA; Kravitz, HM; Tomey, K; Matthews, K; Derby, C; Jackson, EA; Green, R; Ostro, B. (2015). Long-and short-term exposure to air pollution and inflammatory/hemostatic markers in midlife women. Epidemiology 27: 211-220. http://dx.doi.org/10.1097/EDE.0000000000000421.

Halonen, JI; Lanki, T; Tiittanen, P; Niemi, JV; Loh, M; Pekkanen, J. (2009). Ozone and cause-specific cardiorespiratory morbidity and mortality. J Epidemiol Community Health 64: 814-820. http://dx.doi.org/10.1136/jech.2009.087106.

Halvorsen, B; Otterdal, K; Dahl, TB; Skjelland, M; Gullestad, L; Oie, E; Aukrust, P. (2008). Atherosclerotic plaque stability - what determines the fate of a plaque? Prog Cardiovasc Dis 51: 183-194. http://dx.doi.org/10.1016/j.pcad.2008.09.001.

Hanna, AF; Yeatts, KB; Xiu, A; Zhu, Z; Smith, RL; Davis, NN; Talgo, KD; Arora, G; Robinson, PJ; Meng, Q; Pinto, JP. (2011). Associations between ozone and morbidity using the spatial synoptic classification system. Environ Health 10: 49. http://dx.doi.org/10.1186/1476-069X-10-49.

Hatch, GE; Crissman, K; Schmid, J; Richards, JE; Ward, WO; Schladweiler, MC; Ledbetter, AD; Kodavanti, UP. (2015). Strain differences in antioxidants in rat models of cardiovascular disease exposed to ozone. Inhal Toxicol 27: 54-62. http://dx.doi.org/10.3109/08958378.2014.954170.

Heckbert, SR; Kooperberg, C; Safford, MM; Psaty, BM; Hsia, J; McTiernan, A; Gaziano, JM; Frishman, WH; Curb, JD. (2004). Comparison of self-report, hospital discharge codes, and adjudication of cardiovascular events in the Women's Health Initiative. Am J Epidemiol 160: 1152-1158. http://dx.doi.org/10.1093/aje/kwh314.

Henriquez, AR; Snow, SJ; Schladweiler, MC; Miller, CN; Dye, JA; Ledbetter, AD; Richards, JE; Mauge-Lewis, K; Mcgee, MA; Kodavanti, UP. (2017). Adrenergic and glucocorticoid receptor antagonists reduce ozone-induced lung injury and inflammation. Toxicol Appl Pharmacol 339: 161-171. http://dx.doi.org/10.1016/j.taap.2017.12.006.

Henrotin, JB; Besancenot, JP; Bejot, Y; Giroud, M. (2007). Short-term effects of ozone air pollution on ischaemic stroke occurrence: A case-crossover analysis from a 10-year population-based study in Dijon, France. Occup Environ Med 64: 439-445. http://dx.doi.org/10.1136/oem.2006.029306.

Henrotin, JB; Zeller, M; Lorgis, L; Cottin, Y; Giroud, M; Béjot, Y. (2010). Evidence of the role of short-term exposure to ozone on ischaemic cerebral and cardiac events: The Dijon Vascular Project (DIVA). Heart 96: 1990-1996. http://dx.doi.org/10.1136/hrt.2010.200337.

Hoffmann, B; Luttmann-Gibson, H; Cohen, A; Zanobetti, A; de Souza, C; Foley, C; Suh, HH; Coull, BA; Schwartz, J; Mittleman, M; Stone, P; Horton, E; Gold, DR. (2012). Opposing effects of particle pollution, ozone, and ambient temperature on arterial blood pressure. Environ Health Perspect 120: 241-246. http://dx.doi.org/10.1289/ehp.1103647.

Hůnová, I; Malý, M; Rezáčová, J; Braniš, M. (2013). Association between ambient ozone and health outcomes in Prague. Int Arch Occup Environ Health 86: 89-97. http://dx.doi.org/10.1007/s00420-012-0751-y.

Hunova, I, va; Brabec, M; Maly, M; Knobova, V; Branis, M. (2017). Major heat waves of 2003 and 2006 and health outcomes in Prague. Air Qual Atmos Health 10: 183-194. http://dx.doi.org/10.1007/s11869-016-0419-y.

Jerrett, M; Burnett, RT; Beckerman, BS; Turner, MC; Krewski, D; Thurston, G; Martin, RV; van Donkelaar, A; Hughes, E; Shi, Y; Gapstur, SM; Thun, MJ; Pope, CA, III. (2013). Spatial analysis of air pollution and mortality in California. Am J Respir Crit Care Med 188: 593-599. http://dx.doi.org/10.1164/rccm.201303-0609OC.

Jerrett, M; Burnett, RT; Pope, CA, III; Ito, K; Thurston, G; Krewski, D; Shi, Y; Calle, E; Thun, M. (2009). Long-term ozone exposure and mortality. N Engl J Med 360: 1085-1095. http://dx.doi.org/10.1056/NEJMoa0803894.

Kahle, JJ; Neas, LM; Devlin, RB; Case, MW; Schmitt, MT; Madden, MC; Diaz-Sanchez, D. (2015). Interaction effects of temperature and ozone on lung function and markers of systemic inflammation, coagulation, and fibrinolysis: a crossover study of healthy young volunteers. Environ Health Perspect 123: 310-316. http://dx.doi.org/10.1289/ehp.1307986.

Kalantzi, EG; Makris, D; Duquenne, MN; Kaklamani, S; Stapountzis, H; Gourgoulianis, KI. (2011). Air pollutants and morbidity of cardiopulmonary diseases in a semi-urban Greek peninsula. Atmos Environ 45: 7121-7126. http://dx.doi.org/10.1016/j.atmosenv.2011.09.032.

Karoly, ED; Li, Z; Dailey, LA; Hyseni, X; Huang, YCT. (2007). Up-regulation of tissue factor in human pulmonary artery endothelial cells after ultrafine particle exposure. Environ Health Perspect 115: 535-540. http://dx.doi.org/10.1289/ehp.9556.

Khder, Y; Briançon, S; Petermann, R; Quilliot, D; Stoltz, JF; Drouin, P; Zannad, F. (1998). Shear stress abnormalities contribute to endothelial dysfunction in hypertension but not in type II diabetes. J Hypertens 16: 1619-1625. http://dx.doi.org/10.1097/00004872-199816110-00008.

Kilkenny, C; Browne, WJ; Cuthill, IC; Emerson, M; Altman, DG. (2010). Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research [Review]. PLoS Biol 8: e1000412. http://dx.doi.org/10.1371/journal.pbio.1000412.

Kim, H; Kim, J; Kim, S; Kang, SH; Kim, HJ; Kim, H; Heo, J; Yi, SM; Kim, K; Youn, TJ; Chae, IH. (2017). Cardiovascular effects of long-term exposure to air pollution: a population-based study with 900845person-years of follow-up. J Am Heart Assoc 6. http://dx.doi.org/10.1161/JAHA.117.007170.

Klemm, RJ; Lipfert, FW; Wyzga, RE; Gust, C. (2004). Daily mortality and air pollution in Atlanta: two years of data from ARIES. Inhal Toxicol 16 Suppl 1: 131-141. http://dx.doi.org/10.1080/08958370490443213.

Klemm, RJ; Mason, RM, Jr. (2000). Aerosol Research and Inhalation Epidemiological Study (ARIES): air quality and daily mortality statistical modeling--interim results. J Air Waste Manag Assoc 50: 1433-1439.

Klemm, RJ; Thomas, EL; Wyzga, RE. (2011). The impact of frequency and duration of air quality monitoring: Atlanta, GA, data modeling of air pollution and mortality. J Air Waste Manag Assoc 61: 1281-1291. http://dx.doi.org/10.1080/10473289.2011.617648.

Klimisch, HJ; Andreae, M; Tillmann, U. (1997). A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regul Toxicol Pharmacol 25: 1-5. http://dx.doi.org/10.1006/rtph.1996.1076.

Kotecha, D; New, G; Flather, MD; Eccleston, D; Pepper, J; Krum, H. (2012). Five-minute heart rate variability can predict obstructive angiographic coronary disease. Heart 98: 395-401. http://dx.doi.org/10.1136/heartjnl-2011-300033.

Kumarathasan, P; Blais, E; Saravanamuthu, A; Bielecki, A; Mukherjee, B; Bjarnason, S; Guénette, J; Goegan, P; Vincent, R. (2015). Nitrative stress, oxidative stress and plasma endothelin levels after inhalation of particulate matter and ozone. Part Fibre Toxicol 12: 28. http://dx.doi.org/10.1186/s12989-015-0103-7.

Kurhanewicz, N; Mcintosh-Kastrinsky, R; Tong, H; Walsh, L; Farraj, A; Hazari, MS. (2014). Ozone co-exposure modifies cardiac responses to fine and ultrafine ambient particulate matter in mice: Concordance of electrocardiogram and mechanical responses. Part Fibre Toxicol 11: 54. http://dx.doi.org/10.1186/s12989-014-0054-4.

Kusha, M; Masse, S; Farid, T; Urch, B; Silverman, FS; Brook, RD; Gold, DR; Mangat, I; Speck, M; Nair, K; Poku, K; Meyer, C; Mittleman, MA; Wellenius, GA; Nanthakumar, K. (2012). Controlled exposure study of air pollution and t wave alternans in volunteers without cardiovascular disease. Environ Health Perspect 120: 1157-1161. http://dx.doi.org/10.1289/ehp.1104171.

Lahiri, MK; Kannankeril, PJ; Goldberger, JJ. (2008). Assessment of autonomic function in cardiovascular disease. J Am Coll Cardiol 51: 1725-1733. http://dx.doi.org/10.1016/j.jacc.2008.01.038.

Lanzinger, S; Breitner, S; Neas, L; Cascio, W; Diaz-Sanchez, D; Hinderliter, A; Peters, A; Devlin, RB; Schneider, A. (2014). The impact of decreases in air temperature and increases in ozone on markers of endothelial function in individuals having type-2 diabetes. Environ Res 134C: 331-338. http://dx.doi.org/10.1016/j.envres.2014.08.003.

Li, W; Dorans, KS; Wilker, EH; Rice, MB; Ljungman, PL; Schwartz, JD; Coull, BA; Koutrakis, P; Gold, DR; Keaney, JF; Vasan, RS; Benjamin, EJ; Mittleman, MA. (2017). Short-term exposure to ambient air pollution and biomarkers of systemic inflammation: the Framingham Heart Study. Arterioscler Thromb Vasc Biol 37: 1793-1800. http://dx.doi.org/10.1161/ATVBAHA.117.309799.

Li, W; Wilker, EH; Dorans, KS; Rice, MB; Schwartz, J; Coull, BA; Koutrakis, P; Gold, DR; Keaney, JF; Lin, H; Vasan, RS; Benjamin, EJ; Mittleman, MA. (2016). Short-term exposure to air pollution and biomarkers of oxidative stress: the framingham heart study. J Am Heart Assoc 5. http://dx.doi.org/10.1161/JAHA.115.002742.

Liu, WT; Lee, KY; Lee, HC; Chuang, HC; Wu, D; Juang, JN; Chuang, KJ. (2016). The association of annual air pollution exposure with blood pressure among patients with sleep-disordered breathing. Sci Total Environ 543: 61-66. http://dx.doi.org/10.1016/j.scitotenv.2015.10.135.

Ljungman, PL; Wilker, EH; Rice, MB; Schwartz, J; Gold, DR; Koutrakis, P; Vita, JA; Mitchell, GF; Vasan, RS; Benjamin, EJ; Mittleman, MA; Hamburg, NM. (2014). Short-term exposure to air pollution and digital vascular function. Am J Epidemiol 180: 482-489. http://dx.doi.org/10.1093/aje/kwu161.

M, A; H, W; A, D; J, F; S, D; W, C; K, P; JR, B. (2015). Exposure to medium and high ambient levels of ozone causes adverse systemic inflammatory and cardiac autonomic effects. Am J Physiol Heart Circ Physiol 308: H1499-1509. http://dx.doi.org/10.1152/ajpheart.00849.2014.

Maheswaran, R; Pearson, T; Beevers, SD; Campbell, MJ; Wolfe, CD. (2016). Air pollution and subtypes, severity and vulnerability to ischemic stroke-a population based case-crossover study. PLoS ONE 11: e0158556. http://dx.doi.org/10.1371/journal.pone.0158556.

Martinez-Campos, C; Lara-Padilla, E; Bobadilla-Lugo, RA; Kross, RD; Villanueva, C. (2012). Effects of exercise on oxidative stress in rats induced by ozone. ScientificWorldJournal 2012: 1-5. http://dx.doi.org/10.1100/2012/135921.

McIntosh-Kastrinsky, R; Diaz-Sanchez, D; Sexton, KG; Jania, CM; Zavala, J; Tilley, SL; Jaspers, I; Gilmour, MI; Devlin, RB; Cascio, WE; Tong, H. (2013). Photochemically altered air pollution mixtures and contractile parameters in isolated murine hearts before and after ischemia. Environ Health Perspect 121: 1344-1348. http://dx.doi.org/10.1289/ehp.1306609.

Mechtouff, L; Canoui-Poitrine, F; Schott, AM; Nighoghossian, N; Trouillas, P; Termoz, A; Porthault-Chatard, S; David, JS; Chasles, V; Derex, L. (2012). Lack of association between air pollutant exposure and short-term risk of ischaemic stroke in Lyon, France. Int J Stroke 7: 669-674. http://dx.doi.org/10.1111/j.1747-4949.2011.00737.x.

Metzger, KB; Klein, M; Flanders, WD; Peel, JL; Mulholland, JA; Langberg, JJ; Tolbert, PE. (2007). Ambient air pollution and cardiac arrhythmias in patients with implantable defibrillators. Epidemiology 18: 585-592. http://dx.doi.org/10.1097/EDE.0b013e318124ff0e.

Miller, DB; Snow, SJ; Henriquez, A; Schladweiler, MC; Ledbetter, AD; Richards, JE; Andrews, DL; Kodavanti, UP. (2016). Systemic metabolic derangement, pulmonary effects, and insulin insufficiency following subchronic ozone exposure in rats. Toxicol Appl Pharmacol 306: 47-57. http://dx.doi.org/10.1016/j.taap.2016.06.027.

Milojevic, A; Wilkinson, P; Armstrong, B; Bhaskaran, K; Smeeth, L; Hajat, S. (2014). Short-term effects of air pollution on a range of cardiovascular events in England and Wales: Case-crossover analysis of the MINAP database, hospital admissions and mortality. Heart 100: 1093-1098. http://dx.doi.org/10.1136/heartjnl-2013-304963.

Mirowsky, JE; Carraway, MS; Dhingra, R; Tong, H; Neas, L; Diaz-Sanchez, D; Cascio, W; Case, M; Crooks, J; Hauser, ER; Elaine Dowdy, Z; Kraus, WE; Devlin, RB. (2017). Ozone exposure is associated with acute changes in inflammation, fibrinolysis, and endothelial cell function in coronary artery disease patients. Environ Health 16: 126. http://dx.doi.org/10.1186/s12940-017-0335-0.

Montresor-López, JA; Yanosky, JD; Mittleman, MA; Sapkota, A; He, X; Hibbert, JD; Wirth, MD; Puett, RC. (2015). Short-term exposure to ambient ozone and stroke hospital admission: A case-crossover analysis. J Expo Sci Environ Epidemiol 26: 162-166. http://dx.doi.org/10.1038/jes.2015.48.

Muhajarine, N; Mustard, C; Roos, LL; Young, TK; Gelskey, DE. (1997). Comparison of survey and physician claims data for detecting hypertension. J Clin Epidemiol 50: 711-718. http://dx.doi.org/10.1016/S0895-4356(97)00019-X.

Murgia, N; Brisman, J; Claesson, A; Muzi, G; Olin, AC; Torén, K. (2014). Validity of a questionnaire-based diagnosis of chronic obstructive pulmonary disease in a general population-based study. BMC Pulm Med 14: 49. http://dx.doi.org/10.1186/1471-2466-14-49.

Notarius, CF; Butler, GC; Ando, S; Pollard, MJ; Senn, BL; Floras, JS. (1999). Dissociation between microneurographic and heart rate variability estimates of sympathetic tone in normal subjects and patients with heart failure. Clin Sci (Lond) 96: 557-565.

Nuvolone, D; Balzi, D; Pepe, P; Chini, M; Scala, D; Giovannini, F; Cipriani, F; Barchielli, A. (2013). Ozone short-term exposure and acute coronary events: A multicities study in Tuscany (Italy). Environ Res 126: 17-23. http://dx.doi.org/10.1016/j.envres.2013.08.002.

Paffett, ML; Zychowski, KE; Sheppard, L; Robertson, S; Weaver, JM; Lucas, SN; Campen, MJ. (2015). Ozone inhalation impairs coronary artery dilation via intracellular oxidative stress: Evidence for serum-borne factors as drivers of systemic toxicity. Toxicol Sci 146: 244-253. http://dx.doi.org/10.1093/toxsci/kfv093.

Peel, JL; Metzger, KB; Klein, M; Flanders, WD; Mulholland, JA; Tolbert, PE. (2007). Ambient air pollution and cardiovascular emergency department visits in potentially sensitive groups. Am J Epidemiol 165: 625-633. http://dx.doi.org/10.1093/aje/kwk051.

Perepu, RS; Garcia, C; Dostal, D; Sethi, R. (2010). Enhanced death signaling in ozone-exposed ischemic-reperfused hearts. Mol Cell Biochem 336: 55-64. http://dx.doi.org/10.1007/s11010-009-0265-4.

Perepu, RSP; Dostal, DE; Garcia, C; Kennedy, RH; Sethi, R. (2012). Cardiac dysfunction subsequent to chronic ozone exposure in rats. Mol Cell Biochem 360: 339-345. http://dx.doi.org/10.1007/s11010-011-1073-1.

Pilz, V; Wolf, K; Breitner, S; Rückerl, R; Koenig, W; Rathmann, W; Cyrys, J; Peters, A; Schneider, A; group, K-S. (2018). C-reactive protein (CRP) and long-term air pollution with a focus on ultrafine particles. Int J Hyg Environ Health. http://dx.doi.org/10.1016/j.ijheh.2018.01.016.

Poloniecki, JD; Atkinson, RW; Ponce de Leon, A; Anderson, HR. (1997). Daily time series for cardiovascular hospital admissions and previous day's air pollution in London, UK. Occup Environ Med 54: 535-540. http://dx.doi.org/10.1136/oem.54.8.535.

Pradeau, C; Rondeau, V; Lévèque, E; Guernion, PY; Tentillier, E; Thicoipé, M; Brochard, P. (2015). Air pollution and activation of mobile medical team for out-of-hospital cardiac arrest. Am J Emerg Med 33: 367-372. http://dx.doi.org/10.1016/j.ajem.2014.12.007.

Qin, XD; Qian, Z; Vaughn, MG; Trevathan, E; Emo, B; Paul, G; Ren, WH; Hao, YT; Dong, GH. (2015). Gender-specific differences of interaction between obesity and air pollution on stroke and cardiovascular diseases in Chinese adults from a high pollution range area: A large population based cross sectional study. Sci Total Environ 529: 243-248. http://dx.doi.org/10.1016/j.scitotenv.2015.05.041.

Ramanathan, G; Yin, F; Speck, M; Tseng, CH; Brook, JR; Silverman, F; Urch, B; Brook, RD; Araujo, JA. (2016). Effects of urban fine particulate matter and ozone on HDL functionality. Part Fibre Toxicol 13: 26. http://dx.doi.org/10.1186/s12989-016-0139-3.

Ramot, Y; Kodavanti, UP; Kissling, GE; Ledbetter, AD; Nyska, A. (2015). Clinical and pathological manifestations of cardiovascular disease in rat models: the influence of acute ozone exposure. Inhal Toxicol 27: 26-38. http://dx.doi.org/10.3109/08958378.2014.954168.

Rasche, M; Walther, M; Schiffner, R; Kroegel, N; Rupprecht, S; Schlattmann, P; Schulze, PC; Franzke, P; Witte, OW; Schwab, M; Rakers, F. (2018). Rapid increases in nitrogen oxides are associated with acute myocardial infarction: A case-crossover study. Eur J Prev Cardiol2047487318755804. http://dx.doi.org/10.1177/2047487318755804.

Raza, A; Bellander, T; Bero-Bedada, G; Dahlquist, M; Hollenberg, J; Jonsson, M; Lind, T; Rosenqvist, M; Svensson, L; Ljungman, PL. (2014). Short-term effects of air pollution on out-of-hospital cardiac arrest in Stockholm. Eur Heart J 35: 861-867. http://dx.doi.org/10.1093/eurheartj/eht489.

Rich, DQ; Balmes, JR; Frampton, MW; Zareba, W; Stark, P; Arjomandi, M; Hazucha, MJ; Costantini, MG; Ganz, P; Hollenbeck-Pringle, D; Dagincourt, N; Bromberg, PA. (2018). Cardiovascular function and ozone exposure: The Multicenter Ozone Study in oldEr Subjects (MOSES). Environ Int 119: 193-202. http://dx.doi.org/10.1016/j.envint.2018.06.014.

Robertson, S; Colombo, ES; Lucas, SN; Hall, PR; Febbraio, M; Paffett, ML; Campen, MJ. (2013). CD36 mediates endothelial dysfunction downstream of circulating factors induced by O3 exposure. Toxicol Sci 134: 304-311. http://dx.doi.org/10.1093/toxsci/kft107.

Rodopoulou, S; Chalbot, MC; Samoli, E; Dubois, DW; Filippo, B; Kavouras, IG. (2014). Air pollution and hospital emergency room and admissions for cardiovascular and respiratory diseases in Doria Ana County, New Mexico. Environ Res 129: 39-46. http://dx.doi.org/10.1016/j.envres.2013.12.006.

Rodopoulou, S; Samoli, E; Chalbot, MG; Kavouras, IG. (2015). Air pollution and cardiovascular and respiratory emergency visits in Central Arkansas: A time-series analysis. Sci Total Environ 536: 872-879. http://dx.doi.org/10.1016/j.scitotenv.2015.06.056.

Rooney, AA; Boyles, AL; Wolfe, MS; Bucher, JR; Thayer, KA. (2014). Systematic review and evidence integration for literature-based environmental health science assessments. Environ Health Perspect 122: 711-718. http://dx.doi.org/10.1289/ehp.1307972.

Rosenthal, FS; Kuisma, M; Lanki, T; Hussein, T; Boyd, J; Halonen, JI; Pekkanen, J. (2013). Association of ozone and particulate air pollution with out-of-hospital cardiac arrest in Helsinki, Finland: evidence for two different etiologies. J Expo Sci Environ Epidemiol 23: 281-288. http://dx.doi.org/10.1038/jes.2012.121.

Rowan III, WH; Campen, MJ; Wichers, LB; Watkinson, WP. (2007). Heart rate variability in rodents: uses and caveats in toxicological studies. Cardiovasc Toxicol 7: 28-51.

Rudez, G; Janssen, NA; Kilinc, E; Leebeek, FW; Gerlofs-Nijland, ME; Spronk, HM; ten Cate, H; Cassee, FR; de Maat, MP. (2009). Effects of ambient air pollution on hemostasis and inflammation. Environ Health Perspect 117: 995-1001. http://dx.doi.org/10.1289/ehp.0800437.

Sacks, JD; Ito, K; Wilson, WE; Neas, LM. (2012). Impact of covariate models on the assessment of the air pollution-mortality association in a single- and multipollutant context. Am J Epidemiol 176: 622-634. http://dx.doi.org/10.1093/aje/kws135.

Sade, MY; Vodonos, A; Novack, V; Friger, M; Amit, G, uy; Katra, I; Schwartz, J; Novack, L. (2015). Can air pollution trigger an onset of atrial fibrillation: a population-based study. Air Qual Atmos Health 8: 413-420. http://dx.doi.org/10.1007/s11869-014-0295-2.

Sarnat, SE; Suh, HH; Coull, BA; Schwartz, J; Stone, PH; Gold, DR. (2006). Ambient particulate air pollution and cardiac arrhythmia in a panel of older adults in Steubenville, Ohio. Occup Environ Med 63: 700-706. http://dx.doi.org/10.1136/oem.2006.027292.

Sarnat, SE; Winquist, A; Schauer, JJ; Turner, JR; Sarnat, JA. (2015). Fine particulate matter components and emergency department visits for cardiovascular and respiratory diseases in the St. Louis, Missouri-Illinois, metropolitan area. Environ Health Perspect 123: 437-444. http://dx.doi.org/10.1289/ehp.1307776.

Sethi, R; Manchanda, S; Perepu, RS; Kumar, A; Garcia, C; Kennedy, RH; Palakurthi, S; Dostal, D. (2012). Differential expression of caveolin-1 and caveolin-3: potential marker for cardiac toxicity subsequent to chronic ozone inhalation. Mol Cell Biochem 369: 9-15. http://dx.doi.org/10.1007/s11010-012-1363-2.

Silverman, RA; Ito, K; Freese, J; Kaufman, BJ; De Claro, D; Braun, J; Prezant, DJ. (2010). Association of ambient fine particles with out-of-hospital cardiac arrests in New York City. Am J Epidemiol 172: 917-923. http://dx.doi.org/10.1093/aje/kwq217.

Sivagangabalan, G; Spears, D; Masse, S; Urch, B; Brook, RD; Silverman, F; Gold, DR; Lukic, KZ; Speck, M; Kusha, M; Farid, T; Poku, K; Shi, E; Floras, J; Nanthakumar, K. (2011). The effect of air pollution on spatial dispersion of myocardial repolarization in healthy human volunteers. J Am Coll Cardiol 57: 198-206. http://dx.doi.org/10.1016/j.jacc.2010.08.625.

Snow, SJ; Cheng, WY; Henriquez, A; Hodge, M; Bass, V; Nelson, GM; Carswell, G; Richards, JE; Schladweiler, MC; Ledbetter, AD; Chorley, B; Gowdy, KM; Tong, H; Kodavanti, UP. (2018). Ozone-induced vascular contractility and pulmonary injury are differentially impacted by diets enriched with coconut oil, fish oil, and olive oil. Toxicol Sci 163: 5769. http://dx.doi.org/10.1093/toxsci/kfy003.

Spiezia, L; Campello, E; Bon, M; Maggiolo, S; Pelizzaro, E; Simioni, P. (2014). Short-term exposure to high levels of air pollution as a risk factor for acute isolated pulmonary embolism. Thromb Res Suppl 134: 259-263. http://dx.doi.org/10.1016/j.thromres.2014.05.011.

Steinvil, A; Kordova-Biezuner, L; Shapira, I; Berliner, S; Rogowski, O. (2008). Short-term exposure to air pollution and inflammation-sensitive biomarkers. Environ Res 106: 51-61. http://dx.doi.org/10.1016/j.envres.2007.08.006.

Stieb, DM; Szyszkowicz, M; Rowe, BH; Leech, JA. (2009). Air pollution and emergency department visits for cardiac and respiratory conditions: A multi-city time-series analysis. Environ Health 8: 25. http://dx.doi.org/10.1186/1476-069X-8-25.

Stiegel, MA; Pleil, JD; Sobus, JR; Madden, MC. (2016). Inflammatory cytokines and white blood cell counts response to environmental levels of diesel exhaust and ozone inhalation exposures. PLoS ONE 11: e0152458. http://dx.doi.org/10.1371/journal.pone.0152458.

Stiegel, MA; Pleil, JD; Sobus, JR; Stevens, T; Madden, MC. (2017). Linking physiological parameters to perturbations in the human exposome: Environmental exposures modify blood pressure and lung function via inflammatory cytokine pathway. J Toxicol Environ Health A 80: 485-501. http://dx.doi.org/10.1080/15287394.2017.1330578.

Straney, L; Finn, J; Dennekamp, M; Bremner, A; Tonkin, A; Jacobs, I. (2014). Evaluating the impact of air pollution on the incidence of out-of-hospital cardiac arrest in the Perth Metropolitan Region: 2000-2010. J Epidemiol Community Health 68: 6-12. http://dx.doi.org/10.1136/jech-2013-202955.

Suissa, L; Fortier, M; Lachaud, S; Staccini, P; Mahagne, MH. (2013). Ozone air pollution and ischaemic stroke occurrence: a case-crossover study in Nice, France. BMJ Open 3: e004060. http://dx.doi.org/10.1136/bmjopen-2013-004060.

Tallon, LA; Manjourides, J; Pun, VC; Mittleman, MA; Kioumourtzoglou, MA; Coull, B; Suh, H. (2017). Erectile dysfunction and exposure to ambient Air pollution in a nationally representative cohort of older Men. Environ Health 16: 12. http://dx.doi.org/10.1186/s12940-017-0216-6.

Tanaka, T; Narazaki, M; Kishimoto, T. (2014). IL-6 in inflammation, immunity, and disease [Review]. Cold Spring Harbor Perspectives in Biology 6: a016295. http://dx.doi.org/10.1101/cshperspect.a016295.

Tankersley, CG; Georgakopoulos, D; Tang, WY; Sborz, N. (2013). Effects of ozone and particulate matter on cardiac mechanics: role of the atrial natriuretic peptide gene. Toxicol Sci 131: 95-107. http://dx.doi.org/10.1093/toxsci/kfs273.

Tankersley, CG; Peng, RD; Bedga, D; Gabrielson, K; Champion, HC. (2010). Variation in echocardiographic and cardiac hemodynamic effects of PM and ozone inhalation exposure in strains related to Nppa and Npr1 gene knock-out mice. Inhal Toxicol 22: 695-707. http://dx.doi.org/10.3109/08958378.2010.487549.

Thompson, AM; Zanobetti, A; Silverman, F; Schwartz, J; Coull, B; Urch, B; Speck, M; Brook, JR; Manno, M; Gold, DR. (2010). Baseline repeated measures from controlled human exposure studies: Associations between ambient air pollution exposure and the systemic inflammatory biomarkers IL-6 and fibrinogen. Environ Health Perspect 118: 120-124. http://dx.doi.org/10.1289/ehp.0900550.

Thomson, EM; Pal, S; Guénette, J; Wade, MG; Atlas, E; Holloway, AC; Williams, A; Vincent, R. (2016). Ozone inhalation provokes glucocorticoid-dependent and -independent effects on inflammatory and metabolic pathways. Toxicol Sci 152: 17-28. http://dx.doi.org/10.1093/toxsci/kfw061.

Thomson, EM; Vladisavljevic, D; Mohottalage, S; Kumarathasan, P; Vincent, R. (2013). Mapping acute systemic effects of inhaled particulate matter and ozone: multiorgan gene expression and glucocorticoid activity. Toxicol Sci 135: 169-181. http://dx.doi.org/10.1093/toxsci/kft137.

Toren, K; Brisman, J; Jarvholm, B. (1993). Asthma and asthma-like symptoms in adults assessed by questionnaires: A literature review [Review]. Chest 104: 600-608. http://dx.doi.org/10.1378/chest.104.2.600.

Turner, MC; Jerrett, M; Pope, A, III; Krewski, D; Gapstur, SM; Diver, WR; Beckerman, BS; Marshall, JD; Su, J; Crouse, DL; Burnett, RT. (2016). Long-term ozone exposure and mortality in a large prospective study. Am J Respir Crit Care Med 193: 1134-1142. http://dx.doi.org/10.1164/rccm.201508-1633OC.

U.S. EPA (U.S. Environmental Protection Agency). (1991). Guidelines for developmental toxicity risk assessment (pp. 1-71). (EPA/600/FR-91/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=23162.

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

U.S. EPA (U.S. Environmental Protection Agency). (1996b). Guidelines for reproductive toxicity risk assessment (pp. 1-143). (EPA/630/R-96/009). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/sites/production/files/2014-11/documents/guidelines_repro_toxicity.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (1998). Guidelines for neurotoxicity risk assessment [EPA Report] (pp. 1-89). (EPA/630/R-95/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/risk/guidelines-neurotoxicity-risk-assessment.

U.S. EPA (U.S. Environmental Protection Agency). (2005). Guidelines for carcinogen risk assessment [EPA Report] (pp. 1-166). (EPA/630/P-03/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/sites/production/files/2013-09/documents/cancer_guidelines_final_3-25-05.pdf.

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

U.S. EPA (U.S. Environmental Protection Agency). (2008). Integrated science assessment for sulfur oxides: Health criteria [EPA Report]. (EPA/600/R-08/047F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment-RTP. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=198843.

U.S. EPA (U.S. Environmental Protection Agency). (2013a). Integrated science assessment for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-10/076F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment-RTP Division. http://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=247492.

U.S. EPA (U.S. Environmental Protection Agency). (2013b). Toxicological review of trimethylbenzenes (CASRN 25551-13-7, 95-63-6, 526-73-8, and 108-67-8) in support of summary information on the Integrated Risk Information System (IRIS): revised external review draft [EPA Report]. (EPA/635/R13/171a). Washington, D.C.: U.S. Environmental Protection Agency, National Center for Environmental Assessment. http://yosemite.epa.gov/sab/SABPRODUCT.NSF/b5d8a1ce9b0729348525737500701 2b7/ee1e280e77586de985257b65005d37e7!OpenDocument.

U.S. EPA (U.S. Environmental Protection Agency). (2015). Preamble to the integrated science assessments [EPA Report]. (EPA/600/R-15/067). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, RTP Division. https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=310244.

van Rossem, L; Rifas-Shiman, SL; Melly, SJ; Kloog, I; Luttmann-Gibson, H; Zanobetti, A; Coull, BA; Schwartz, JD; Mittleman, MA; Oken, E; Gillman, MW; Koutrakis, P; Gold, DR. (2015). Prenatal air pollution exposure and newborn blood pressure. Environ Health Perspect 123: 353-359. http://dx.doi.org/10.1289/ehp.1307419.

Vanos, JK; Hebbern, C; Cakmak, S. (2014). Risk assessment for cardiovascular and respiratory mortality due to air pollution and synoptic meteorology in 10 Canadian cities. Environ Pollut 185: 322-332. http://dx.doi.org/10.1016/j.envpol.2013.11.007.

Vencloviene, J; Braziene, A; Dedele, A; Lopatiene, K; Dobozinskas, P. (2017). Associations of short-term exposure to ambient air pollutants with emergency ambulance calls for the exacerbation of essential arterial hypertension. Int J Environ Health Res 27: 509-524. http://dx.doi.org/10.1080/09603123.2017.1405246.

Vidale, S; Arnaboldi, M; Bosio, V; Corrado, G; Guidotti, M; Sterzi, R; Campana, C. (2017). Short-term air pollution exposure and cardiovascular events: A 10-year study in the urban area of Como, Italy. Int J Cardiol 248: 389-393. http://dx.doi.org/10.1016/j.ijcard.2017.06.037.

Villeneuve, PJ; Chen, L; Stieb, D; Rowe, BH. (2006). Associations between outdoor air pollution and emergency department visits for stroke in Edmonton, Canada. Eur J Epidemiol 21: 689-700. http://dx.doi.org/10.1007/s10654-006-9050-9.

von Elm, E; Altman, DG; Egger, M; Pocock, SJ; Gøtzsche, PC; Vandenbroucke, JP. (2007). The strengthening the reporting of observational studies in epidemiology (strobe) statement: guidelines for reporting observational studies [Review]. PLoS Med 4: e296. http://dx.doi.org/10.1371/journal.pmed.0040296.

von Klot, S; Peters, A; Aalto, P; Bellander, T; Berglind, N; D'Ippoliti, D; Elosua, R; Hormann, A; Kulmala, M; Lanki, T; Lowel, H; Pekkanen, J; Picciotto, S; Sunyer, J; Forastiere, F. (2005). Ambient air pollution is associated with increased risk of hospital cardiac readmissions of myocardial infarction survivors in five European cities. Circulation 112: 3073-3079. http://dx.doi.org/10.1161/CIRCULATIONAHA.105.548743.

Wagner, JG; Allen, K; Yang, HY; Nan, B; Morishita, M; Mukherjee, B; Dvonch, JT; Spino, C; Fink, GD; Rajagopalan, S; Sun, Q; Brook, RD; Harkema, JR. (2014). Cardiovascular depression in rats exposed to inhaled particulate matter and ozone: effects of diet-induced metabolic syndrome. Environ Health Perspect 122: 27-33. http://dx.doi.org/10.1289/ehp.1307085.

Wang, G; Jiang, R; Zhao, Z; Song, W. (2013). Effects of ozone and fine particulate matter (PM(2.5)) on rat system inflammation and cardiac function. Toxicol Lett 217: 23-33. http://dx.doi.org/10.1016/j.toxlet.2012.11.009.

Wang, X; Kindzierski, W; Kaul, P. (2015a). Air pollution and acute myocardial infarction hospital admission in Alberta, Canada: a three-step procedure case-crossover study. PLoS ONE 10: e0132769. http://dx.doi.org/10.1371/journal.pone.0132769.

Wang, X; Kindzierski, W; Kaul, P. (2015b). Comparison of transient associations of air pollution and AMI hospitalisation in two cities of Alberta, Canada, using a case-crossover design. BMJ Open 5: e009169. http://dx.doi.org/10.1136/bmjopen-2015-009169.

Weakley, J; Webber, MP; Ye, F; Zeig-Owens, R; Cohen, HW; Hall, CB; Kelly, K; Prezant, DJ. (2013). Agreement between obstructive airways disease diagnoses from self-report questionnaires and medical records. Prev Med 57: 38-42. http://dx.doi.org/10.1016/j.ypmed.2013.04.001.

Wing, JJ; Adar, SD; Sánchez, BN; Morgenstern, LB; Smith, MA; Lisabeth, LD. (2015). Ethnic differences in ambient air pollution and risk of acute ischemic stroke. Environ Res 143: 62-67. http://dx.doi.org/10.1016/j.envres.2015.09.031.

Wing, JJ; Adar, SD; Sánchez, BN; Morgenstern, LB; Smith, MA; Lisabeth, LD. (2017a). Short-term exposures to ambient air pollution and risk of recurrent ischemic stroke. Environ Res 152: 304-307. http://dx.doi.org/10.1016/j.envres.2016.11.001.

Wing, JJ; Sánchez, BN; Adar, SD; Meurer, WJ; Morgenstern, LB; Smith, MA; Lisabeth, LD. (2017b). Synergism of short-term air pollution exposures and neighborhood disadvantage on initial stroke severity. Stroke 48: 3126-3129. http://dx.doi.org/10.1161/STROKEAHA.117.018816.

Winquist, A; Klein, M; Tolbert, P; Flanders, WD; Hess, J; Sarnat, SE. (2012). Comparison of emergency department and hospital admissions data for air pollution time-series studies. Environ Health 11: 70. http://dx.doi.org/10.1186/1476-069X-11-70.

Xu, X; Sun, Y; Ha, S; Talbott, EO; Lissaker, CTK. (2013). Association between ozone exposure and onset of stroke in Allegheny County, Pennsylvania, USA, 1994-2000. Neuroepidemiology 41: 2-6. http://dx.doi.org/10.1159/000345138.

Yang, BY; Qian, ZM; Vaughn, MG; Nelson, EJ; Dharmage, SC; Heinrich, J; Lin, S; Lawrence, WR; Ma, H; Chen, DH; Hu, LW; Zeng, XW; Xu, SL; Zhang, C; Dong, GH. (2017). Is prehypertension more strongly associated with long-term ambient air pollution exposure than hypertension? Findings from the 33 Communities Chinese Health Study. Environ Pollut 229: 696-704. http://dx.doi.org/10.1016/j.envpol.2017.07.016.

Yang, CL; To, T; Foty, RG; Stieb, DM; Dell, SD. (2011). Verifying a questionnaire diagnosis of asthma in children using health claims data. BMC Pulm Med 11. http://dx.doi.org/10.1186/1471-2466-11-52.

Ying, Z; Allen, K; Zhong, J; Chen, M; Williams, KM; Wagner, JG; Lewandowski, R; Sun, Q; Rajagopalan, S; Harkema, JR. (2016). Subacute inhalation exposure to ozone induces systemic inflammation but not insulin resistance in a diabetic mouse model. Inhal Toxicol 28: 155-163. http://dx.doi.org/10.3109/08958378.2016.1146808.

Zanobetti, A; Canner, MJ; Stone, PH; Schwartz, J; Sher, D; Eagan-Bengston, E; Gates, KA; Hartley, LH; Suh, H; Gold, DR. (2004). Ambient pollution and blood pressure in cardiac rehabilitation patients. Circulation 110: 2184-2189. http://dx.doi.org/10.1161/01.cir.0000143831.33243.d8.

Zanobetti, A; Luttmann-Gibson, H; Horton, ES; Cohen, A; Coull, BA; Hoffmann, B; Schwartz, JD; Mittleman, MA; Li, Y; Stone, PH; de Souza, C; Lamparello, B; Koutrakis, P; Gold, DR. (2014). Brachial artery responses to ambient pollution, temperature, and humidity in people with type 2 diabetes: a repeated-measures study. Environ Health Perspect 122: 242-248. http://dx.doi.org/10.1289/ehp.1206136.

Zhao, Y; Qian, Z; Wang, J; Vaughn, MG; Liu, Y; Ren, W; Dong, G. (2013). Does obesity amplify the association between ambient air pollution and increased blood pressure and hypertension in adults? Findings from the 33 Communities Chinese Health Study [Letter]. Int J Cardiol 168: E148-E150. http://dx.doi.org/10.1016/j.ijcard.2013.08.071.

Zhong, J; Allen, K; Rao, X; Ying, Z; Braunstein, Z; Kankanala, SR; Xia, C; Wang, X; Bramble, LA; Wagner, JG; Lewandowski, R; Sun, Q; Harkema, JR; Rajagopalan, S. (2016). Repeated ozone exposure exacerbates insulin resistance and activates innate immune response in genetically susceptible mice. Inhal Toxicol 28: 383-392. http://dx.doi.org/10.1080/08958378.2016.1179373.

Zychowski, KE; Lucas, SN; Sanchez, B; Herbert, G; Campen, MJ. (2016). Hypoxia-induced pulmonary arterial hypertension augments lung injury and airway reactivity caused by ozone exposure. Toxicol Appl Pharmacol 305: 40-45. http://dx.doi.org/10.1016/j.taap.2016.06.003.

APPENDIX 5 HEALTH EFFECTS—METABOLIC EFFECTS

Summary of Causal Determinations for Short- and Long-Term Metabolic Health Effects

This Appendix characterizes the scientific evidence that supports causality determinations for short- and long-term ozone exposure and metabolic effects. The types of studies evaluated within this Appendix are consistent with the overall scope of the ISA as detailed in the Preface. In assessing the overall evidence, the strengths and limitations of individual studies were evaluated based on scientific considerations detailed in the Annex for Appendix 5. More details on the causal framework used to reach these conclusions are included in the Preamble to the ISA (U.S. EPA, 2015).

Exposure Duration	Causality Determination
Short-term exposure	Likely to be causal
Long-term exposure	Likely to be causal

5.1 Short-Term Ozone Exposure—Introduction, Summary from the 2013 Ozone ISA, and Scope for Current Review

The evidence relevant to metabolic effects that was reviewed in the 2013 Ozone ISA included a small number of studies that examined glucose and insulin homeostasis, lipids, cholesterol, liver, and obesity health endpoints (U.S. EPA, 2013). These studies provided evidence for modes of action and were discussed alongside cardiovascular biomarkers. There was no causality determination for metabolic effects in the 2013 Ozone ISA.

The metabolic effects reviewed in this Appendix include metabolic syndrome and its components, diabetes, metabolic disease mortality, and effects of metabolic indicators that underlie metabolic and cardiovascular diseases (see Appendix 4). These effects include alterations in glucose and insulin homeostasis (Section 5.1.3.1), inflammation, and changes in liver function, neuroendocrine signaling, and serum lipids, among other endpoints (Section 5.1.3.3).

Metabolic syndrome is a term used to describe a collection of risk factors that include high blood pressure, dyslipidemia (elevated triglycerides and low levels of high density lipoprotein [HDL] cholesterol), obesity (particularly central obesity), and increased fasting blood glucose [FBG; Alberti et al. (2009)]. The presence of these risk factors may predispose one to an increased risk of type 2 diabetes and cardiovascular disease (see Appendix 4).

Diabetes is characterized by a continuum of hyperglycemia (i.e., elevated glucose level) resulting from defects in insulin signaling, secretion, or both. Several types of diabetes have been classified by the American Diabetes Association (ADA, 2014). Type 1 diabetes (T1D) is caused by β-cell dysfunction or destruction that leads to insulin deficiency, while type 2 diabetes is characterized by defects in insulin secretion in an insulin resistant environment. Gestational diabetes mellitus (GDM) is generally diagnosed during the second or third trimester of pregnancy.

The subsections below provide an evaluation of the most policy-relevant scientific evidence relating short-term ozone exposure to metabolic health effects. These sections focus on studies published since the completion of the 2013 Ozone ISA. There are a limited number of recent epidemiologic studies examining the effects of short-term ozone exposure on glucose tolerance, insulin sensitivity, and diabetes control. In addition, multiple animal toxicological studies evaluate ozone-mediated effects, and these studies indicate that short-term exposure to ozone affects glucose homeostasis and other factors that contribute to metabolic syndrome.

5.1.1 Population, Exposure, Comparison, Outcome, and Study Design (PECOS) Tool

The scope of this section is defined by a scoping tool that generally defines the relevant Population, Exposure, Comparison, Outcome, and Study Design (PECOS). The PECOS tool defines the parameters and provides a framework to help identify the relevant evidence in the literature to inform the ISA. Because the 2013 Ozone ISA did not make a causality determination for short-term ozone exposure and metabolic health effects, the epidemiologic studies evaluated are less limited in scope and not targeted towards specific study locations, as reflected in the PECOS tool. The studies evaluated and subsequently discussed within this section were identified using the following PECOS tool:

Experimental Studies:

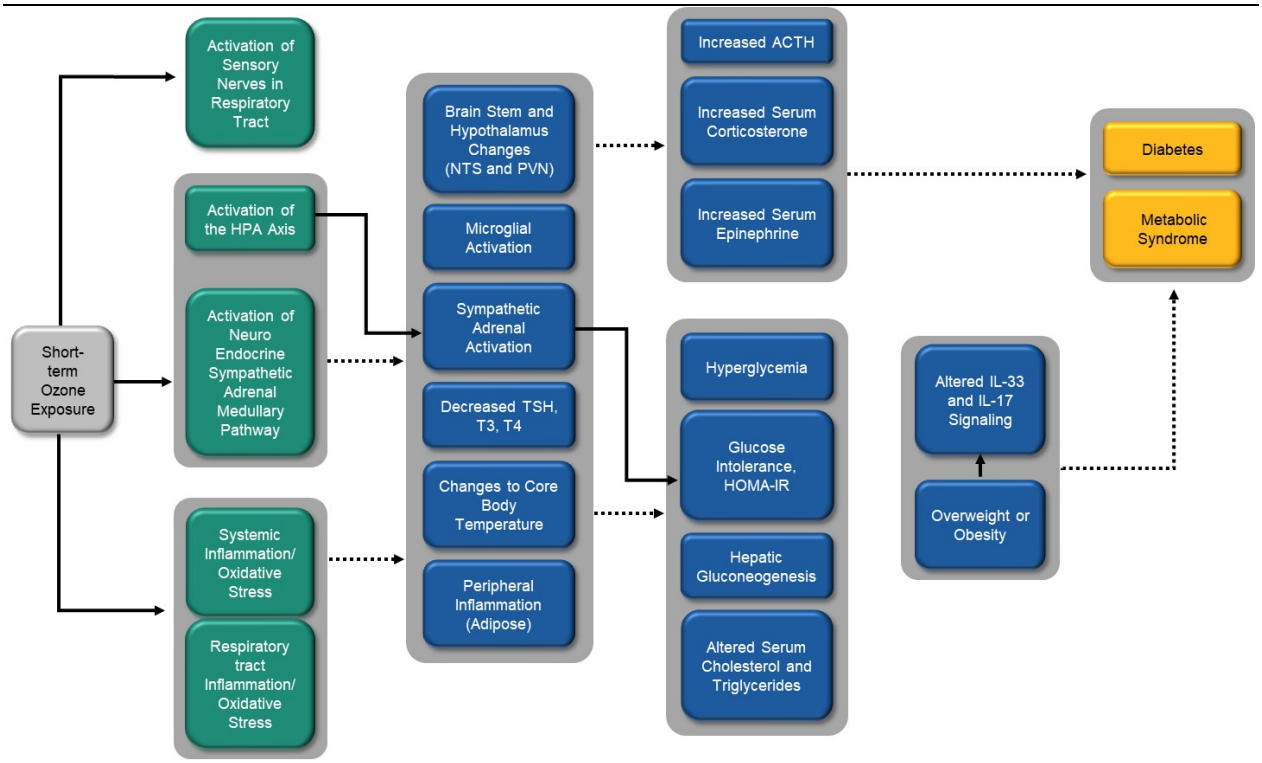
- Population: Study populations of any controlled human exposure or animal toxicological study of mammals at any lifestage
- Exposure: Short-term (in the order of minutes to weeks) inhalation exposure to relevant ozone concentrations (i.e., 0.4 ppm or below for humans, 2 ppm or below for other mammals)
- Comparison: Human subjects that serve as their own controls with an appropriate washout period or when comparison to a reference population exposed to lower levels is available, or, in toxicological studies of mammals, an appropriate comparison group that is exposed to a negative control (i.e., clean air or filtered air control)
- Outcome: Metabolic effects (e.g., diabetes, metabolic syndrome, dyslipidemia, glucose intolerance, insulin resistance, overweight, obesity)
- Study Design: Controlled human exposure (e.g., chamber) studies; in vivo acute, subacute, or repeated-dose toxicity studies in mammals, immunotoxicity studies

Epidemiologic Studies:

- Population: Any population, including populations or lifestages that might be at increased risk
- Exposure: Ambient ozone from any source measured as short-term (hours to days)
- Comparison: Per unit increase (in ppb)
- Outcome: Change in risk (incidence/prevalence) of metabolic effects
- Study Design: Epidemiologic studies consisting of panel, case-crossover, time-series studies, and case-control studies; cross-sectional studies with appropriate timing of exposure for the health endpoint of interest

5.1.2 Biological Plausibility

This section describes biological pathways that potentially underlie metabolic effects resulting from short-term exposure to ozone. Figure 5-1 graphically depicts the proposed pathways as a continuum of upstream events, connected by arrows, that may lead to downstream events observed in epidemiologic studies. This discussion of "how" exposure to ozone may lead to metabolic effects contributes to an understanding of the biological plausibility of epidemiologic results evaluated later. Additionally, most studies cited in this subsection are discussed in greater detail throughout this Appendix.



Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence related to ozone exposure, and the arrows indicate a proposed relationship between those effects. Solid arrows denote evidence of essentiality as provided, for example, by an inhibitor of the pathway or a genetic knockout model used in an experimental study involving ozone exposure. Shading around multiple boxes is used to denote a grouping of these effects. Arrows may connect individual boxes, groupings of boxes, and individual boxes within groupings of boxes. Progression of effects is generally depicted from left to right and color-coded (gray, exposure; green, initial effect; blue, intermediate effect; orange, effect at the population level or a key clinical effect). Here, population level effects generally reflect results of epidemiologic studies. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below.

Figure 5-1 Potential biological pathways for metabolic outcomes following short-term ozone exposure.

Ozone inhalation can contribute to metabolic syndrome or diabetic health outcomes starting with upstream events that impact the nervous system leading to changes in HPA axis that impact overall endocrine and energy homeostasis. Ozone inhalation interacts with the central nervous system in the respiratory tract activating a stress response. Specifically, the pulmonary irritant ozone stimulates nasopharyngeal and pulmonary nerves and receptors including the trigeminal and vagal nerves, which induces downstream effects to the autonomic nervous system. The sensory nerves innervate the lungs and also communicate with brain regions and other areas of the body like the vagus jugular or nodose ganglions and is sensitive to this ozone irritant response. Sensory nerve activation can affect metabolic pathways as well as the pulmonary and cardiovascular systems. The developing rat nodose and jugular ganglions are structurally altered with early-life ozone exposure, with fewer neurons present after ozone exposure (Zellner et al., 2011). The communication through the nodose and jugular ganglion is transmitted to the hypothalamic paraventricular nucleus [PVN; Gackière et al. (2011)] and the brainstem's

nucleus tractus solitarius (NTS) where ozone can have an effect as seen with increased c-fos staining, a marker for neuronal activation Gackière et al. (2011). Also, the paratrigeminal nucleus (Pa5) can potentially signal to the hypothalamus via norepinephrine (NE) and second-order neurons or via glucagon-like peptide-1, or it can communicate back to the heart or airways. These studies show that ozone exposure induces neuronal communication from the lung to the brain affecting areas including the brainstem and the hypothalamus. Hypothalamic effects of ozone have been demonstrated in multiple studies (Mumaw et al., 2016; Dorado-Martinez et al., 2001). The hypothalamus is the control center for stress activation in the brain and it activates downstream targets including the pituitary and the adrenals contributing further to the stress axis activation through the sympathetic adrenomedullary (SAM) pathway. For example, ozone induces neuro-inflammatory activation of brain microglia 4 hours after exposure and out to 24 hours post-exposure. These changes are mediated by soluble factors in the blood (TNF-α, H2O2) as seen with microglial activation during ex vivo testing of the bioactivity of serum from ozone-exposed animals (Mumaw et al., 2016). Thus, ozone exposure along the lung-brain axis involves initial neuronal irritant activation in the lungs that then activates multiple downstream pathways in the brain that contribute directly to activation of the neuroendocrine system starting with the hypothalamus, which is detailed further below as it progresses to the pituitary and adrenals and other stress pathways that contribute to perturbed energy homeostasis.

Ozone exposure also induces activation of the neuro-endocrine hypothalamic pituitary adrenal (HPA) axis stimulating the sympathetic adrenal medullary (SAM) pathways. The hypothalamus, pituitary, and adrenals respectively release CRH, ACTH and cortisol forming the neuro-endocrine system that controls and mediates reactions to stress and regulates body systems accordingly. There is direct evidence of activation of the neuro-endocrine pathways in the brain, lung, and metabolic organs mediated through the release of stress hormones [ACTH, norepinephrine, cortisol, and corticosterone; Miller et al. (2016a); Miller et al. (2015); Bass et al. (2013); Thomson et al. (2013)] into the circulation of rats with ozone exposure. The upstream mediators like adrenocorticotropin hormone (ACTH) from the pituitary (Thomson et al., 2013) are released into the circulation of rats (Miller et al., 2016a) and contribute to a multiorgan stress response upon ozone exposure. Diabetes and metabolic syndrome are disorders of the autonomic nervous system. Ozone exposure increases stress hormones and multiple downstream metabolic effects including glucose intolerance, fasting hyperglycemia, and hepatic gluconeogenesis (Miller et al., 2016b; Zhong et al., 2016; Miller et al., 2015; Bass et al., 2013). With ozone exposure, changes in the biomarkers of glucose intolerance and insulin resistance, including HOMA-IR (Li et al., 2017; Miller et al., 2016a; Kim and Hong, 2012) or HbA1c (Chuang et al., 2011), as well as increased ketone-body formation (Miller et al., 2016a), have been noted in humans. Impaired insulin signaling is a pathophysiological effect leading to clinical outcomes such as insulin resistance, increased blood glucose, and increased blood lipids. Specifically, insulin stimulates sensitive tissues to take up glucose, lipids, and amino acids. In muscle, insulin stimulates glucose oxidation or storage as glycogen and protein synthesis; in liver, insulin stimulates glycogen synthesis; and in adipose tissue, insulin stimulates lipid synthesis and storage. During a fast (overnight) plasma glucose and insulin levels are low; glucagon levels rise, and lipids are mobilized from adipose tissue into the circulation; glycogenolysis and gluconeogenesis increase

in the liver; and striated muscle metabolizes lipids and degrades proteins into amino acids (Boron and Boulpaep, 2017). When individuals do not respond properly to glucose and insulin levels (as in T2D), body fuels (glucose, lipid, and amino acid) are mobilized into the blood, putting a burden on liver, kidney, and vascular function.

Other biomarkers of ozone-dependent altered energy homeostasis are elevated triglycerides in animals (Miller et al., 2016c; Bass et al., 2013) and humans (Chuang et al., 2011), increased levels of circulating free fatty acids in both humans and animal toxicological models (Miller et al., 2016a; Miller et al., 2015), and altered cholesterol levels (Thomson et al., 2013). Skeletal muscle insulin resistance develops with ozone exposure (Vella et al., 2014).

Further verification of the importance of the HPA axis in ozone-induced metabolic perturbations (denoted by the solid line in the Figure 5-1) comes from the attenuation or amelioration of ozone-induced metabolic effects (glucose intolerance, hyperglycemia, elevated stress hormones epinephrine and cortisol) after surgical removal of the adrenal glands (Miller et al., 2016c; Miller et al., 2015). As mentioned earlier, under normal physiological conditions, the hypothalamus, pituitary and adrenals work together to respond to a potential stressor with cortisol or corticosterone produced by the adrenal cortex. Administration of glucocorticoid receptor antagonists reduces ozone-dependent inflammation (Henriquez et al., 2017b).

In addition to effects mediated by the HPA axis, there are also immediate changes to baseline metabolic rate in animals after ozone exposure as well as changes to the thyroid and the pituitary. Adult male rats immediately become hypothermic with an associated bradycardia during exposure (Mautz and Bufalino, 1989). Once exposure ceases, there is a delayed daytime hyperthermia that manifests a couple of days after exposure stops (Gordon et al., 2014). Baseline metabolism and thermoregulation can be influenced by thyroid function, and people with thyroid disease are at increased risk of developing type 2 diabetes (Chaker et al., 2016). Under normal physiological conditions, the thyroid regulates metabolism and thermoregulation; thyroid hormone status is associated with body weight and energy expenditure (Chaker et al., 2016). Hyperthyroidism or excess thyroid hormone production causes a hypermetabolic state with increased resting energy expenditure, decreased body weight, reduced cholesterol levels, increased lipolysis, and increased gluconeogenesis. Alternatively, hypothyroidism or decreased thyroid hormone levels is associated with decreased metabolism and reduced resting energy expenditure, increased body weight, elevated serum cholesterol, decreased lipolysis, and decreased gluconeogenesis. Changes in thyroid function are seen after acute ozone exposure (1 hour, 1 ppm, adult male rodents): circulating serum TSH levels significantly decreased, thyroid hormone (T3 and T4) levels significantly decreased, circulating protein-bound iodine concentrations significantly decreased, and thyroid weight went down. Circulating prolactin was significantly increased. Pituitary TSH and prolactin content were considerably increased, but only TSH was statistically significantly increased in the pituitary, demonstrating perturbation of the pituitary-thyroid axis following ozone exposure (Clemons and Garcia, 1980) and responsive upregulation of pituitary TSH in response to the drop in serum thyroid hormone

levels. The pituitary-thyroid axis may be depressed with acute ozone exposure by decreasing hypothalamic stimulation by thyrotropin releasing hormone while at the same time removing the hypothalamic catecholamine inhibition of prolactin release. In an adjacent organ, histological analysis of the parathyroid gland is consistent with hyperactivity of the parathyroid gland after ozone exposure in rabbits [4−8 hours exposure to 0.75 ppm ozone; Atwal and Wilson (1974)].

In summary, short-term ozone exposure has extrapulmonary effects that contribute to metabolic disturbances, including hypothermia, decreased metabolic rate, increased corticosterone, and hyperglycemia. These pathways may be initially stimulated by irritant receptors in the pulmonary tract. The thyroid system is also affected with decreased circulating TSH, T4, and T3. Cytokines, including IL-33 and IL-17a, contribute to development of metabolic syndrome in animal models of obesity. The entire cascade begins when pulmonary signaling at irritant receptors and pulmonary nerves (trigeminal and vagus nerve) is activated by ozone exposure. The HPA axis is then stimulated and systemic inflammation and oxidative stress ensues. After these initial events, downstream events are activated, including microglial activation, hypothalamic changes at the level of the PVN and brainstem changes in the NTS, decreased TSH and thyroid hormones, and changes to core body temperature. Serum triglycerides increase with ozone exposure. Serum cholesterol can also be affected by ozone exposure, but varies by model; free fatty acids increase in serum with ozone exposure. ACTH is elevated with ozone exposure as is its downstream corticosterone or cortisol. The sympathetic activation also includes increased levels of norepinephrine. Hyperglycemia, glucose intolerance, and hepatic gluconeogenesis follow. Recent epidemiologic studies provide evidence for impaired glucose regulation and altered HbA1c, and human clinical studies show increased ketone body formation in participants exposed to ozone. All of these upstream factors of autonomic activation and homeostatic imbalance can contribute to an animal model or humans being at a greater risk for developing metabolic syndrome or diabetes with ozone exposure. Together, these proposed pathways provide biological plausibility for epidemiologic evidence of metabolic syndrome and/or diabetes with ozone exposure and will be used to inform a causality determination, which is discussed later in this Appendix.

5.1.3 Glucose and Insulin Homeostasis

Insulin is secreted by β-cells within the pancreas in response to glucose levels. When glucose levels rise, depolarization of the pancreatic β-cells or modulation by other hormones stimulate insulin secretion. Thus, during feeding, blood insulin levels rise, stimulating glucose uptake and replenishing body fuel reserves in the form of triglycerides and glycogen. When insulin levels decrease (e.g., during fasting), fuels, such as lipids from adipose tissue and amino acids from muscle, are mobilized to the bloodstream where they are used by the liver to synthesize glucose.

Clinical outcomes of impaired glucose regulation include diabetic ketoacidosis and diabetic coma. Diabetic ketoacidosis, which is usually seen in type 1 diabetics, can result in unconsciousness from

a combination of a severely increased blood sugar level, dehydration, and accumulation of ketones or acids that were formed as the diabetic body used fat for fuel instead of sugar. Diabetic coma is a reversible form of coma found in people with diabetes which involves extremely low blood sugar.

The effects of short-term exposure to ozone on glucose and insulin homeostasis are characterized below and utilize various techniques. The glucose tolerance test (GTT) involves the sampling of blood glucose levels at multiple time points after glucose injection or ingestion to measure the body's response to glucose and is used to diagnose or monitor diabetes or gestational diabetes. The insulin tolerance test (ITT) involves insulin injection to fasting animals and glucose monitoring after injection. Ketone bodies can be formed in diabetic ketoacidosis when energy production pathways are altered and higher levels of ketones are generated in response to low insulin. The Homeostatic Model Assessment (HOMA) is a method for assessing β-cell function and insulin resistance.

5.1.3.1 Epidemiologic Studies

One epidemiologic study of short-term ozone exposure and glucose or insulin homeostasis was reviewed in the 2013 Ozone ISA. Chuang et al. (2010) found increases in fasting glucose 5 days following increased exposure to ozone. Recent epidemiologic studies provide some evidence of associations between short-term ozone exposure and these endpoints (Table 5-5). Specifically:

- Kim and Hong (2012) found increases in fasting glucose (0.19%; 95% CI: 0.09, 0.28%), insulin (0.71%; 95% CI: 0.02, 1.38%)[1], and HOMA (0.30%; 95% CI: 0.06, 0.53%) in the Korean Elderly Environmental Panel (KEEP). The KEEP cohort consisted of 560 Koreans over 60 years old. The association of 5-day avg ozone concentration with glucose, insulin, and HOMA-IR was approximately threefold larger in people with a previous diagnosis of type 2 diabetes (glucose [0.68%; 95% CI: 0.28, 1.07%], insulin [2.76%; 95% CI: 0.78, 4.75%], HOMA [1.21%; 95% CI: 0.44, 1.99%]). In subjects without type 2 diabetes, an association with glucose was observed (0.09%; 95% CI: 0.02, 0.16%), while associations with insulin and HOMA were not observed. Copollutant models with NO_2 and PM_{10} were also evaluated. The associations with glucose remained after adjustment for NO_2 (0.16%; 95% CI: 0.06, 0.25%) and PM_{10} (0.15%; 95% CI: 0.01, 0.14%).
- Using 5,958 participants from the Framingham Offspring Cohort and Third Generation Cohort, Li et al. (2017) completed a panel study evaluating the association of fasting glucose, insulin, HOMA-IR, and other metabolic endpoints with 1- to 7-day moving avg ozone concentrations. Decreases in fasting glucose were observed at 3-, 5-, and 7-day moving avg. No other endpoints differed based on the qualitative results presented in the study.
- In a study of 1,023 Mexican Americans in southern California, Chen et al. (2016b) evaluated changes to HOMA-IR, fasting glucose, and insulin resulting from short-term exposure to ozone. The study used cumulative averages of daily ozone concentrations from 0−90 days prior to

[1] All epidemiologic results standardized to a 15-ppb increase in 24-hour avg, 20-ppb increase in 8-hour daily max, 25-ppb increase in 1-hour daily max ozone concentrations, or a 10-ppb increase in seasonal/annual ozone concentrations to facilitate comparability across studies.

testing. No associations were reported for any metabolic outcomes. Results were presented qualitatively.

- One study evaluated hospital admissions for diabetic ketoacidosis and diabetic coma in the Santiago region of Chile (Dales et al., 2012). Insulin resistance may lead to these hospitalizations in both type 1 and 2 diabetics. Using a 6-day distributed lag, a null association for the relationships of hospital admissions for diabetic ketoacidosis or diabetic coma (RR: 1.02; 95% CI: 0.996, 1.04) was observed. The effect remained null when divided into subregions of Santiago and when CO, PM_{10}, $PM_{2.5}$, or SO_2 were individually added to evaluate two-pollutant models.

5.1.3.2 Controlled Human Exposure Studies

Controlled human exposure studies of short-term ozone and glucose or insulin homeostasis were not reviewed in the 2013 Ozone ISA. A recent study (Table 5-6) did not show evidence that short-term ozone exposure affected these endpoints. In a study of 24 health volunteers aged 22−30 years, Miller et al. (2016a) randomly exposed subjects to ozone (0.3 ppm) or filtered air over 2 hours while alternating 15 minutes of exercise and 15 minutes of rest during two clinic visits. After a 2-week wash-out period, the subjects had the alternate exposure. Serum samples found no change in HOMA-IR or insulin levels immediately following ozone exposure when compared with HOMA-IR and insulin levels immediately following exposure to filtered air.

5.1.3.3 Animal Toxicological Studies

No animal toxicological studies of short-term ozone and glucose or insulin homeostasis were reviewed in the 2013 Ozone ISA. A number of recent animal toxicological studies provide evidence for changes in glucose and insulin homeostasis following short-term ozone exposure. As detailed below and in the evidence inventory table that follows (Table 5-7), short-term ozone exposure is associated with elevated fasting blood glucose, hyperglycemia, and glucose intolerance, endpoints which are controlled by adrenal-cortex derived hormones, as demonstrated by the fact that removal of the adrenals attenuates the ozone-dependent metabolic dysfunction (Miller et al., 2016c). Exercise attenuates the ozone-dependent glucose intolerance. Also, insulin homeostasis is altered by ozone exposure and varies by animal model with mixed effects including some studies showing insulin resistance and others null effects. Ozone exposure also induces impaired insulin secretion from β-cells. Multiple metabolic indicators demonstrate that ozone exposure impairs glucose and insulin homeostasis in animal toxicological studies. Evidence inventory tables provide detailed information on experimental design and the studies are characterized in greater detail below.

- In multiple studies of healthy animals, ozone induced hyperglycemia and impaired glucose tolerance (glucose tolerance test) have been noted. Ozone induced hyperglycemia and glucose intolerance after acute ozone exposure [Brown Norway rats, 1.0 ppm, 6 hours/day for 2 days; Bass et al. (2013)] or [Wistar Kyoto rats, 6 hours/day, 0, 0.5, or 1.0 ppm ozone; Miller et al. (2015)]. These ozone effects were slightly reduced in animals that were intermittently exposed to

ozone for 13 weeks. In a separate study, after 1 day of ozone exposure (5 hours/day, 1 or 3 days, 1.0 ppm exposure), adult male Wistar Kyoto rats had fasting hyperglycemia (Miller et al., 2016b) and glucose tolerance testing demonstrated that the rats had glucose intolerance across time (statistically significantly increased area under the curve [AUC] over the time course of 2 hours, measurements taken every 30 minutes). Adult male Fischer 344 rats that were acutely exposed to ozone (4 hours/day, 1 day, 0.8 ppm ozone) had impaired glucose tolerance with the GTT (Thomson et al., 2018); specifically, peak glucose levels in ozone-exposed animals (30 minutes post-glucose injection) were significantly higher than air-exposed controls.

- Ozone is known to increase circulating corticosterone in rats and humans (Miller et al., 2016c; Miller et al., 2016a), and removal of the adrenal corticosterone (bilateral total adrenalectomy [ADX] or bilateral adrenal medullar ablation [AMX] in male Wistar Kyoto rats [12−13 weeks of age, 1.0 ppm ozone, 4 hours/day for 1 or 2 days]) significantly attenuates or ameliorates the perturbed metabolic response to ozone (Miller et al., 2016c). With ozone exposure, hyperglycemia and glucose intolerance were significantly ameliorated with ADX and significantly attenuated with AMX (Miller et al., 2016c).

- Exercise's effect on glucose use after ozone challenge (0.25, 0.5, or 1.0 ppm, exercising or sedentary female Long-Evans [LE] rats): The study showed that all ozone-exposed animals (1.0 ppm) had elevated blood glucose after ozone exposure (Gordon et al., 2017b). Also, glucose tolerance was impaired in ozone-exposed animals; however, the exercising rats (0.25 and 0.5 ppm ozone) recovered better from the glucose challenge 30 minutes post-exposure than did the sedentary animals with a smaller glucose peak. Exercise confounded the effect of ozone on glucose tolerance, and the highest dose of ozone increased the time required for serum glucose levels to return to baseline, as measured over 2 hours after an initial glucose challenge.

- The effect of ozone on insulin homeostasis was measured in multiple toxicology studies of healthy animals. In one study, ozone exposure decreased serum insulin (2 days of ozone exposure 1.0 ppm), but insulin levels returned to baseline after the period of recovery [18 hours later; Miller et al. (2015)]. In a separate study using the insulin tolerance test (ITT), adult male Wistar Kyoto rats exposed to ozone (5 hours/day, 1 day, 1.0 ppm exposure) had fasting hyperglycemia (Miller et al., 2016b), and glucose remained significantly elevated at the first measurements (30 minutes after insulin injection), showing initial insulin resistance or residual hyperglycemia that resolved and returned to baseline over the remainder of the testing period (every 30 minutes out to 2 hours) indicating no prolonged insulin resistance. The ITT addresses pituitary or adrenal function. Another endpoint studied (Miller et al., 2016b) also demonstrated that ozone exposure caused decreased glucose-stimulated insulin secretion in response to glucose injection with the β-cell function test (serum insulin measurement, 30 minutes after glucose injection to fasting animals) suggesting impairment of insulin secretion which has been linked to stress mediated changes in metabolic response. In another study, ozone exposure (0.8 ppm for 16 hours) induced systematic and peripheral insulin resistance (HOMA-IR, ITT, and the EH clamp technique) and impaired insulin sensitivity in skeletal muscle (Vella et al., 2014). Glucagon and insulin are hormones secreted by the pancreas that counterbalance glucose changes. Glucagon keeps blood glucose from dropping too low by stimulating the release of glucose into the bloodstream from storage depots in the body. Whereas insulin controls glucose by signaling the body (liver and adipose) to move glucose from the serum to storage depots. Nose-only ozone exposure (4 hours, 0.8 ppm) to male Fischer 344 rats resulted in significantly decreased plasma glucagon (Thomson et al., 2016).

- Multiple distinct diabetic and overweight rodent models have been used to explore the effects of ozone in overweight or obese animals. KKAy mice are obese, diabetic, and have severe hyperglycemia and insulin resistance at baseline in control filtered-air animals; with ozone exposure (males, 0.5 ppm ozone for 13 consecutive weekdays), there were significant decreases

in fasting plasma insulin and HOMA-IR, but not altered insulin resistance (ITT, AUC); muscle insulin signaling increased (Ying et al., 2016). In a separate study by the same lab, another strain of diabetes-prone mice (adult male KK mice) were exposed to ozone for 13 consecutive weekdays [0.5 ppm, 4 hours/day; Zhong et al. (2016)]. While the fasting glucose levels were unchanged between ozone and filtered-air controls, fasting insulin was significantly decreased, insulin resistance was significantly elevated (AUC ITT), and β-cell insulin secretory function (HOMA-%B) was significantly decreased. In summary, the KKAY obese diabetic mice have decreased HOMA-IR, no change in insulin resistance and decreased fasting insulin with ozone exposure. In a separate model, the KK diabetes-prone mice, weekday ozone exposure exacerbated insulin resistance and impaired β-cell insulin secretion.

- The effects of age on metabolic response to ozone (1.0 ppm ozone, 6 hours/day for 2 days) were studied by exploring effects in rats (male brown Norway rats) at ages 1, 4, 12, and 24-months. Bass et al. (2013) reported reduced glucose intolerance in rats exposed to ozone across all age groups. Also, ozone induced hyperglycemia in fasting rats at 1, 12, and 24 months of age; 4-month-old rats were refractory to hyperglycemia with ozone exposure.

5.1.3.4 Summary

Recent evidence from the epidemiologic literature shows associations between short-term ozone exposure and fasting glucose levels, although some studies showed opposing associations. The one controlled human exposure study showed no changes in glucose and insulin homeostasis with ozone exposure to healthy volunteers but there were marked changes in lipid metabolism that were associated with increased plasma cortisol. It was concluded that intermittent exercise during ozone exposure lead to the lack of hyperglycemia. However, the animal toxicological literature shows short-term ozone exposure induces hyperglycemia, impaired glucose tolerance, increased gluconeogenesis, β-cell dysfunction, and decreased glucagon levels. Further, animal toxicological literature shows that these ozone-dependent effects can be ameliorated with removal of the adrenal glands, indicating the importance of the neuro-endocrine system's sympathetic adrenal medullary axis and the hypothalamic-pituitary-adrenal axis on the ozone-mediated effects. Despite limited epidemiologic and controlled human exposure literature, the expanding animal toxicological literature shows robust evidence of short-term ozone exposure contributing to impairment of glucose and insulin homeostasis.

5.1.4 Overweight and Obesity

5.1.4.1 Animal Toxicological Studies

The 2013 Ozone ISA included studies in overweight/obese animals demonstrated altered respiratory responses and respiratory inflammation with ozone exposure compared to lean controls. Genetically obese mice had airway hyper-responsiveness and responded more vigorously to acute ozone

exposure than did lean controls (Shore, 2007). Studies done at the U.S. EPA examining effects of ozone at various concentrations (0.25, 0.5, 1.0 ppm) in healthy and obese rat models with leptin receptor mutation and associated cardiovascular disease demonstrated low sensitivity to ozone-induced lung injury and neutrophilic inflammation (Kodavanti, 2015). Pulmonary inflammation and injury in response to ozone were also enhanced [2 ppm ozone for 3 hours; Shore (2007)]. The 2013 Ozone ISA also included studies in diet-induced obese animals, showing obesity-augmented inflammation and injury, as measured by BALF markers, and enhanced AHR in mice exposed acutely to ozone [2 ppm ozone for 3 hours; Johnston et al. (2008)]. Another study from the 2013 Ozone ISA at a lower exposure level (0.3 ppm ozone, 72 hours) with the same genetically obese mice reported the inflammatory response following exposure to ozone was dampened by obesity (Shore et al., 2009). The ozone-dependent pulmonary injury and inflammation (PMNs in the lung), and reduced pulmonary compliance seen in lean mice was attenuated or absent in the obese animals (Shore et al., 2009). Recent toxicological studies provided some evidence that ozone may impair metabolism and affect body weight, BMI, and body composition, as well as effect caloric intake. More detailed information on these studies is contained in the evidence inventory (Table 5-8).

- Male Brown Norway rats exposed to ozone consumed more food and water than did air control animals. High fat and high fructose were included in diet to determine whether animals with ozone exposure on different diets had similar eating patterns (0.8 ppm ozone, 4 day/week for 3 weeks). Ozone exposure caused males on the control and high-fat diets to eat statistically significantly more food and trended toward statistically significant increases on high fructose diet (Gordon et al., 2016). Ozone exposure caused males on normal diet and high-fructose diets to drink statistically significantly more water. Ozone exposure caused animals on high-fat diets to statistically significantly increase caloric intake compared with filtered-air controls on a high-fat diet. Females were refractory to ozone-dependent changes. Other rodent strains developed metabolic syndrome with high-fat or high-fructose diets, but Brown Norway rats less susceptible to this.
- A diabetic mouse model (male KKAy mice, 0.5 ppm ozone for 13 consecutive weekdays) provided evidence for reduced body-weight gain with ozone exposure (Ying et al., 2016). KKAy mice are diabetic, obese, and have severe hyperglycemia and insulin resistance at baseline; they are a genetic model of obese type 2 diabetes, as described above. Reduction in body weight gain has also been noted in healthy nondiabetic rats exposed to ozone (Henriquez et al., 2018).
- Obesity is a risk factor for the development of type 2 diabetes and exercise can improve glucose tolerance. To determine the role of maternal exercise and diet on ozone's effect on glucose homeostasis and obesity in offspring, a study was conducted with multiple diet and exercise options. A control (CD) or high-fat diet (HF) with or without exercise (run wheel, RW) was provided to pregnant dams creating four exposure groups (CD, CD-RW, HF, and HF-RW). The dams on the high-fat diet weighed more at the onset of pregnancy versus control dams and produced offspring that weighed more at weaning (PND 27) but not in adulthood (PND 133), independent of exercise status. When these offspring were challenged with ozone in adulthood (0.8 ppm 4 hours/day, PND 161−162), baseline glucose levels in ozone-exposed males were increased; females were refractory at baseline. Male and female offspring in all four exposure groups were statistically significantly glucose intolerant at one or two time points over the 2-hour glucose tolerance test when compared to filtered-air animals on the same diet and exercise regimen(Gordon et al., 2017a). Glucose area under the curve during the glucose tolerance test

was not measured, and comparisons were not made across groups. With a glucose challenge in the glucose tolerance test, male and female animals on various diets, whether exercising or sedentary had significantly elevated glucose versus air exposed animals; baseline glucose levels were only elevated in male ozone-exposed animals.

- Genetically obese mice (dg/db) that were exposed short-term to ozone showed increased pulmonary inflammation after ozone exposure (2 ppm ozone, 3 hours) compared with lean, wild-type mice with mechanistic contribution from IL-17a and gastrin releasing peptide receptor (Mathews et al., 2018). It was conclude that the type 2 inflammatory/cytokine reaction that contributed to increased effects of ozone in obese mice may be driven by IL-33 (Mathews et al., 2017b). Further work examined how the metabolome differed between these lean and obese mice and explored those differences with ozone exposure [2 ppm for 3 hours; Mathews et al. (2017a)]. The lung metabolomes of the lean versus the obese mice differed at baseline, and pathways like the glutathione pathway were differentially altered with ozone exposure. More information on these studies is included in Appendix 3—Respiratory Health Effects.

In summary, ozone exposure changes eating patterns in control rodents on various diets, leading males to eat more food and drink more water. Ozone induces glucose intolerance in multiple animal models independent of diet (high-fat or control diet) or exercise status (exercising or sedentary). In one genetic model of severe type 2 diabetes and overweight status, ozone exposure caused the animals to lose weight as is seen with acute ozone exposure in healthy nonobese animals. Obese and diabetic animals have a different pulmonary inflammatory response to ozone than lean animals.

5.1.5 Other Indicators of Metabolic Function

5.1.5.1 Inflammation

It is widely believed that inflammation plays a critical role in the development of type 2 diabetes and atherosclerosis leading to CHD. As outlined in the Section 5.1.3 (Biological Plausibility), inflammation may promote a peripheral inflammatory response in organs and tissues, such as liver and adipose tissues. The role of systemic inflammation after acute ozone exposure may be seen in some but not all strains of rodents used in animal toxicology studies. The role of systemic inflammation in ozone exposure is covered in Section 4.1.11, of the Cardiovascular appendix. New evidence for peripheral inflammation in adipose tissue following short-term exposure to ozone is presented below.

5.1.5.1.1 Animal Toxicological Studies

Inflammatory markers in adipose tissue are significantly elevated with ozone exposure in obese and diabetic animals. Specific examples are detailed below and in the evidence inventory tables that follow (Table 5-10). The inflammatory effects of ozone reach peripheral tissue like adipose.

- Obesity-prone mice (adult male KK mice) were exposed to ozone for 13 consecutive weekdays [4 hours/day; Zhong et al. (2016)]. Epididymal adipose showed significantly increased inflammation (increased monocytes/macrophages), increased expression of the chemokine CXC-11, and significant increases in inflammatory gene expression (Ifn-g, IL-12, iNOS, cd56). Ozone exposure to obesity-prone mice leads to increased visceral adipose inflammation as measured by multiple aforementioned biomarkers.
- Inflammatory and oxidative stress biomarkers (Tnf-α, Mcp-1) were upregulated and anti-inflammatory genes were downregulated (IL-10) in epicardial and perirenal adipose tissue in rats (8-week-old Male Sprague-Dawley rats were fed a normal diet [ND] or high fructose diet [HFr] for 8 weeks) exposed to ozone [0.5 ppm, 8 hours/day, 5 days/week, for 9 days over 2 weeks; Sun et al. (2013)]. There was significantly increased infiltration of macrophages that was associated with increased expression of tumor necrosis factor α and iNOS.
- Inhalation exposure to ozone increased proinflammatory macrophages in adipose tissue of a diabetic mouse model [male KKAy mice, 0.5 ppm ozone for 13 consecutive weekdays; Ying et al. (2016)].

A limited number of animal toxicological studies provide additional evidence that short-term exposure to ozone may result in inflammation of the visceral or perirenal adipose tissue, which is particularly relevant to metabolic function and a risk factor for metabolic syndrome.

5.1.5.2 Liver Outcomes

The liver, which is between the portal and systemic circulation, is the site for primary energy and xenobiotic metabolism (Boron and Boulpaep, 2017). The liver is a crucial organ for the maintenance of glucose homeostasis. It can be stimulated to increase blood glucose by inducing gluconeogenesis during fasting or to store glucose after feeding. The liver can also synthesize and degrade protein, carbohydrates, and lipids for distribution to extrahepatic tissues depending on energy needs. Finally, the liver regulates whole-body cholesterol balance via biliary excretion of cholesterol, cholesterol conversion to bile acids, and by regulating cholesterol synthesis (Boron and Boulpaep, 2017). The liver is also the site of generation of ketone bodies, which are a biomarker for diabetes, because the diabetic body can switch to using fats as its fuel source. Consequently, the liver is an essential regulator of whole-body metabolism and energy homeostasis.

Acute-phase liver proteins, such as CRP, can act as sensors of liver function and are discussed in more detail in Appendix 4, Section 4.1.11. An epidemiologic study found associations between CRP, a protein that is produced in response to acute systemic inflammation, and ozone exposure. These proteins, in combination with other liver enzymes can give information about overall health, including liver function.

5.1.5.2.1 Controlled Human Exposure Studies

No controlled human exposure studies of liver outcomes were evaluated in the 2013 Ozone ISA. One liver outcome measured in humans is ketone body formation; more information is available in the evidence inventory (Table 5-6). Ketone body formation is a biomarker for diabetes and ketone bodies are formed by the liver from fatty acids as a result of gluconeogenesis. In one recent controlled human exposure study, healthy adult human volunteers exercised intermittently and were exposed separately to ozone and fresh air during two visits to the clinic (2 hours at 0.3 ppm ozone or fresh air exposure with 15 minute on/off exercise in a controlled chamber). Ozone exposure was associated with increased carnitine conjugates of long-chain FFA and acetyl carnitine suggestive of accelerated β-oxidation and increased ketone body generation(Miller et al., 2016a).

5.1.5.2.2 Animal Toxicological Studies

Ozone exposure in animal models impacts various pathways that are mediated through the liver, including increasing hepatic glucose production through gluconeogenesis (pyruvate tolerance test), decreasing bile acid production, altering gut microbiome, impairing glycolytic pathways, altering β-oxidation, and altering expression of hepatic metabolism-related genes in the liver. More detailed information on how ozone exposure affects metabolic outcomes in the liver follows below, but like other pathways, the liver contributes to increased blood glucose with ozone exposure.

- Ozone induced hyperglycemia, impaired glucose tolerance, and altered cholesterol after 1 or 2 days of ozone exposure [Wistar Kyoto rats or Brown Norway rats, 6 hours/day, 0, 0.25, 0.5, or 1.0 ppm ozone; Miller et al. (2015); Bass et al. (2013)], and pathways that may contribute to this in the liver were delineated with metabolomic analysis of serum. Bile acids are made in the liver from cholesterol, further processed by the gut microbiome, and released to the intestine to facilitate absorption of dietary fat. Serum cholesterol and bile acid metabolites were significant decreased by ozone exposure. Ozone increased circulating free fatty acids. Ozone also impaired glucose homeostasis by perturbing glycolytic pathways (decreased anhydro glucitol [a biomarker of glycemic control], increased fructose levels, increased pyruvate [Day 1], and decreased lactate [Day 2]-glycolysis/glycolytic pathways). Mitochondrial β-oxidation metabolites were reduced with ozone exposure (Miller et al., 2015). This metabolomic analysis demonstrates multiple pathways that are affected by ozone exposure.
- Acute exposure of male Wistar Kyoto Rats to 1 ppm ozone (5 hours/day for 1 day) resulted in hyperglycemia (Miller et al., 2016b). To determine whether this ozone-dependent hyperglycemia was controlled by liver gluconeogenesis, a pyruvate tolerance test (PTT) was performed where pyruvate was injected and blood glucose was measured over time. The PTT showed statistically significant increased blood glucose with ozone exposure (1.0 ppm) compared with filtered-air controls, confirming the stimulation of gluconeogenesis with ozone exposure. Further, at 1.0 ppm ozone, glucose AUC was statistically significantly increased, confirming these findings.
- Short-term exposure to ozone (8 hours/day for 5 days to male Sprague-Dawley rats) is associated with altered expression of certain proteins in the liver that can modulate hepatic metabolic function, including glucose-regulated protein 78 or GRP-78 (post-translationally modified

GRP-78 is a novel autoantigen in human type 1 diabetes), protein disulfide isomerase, and glutathione S-transferase M1 (Theis et al., 2014).

5.1.5.2.3 Summary

Multiple metabolic indicators from the liver provide evidence that ozone exposure induces changes within the liver, affecting glucose homeostasis. Healthy volunteers who exercised with ozone exposure in controlled human exposure studies had increased ketone body formation. In animal toxicological studies, ozone exposure induced changes to the liver including hepatic gluconeogenesis, altered bile acid profile, alterations to β-oxidation, and alterations to proteins in hepatic metabolic pathways.

5.1.5.3 Endocrine Hormones

Ozone exposure activates the autonomic sensory pathway, which triggers central neuroendocrine stress response including responses like increased corticosterone, cortisol, or epinephrine (Snow et al., 2018). Ozone acts as a pulmonary irritant and stimulates nasopharyngeal and pulmonary nerves and receptors, including the trigeminal and vagal nerves, which induces downstream effects to the autonomic nervous system and increases the levels of epinephrine (Snow et al., 2018). The hypothalamus and adrenals are activated with ozone exposure, and removal of the adrenal pathway with adrenalectomy or pharmacologically can ameliorate the ability of ozone to induce metabolic homeostatic changes in rodents.

5.1.5.3.1 Epidemiologic Studies

No epidemiologic studies in the 2013 Ozone ISA assessed the association between short-term ozone exposure and endocrine hormones. As noted in Table 5-9, one recent study evaluated the association between short-term ozone exposure and endocrine hormones. Using 5,958 participants from the Framingham Offspring Cohort and Third Generation Cohort, Li et al. (2017) completed a panel study evaluating adiponectin, leptin, and resistin over a 1- to 7-day moving avg. Based on the published qualitative results, there were no changes due to short-term exposure to ozone, but adiponectin had a positive trend and resistin had a negative trend.

5.1.5.3.2 Controlled Human Exposure Studies

No controlled human exposure studies of short-term ozone and endocrine hormones were reviewed in the 2013 Ozone ISA. One recent study (Table 5-6) used healthy adult human volunteers who

were intermittently exercised and exposed separately to ozone and fresh air during two visits to the clinic (2 hours, 0.3 ppm ozone or fresh air exposure with 15 minute on/off exercise in a controlled chamber). Acute ozone exposure increased circulating stress hormones (cortisol and corticosterone) in these volunteers (Miller et al., 2016a).

5.1.5.3.3 Animal Toxicological Studies

Ozone exposure activates the hypothalamic-pituitary adrenocortical stress pathway and its associated release of stress hormones into the circulation (adrenaline, epinephrine, and cortisol/corticosterone) in animal studies. Ozone also affects the hormones leptin and ghrelin, which are related to energy balance and hunger/satiety control. Specific details of these studies are included in the Evidence Inventory (Table 5-10).

- Circulating adrenaline, epinephrine, and cortisol/corticosterone are significantly increased in laboratory animals after acute ozone exposure (Henriquez et al., 2017a; Henriquez et al., 2017b; Miller et al., 2016c; Miller et al., 2016a; Miller et al., 2015; Bass et al., 2013; Thomson et al., 2013). Removal of input from the adrenals or the adrenal medullary system significantly ameliorates or attenuates the metabolic effects of ozone exposure, respectively (Henriquez et al., 2017a; Henriquez et al., 2017b; Miller et al., 2016c).
- In healthy rodent models (Thomson et al., 2016), the adrenocorticoid axis's contribution to ozone-induced metabolic changes was monitored using metyrapone, a glucocorticoid synthesis inhibitor. Ghrelin was statistically significantly decreased with ozone exposure, but pretreatment with metyrapone did not alter the effect of ozone on ghrelin. Thus, ghrelin is significantly decreased with ozone exposure, and this effect is independent of modification of the adrenocortical pathway. Likewise; ozone-induced hypothermia which might be linked to global metabolic changes was also not prevented by adrenalectomy (Henriquez et al., 2017a) suggesting that multiple neuroendocrine mechanisms might be altered after ozone exposure.
- In healthy rodent models, short-term ozone exposure was associated with either elevated serum leptin (Miller et al., 2015; Bass et al., 2013; Sun et al., 2013) or a trend toward increased leptin (Gordon et al., 2017b). In obese animals (Zhong et al., 2016) and diabetic animals (Ying et al., 2016), there was significantly decreased serum leptin with ozone exposure. Thus, healthy and diseased animal models have significantly different leptin responses to ozone exposure or the temporality differences between studies might explain the directionality differences.

5.1.5.3.4 Summary

Recent evidence shows that neuroendocrine activation is essential to the perturbed metabolic pathways that develop after ozone exposure. Elevated circulating stress hormones are consistently observed in animal models and in controlled human exposure studies after short-term ozone exposure. Removal of the neuroendocrine input by surgically removing the adrenal glands removes the neuroendocrine stress activation, ameliorates the stress hormone response and attenuates glucose intolerance and other factors that contribute to metabolic syndrome in rodents exposed to ozone. Thus,

neuroendocrine stress activation is essential to the development of adverse metabolic outcomes after short-term ozone exposure.

5.1.5.4 Serum Lipids

In the 2013 Ozone ISA, one epidemiologic study provided evidence of ozone exposure association with altered blood lipids. Chuang et al. (2010) conducted a population-based cross-sectional analysis of data collected on 7,578 participants during the Taiwanese Survey on Prevalence of Hyperglycemia, Hyperlipidemia, and Hypertension in 2001. Apolipoprotein B (ApoB), as a lipid carrier which transports triglycerides and cholesterol around the body, was associated with 3-day avg ozone concentration. The 5-day mean ozone concentration was associated with increased fasting glucose levels and triglycerides. In addition, the 1-, 3-, and 5-day mean ozone concentrations were associated with increased HbA1c levels (a marker used to monitor the degree of control of glucose metabolism). No association was observed between ozone concentration and ApoA1. Recent studies support the findings that ozone exposure is associated with changes to serum lipids in animal and human studies.

5.1.5.4.1 Epidemiologic Studies

As noted above, Chuang et al. (2010) reported associations between altered serum lipids and short-term ozone exposure. Since then, one epidemiologic study (Table 5-9) evaluated the effects of short-term ozone exposure on blood lipids. Chen et al. (2016a) used the β-Gene cohort of 1,023 Mexican Americans living in southern California. The study considered LDL levels and HDL-to-LDL ratios. The study used cumulative averages of daily concentrations from 0−90 days prior to testing. No outcomes were reported for any metabolic endpoints evaluated with short-term increases of ozone exposure. Results were presented qualitatively.

5.1.5.4.2 Controlled Human Exposure Studies

In the 2013 Ozone ISA, no controlled human exposure studies evaluated short-term ozone and serum lipids. As indicated in Table 5-6, there is one study of healthy adult human volunteers (n = 24) who were exercised intermittently and exposed separately to ozone and fresh air during two visits to the clinic (2 hours, 0.3 ppm ozone or fresh air exposure with 15 minutes on off exercise in a controlled chamber). Ozone exposure was associated with increased medium and long-chain FFA and plasma glycerol consistent with enhanced lipolysis (Miller et al., 2016a).

5.1.5.4.3 Animal Toxicological Studies

Animal toxicology studies demonstrate that ozone exposure induces changes in serum lipids including cholesterol, free fatty acids, and triglycerides. Animals exposed to ozone show increased serum triglycerides, elevated free fatty acids, and altered serum cholesterol levels. The majority of the studies mentioned below use male rodents and some study these outcomes in rodents that are obese, diabetic, or have cardiovascular disease (CVD). More specific information follows below and detailed study design can be found in the table that follows (Table 5-10).

- Ozone exposure to rodents can alter serum lipids, and this may differ by strain of rodent or by rodent disease model (Ramot et al., 2015). Some rodent models have significantly elevated serum lipids before ozone challenge at baseline versus other rodents, especially the rodent models of CVD, diabetes, or obesity.
- Ozone exposure has been associated with changes to serum triglycerides. In healthy brown Norway rats, ozone induced increased serum triglycerides in a dose-dependent manner with increasing age of the animal; the statistically significant triglyceride changes were highest in the oldest animals exposed to ozone (1-, 4-, 12-, and 24-month-old males) which was measured immediately after an exposure of 6 hours/day for 2 days to 1 ppm ozone (Bass et al., 2013). In healthy animals, ozone-induced statistically significantly increased triglycerides were ameliorated with adrenalectomy or demedularization of the adrenal glands, which removes the section of the adrenal gland that produces stress hormones [Wistar Kyoto rats, 1.0 ppm ozone, 4 hours/day for 2 days; Miller et al. (2016c)]. In animal models of CVD, ozone exposure increased serum triglycerides [0.3 ppm ozone, 3 hours, 1 day, 12-week-old male spontaneously hypertensive (SH) rats; Farraj et al. (2016)].
- Ozone exposure can alter serum cholesterol. In healthy animal models, ozone exposure (1.0 ppm, 6 hours/day for 2 days, 10-week-old male Wistar Kyoto rats) resulted in statistically significantly increased LDL cholesterol (Miller et al., 2015). In animal models of CVD, ozone induced statistically significant decreases in HDL cholesterol [0.8 ppm ozone, 4 hours; 12-week-old male spontaneously hypertensive rats; Farraj et al. (2012); 1.0 ppm ozone, obese FHH rats, and obese diabetic JCR rats Ramot et al. (2015)]. But some animals are refractory to cholesterol changes with ozone exposure; ozone exposure did not significantly affect HDL or LDL cholesterol [0.3 ppm ozone, 3 hours, 1 day exposure of 12-week-old male SH rats; Farraj et al. (2016)], or HDL cholesterol [0.2 ppm ozone, 4 hours, 12-week-old male SH rats; Farraj et al. (2012)]. In CVD animal models, LDL cholesterol was statistically significantly decreased with a greater ozone exposure [SH rats, 0.5 and 1.0 ppm ozone, 4 hours; Ramot et al. (2015)]. Ozone exposure alters serum cholesterol in multiple animal models.
- Ozone exposure affects serum lipids immediately after exposure and can continue to have more prolonged effects after a period of recovery. After a period of recovery from ozone exposure, healthy animals had statistically significantly increased LDL [male WKY rats, 20 hours recovery after 4 hours 1.0 ppm ozone; Ramot et al. (2015)]. Effects in healthy rodents of different ages (male brown Norway rats; 1-, 4-, 12-, and 24-month-old males, 6 hours/day for 2 consecutive days, 1 ppm ozone) included statistically significantly increased levels of serum HDL in 12-month-old animals with 1 ppm ozone exposure compared with filtered-air control measured after 18 hours of recovery from ozone exposure; all other endpoints (HDL, LDL, and total cholesterol), ages, and doses (0.25 ppm ozone) were refractory to change (Bass et al., 2013). Animals with CVD maintained altered cholesterol levels, including statistically significantly decreased HDL [0.5 and 1.0 ppm ozone 4 hours, diabetic obese male JCR rats; 1.0 ppm 4 hours

ozone, obese male FHH rats, 20 hours recovery after ozone exposure; Ramot et al. (2015)], and statistically significantly increased total cholesterol [male SH rats, 4 hours exposure to 1.0 ppm ozone, 20 hours recovery; Ramot et al. (2015)]. In healthy animals, there were significant increases in all types of cholesterol measured [total, LDL and HDL; 1.0 ppm, 5 hours/day for 1 or 2 days, 10-week-old male Wistar Kyoto rats, measured 18 hours after exposure; Miller et al. (2015)]. These studies show that cholesterol does not recover to baseline levels after recovery from ozone exposure in these animals.

- The effect of exercise on ozone-dependent changes in serum lipids was examined in female LE rats, specifically, the role of exercise training (active vs. sedentary lifestyle) in its contribution to cholesterol changes after a 1-day ozone challenge. Rats exercised or remained sedentary from weaning to age 10 weeks, whereupon they were exposed to ozone (0.8 ppm ozone, for 5 hours) and their cholesterol levels measured (Gordon et al., 2017b). All serum cholesterol measurements showed no significant changes in cholesterol with ozone exposure (total cholesterol, HDL). Most studies of the effects of ozone on serum lipids were conducted in male animals. Thus, female LE rats were refractory to ozone-dependent changes in serum cholesterol. Interestingly, there was a statistically significant decrease in running wheel activity the night after ozone exposure, demonstrating changed behavior after ozone exposure.
- The effect of high-fat and high-fructose diets was tested in male brown Norway rats. With ozone exposure (0.8 ppm ozone, 4 days/week for 3 weeks), there was significantly decreased serum cholesterol in animals on control diet, an effect which was ameliorated with high-fat or high-fructose diets (Gordon et al., 2016). In fact, ozone induced statistically significantly increased cholesterol in male animals on the high-fat diet versus high fat filter air controls. Serum triglycerides were significantly increased in ozone-exposed male rodents on the control or high-fat diets versus filter air controls. Females were refractory to change.

5.1.5.4.4 Summary

Multiple studies provide additional evidence that short-term exposure to ozone may result in altered lipid homeostasis (cholesterol and triglycerides). Additionally, increases in free-fatty acid release into the circulation, an indicator that the body has shifted toward using fats as its source of fuel in place of glucose, as can be seen in diabetics, demonstrates neuroendocrine activation with ozone exposure. In animal toxicology studies, removal of the neuroendocrine activation by adrenalectomy ameliorates the ozone-dependent dyslipidemia. Ozone exposure induced metabolic changes in humans and animals, including neuroendocrine activation and altered lipid metabolism, which is particularly relevant to metabolic function and a risk factor for metabolic syndrome, especially with chronic exposure.

5.1.5.5 Blood Pressure

Short-term ozone exposure mediated effects on blood pressure are discussed in detail in the Cardiovascular Appendix (see Appendix 4) (Akcilar et al., 2015; Wagner et al., 2014; Gordon et al., 2013; Uchiyama and Yokoyama, 1989; Uchiyama et al., 1986). Hypertension is a clinically relevant consequence of chronically high blood pressure, which typically develops over years. High blood pressure, dyslipidemia, increased fasting blood glucose, and obesity are criteria for metabolic syndrome,

which is a risk factor for heart disease, stroke, and diabetes. Recent epidemiologic evidence and human exposure evidence is limited in number and generally inconsistent. Animal toxicological studies show some evidence to suggest that short-term exposure to ozone can result in changes in blood pressure in animals. However, some results also suggest that changes in diet may mediate these effects (see Section 4.1.8).

5.1.6 Potential Copollutant Confounding of the Ozone-Metabolic Effects Relationship

Few studies have examined potential short-term ozone exposure copollutant confounding with $PM_{2.5}$ or PM_{10}, or gaseous copollutants. When associations were noted, the association with ozone remained, and with null associations, the null effect also remained. This suggests that these findings may not be substantially impacted by copollutant confounding.

- Kim and Hong (2012) analyzed the KEEP cohort consisting of 560 Koreans over 60 years old and observed increases in fasting glucose (0.19%; 95% CI: 0.09, 0.28%). Copollutant models of NO_2 and PM_{10} were also evaluated. The associations with glucose remained after adjustment for NO_2 (0.16%; 95% CI: 0.06, 0.25%) and PM_{10} (0.15%; 95% CI: 0.01, 0.14%).
- One study evaluated hospital admissions for diabetic ketoacidosis and diabetic coma in Chile (Dales et al., 2012). Using a 6-day distributed lag for ozone, a weak, positive association was observed for the risk of hospital admissions for diabetic ketoacidosis or diabetic coma in the greater Santiago area (RR: 1.02; 95% CI: 1.00, 1.04). The effect remained relatively unchanged when divided into subregions of Santiago and when the model added CO, PM_{10}, $PM_{2.5}$, and SO_2.

5.1.7 Effect Modification of the Ozone-Metabolic Effects Relationship

5.1.7.1 Lifestage

The 1996 and 2006 Ozone AQCDs identified children, especially those with asthma, and older adults as at-risk populations (U.S. EPA, 2006, 1996). In addition, the 2013 Ozone ISA confirmed that there was adequate evidence to conclude that children and older adults are at increased risk of ozone-related health effects (U.S. EPA, 2013). Collectively, the majority of evidence for older adults has come from studies of short-term ozone exposure and mortality. A couple of recent studies of short-term ozone exposure and metabolic effects compared associations between different age groups. One epidemiologic study did not report consistent evidence that older adults are at increased risk for metabolic effects; however, the animal toxicology study did see greater effects in aged animals.

- One study evaluated associations of short-term ozone exposure and hospital admissions for diabetic ketoacidosis and diabetic coma in the Santiago region of Chile (Dales et al., 2012). Using

a 6-day distributed lag, a null association was observed for the relationships of hospital admissions for diabetic ketoacidosis or diabetic coma (1.02; 95% CI: 1.00, 1.04). However, the effect increased in populations aged 75−84 (1.08; 95% CI: 1.01,1.15) and over 85 years (1.08; 95% CI: 1.01,1.1). While increases were noted in the higher age brackets, the risks were not higher in other age groups (<64 or 65−74).

- Gordon et al. (2013) compared young Brown Norway rats (4 months of age) to aged or senescent rats (20 months of age). With ozone exposure, both age groups had significant metabolic responses, including increased triglycerides and serum insulin, but the response was greater in the aged animals. Ozone induces glucose intolerance in young and aged brown Norway rats (Bass et al., 2013). Ozone-induced elevated blood glucose area under the curve is increased in an age-dependent manner in rats with the greatest glucose elevation seen in the oldest animals (age 1, 4, 12, and 24 months).

5.1.7.2 Pre-existing Disease

Individuals with certain pre-existing diseases may be considered at greater risk of an air pollution-related health effect because their health is compromised depending on the type and severity of their disease. The 2013 Ozone ISA concluded that there was adequate evidence for increased ozone-related health effects among individuals with asthma (U.S. EPA, 2013). The results of controlled human exposure studies, as well as epidemiologic and animal toxicological studies, contributed to this evidence. Studies of short-term ozone exposure and mortality provided limited evidence for stronger associations among individuals with pre-existing cardiovascular disease or diabetes.

A limited number of recent studies provides some evidence that individuals with pre-existing diseases may be at greater risk of cardiovascular health effects associated with short-term ozone exposure. These studies focus on specific diseases of varying severity (e.g., previous CVD events, type 2 diabetes). Specifically:

- Kim and Hong (2012) found increases in fasting glucose (0.19; 95% CI: 0.09, 0.28), insulin (0.71%; 95% CI: 0.02, 1.38%), and HOMA (0.30%; 95% CI: 0.06, 0.53%) in the Korean Elderly Environmental Panel (KEEP). The KEEP cohort consisted of 560 Koreans over 60 years old. The association of 5-day avg ozone concentration with glucose, insulin, and HOMA-IR was approximately threefold larger in people with a previous diagnosis of type 2 diabetes (glucose [0.68%; 95% CI: 0.28, 1.07%], insulin [2.76%; 95% CI: 0.78, 4.75%], and HOMA [1.21%; 95% CI: 0.44, 1.99%]). In subjects without type 2 diabetes, an association with glucose was observed (0.09%; 95% CI: 0.02, 0.16%), while associations with insulin and HOMA were not found in those without pre-existing type 2 diabetes.

  Animal toxicological studies with animal models of cardiovascular disease with or without obesity have shown differences in sensitivity to ozone in terms of circulating triglycerides and cholesterol (Ramot et al., 2015) and changes in transcriptional profile of the lung metabolic pathways indicating animal model and disease specific expression signatures at baseline and after ozone exposure in rat models of obesity with or without CVD (Ward and Kodavanti, 2015).

5.1.8 Summary and Causality Determination

There were no causality conclusions for metabolic effects in the 2013 Ozone ISA (U.S. EPA, 2013). The literature pertaining to outcomes from short-term exposure to ozone and metabolic effects has expanded substantially since the 2013 Ozone ISA (U.S. EPA, 2013), with multiple epidemiologic and experimental studies and a few human clinical studies currently available for review. Findings from animal toxicological studies of metabolic effects showed short-term ozone exposure impaired glucose and insulin homeostasis (glucose intolerance, hyperglycemia, dyslipidemia of triglycerides, altered blood pressure, impaired β-cell function, increased hepatic gluconeogenesis, and neuroendocrine activation contributing to altered metabolic function). Controlled human exposure to ozone in exercising participants confirmed activation of the neuroendocrine system and showed formation of ketone bodies, a biomarker of diabetes. Previous epidemiologic evidence demonstrates elevated HbA1c (a biomarker of diabetes and an indicator of the degree of glycemic control in diabetics), increased triglycerides, altered serum cholesterol, increased HOMA-IR, and fasting glucose level instability associated with short-term ozone exposure. The information pertaining to the relationship between short-term exposure to ozone and metabolic effects is summarized in Table 5-1, using the framework for causality determinations described in the Preamble to the ISAs (U.S. EPA, 2015).

The strongest evidence for an effect of short-term exposure to ozone on metabolic effects is provided by animal toxicological studies that show impaired glucose tolerance, increased triglycerides, fasting hyperglycemia, decreased insulin, and increased hepatic gluconeogenesis in various strains of animals across multiple labs following short-term exposure to ozone. Biological plausibility is indicated by results from controlled human exposure studies and animal studies that show ozone activates the autonomic sensory pathway, which triggers central neuroendocrine stress response including responses like increased corticosterone, cortisol or epinephrine, as noted in the controlled human exposure study. Ketone body formation, a biomarker of diabetes, is induced in controlled human exposure studies with ozone exposure. This begins when ozone acts as a pulmonary irritant and stimulates nasopharageal and pulmonary nerves and receptors including the trigeminal and vagal nerves, which induces downstream effects on the autonomic nervous system. The sympathetic adrenal-medullary (SAM) and hypothalamus-pituitary-adrenal (HPA) axes are activated with ozone exposure and removal of the adrenal pathway with adrenalectomy or pharmacologically can ameliorate the ability of ozone to induce metabolic syndrome in rodents. Despite limited epidemiologic and controlled human exposure literature, the expanding animal toxicological literature shows robust evidence of short term ozone exposure contributing to impairment of glucose and insulin homeostasis. These findings are coherent with epidemiologic studies that report associations with perturbations to glucose and insulin homeostasis with ozone exposure. **Overall, the collective evidence is sufficient to conclude that a likely to be causal relationship exists between short-term ozone exposure and metabolic effects**.

Table 5-1 Summary of evidence for a likely to be causal relationship between short-term ozone exposure and metabolic effects.

Rationale for Causality Determination	Key Evidence	Key References	Ozone Concentrations Associated with Effects
Consistent animal toxicological evidence from multiple, high quality studies at relevant ozone concentrations	Animal toxicological studies of impaired glucose tolerance, fasting hyperglycemia, dyslipidemia, hepatic gluconeogenesis, and activation of the neuroendocrine pathway with ozone exposure	Section 5.1. Miller et al. (2016c), Miller et al. (2015) Miller et al. (2016b), Thomson et al. (2018)	0.25−1 ppm
	Animal toxicological evidence of increased inflammation	Ying et al. (2016); Zhong et al. (2016); Sun et al. (2013)	0.25−1 ppm
	Animal toxicological evidence of dyslipidemia	Bass et al. (2013), Farraj et al. (2012), Farraj et al. (2016), Gordon et al. (2016), Miller et al. (2016c), Ramot et al. (2015)	0.25−1 ppm
	Animal toxicological evidence of liver-mediated effects	Miller et al. (2016b); Miller et al. (2015); Theis et al. (2014)	0.25−1 ppm
Consistent epidemiologic evidence of increased risk diabetes or metabolic syndrome	Epidemiologic evidence for positive associations between short-term ozone exposure and increased indicators of impaired glucose and insulin homeostasis, including HOMA-IR, dyslipidemia, elevated HbA1c, and increased fasting glucose	Chuang et al. (2011)	Section 5.1.4.1
Limited epidemiologic evidence from case-crossover and panel studies of metabolic endpoints	Limited number of studies with generally null associations (glucose, HOMA-IR, Insulin) observed among populations with or without pre-existing disease	Li et al. (2017); Chen et al. (2016b); Dales et al. (2012); Kim and Hong (2012)	Section 5.1.3.1
Controlled human exposure evidence of increased metabolic changes with ozone exposure at relevant concentrations	A limited number of studies observed ketone body formation and neuroendocrine system activation with ozone exposure	Miller et al. (2016a)	0.3 ppm

Table 5-1 (Continued): Summary of evidence for a likely to be causal relationship between short-term ozone exposure and metabolic effects.

Rationale for Causality Determination	Key Evidence	Key References	Ozone Concentrations Associated with Effects
Limited epidemiologic evidence from copollutant models provides some support for an independent ozone association	The magnitude of ozone associations remains relatively unchanged in a limited number of studies evaluating copollutant models, including $PM_{2.5}$ and other gaseous pollutants	Kim and Hong (2012) Dales et al. (2012)	Section 5.1.6
Biological plausibility	Experimental studies provide evidence of metabolic syndrome mediated by pulmonary irritant receptor stimulation and activation of the neuroendocrine system with short-term ozone exposure provides biological plausibility to the effects of ozone on metabolic syndrome and diabetes	Section 5.1.2	0.3−2.0 ppm

HbA1c = hemoglobin A1c; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; $PM_{2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 2.5 μm; ppm = parts per million.

5.2 Long-Term Ozone Exposure—Introduction, Summary from the 2013 Ozone ISA, and Scope for Current Review

Metabolic effects were not included in the 2013 Ozone ISA as a distinct section because there were limited studies evaluating the effects of long-term ozone exposure on metabolic effects. One study presented in the cardiovascular disease appendix evaluated the association between long-term exposure of ozone and effects in blood lipids and glucose homeostasis (Chuang et al., 2011); it reported increases in total cholesterol, fasting glucose, and hemoglobin A1c. Multiple experimental animal studies have evaluated ozone-mediated effects, and these studies indicate that long-term exposure to ozone may affect glucose homeostasis and factors that may contribute to metabolic syndrome.

The metabolic effects from long-term ozone exposure reviewed here include indicators of metabolic function that underlie metabolic and cardiovascular diseases. These include glucose and insulin homeostasis, adiposity, weight gain, metabolic syndrome, type 1 and 2 diabetes, and mortality from diabetes or cardiometabolic diseases. The subsections below provide an evaluation of the most policy-relevant scientific evidence relating long-term ozone exposure to metabolic effects. These sections focus on studies published since the completion of the 2013 Ozone ISA.

5.2.1 Population, Exposure, Comparison, Outcome, and Study Design (PECOS) Tool

The scope of this section is defined by a scoping tool that generally defines the relevant Population, Exposure, Comparison, Outcome, and Study Design (PECOS). The PECOS tool defines the parameters and provides a framework to help identify the relevant evidence in the literature to inform the ISA. Because the 2013 Ozone ISA did not make a causality determination for long-term ozone exposure and metabolic health effects, the epidemiologic studies evaluated are less limited in scope and not targeted towards specific study locations, as reflected in the PECOS tool. The studies evaluated and subsequently discussed within this section were identified using the following PECOS tool:

Experimental Studies:

- Population: Study populations of any controlled human exposure or animal toxicological study of mammals at any lifestage
- Exposure: Long-term (over 30 days) inhalation exposure to relevant ozone concentrations (i.e., ≤2 ppm for mammals)
- Comparison: In toxicological studies of mammals and in controlled human exposures, an appropriate comparison group that is exposed to a negative control (i.e., clean air or filtered-air control)
- Outcome: Metabolic effects

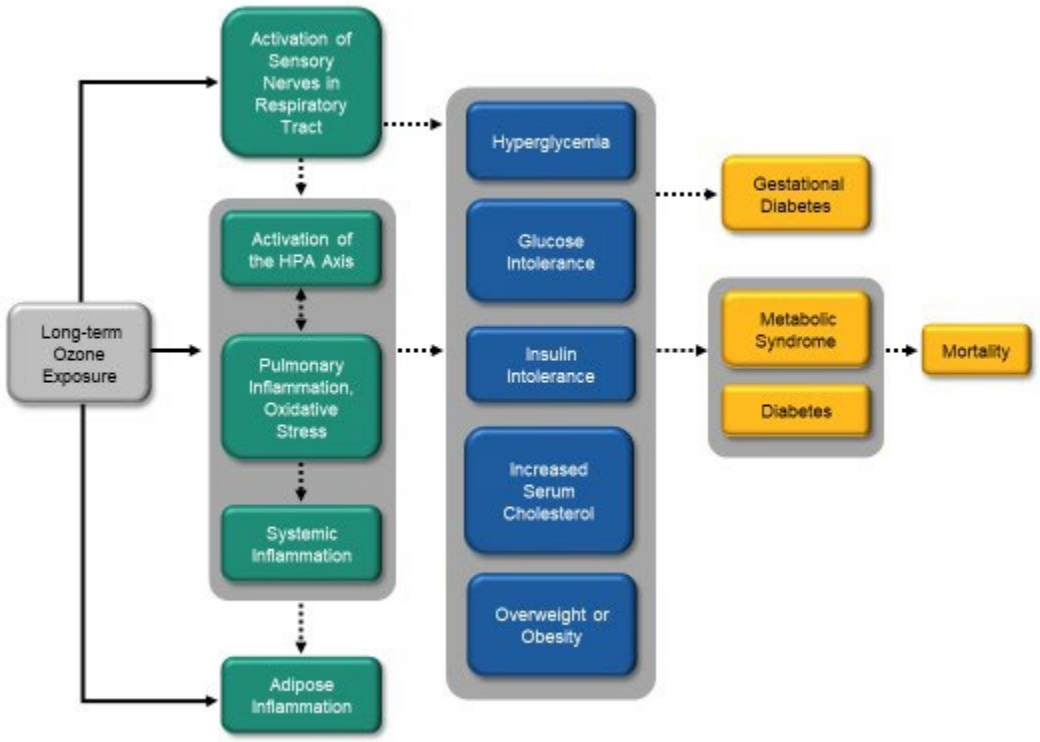
- Study Design: In vivo acute, subacute, or repeated-dose toxicity studies in mammals, immunotoxicity studies

Epidemiologic Studies:

- Population: Any population, including populations or lifestages that might be at increased risk
- Exposure: Long-term (months to years) exposure to ambient concentrations of ozone
- Comparison: Per unit increase (in ppb)
- Outcome: Change in risk (incidence/prevalence) of metabolic effects
- Study Design: Epidemiologic studies consisting of panel, case-crossover, time-series studies, and case-control studies; cross-sectional studies with appropriate timing of exposure for the health endpoint of interest

5.2.2 Biological Plausibility

This section describes biological pathways that potentially underlie metabolic effects resulting from long-term exposure to ozone. Figure 5-2 graphically depicts the proposed pathways as a continuum of upstream events, connected by arrows that may lead to downstream events observed in epidemiologic studies. This discussion of "how" exposure to ozone may lead to metabolic health effects contributes to an understanding of the biological plausibility of epidemiologic results evaluated later in Section 5.2.4.



Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence related to ozone exposure, and the arrows indicate a proposed relationship between those effects. Solid arrows denote evidence of essentiality as provided, for example, by an inhibitor of the pathway or a genetic knockout model used in an experimental study involving ozone exposure. Shading around multiple boxes is used to denote a grouping of these effects. Arrows may connect individual boxes, groupings of boxes, and individual boxes within groupings of boxes. Progression of effects is generally depicted from left to right and color-coded (gray, exposure; green, initial effect; blue, intermediate effect; orange, effect at the population level or a key clinical effect). Here, population level effects generally reflect results of epidemiologic studies. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below.

Figure 5-2 Potential biological pathways for metabolic outcomes following long-term ozone exposure.

Ozone exposure can induce irritant signaling, both nasopharyngeal and pulmonary, and activate the trigeminal and vagus nerves. Ozone exposure can also directly lead to hypothalamic-pituitary-adrenal axis activation or this pathway can be activated through irritant signaling as further described in the pulmonary appendix. The ozone-dependent activation of the HPA axis has been shown to occur with short-term ozone exposure (Miller et al., 2016c). As in the short-term controlled human exposure studies and epidemiologic studies, ozone exposure in animals activates the autonomic sensory pathway, which triggers central neuroendocrine stress response including responses like increased corticosterone, cortisol, or epinephrine. Ozone acts as a pulmonary irritant and stimulates nasopharyngeal and pulmonary nerves and receptors, including the trigeminal and vagal nerves, which induces downstream effects to the autonomic nervous system. The hypothalamus and adrenals are activated with ozone exposure and

removal of the adrenal pathway either with adrenalectomy or pharmacologically can ameliorate the ability of ozone to induce metabolic syndrome in rodents.

With long-term ozone exposure, animals develop hyperglycemia, glucose intolerance, peripheral muscle insulin resistance, decreases circulating insulin, and inhibition of glucose-dependent insulin release [Section 5.2.3; Miller et al. (2016b); Bass et al. (2013)]. Studies also show dyslipidemia (elevated triglycerides and decreased HDL cholesterol) with ozone exposure (Bass et al., 2013). Fewer animal toxicological studies exist on long-term ozone exposure but outcomes studied in both short- and long-term studies both show consistently impaired metabolic homeostasis with ozone exposure.

The animal toxicology demonstrates the pathways of disruption, provides a plausibility for the long-term adverse human health effects, including diagnosis of metabolic syndrome, diabetes, and mortality resulting from diabetes or cardiometabolic diseases.

5.2.3 Glucose and Insulin Homeostasis

Insulin is secreted by β-cells within the pancreas in response to glucose levels. When glucose levels rise, depolarization of the pancreatic β-cells or modulation by other hormones stimulate insulin secretion. Thus, during feeding, blood insulin levels rise stimulating glucose uptake and replenishing the body's fuel reserves in the form of triglycerides and glycogen. When insulin levels decrease (e.g., during fasting) fuels such as lipids from adipose tissue and amino acids from muscle are mobilized to the bloodstream where they are used by the liver to synthesize glucose. Ozone has been shown to impair glucose and insulin homeostasis in health animals. Details of these studies follow below.

5.2.3.1 Animal Toxicological Studies

The 2013 Ozone ISA did not contain information on long-term ozone exposure and metabolic effects. Since that time, several new animal toxicology studies have been published showing the effects of long-term ozone on glucose and insulin homeostasis (e.g., glucose tolerance test, insulin tolerance test fasting glucose and insulin, blood glucose and insulin levels, β-cell insulin secretion test). With long-term exposure, healthy animals develop hyperglycemia, glucose intolerance, and insulin resistance, and inhibition of glucose-dependent insulin release. Senescent animals were more sensitive to ozone-dependent serum insulin changes in a study that examined an age by ozone effect. Specific information is detailed below and in the evidence inventory tables that follow (Table 5-13).

- Subchronic ozone-induced glucose intolerance was evaluated in young and old male brown Norway rats (4, 12, and 24 months of age) exposed 2 days/week for 13 weeks [0.25 or 1.0 ppm ozone; Bass et al. (2013)]. Glucose tolerance testing was performed immediately after the last ozone exposure. Glucose tolerance was statistically significantly impaired in all age groups (1.0 ppm ozone). AUC was not measured in this study, but in the 12-month-old animals,

0.25 ozone exposure trended toward glucose intolerance with higher blood glucose levels and a longer decay to baseline compared with control animals. This study compared acute exposure (Section 5.1.2) with this subchronic exposure, and the acutely exposed animals had greater glucose intolerance than did animals with subchronic ozone exposure. Nonetheless, all of the animals exposed to ozone had impaired glucose tolerance.

- Ozone-induced glucose intolerance was followed in adult male Wistar Kyoto rats (250−300 g) after 12 weeks of ozone exposure [1.0 ppm, 5 hours/day for 3 consecutive days/week; Miller et al. (2016b)]. With ozone exposure, these animals had fasting hyperglycemia and statistically significant glucose intolerance. With the glucose tolerance test, glucose AUC was statistically significantly increased with ozone exposure versus filtered-air exposure. With the insulin tolerance test, ozone-exposed fasting animals were hyperglycemic at baseline versus air control and remained significantly elevated at the first two measurements (30 and 60 minutes after insulin injection during the insulin tolerance test but not at 1 and 2 hours), but ozone AUC was not significantly increased over control air animals, demonstrating that insulin resistance did not remain over the 2-hour time course. Ozone caused an impaired insulin response with significantly decreased serum insulin as measured with the β-cell insulin secretion test (serum insulin measurement, 30 minutes after glucose injection to fasting animals, 12 weeks after ozone exposure). Effects were not seen in animals exposed to 0.25 ppm ozone. Long-term ozone (1.0 ppm) significantly lowered circulating insulin and significantly impaired glucose-stimulated β-cell insulin secretion. Ozone-exposed animals had fasting hyperglycemia and were less able to respond to a glucose challenge.
- Permanence of effects in these same animals was tested by 1 week of recovery with filtered air after 13 weeks of ozone exposure (Miller et al., 2016b). Glucose intolerance was ameliorated 1 week after the end of ozone exposure; ozone-exposed animals were no longer hyperglycemic at baseline, and glucose tolerance testing was no different from air controls. Thus, ozone-dependent systemic metabolic change was reversible after a period of recovery or exposure to clean air.
- The effect of ozone on metabolism was assessed in aged animals versus young adult animals. Ozone-exposed aged (senescent) males had significantly increased serum insulin versus aged filtered-air controls and aged ozone-exposed animals had significantly increased insulin versus adult ozone-exposed animals [male brown Norway rats chronically exposed to ozone, 6 hours/day, 1 day/week for 17 weeks; 4-month olds or aged animals 20 months old; Gordon et al. (2013)]. In the same study, 4-month-old rodents exposed to ozone did not have significant changes in serum insulin with ozone exposure (Gordon et al., 2013). Thus, age contributes to the insulin response to ozone, with aged animals producing statistically significantly increased levels of insulin with ozone exposure, an effect not present in nonsenescent younger adult rodents.

5.2.4 Adiposity, Weight Gain, and Obesity

Adiposity (particularly visceral adiposity) and weight gain are risk factors for metabolic syndrome, type 2 diabetes, and cardiovascular disease. Although most epidemiologic studies consider BMI as a potential confounder or modifier of the association between ozone and cardiovascular disease, there were no studies of the association of long-term exposure to ozone with adiposity or weight gain reviewed in the 2013 Ozone ISA.

5.2.4.1 Epidemiologic Studies

No epidemiologic studies in the 2013 Ozone ISA evaluated the relationship between long-term ozone exposure and adiposity, weight gain, or obesity. Recent evidence is limited, but provides some evidence that long-term exposure to ozone is associated with increased weight gain and obesity (Table 5-11). Specifically:

- White et al. (2016) analyzed data from 38,374 women from the Black Women's Health Study Cohort in a prospective study of weight gain. The women lived within 56 metropolitan areas, weighed between 80−300 pounds, were under 55 years old, had not had gastric bypass surgery, and had not given birth in the previous 2 years. Ozone was estimated using the CMAQ model 8-hour max concentration for the centroid of the census tract of residence. The study used a 16-year follow-up and found no weight change associated with an increased exposure to ozone (0.23 kg; 95% CI: −0.16, 0.64) in a large cohort of African American adult women.
- Two studies evaluated the prevalence of being overweight or obese related to ambient concentrations of ozone. In a study by Dong et al. (2014), 30,056 children were recruited from seven cities in northeast China. Body mass index (BMI) was calculated according to World Health Organization (WHO) protocol and the Center for Disease Control and Prevention (CDC) definition of overweight and obese were used to categorize status. Ozone exposure was determined using the 3-year avg of the 8-hour max concentration of the monitor closest to the school children attended. Increased odds for children being overweight (OR: 1.16; 95% CI: 1.05, 1.27) or obese (OR: 1.26; 95% CI: 1.07, 1.45) were observed. Additionally, the study did not report correlations for the copollutants, making it difficult to assess the ozone-specific outcomes.
- The second study evaluated adults from the 33 Communities Chinese Health Study Cohort in participants that were 18−74 years of age and had lived in the same location for more than 5 years (Li et al., 2015). The sample included 24,845 participants and used a 3-year avg of the daily 8-hour avg exposure recorded at the monitor nearest to their home. There were increased odds of being overweight (OR: 1.08; 95% CI: 1.04, 1.12) and obese (OR: 1.09; 95% CI: 1.01, 1.18) associated with long-term exposure to ozone. Both males (1.09; 95% CI: 1.03, 1.15) and females (OR: 1.05; 95% CI: 1.00, 1.12) had increased odds of becoming overweight, while only females had increased odds of becoming obese (OR: 1.12; 95% CI: 1.01, 1.26). Similar to the other study, copollutant correlations were not reported, and both PM_{10} and SO_2 observations were high in the 33 communities, so it is difficult to estimate the level of confounding from other ambient pollutant exposures.

5.2.4.2 Animal Toxicological Studies

Elevated body weight, BMI, and adiposity are risk factors for metabolic syndrome as is dyslipidemia (altered serum cholesterol or triglycerides). The 2013 Ozone ISA contained no animal toxicological studies on these endpoints with ozone exposure. The effect of long-term exposure to ozone on body weight was studied in one recent animal toxicological study and the rodents displayed no ozone-dependent changes to body weight or body composition. Serum lipids (triglycerides and HDL cholesterol) were significantly changed with ozone exposure in aged animals [24-month-old males,

0.25 ppm ozone, 6 hours/day, 2 days/week for 13 weeks; Bass et al. (2013)], an effect not seen in younger animals with the same exposure.

- Ozone had no effect on body composition and body weight of brown Norway rats (young adult 4 months old and aged 20 months old) with long-term ozone exposure [6 hours/day, 1 day/week for 17 weeks; Gordon et al. (2013)]; also these animals displayed no changes to body composition (fat or lean mass) with ozone exposure (Gordon et al., 2013). Brown Norway rats tend to be less susceptible to metabolic changes than do some other strains of rodents.

5.2.5 Metabolic Syndrome and Type 2 Diabetes

Criteria for metabolic syndrome include high blood pressure, dyslipidemia (elevated triglycerides and low levels of high density lipoprotein [HDL] cholesterol), obesity (particularly central obesity), and increased fasting blood glucose (FBG). Table 5-2 provides the criteria for a clinical diagnosis for metabolic syndrome.

Table 5-2 Criteria for clinical diagnosis of metabolic syndrome.

Risk Factor	Threshold
Waist circumference	≥89 cm in women and ≥102 cm in males
Triglycerides[a]	≥150 mg/dL (1.7 mmol/L)
HDL-C[a]	<40 mg/dL (1.0 mmol/L in males); <50 mg/dL (1.3 mmol/L) in females
Blood pressure[b]	Systolic ≥130 and/or diastolic ≥85 mm Hg
Fasting glucose[c]	≥100 mg/dL (5.6 mmol/L)

HDL-C = HDL cholesterol; mg/dL = milligrams per deciliter; mm Hg = millimeters of mercury; mmol/L = millimoles per liter.
[a]A person taking drugs used to lower triglycerides or raise HDL-C is considered to exceed the threshold.
[b]A person taking blood pressure medication is considered to exceed the threshold.
[c]A person taking glucose-regulating medication is considered to exceed the threshold.
Source: Permission pending. Adapted from Alberti et al. (2009).

The diagnostic testing criteria for diabetes are listed in Table 5-3. The A1c, which is also known as the hemoglobin A1c, HbA1c, or glycohemoglobin, is a blood test that provides information about a person's average blood glucose over the past 3 months by measuring the percentage of hemoglobin (i.e., a blood protein with a 3-month lifespan) modified by glucose. In controlled human exposure, animal toxicological, and epidemiologic studies, the homeostasis model assessment (HOMA) has been widely used to quantify insulin resistance (HOMA-IR) and pancreatic β-cell (HOMA-β) function and used to

infer diabetes risk. The HOMA-IR index is given by the product of basal insulin and glucose levels divided by 22.5, whereas the HOMA-β index is derived from the product of 20 and basal insulin levels divided by glucose concentration minus 3.5 (Wallace et al., 2004; Matthews et al., 1985).

Table 5-3 Criteria for clinical diagnosis of diabetes.

Test	Criteria
A1c	≥6.5%a
	OR
Fasting plasma glucose (FPG)	≥126 mg/dL (7 mmol/L). Fasting is defined as no caloric intake for at least 8 h.[a]
	OR
Oral glucose tolerance test (OGTT)	2-hour plasma glucose ≥200 mg/dL (11.1 mmol/L) during OGTT. The test should be performed as described by the World Health Organization using a glucose load containing the equivalent of 75 g of anhydrous glucose dissolved in water.[a]
	OR
Random glucose test	≥200 mg/dL (11.1 mmol/L) in a person with classical symptoms of hyperglycemia or hyperglycemic crisis

mg/dL = milligrams per deciliter; mmol/L = millimoles per liter.

[a]In the absence of unequivocal hyperglycemia, Criteria 1–3 should be confirmed by repeat testing.

Source: Test criteria extracted from ADA (2014).

5.2.5.1 Epidemiologic Studies

No long-term epidemiologic studies of metabolic syndrome or type 2 diabetes were evaluated in the 2013 Ozone ISA. Recent studies, which are listed in Table 5-12, include large cohort studies around the world; they provide evidence for increased incidence for type 2 diabetes and metabolic syndrome. Specifically:

- Jerrett et al. (2017) analyzed data from the Black Women's Health Study Cohort in a prospective study of type 2 diabetes. The 43,003 women were greater than 30 years old, resided in 56 metropolitan areas, and had BMI information at baseline. Ozone was estimated using the CMAQ model 8-hour max concentration for the centroid of the census tract of residence between 2007–2008 to approximate long-term averages. The study observed increased hazard ratios for incident diabetes (1.28; 95% CI: 1.06, 1.55); however, when adjusted for NO_2, this relationship was slightly weaker and had wider confidence intervals (1.20; 95% CI: 0.96, 1.50).

- Using the Rome Longitudinal Study Cohort, Renzi et al. (2017) evaluated the effects of ozone exposure in over one million subjects over 35 years old without diabetes at baseline. The study used the Flexible Air Quality Regional Model (FARM) with a 1-km grid dispersion and 2005 seasonal ozone (May−September 8-hour avg) to predict the spatial distribution of ozone in Rome between 2008−2013. The study showed increased hazard ratios for incidence of diabetes for those living in Rome (1.01; 95% CI: 1.00, 1.02). Additionally, when the ozone model was adjusted for NO_X, the increased incidence remained significant (1.02; 95% CI: 1.00, 1.03).
- Yang et al. (2018) looked at the odds of developing metabolic syndrome due to exposure to ozone in adults from the 33 Communities Chinese Health Study Cohort in participants that were 18−74 years of age and had lived in the same location for more than 5 years. Ozone exposure was measured at municipal air monitoring stations, and the 8-hour daily mean concentrations were aggregated into a 3-year avg. In a study population of 15,477, odds of metabolic syndrome increased (1.16; 95% CI: 1.12, 1.23) according to the American Heart Association definition. The study reported high correlations of ozone with PM_{10} (0.81 ± 0.002 SD) and SO_2 (0.84 ± 0.001 SD); these high correlations provide potential for copollutant confounding, and are a source of uncertainty in estimating the direct effect of ozone on metabolic syndrome.

5.2.6 Type 1 Diabetes

Type 1 diabetes mellitus (T1D), which typically affects children and young adults, is a chronic condition that results when the pancreas fails to produce the insulin needed for glucose homeostasis. There were no epidemiologic studies of TID in the 2013 Ozone ISA. The evidence relating to the effect of long-term exposure to ozone on T1D is limited to a prospective study in Scania, Sweden [Malmqvist et al. (2015); Table 5-14]. The study evaluated prenatal exposure during first, second, and third trimesters of pregnancies for children born between 1999−2005. Ozone exposure was measured by the nearest monitoring station, averaging the 24-hour ozone concentrations and aggregating them into trimester averages. The levels were categorized in quartiles with the reference exposure being set at a level less than 22 ppb and the highest quartile exposure over 30.6 ppb. There were elevated ORs for type 1 diabetes in the highest quartile of ozone concentrations in the first trimester (1.52; 95% CI: 0.88, 2.61) and second trimester (1.62; 95% CI: 0.99, 2.65), although confidence intervals were wide. There was no evidence of association with third-trimester exposure levels.

5.2.7 Gestational Diabetes

Several studies of gestational diabetes were conducted. Generally, the results of the studies were inconsistent, although several reported positive associations with gestational diabetes or impaired glucose tolerance with ozone exposures during the second trimester. While the evidence base for gestational diabetes is growing, it is still limited to a relatively small number of studies which report generally inconsistent results (see the "Pregnancy and Birth Outcomes" section for more details [Section 7.1.3]).

5.2.8 Metabolic Disease Mortality

Studies that examine the association between long-term ozone exposure and cause-specific mortality outcomes, such as diabetes or other metabolic disease mortality, provide additional evidence for ozone-related metabolic effects, specifically whether there is evidence of an overall continuum of effects.

There were no studies that evaluated the relationship between long-term ozone exposure and mortality due to diabetes or cardiometabolic disease in the 2013 Ozone ISA. However, recent analyses from the ACS cohort in the U.S. and the CanCHEC cohort in Canada provide consistent evidence for positive associations between long-term ozone exposure and mortality due to diabetes or cardiometabolic diseases [Turner et al. (2016); Crouse et al. (2015); see Section 6.2.3.2, Figure 6-10 for more details].

5.2.9 Potential Copollutant Confounding of the Ozone-Metabolic Effects Relationship

The evaluation of potential confounding effects of copollutants on the relationship between long-term ozone exposure and metabolic effects allows for examination of whether ozone risks are changed in copollutant models. Recent studies examined the potential for copollutant confounding by evaluating copollutant models that included $PM_{2.5}$, PM_{10}, and NO_2. These recent studies help inform the extent to which effects associated with long-term ozone exposure are independent of coexposure to correlated copollutants in long-term analyses.

- Using the Rome Longitudinal Study Cohort, Renzi et al. (2017) evaluated the effects of long-term ozone exposure in over one million subjects over 35 years old without diabetes at baseline. The study showed increased hazard ratios for incidence of diabetes for those living in Rome (1.01; 95% CI: 1.00, 1.02). Additionally when the ozone model was adjusted for NO_X, the increased incidence remained (1.02; 95% CI: 1.00, 1.03).
- Jerrett et al. (2017) analyzed data from the Black Women's Health Study Cohort in a prospective study of type 2 diabetes. The authors observed increased hazard ratios for incident diabetes (1.28; 95% CI: 1.06, 1.55), however when adjusted for $PM_{2.5}$ it further increased (1.31; 95% CI: 1.08, 1.60), but when adjusted for NO_2 the estimate was slightly attenuated and less precise (1.20; 95% CI: 0.96, 1.50).

5.2.10 Effect Modification of the Ozone-Metabolic Effects Relationship

5.2.10.1 Lifestage

The 1996 and 2006 Ozone AQCDs identified children, especially those with asthma, and older adults as at-risk populations (U.S. EPA, 2006, 1996). In addition, the 2013 Ozone ISA confirmed that

there was adequate evidence to conclude that children and older adults are at increased risk of ozone-related health effects (U.S. EPA, 2013). Collectively, the majority of evidence for older adults has come from studies of short-term ozone exposure and mortality. One recent study of short-term ozone exposure and metabolic health effects compared associations between different age groups (Section 5.1.6), but it does not report consistent evidence that older adults are at increased risk. Long-term exposure to ozone associations with lifestage are described below.

- Using the Rome Longitudinal Study Cohort, Renzi et al. (2017) evaluated the effects of ozone exposure in over one million subjects over the 35 years old without diabetes at baseline. When stratified by age, the study showed increased hazard ratios for incidence of diabetes for those under 50 (1.05; 95% CI: 1.02, 1.08) but not those from 50−60 (1.02; 95% CI: 0.99, 1.04), or over 60 (1.00; 95%: 0.98, 1.02).
- Jerrett et al. (2017) analyzed data from the Black Women's Health Study Cohort in a prospective study of type 2 diabetes. When the population was further analyzed by age, increased hazard ratios for incident diabetes was seen in women aged 40−54 (1.33; 95% CI: 1.03, 1.72), was higher, although less precise, for those under 40 (1.43; 95% CI: 0.90, 2.25), and lower for those over 55 (1.24; 95% CI: 0.90, 1.72). There was no difference between the groups.
- In a study by Dong et al. (2014), 30,056 children were recruited from seven cities in northeast China. Body mass index (BMI) was calculated according to World Health Organization (WHO) protocol and the Center for Disease Control and Prevention (CDC) definition of overweight and obese were used to categorize status. Ozone exposure was determined using the 3-year avg of the 8-hour max concentration of the monitor closest to the school children attended. Increased odds for children being overweight (1.16; 95% CI: 1.05, 1.27) or obese (1.26; 95% CI: 1.07, 1.45) were observed. Li et al. (2015) used the 33 Communities Chinese Health Study Cohort in participants that were 18−74 years of age and had lived in the same location for more than 5 years. When the population was stratified for age (over or under 50), the population over 50 had increased odds of being overweight (1.12; 95% CI: 1.05, 1.19), and females over 50 also had increased odds of obesity (1.23; 95% CI: 1.04, 1.44). There were no differences found in the under 50 age group for increased odds of being overweight or obese.
- A recent study examined the effect of age on health outcomes in rodents. Senescent or aged animals were more sensitive to ozone-dependent serum insulin changes. Ozone-exposed senescent males had significantly increased serum insulin versus aged filtered-air controls [male brown Norway rats ozone, 6 hours/day, 1 day/week for 17 weeks; 4-month-olds or aged animals 20 months old; Gordon et al. (2013)]. In the same study, 4-month-old adult rodents exposed to ozone did not have significant changes in serum insulin with ozone exposure (Gordon et al., 2013). Thus, age contributes to the insulin response to ozone, with aged animals producing statistically significantly increased levels of insulin with ozone exposure, an effect not present in younger adult rodents.

5.2.10.2 Pre-existing Disease

Individuals with certain pre-existing diseases may be considered at greater risk of an air pollution-related health effect because they are likely in a compromised biological state varying with the disease and severity. The 2013 Ozone ISA concluded that there was adequate evidence for increased ozone-related health effects among individuals with asthma (U.S. EPA, 2013). The results of controlled

human exposure studies, as well as epidemiologic and animal toxicological studies, contributed to this evidence. No studies evaluated in the 2013 Ozone ISA evaluated the potential of pre-existing disease to modify the relationship between long-term ozone exposure and metabolic health effects. Recent epidemiologic studies evaluated the potential for pre-existing diseases to modify associations between long-term ozone exposure and metabolic effects.

- Using the Rome Longitudinal Study Cohort, Renzi et al. (2017) evaluated the effects of ozone exposure in over one million subjects over the 35 years old without diabetes at baseline. When stratified by subjects that had comorbidities (myocardial infarction, COPD, hypertension, or hyperlipidemia) had an increased incidence of diabetes (1.02; 95% CI: 1.00, 1.03), but did not differ from those without comorbidities had an increased HR for diabetes (1.01; 95% CI: 1.00, 1.05).
- Jerrett et al. (2017) analyzed data from the Black Women's Health Study Cohort in a prospective study of type 2 diabetes. The study found increased hazard ratios for incident diabetes (1.28; 95% CI: 1.06, 1.55); with pre-existing hypertension the effect increased (1.35, 95% CI: 1.03, 1.76) but was attenuated without the presence of hypertension (1.15; 95% CI: 0.85, 1.53).

5.2.11 Summary and Causality Determination

There were no causality determinations for metabolic effects in the 2013 Ozone ISA (U.S. EPA, 2013). The recent literature pertaining to long-term exposure to ozone and metabolic effects has expanded substantially since the 2013 Ozone ISA, with multiple epidemiologic and experimental studies currently available for review. In prospective cohort studies in the U.S. and Europe increased incidence of type 2 diabetes was observed with long-term exposure to ozone. In China, the odds of metabolic syndrome increased as well. These findings are consistent with two long-term ozone exposure studies in China, one in adults and one in children, presented increased odds of obesity in both adults and children as obesity is a risk factor for type 2 diabetes (T2D). Positive associations between long-term exposure to ozone and diabetes-related mortality were observed in well-established cohorts in the U.S. and Canada. The mortality findings are supported by epidemiologic and experimental studies reporting effects on glucose homeostasis and serum lipids, as well as other indicators of metabolic function (e.g., peripheral inflammation and neuroendocrine activation). Findings from the one epidemiologic study of metabolic disease showed increases in metabolic syndrome for both the Joint International and American Heart Association criteria in 33 communities in China. Additionally, in prospective cohort studies in the U.S. and Europe, increased incidence of type 2 diabetes was observed with ozone exposure. The information pertaining to the relationship between long-term exposure to ozone and metabolic effects is summarized in Table 5-4, using the framework for causality determination described in the Preamble to the ISAs (U.S. EPA, 2015).

Experimental animal studies address some of the uncertainty in the epidemiologic evidence related to the independent effect of ozone exposure by providing evidence of direct effects on metabolic function. The animal toxicological studies provided evidence that long-term ozone exposure resulted in

impaired insulin signaling, glucose intolerance, hyperglycemia, and insulin resistance (Section 5.2.3.1). In addition, these pathophysiological changes were often accompanied by increased inflammatory markers in peripheral tissues, and activation of the neuroendocrine system (Section 7.2.1.5). A limited number of epidemiologic studies have evaluated potential copollutant cofounding for PM or NO_X [Jerrett et al. (2017); Renzi et al. (2017); Section 5.2.3]. Importantly, short-term ozone exposure studies also provided evidence that ozone exposure could contribute to the development of metabolic syndrome and show consistency with the evidence that long-term ozone exposure could lead to development or worsening of metabolic syndrome or its risk factors. **Overall, the collective evidence is sufficient to conclude that a likely to be causal relationship exists between long-term ozone exposure and metabolic effects**.

Table 5-4 Summary of evidence to support a likely to be causal relationship between long-term ozone exposure and metabolic effects.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Consistent animal toxicology evidence from multiple, high-quality studies at relevant ozone concentrations	Animal toxicological studies of impaired glucose tolerance, fasting hyperglycemia, dyslipidemia, insulin resistance, and activation of the neuroendocrine pathway with ozone exposure	Section 5.2.3 Miller et al. (2016b); Bass et al. (2013)	0.25, 1.0 ppm
Consistent epidemiologic evidence of increased risk diabetes or metabolic syndrome	Increased odds of metabolic syndrome, increased hazard ratio for incidence of diabetes, increased hazard ratio for incident diabetes in U.S. cohort	Yang et al. (2018) Jerrett et al. (2017); Renzi et al. (2017)	See Section 5.2.5.1 for exposure information
	Increased odds of developing of gestational diabetes with ozone exposure in the second trimester. Elevated ORs for type 1 diabetes with higher ozone concentrations in first and second trimester.	Malmqvist et al. (2015)	See Section 7.1.3
Epidemiologic evidence of increased diabetes associated mortality	A limited number of studies observed positive associations between long-term ozone exposure and mortality from diabetes and cardiometabolic diseases	Turner et al. (2016) Crouse et al. (2015)	Section 5.2.8
Limited epidemiologic evidence from copollutant models provides some support for an independent ozone association	Limited number of epidemiologic studies evaluate potential copollutant cofounding for PM or NO_X	Jerrett et al. (2017); Renzi et al. (2017)	Section 5.2.3
Biological plausibility	Experimental studies provide evidence of metabolic syndrome mediated by neuroendocrine activation with long-term ozone exposure provides biological plausibility to the effects of ozone on metabolic syndrome and diabetes	Section 5.2.2	

OR = odds ratio; ppm = parts per million.

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble to the ISAs U.S. EPA (2015).

[b]Describes the key evidence and references, supporting or contradicting, contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where full body of evidence is described.

[c]Describes the ozone concentrations with which the evidence is substantiated.

5.3 Evidence Inventories—Data Tables to Summarize Study Details

Table 5-5 Epidemiologic studies of short-term exposure to ozone and glucose/insulin homeostasis.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Kim and Hong (2012) Seongbuk-Gu, Seoul, South Korea Ozone: 2008−2010 Panel study Lags examined: 0−10	KEEP n = 560 Participants over 60 years old in the Seongbuk-Gu area of Seoul, South Korea	Daily mean concentration of monitor nearest residence 24-h avg	Mean: 19.38 Median: 19.34 75th: 26.67 90th: 29.56 95th: 31.33	Correlation (*r*): NO_2: −0.35; SO_2: −0.3; Other: PM_{10}: −0.12 Copollutant models with: NO_2, PM_{10} Using O_3 lag 5, NO_2 lag Day 7, and PM_{10} lag Day 4	Lag 5: Percentage increase in glucose 0.19 (0.09, 0.28) Without pre-existing T2D: 0.09 (0.02, 0.16) With pre-existing T2D: 0.68 (0.28, 1.07) Adjusted for PM_{10}: 0.15 (0.05, 0.25) Adjusted for NO_2: 0.16 (0.06, 0.25) Percentage increase in HOMA: 0.30 (0.06, 0.53) Without pre-existing T2D: 0.12 (−0.11, 0.35) With pre-existing T2D: 1.21 (0.44, 1.99) Adjusted for PM_{10}: 0.25 (−0.001, 0.49) Adjusted for NO_2: 0.21 (−0.02, 0.45) Percentage increase in insulin: 0.71 (0.02, 1.38) Without pre-existing T2D: 0.32 (−0.39, 1.02) Pre-existing T2D: 2.76 (0.78, 4.75) Adjusted for PM_{10}: 0.67 (−0.06, 1.39) Adjusted for NO_2: 0.49 (−0.20, 1.19)

Table 5-5 (Continued): Epidemiologic studies of short-term exposure to ozone and glucose/insulin homeostasis.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Chen et al. (2016b) California, U.S. Ozone: 2002−2008 Panel study	β-Gene n = 1,023 Mexican American women with a previous diagnosis of GDM within previous 5 yr, siblings and cousins (both sexes) all with fasting glucose levels <7 mmol/L	Daily average of monitored air quality data spatially mapped to residence locations using inverse distance squared interpolation with a maximum radius of 50 km 24-h avg	Mean: 30-day cumulative: 43.4 ppb; Annual average: 40.8 ppb	Correlation (*r*): $PM_{2.5}$: 30 day: −0.02; annual: 0.04; NO_2: 30 day: −0.37; annual: −0.31 Copollutant models with: NR	Qualitative results only, no change in fractional disappearance rate Glucose HOMA-IR Insulin Metabolic clearance Insulin sensitivity
†Li et al. (2017) Northeastern U.S. Ozone: 2002−2005 and 2008−2011 Panel study	Framingham Offspring Cohort and Third Generation Cohort n = 4,116 Residents within 50 km of Harvard Supersite excluding patients with diabetes at the time of examination visits (fasting glucose >126 mg/dL)	Daily averages of two ozone monitors in the greater Boston, MA area 24-h avg	Mean: 23.7	Correlation (*r*): $PM_{2.5}$: 0.01; NO_2: −0.54; SO_2: 0.13; Other: BC: −0.26 Copollutant models with: NR	Qualitative Results only: Decrease in percentage glucose at 24-h, 3- and 7-day avg Negative trend for percentage change in Insulin HOMA-IR at 24-h, 3- and 7-day avg
†Dales et al. (2012) Santiago Province, Chile Ozone: 2001−2008 Cross-sectional study	n = general population of five sectors was 5 million Daily hospital admissions where diabetes was the principal diagnosis (insulin dependent and noninsulin-dependent) with coma or ketoacidosis	Daily averaged monitor(s) in the sector of residence 24-h avg	Mean: 64.41	Correlation (*r*): $PM_{2.5}$: −0.31; NO_2: −0.31; SO_2: −0.08 Copollutant models with: NR	Increased risk for hospital admission for diabetic coma or diabetic ketoacidosis: 1.02 (1.00, 1.04)

BC = black carbon; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; mg/dL = milligrams per deciliter; mmol/L = millimoles per liter; NO_2 = nitrogen dioxide; O_3 = ozone; $PM_{2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 2.5 μm; PM_{10} = particulate matter with a nominal aerodynamic diameter less than or equal to 10 μm; ppb = parts per billion; SO_2 = sulfur dioxide; T2D = type 2 diabetes.

†Studies published since the 2009 PM ISA.

Table 5-6 Controlled human exposure study of short-term exposure to ozone and glucose/insulin homeostasis and other metabolic indicators.

Study	Population N, Sex, Age (Range or Mean ± SD)	Exposure Details (Concentration, Duration)	Endpoints Examined
Miller et al. (2016a)	Healthy young adults n = 20 males, 4 females Age: 25.6 ± 3.8	0.3 ppm, 2 h (15 min of exercise alternating with 15 min of rest)	HOMA-IR, insulin, cortisol, corticosterone, cortisone, leptin, ketone bodies, free fatty acids

Table 5-7 Study-specific details from animal toxicological studies of short-term exposure to ozone and glucose/insulin homeostasis.

Study	Species (Strain), N, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Wagner et al. (2014)	Rats (S-D) n = 4−8/group males, 0 females Age: 8 weeks	0.5 ppm, 8 h/day for 2 weeks (O_3 and O_3 + CAPs; high fructose or normal diet for 8 weeks prior) for 9 consecutive weekdays	Blood pressure, insulin resistance, fasting levels of blood glucose and triglycerides, HOMA-IR, body weight, heart rate (24 h PE)
Bass et al. (2013)	Rats (BN) n = 4−21/group males, 0 females Age: 1, 4, 12, and 24 mo old Rats (BN) n = 4−21/group males, 0 females Age: 1, 9, and 21 mo	0.25 ppm, 6 h/day for 2 days 0.25 ppm, 6 h/day, 2 days/week for 13 weeks 1 ppm, 6 h/day for 2 days 1 ppm, 6 h/day, 2 days/week for 13 weeks	GTT, AUC, epinephrine, cholesterol (total, HDL, LDL), triglycerides, serum leptin, IL-6, Insulin, mRNA biomarkers in liver and adipose (NR)
Vella et al. (2014)	Rats (Wistar) n = 4−10 males, 0 females Age: adult (400−450 g)	0.8 ppm, 16 h (with and without pretreatment of N-acetylcysteine)	HOMA-IR, glucose (fasting glucose, insulin, ITT, triacylglycerols, total cholesterol, IST [l-arginine]), serum oxidative stress biomarkers (GSH/GSSG, MDA), glucose-dep JNK, AKT, or ER pathways PE
Zhong et al. (2016)	Mice (KK; obesity-prone develops moderate degrees of obesity, insulin resistance, and diabetes) n = 8/group males, no females Age: adult	0.5 ppm, 4 h/day for 3 consecutive days	Glucose metabolic hormones (insulin, leptin, adiponectin), visceral adipose characterization (oil-red-o stain), inflammatory genes in adipose (CXCL-11, IFN-g, TNF-α, IL-12, and iNOS) 22 h PE Insulin tolerance test (IP) 2 h PE
Thomson et al. (2016)	Rats (F344) n = NR males, 0 females Age: adult (200−250 g)	0.8 ppm, 4 h	Glucose met hormones (glucagon, insulin, ghrelin, PAI-1; NR)
Miller et al. (2016c)	Rats (WKY) n = 5/group males, 0 females Age: adult	1 ppm, 4 h/day for 1 or 2 days (rats underwent bilateral adrenal demedullation [DEMED], total bilateral adrenalectomy; ADREX), or sham surgery (SHAM)	GTT (blood glucose, AUC) Immediately PE, lipids, free fatty acids, branched chain amino acids, leptin

Table 5-7 (Continued): Study-specific details from animal toxicological studies of short-term exposure to ozone and glucose/insulin homeostasis.

Study	Species (Strain), N, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Gordon et al. (2017b)	Rats (LE) n = 0 males, 10/group females Age: 22 days at start of exercise regimen	0.25 ppm, 5 h/day for 2 days 0.5 ppm, 5 h/day for 2 days 1 ppm, 5 h/day for 2 days	Glucose tolerance test, body composition (lean, fat, fluid percentage), BALF, EMKA plethysmography, Beta cell insulin secretion test-inhibition, insulin resistance test in liver, insulin resistance test muscle. Immediately PE Day 1
Miller et al. (2016b)	Rats (WKY) n = 8−10/group males, 0 females Age: adult (250−300 g)	0.25 ppm, 5 h/day for 3 consecutive days/week for 13 weeks 1 ppm, 5 h/day for 3 consecutive days/week for 13 weeks	GTT (blood glucose, AUC), insulin tolerance test, pyruvate tolerance test (hepatic gluconeogenesis) cholesterol, catecholamines, adrenaline and noradrenaline, AKT (NR)
Miller et al. (2015)	Rats (WKY) Male Age: 10 weeks	0.25, 0.50, or 1.0 ppm ozone, 6 h/day for 2 days	Cholesterol, LDL
Farraj et al. (2012)	Rats (SH) Male Age: 12 weeks	0.8 ppm ozone, 4 h, whole body exposure	Cholesterol, HDL
Farraj et al. (2016)	Rats (SH) Male Age: 12 weeks	0.3 ppm ozone, 3 h, 1 day whole body exposure	Cholesterol
Ramot et al. (2015)	Rats (WKY) Male	1.0 ppm ozone, 4 h, 1 day whole body exposure	Cholesterol, LDL
Thomson et al. (2018)	Rats (Fisher-344) n = 6−8/group males, 0 females Age: adult	0.8 (with or without metyrapone) ppm, 4 h, whole body exposure	Glucose tolerance test, HOMA IR, plasma triglycerides, HPA axis (cort synth inhibitor metyrapone, or exogenous cort), glucose met hormones (glucagon, insulin, leptin, GLP-1, ghrelin, cort), inf cytokines (TNF, IL-6, VEGF, PAI-1; NR)

AKT = protein kinase B; AUC = area under the curve; BALF = bronchoalveolar lavage fluid; BN = brown Norway; CAP = criteria air pollutants; ER = estrogen receptor; F344 = Fischer 344; GSH/GSSG = ratio of reduced to oxidized glutathione; GTT = glucose tolerance test; HDL = high density lipoproteins; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; HPA = hypothalamic-pituitary-adrenal; ITT = insulin tolerance test; JNK = c-Jun N-terminal kinase; LDL = low density lipoproteins; LE = Long-Evans; MDA = malondialdehyde; NR = not reported; O_3 = ozone; PE = post-exposure; ppm = parts per million; S-D = Sprague-Dawley; SH = spontaneously hypertensive.

Table 5-8 Study-specific details from animal toxicological studies of short-term overweight and obesity.

Study	Species (Strain), N, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Ying et al. (2016)	Mice (KKAy, diabetic prone) n = 8/group males, 0 females Age: 4−5 weeks	0.5 ppm, 4 h/day for 13 consecutive days	HOMA-IR, ITT, inflammatory cytokines, body weight, leptin, hyperglycemia, in vitro insulin treatment, GTT, area under the curve, plasma insulin, plasma glucose, white adipose cell inflammation (NR)
Gordon et al. (2016)	Rats (BN) n = 10/group males, 10/group females Age: 30 days	0.8 ppm, 5 h (high fructose or high fat diet for 12 weeks prior) 0.8 ppm, 5 h/day, 1 day/week for 4 weeks (high fructose or high fat diet for 12 weeks prior)	Serum cholesterol, triglycerides, body weight, effect of diet and exercise on endpoints, changes in body composition (fat, lean, liquid mass) 18 h PE
Mathews et al. (2017b)	Mice (wild type lean or obese; dg/db)	2 ppm ozone, 3 h, whole body exposure	Gastrin releasing peptide receptor, IL-17a and IL-33 signaling
Mathews et al. (2017a)	Mice (wild type lean or obese; dg/db)	2 ppm ozone, 3 h, whole body exposure	Lung metabolome, antioxidant signaling in lung (glutathione pathway)
Gordon et al. (2017a)	Rats (LE) n = 8 offspring total, four males, four females when possible. 10 dams/treatment group.	0.8 ppm, 4 h/day for 2 consecutive days, Age: adult (30 days) offspring ozone challenge, PND 161−162 ozone exposure Pregnant females and offspring (control diet [CD]-sedentary [SED]; CD-run wheel [RW]; high fat diet-SED; HFD-RW); begin diet 6 weeks prior to mating/conception	Glucose tolerance test, ventilation, BALF counts (PND 162, offspring measurements)
Mathews et al. (2018)	Mice (wild type lean or obese; dg/db)	2 ppm ozone, 3 h, whole body exposure	Gastrin releasing peptide receptor, IL-17a and IL-33 signaling

BALF = bronchoalveolar lavage fluid; BN = brown Norway; GTT = glucose tolerance test; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; ITT = insulin tolerance test; NR = not reported; PND = postnatal day; ppm = parts per million.

Table 5-9 Epidemiologic studies of short-term exposure to ozone and other indicators.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Chen et al. (2016b) California, U.S. Ozone: 2002−2008 Follow-up: panel study	β-Gene n = 1,023 Mexican American women with a previous diagnosis of GDM within previous 5 yr, siblings and cousins (both sexes) all with fasting glucose levels <7 mmol/L	Daily average of monitored air quality data spatially mapped to residence locations using inverse distance squared interpolation with a maximum radius of 50 km 24-h avg	Mean: 30-day cumulative: 43.4 ppb; annual average: 40.8 ppb	Correlation (*r*): $PM_{2.5}$: 30 day: −0.02; annual: 0.04; NO_2: 30 day: −0.37; annual: −0.31 Copollutant models with: NR	Qualitative results No change in: HDL-to-LDL ratio, LDL
†Li et al. (2017) Northeastern U.S. Ozone: 2002−2005 and 2008−2011 Follow-up: panel study	Framingham Offspring Cohort and Third Generation Cohort n = 4,116 Residents within 50 km of Harvard Supersite excluding patients with diabetes at the time of examination visits (fasting glucose >126 mg/dl)	Daily averages of two ozone monitors in the greater Boston, MA area 24-h avg	Mean: 23.7	Correlation (*r*): $PM_{2.5}$: 0.01; NO_2: −0.54; SO_2: 0.13; Other: BC: −0.26 Copollutant models with: NR	Negative trend nonsignificant; qualitative results only: resistin No trend; qualitative results: leptin Positive trend nonsignificant; qualitative results only: adiponectin

BC = black carbon; GDM = gestational diabetes mellitus; HDL = high-density lipoproteins; LDL = low-density lipoproteins; mg/dL = milligrams per deciliter; mmol/L = millimoles per liter; NO_2 = nitrogen dioxide; NR = not reported; $PM_{2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 2.5 μm; ppb = parts per billion; SO_2 = sulfur dioxide.

†Studies published since the 2009 PM ISA.

Table 5-10 Study-specific details from animal toxicological studies of short-term, other metabolic indicators.

Study	Species (Strain), N, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Thomson et al. (2013)	Rats (F344) n = 4−6/group males, 0 females Age: adult (200−250 g)	0.4 ppm, 4 h (nose only) 0.8 ppm, 4 h (nose only)	Multiorgan gene expression (mRNA pathway analysis antioxidant response, xenobiotic metabolism, inflammatory signaling, and endothelial dysfunction) and glucocorticoid activity (plasma levels of adrenocorticotropic hormone and the glucocorticoid corticosterone) Immediately PE and after 24 h FA recovery
Sun et al. (2013)	Rats (S-D) n = 4−8 males, 0 females Age: 8 weeks	0.5 ppm, 2 weeks (O_3 and O_3 + CAPs; high fructose or normal diet for 8 weeks prior)	Perirenal and epicardial adipose tissue (PAT and EAT), inflammation in PAT and EAT, body-weight changes with ozone ± diet modification, characterization of fat depots (brown adipose vs. white adipose), histology of fat tissue (morphology changes), tissue adiponectin concentration 24 h PE
Bass et al. (2013)	Rats (BN) n = 4−21/group males, 0 females Age: 1, 4, 12, and 24 mo old Rats (BN) n = 4−21/group males, 0 females Age: 1, 9, and 21 mo	0.25 ppm, 6 h/day for 2 days 0.25 ppm, 6 h/day, 2 days/week for 13 weeks 1 ppm, 6 h/day for 2 days 1 ppm, 6 h/day, 2 days/week for 13 weeks	GTT, AUC, epinephrine, cholesterol (total, HDL, LDL), triglycerides, serum leptin, IL-6, insulin, mRNA biomarkers in liver and adipose (NR)
Miller et al. (2015)	Rats (WKY) n = 6−8/group males, 0 females Age: 10 weeks (250−300 g)	0.25 ppm, 6 h/day for 2 days 1 ppm, 6 h/day for 2 days	GTT, insulin, leptin, IL-6, cholesterol (total, LDL, HDL), metabolomics, liver transcriptomics
Theis et al. (2014)	Rats (S-D) n = 6/group males, 0 females Age: adult	0.5 ppm, 8 h/day for 5 days	Liver endpoints (liver enzymes, liver proteomics [stress responsive proteins, glucose-regulated protein 78, and protein disulfide isomerase, glutathione s-transferase M1, hemeoxygenase-1]; NR)

Table 5-10 (Continued): Study-specific details from animal toxicological studies of short-term, other metabolic indicators.

Study	Species (Strain), N, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Ying et al. (2016)	Mice (KKAy, diabetic prone) n = 8/group males, 0 females Age: 4−5 weeks	0.5 ppm, 4 h/day for 13 consecutive days	HOMA-IR, ITT, inflammatory cytokines, body weight, leptin, hyperglycemia, in vitro insulin treatment, GTT, area under the curve, plasma insulin, plasma glucose, white adipose cell inflammation (NR)
Gordon et al. (2016)	Rats (BN) n = 10/group males, 10/group females Age: 30 days	0.8 ppm, 5 h (high fructose or high fat diet for 12 weeks prior) 0.8 ppm, 5 h/day, 1 day/week for 4 weeks (high-fructose or high-fat diet for 12 weeks prior)	Serum cholesterol, triglycerides, body weight, effect of diet and exercise on endpoints, changes in body composition (fat, lean, liquid mass) 18 h PE
Zhong et al. (2016)	Mice (KK; obesity-prone develops moderate degrees of obesity, insulin resistance, and diabetes) n = 8/group males, no females Age: adult	0.5 ppm, 4 h/day for 3 consecutive days	Glucose metabolic hormones (insulin, leptin, adiponectin), visceral adipose characterization (oil-red-o stain), inflammatory genes in adipose (CXCL-11, IFN-g, TNF-α, IL-12, and iNOS) 22 h PE Insulin tolerance test (IP) 2 h PE
Miller et al. (2016c)	Rats (WKY) n = 5/group males, 0 females Age: adult	1 ppm, 4 h/day for 1 or 2 days (rats underwent bilateral adrenal demedullation [DEMED], total bilateral adrenalectomy; ADX), or sham surgery (SHAM)	GTT (blood glucose, AUC) Immediately PE
Gordon et al. (2017b)	Rats (LE) n = 0 males, 10/group females Age: 22 days at start of exercise regimen	0.25 ppm, 5 h/day for 2 days 0.5 ppm, 5 h/day for 2 days 1 ppm, 5 h/day for 2 days	Glucose tolerance test, body composition (lean, fat, fluid percentage), BALF, EMKA plethysmography Immediately PE Day 1
Miller et al. (2016b)	Male Rats (WKY) n = 8−10/group Age: adult (250−300 g)	0.25 ppm, 5 h/day for 3 consecutive days/week for 13 weeks 1 ppm, 5 h/day for 3 consecutive days/week for 13 weeks	GTT (blood glucose, AUC), Insulin tolerance test, beta cell insulin secretion test, pyruvate tolerance test (hepatic gluconeogenesis) cholesterol, catecholamines, adrenaline and noradrenaline, corticosterone, insulin resistance-AKT (NR)

Table 5-10 (Continued): Study-specific details from animal toxicological studies of short-term, other metabolic indicators.

Study	Species (Strain), N, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Henriquez et al. (2017b)	Male Rats (WKY) Age: 12 weeks old, n = 6−8/group	Rodents were pretreated daily for 7 days with propranolol (PROP; a nonselective β adrenergic receptor antagonist), mifepristone (a glucocorticoid receptor antagonist), both drugs, or respective vehicles, and then exposed to air or ozone (0.8 ppm), 4 h/day for 1 or 2 consecutive days while continuing drug treatment	Inflammation, epinephrine, cortisol, lung transcriptomic assessment

AKT = protein kinase B; AUC = area under the curve; BALF = bronchoalveolar lavage fluid; BN = brown Norway; FA = filtered air; HDL = high-density lipoproteins; LDL = low-density lipoproteins; NR = not reported; O_3 = ozone; PE = post-exposure; ppm = parts per million; S-D = Sprague-Dawley; WKY = Wistar Kyoto.

Table 5-11 Epidemiologic studies of long-term exposure to ozone and overweight and obesity.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Dong et al. (2014) Shenyang, Dalian, Anshan, Fushun, Benxi, Liaoyang, and Yingkou, China Ozone: 2006−2008 Follow-up: cross-sectional study	n = 30,056 Children aged 2−14 yr living in seven cities in northeast China attending schools within 1 km of a monitoring site	Monitors within 1 km of school 8-h max	Mean: 27.4 Maximum: 44.5	Correlation (*r*): NR Copollutant models with: NR	Increased odds of obesity in children: 1.26 (1.07, 1.45) Increased odds of overweight children: 1.16 (1.05, 1.28) Increased odds of obese or overweight children: 1.26 (1.11, 1.41)
†Li et al. (2015) Shenyang, Anshan, and Jinzhou, China Ozone: 2006−2008 Follow-up: 2009 Cross-sectional study	33CCHS n = 24,845 Participants 18−74 yr of age in 11 districts in three Chinese cities (Shenyang, Anshan, Jinzhou)	Monitor within 1 km of the household, using the daily 8-h avg to create a 3-yr avg concentration 8-h avg	Mean: 25.1 Maximum: 36.0	Correlation (*r*): NR Copollutant models with: NR	Increased odds of being overweight: 1.08 (1.04, 1.12) Increased odds of males being overweight: 1.09 (1.03 1.15) Increased odds of females being overweight: 1.05 (1.00, 1.12) Increased odds of obesity: 1.09 (1.01, 1.18) Increased odds of males obesity: 0.99 (0.88, 1.12) Increased odds of females obesity: 1.12 (1.01, 1.26)
†White et al. (2016) 56 metropolitan areas, U.S. Ozone: 2007−2008 Follow-up: 1995−2011 Cohort study	Black Women's Health Study n = 38,374 Black women living in 56 metropolitan areas in the U.S., under 55 yr of age, without history of cancer or gastric bypass surgery, and had not given birth within the past 2 yr	CMAQ model with a resolution of 12 km. Estimates were made at the centroid of each census tract. 8-h max	Mean: 37.5	Correlation (*r*): NR Copollutant models with: NR	Absolute change in weight (kg): 0.24 (−0.16, 0.64)

33CCHS = 33 Communities Chinese Health Study; CMAQ = Community Multiscale Air Quality; HR = hazard ratio; NR = not reported.

†Studies published since the 2009 PM ISA.

Table 5-12 Epidemiologic studies of long-term exposure to ozone and metabolic syndrome and type 2 diabetes.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Yang et al. (2018) Shenyang, Anshan, and Jinzhou, China Ozone: 2006−2008 Follow-up: 2009 Cross-sectional study	33CCHS n = 15,477 Participants 18−74 yr of age in 11 districts in three Chinese cities (Shenyang, Anshan, Jinzhou)	Monitor within 1 km of the household, using the daily 8-h avg to create a 3-yr avg concentration 8-h avg	Mean: 25.1 Maximum: 36.0	Correlation (*r*): $PM_{2.5}$: 0.45; NO_2: 0.45; SO_2: 0.84; Other: PM_{10} 0.81 Copollutant models with: NR	Increased odds of metabolic syndrome diagnosis American Heart Association criteria: 1.164 (1.12, 1.23) Joint international criteria: 1.21 (1.02, 1.39)
†Jerrett et al. (2017) 56 metropolitan areas, U.S. Ozone: 2007−2008 Follow-up: 1995−2011 Cohort study	Black Women's Health Study n = 43,003 Black women living in 56 metropolitan areas in the U.S., aged 30 and over at the time of follow-up without prevalent diabetes at baseline	CMAQ model with a resolution of 12 km. Estimates were made at the centroid of each census tract. 8-h max	Mean: 37.5	Correlation (*r*): $PM_{2.5}$: −0.29; NO_2: −0.57 Copollutant models with: NR	Increased HR for T2D diagnosis: 1.28 (1.06, 1.55) Increased HR for T2D diagnosis adjusted for NO_2: 1.20 (0.96, 1.50) Increased HR for T2D diagnosis adjusted for $PM_{2.5}$: 1.31 (1.08, 1.60) Increased HR for T2D diagnosis under age 40 yr: 1.43 (0.90, 2.25) Increased HR for T2D diagnosis age 40−54 yr: 1.33 (1.03, 1.72) Increased HR for T2D diagnosis over age 55: 1.25 (0.90, 1.72) Increased HR for T2D diagnosis without presence of hypertension: 1.15 (0.85, 1.53) Increased HR for T2D diagnosis with presence of hypertension: 1.35 (1.03, 1.76)

Table 5-12 (Continued): Epidemiologic studies of long term exposure to ozone and metabolic syndrome and type 2 diabetes.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Renzi et al. (2017) Rome, Italy Ozone: 2005 Follow-up: 2008−2013 Cohort study	Rome Longitudinal Study n = 1,319,193 Individuals over 35 yr of age without prevalent diabetes at baseline	Flexible Air Quality Regional Model (FARM) using a 1-km grid dispersion model 8-h avg	Mean: 49.4 Maximum: 57.3	Correlation (*r*): $PM_{2.5}$: −0.01; NO_2: −0.16 Copollutant models with: NR	Increased prevalence of diabetes at baseline: 1.001 (0.991, 1.012) Increased incidence of diabetes: 1.012 (1, 1.024) Increased HR of incident diabetes adjusted for NO_X: 1.02 (1.00, 1.03) Increased HR for incident diabetes female: 1.03 (1.01, 1.05) Increased HR for incident diabetes male: 0.99 (0.98, 1.01) Increased HR for incident diabetes under age 50 yr: 1.05 (1.02, 1.08) Increased HR for incident diabetes age 50−60 yr: 1.02 (0.99, 1.04) Increased HR for incident diabetes over age 60 yr: 1.00 (0.98, 1.02) Increased HR for incident diabetes with comorbidities: 1.02 (1.00, 1.03) Increased HR for incident diabetes without comorbidities: 1.02 (1.00, 1.05)

33CCHS = 33 Communities Chinese Health Study; CMAQ = Community Multiscale Air; HR = hazard ratio; NO_2 = nitrogen dioxide; NR = not reported; $PM_{2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 2.5 μm; PM_{10} = particulate matter with a nominal aerodynamic diameter less than or equal to 10 μm; ppb = parts per billion; SO_2 = sulfur dioxide; T2D = type 2 diabetes.

†Studies published since the 2009 PM ISA.

Table 5-13 Study-specific details from long-term animal toxicological studies of glucose and insulin homeostasis.

Study	Species (Strain), N, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Bass et al. (2013)	Rats (BN) n = 4−21/group males, 0 females Age: 1, 4, 12, and 24 mo old Rats (BN) n = 4−21/group males, 0 females Age: 1, 9, and 21 mo	0.25 ppm, 6 h/day for 2 days 0.25 ppm, 6 h/day, 2 days/week for 13 weeks 1 ppm, 6 h/day for 2 days 1 ppm, 6 h/day, 2 days/week for 13 weeks	GTT, AUC, epinephrine, cholesterol (total, HDL, LDL), triglycerides, serum leptin, IL-6, insulin, mRNA biomarkers in liver and adipose (NR)
Gordon et al. (2013)	Rats (BN) n = 7−10/group males, 0 females Age: adult (300 g) Rats (LE, S-D, and WKY) n = 7−16 pups/group males, 7−16 pups/group females Age: PND 14, PND 21, or PND 28	0, 0.8 ppm, NR 1 ppm, 2 h	HOMA-IR, glucose (fasting glucose, insulin, ITT, triacylglycerols, total cholesterol, IST [l-arginine]), serum oxidative stress biomarkers (GSH/GSSG, MDA), glucose-dep JNK, AKT, or ER pathways (1 day PE) Pre- and post-ozone levels of lung antioxidants (e.g., total glutathione, ascorbic acid, uric acid, alpha-tocopherol), superoxide dismutase (SOD) and enzyme content/activity related to glutathione recycling and differences across strains (NR)
Miller et al. (2016b)	Rats (WKY) n = 8−10/group males, 0 females Age: adult (250−300 g)	0.25 ppm, 5 h/day for 3 consecutive days/week for 13 weeks 1 ppm, 5 h/day for 3 consecutive days/week for 13 weeks	GTT (blood glucose, AUC), insulin tolerance test, pyruvate tolerance test (hepatic gluconeogenesis), cholesterol, catecholamines, adrenaline and noradrenaline, AKT (NR)

AKT = protein kinase B; AUC = area under the curve; BN = brown Norway; ER = estrogen receptor; GSH/GSSG = ratio of reduced to oxidized glutathione; GTT = glucose tolerance test; HDL = high-density lipoproteins; ITT = insulin tolerance test; JNK = c-Jun N-terminal kinase; LDL = low-density lipoproteins; LE = Long-Evans; MDA = malondialdehyde; NR = not reported; PE = post-exposure; ppm = parts per million; S-D = Sprague-Dawley; WKY = Wistar Kyoto.

Table 5-14 Epidemiologic studies of long-term exposure to ozone and type 1 diabetes.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Malmqvist et al. (2015) Scania, Sweden Ozone: 1999−2005 Follow-up: case-control study	n = 930 Gene-matched, case-control study of children with or without T1D born within 2 yr of each other	Monitor within 32 km of residence, average distance 8.5 km 24-h avg		Correlation (*r*): NR Copollutant models with: NR	Quartile 4 vs. reference exposure

HR = hazard ratio; NR = not reported; T1D= type 1 diabetes.

†Studies published since the 2009 PM ISA.

Annex for Appendix 5: Evaluation of Studies on Health Effects of Ozone

This annex describes the approach used in the Integrated Science Assessment (ISA) for Ozone and Related Photochemical Oxidants to evaluate study quality in the available health effects literature. As described in the Preamble to the ISA (U.S. EPA, 2015), causality determinations were informed by the integration of evidence across scientific disciplines (e.g., exposure, animal toxicology, epidemiology) and related outcomes and by judgments of the strength of inference in individual studies. Table Annex 4-1 describes aspects considered in evaluating study quality of controlled human exposure, animal toxicological, and epidemiologic studies. The aspects found in Table Annex 4-1 are consistent with current best practices for reporting or evaluating health science data.1 Additionally, the aspects are compatible with published U.S. EPA guidelines related to cancer, neurotoxicity, reproductive toxicity, and developmental toxicity (U.S. EPA, 2005, 1998, 1996b, 1991).

These aspects were not used as a checklist, and judgments were made without considering the results of a study. The presence or absence of particular features in a study did not necessarily lead to the conclusion that a study was less informative or to exclude it from consideration in the ISA. Further, these aspects were not used as criteria for determining causality in the five-level hierarchy. As described in the Preamble, causality determinations were based on judgments of the overall strengths and limitations of the collective body of available studies and the coherence of evidence across scientific disciplines and related outcomes. Table Annex 4-1 is not intended to be a complete list of aspects that define a study's ability to inform the relationship between ozone and health effects, but it describes the major aspects considered in this ISA to evaluate studies. Where possible, study elements, such as exposure assessment and confounding (i.e., bias due to a relationship with the outcome and correlation with exposures to ozone), are considered specifically for ozone. Thus, judgments on the ability of a study to inform the relationship between an air pollutant and health can vary depending on the specific pollutant being assessed.

[1] For example, NTP OHAT approach (Rooney et al., 2014), IRIS Preamble (U.S. EPA, 2013b), ToxRTool (Klimisch et al., 1997), STROBE guidelines (von Elm et al., 2007), and ARRIVE guidelines (Kilkenny et al., 2010).

Table Annex 5-1 Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Study Design
Controlled Human Exposure:
Studies should clearly describe the primary and any secondary objectives of the study or specific hypotheses being tested. Study subjects should be randomly exposed without knowledge of the exposure condition. Preference is given to balanced crossover (repeated measures) or parallel design studies which include controlled exposures (e.g., to clean filtered air). In crossover studies, a sufficient and specified time between exposure days should be provided to avoid carry over effects from prior exposure days. In parallel design studies, all arms should be matched for individual characteristics such as age, sex, race, anthropometric properties, and health status. In studies evaluating effects of disease, appropriately matched healthy controls are desired for interpretative purposes.
Animal Toxicology:
Studies should clearly describe the primary and any secondary objectives of the study or specific hypotheses being tested. Studies should include appropriately matched controlled exposures (e.g., to clean filtered air, time matched) and use methods to limit differences in baseline characteristics of control and exposure groups. Studies should randomize assignment to exposure groups and where possible conceal allocation to research personnel. Groups should be subjected to identical experimental procedures and conditions; animal care including housing, husbandry, etc. should be identical between groups. Blinding of research personnel to study group may not be possible due to animal welfare and experimental considerations; however, differences in the monitoring or handling of animals in all groups by research personnel should be minimized.
Epidemiology:
Inference is stronger for studies that clearly describe the primary and any secondary aims of the study or specific hypotheses being tested. For short-term exposure, time-series, case-crossover, and panel studies are emphasized over cross-sectional studies because they examine temporal correlations and are less prone to confounding by factors that differ between individuals (e.g., SES, age). Panel studies with scripted exposures, in particular, can contribute to inference because they have consistent, well-defined exposure durations across subjects, measure personal ambient pollutant exposures, and measure outcomes at consistent, well-defined lags after exposures. Studies with large sample sizes and those conducted over multiple years are considered to produce more reliable results. Additionally, multicity studies are preferred over single-city studies because they examine associations for large diverse geographic areas using a consistent statistical methodology, avoiding the publication bias often associated with single-city studies.[a] If other quality parameters are equal, multicity studies carry more weight than single-city studies because they tend to have larger sample sizes and lower potential for publication bias. For long-term exposure, inference is considered to be stronger for prospective cohort studies and case-control studies nested within a cohort (e.g., for rare diseases) than cross-sectional, other case-control, or ecological studies. Cohort studies can better inform the temporality of exposure and effect. Other designs can have uncertainty related to the appropriateness of the control group or validity of inference about individuals from group-level data. Study design limitations can bias health effect associations in either direction.

Table Annex 5-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Study Population/Test Model
Controlled Human Exposure:
In general, the subjects recruited into study groups should be similarly matched for age, sex, race, anthropometric properties, and health status. In studies evaluating effects of specific subject characteristics (e.g., disease, genetic polymorphism, etc.), appropriately matched healthy controls are preferred. Relevant characteristics and health status should be reported for each experimental group. Criteria for including and excluding subjects should be clearly indicated. For the examination of populations with an underlying health condition (e.g., asthma), independent, clinical assessment of the health condition is ideal, but self-report of physician diagnosis generally is considered to be reliable for respiratory and cardiovascular disease outcomes.[b] The loss or withdrawal of recruited subjects during the course of a study should be reported. Specific rationale for excluding subject(s) from any portion of a protocol should be explained.
Animal Toxicology:
Ideally, studies should report species, strain, substrain, genetic background, age, sex, and weight. Unless data indicate otherwise, all animal species and strains are considered appropriate for evaluating effects of ozone exposure. It is preferred that the authors test for effects in both sexes and multiple lifestages and report the result for each group separately. All animals used in a study should be accounted for, and rationale for exclusion of animals or data should be specified.
Epidemiology:
There is greater confidence in results for study populations that are recruited from and representative of the target population. Studies that have high participation, have low drop-out over time, and are not dependent on exposure or health status are considered to have low potential for selection bias. Clearly specified criteria for including and excluding subjects can aid assessment of selection bias. For populations with an underlying health condition, independent, clinical assessment of the health condition is valuable, but self-report of physician diagnosis generally is considered to be reliable for respiratory and cardiovascular diseases.[b] Comparisons of groups with and without an underlying health condition are more informative if groups are from the same source population. Selection bias can influence results in either direction or may not affect the validity of results but rather reduce the generalizability of findings to the target population.
Pollutant
Controlled Human Exposure:
The focus is on studies testing ozone exposure.
Animal Toxicology:
The focus is on studies testing ozone exposure.
Epidemiology:
The focus is on studies testing ozone exposure.

Table Annex 5-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Exposure Assessment or Assignment
Controlled Human Exposure:
For this assessment, the focus is on studies that use ozone concentrations <0.4 ppm. Studies that use higher exposure concentrations may provide information relevant to biological plausibility, dosimetry, or inter-species variation. Studies should have well-characterized pollutant concentration, temperature, and relative humidity and/or have measures in place to adequately control the exposure conditions. Preference is given to balanced crossover or parallel design studies which include control exposures (e.g., to clean filtered air). Study subjects should be randomly exposed without knowledge of the exposure condition. Method of exposure (e.g., chamber, facemask, etc.) should be specified and activity level of subjects during exposures should be well characterized.
Animal Toxicology:
For this assessment, the focus is on studies that use ozone concentrations <2 ppm. Studies that use higher exposure concentrations may provide information relevant to biological plausibility, dosimetry, or inter-species variation. Studies should characterize pollutant concentration, temperature, and relative humidity and/or have measures in place to adequately control the exposure conditions. The focus is on inhalation exposure. Noninhalation exposure experiments (i.e., intra-tracheal instillation [IT]) are informative for size fractions that cannot penetrate the airway of a study animal and may provide information relevant to biological plausibility and dosimetry. In vitro studies may be included if they provide mechanistic insight or examine similar effects as in vivo studies but are generally not included. All studies should include exposure control groups (e.g., clean filtered air).
Epidemiology:
Of primary relevance are relationships of health effects with the ambient component of ozone exposure. However, information about ambient exposure rarely is available for individual subjects; most often, inference is based on ambient concentrations. Studies that compare exposure assessment methods are considered to be particularly informative. Inference is stronger when the duration or lag of the exposure metric corresponds with the time course for physiological changes in the outcome (e.g., up to a few days for symptoms) or latency of disease (e.g., several years for cancer). Ambient ozone concentration tends to have low spatial heterogeneity at the urban scale, except near roads where ozone concentration is lower because ozone reacts with emitted nitric oxide. For studies involving individuals with near-road or on-road exposures to ozone in which ambient ozone concentrations are more spatially heterogeneous and relationships between personal exposures and ambient concentrations are potentially more variable, validated methods that capture the extent of variability for the epidemiologic study design (temporal vs. spatial contrasts) and location carry greater weight. Fixed-site measurements, whether averaged across multiple monitors or assigned from the nearest or single available monitor, typically have smaller biases and smaller reductions in precision compared with spatially heterogeneous air pollutants. Concentrations reported from fixed-site measurements can be informative if correlated with personal exposures, closely located to study subjects, highly correlated across monitors within a location, or combined with time-activity information. Atmospheric models may be used for exposure assessment in place of or to supplement ozone measurements in epidemiologic analyses. For example, grid-scale models (e.g., CMAQ) that represent ozone exposure over relatively large spatial scales (e.g., typically greater than 4- × 4-km grid size) often do provide adequate spatial resolution to capture acute ozone peaks that influence short-term health outcomes. Uncertainty in exposure predictions from these models is largely influenced by model formulations and the quality of model input data pertaining to precursor emissions or meteorology, which tends to vary on a study-by-study basis. In studies of short-term exposure, temporal variability of the exposure metric is of primary interest. For long-term exposures, models that capture within-community spatial variation in individual exposure may be given more weight for spatially variable ambient ozone. Given the low spatial variability of ozone at the urban scale, exposure measurement error typically causes health effect estimates to be underestimated for studies of either short-term or long-term exposure. Biases and decreases in the precision of the association (i.e., wider 95% CIs) tend to be small. Even when spatial variability is higher near roads, the reduction in ozone exposure would cause the exposure to be overestimated at a monitor distant from the road or when averaged across a model grid cell, so that health effects would likely be underestimated.

Table Annex 5-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Outcome Assessment/Evaluation
Controlled Human Exposure:
Endpoints should be assessed in the same manner for control and exposure groups (e.g., time after exposure, methods, endpoint evaluator) using valid, reliable methods. Blinding of endpoint evaluators is ideal, especially for qualitative endpoints (e.g., histopathology). For each experiment and each experimental group, including controls, precise details of all procedures carried out should be provided including how, when, and where. Time of the endpoint evaluations is a key consideration that will vary depending on endpoint evaluated. Endpoints should be assessed at time points that are appropriate for the research questions.
Animal Toxicology:
Endpoints should be assessed in the same manner for control and exposure groups (e.g., time after exposure, methods, endpoint evaluator) using valid, reliable methods. Blinding of endpoint evaluators is ideal, especially for qualitative endpoints (e.g., histopathology). For each experiment and each experimental group, including controls, precise details of all procedures carried out should be provided including how, when, and where. Time of the endpoint evaluations is a key consideration that will vary depending on endpoint evaluated. Endpoints should be assessed at time points that are appropriate for the research questions.
Epidemiology:
Inference is stronger when outcomes are assessed or reported without knowledge of exposure status. Knowledge of exposure status could produce artefactual associations. Confidence is greater when outcomes assessed by interview, self-report, clinical examination, or analysis of biological indicators are defined by consistent criteria and collected by validated, reliable methods. Independent, clinical assessment is valuable for outcomes such as lung function or incidence of disease, but report of physician diagnosis has shown good reliability.[b] When examining short-term exposures, evaluation of the evidence focuses on specific lags based on the evidence presented in individual studies. Specifically, the following hierarchy is used in the process of selecting results from individual studies to assess in the context of results across all studies for a specific health effect or outcome: • Distributed lag models; • Multiple days (e.g., 0−2) are averaged; • Effect estimates are presented for lag days selected a priori by the study authors; or • If a study focuses on only a series of individual lag days, expert judgment is applied to select the appropriate result to focus on considering the time course for physiologic changes for the health effect or outcome being evaluated. When health effects of long-term exposure are assessed by acute events such as symptoms or hospital admissions, inference is strengthened when results are adjusted for short-term exposure. Validated questionnaires for subjective outcomes such as symptoms are regarded to be reliable,[c] particularly when collected frequently and not subject to long recall. For biological samples, the stability of the compound of interest and the sensitivity and precision of the analytical method is considered. If not based on knowledge of exposure status, errors in outcome assessment tend to bias results toward the null.
Potential Copollutant Confounding
Controlled Human Exposure:
Exposure should be well characterized to evaluate independent effects of ozone.
Animal Toxicology:
Exposure should be well characterized to evaluate independent effects of ozone.

Table Annex 5-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Epidemiology:
Not accounting for potential copollutant confounding can produce artifactual associations; thus, studies that examine copollutant confounding carry greater weight. The predominant method is copollutant modeling (i.e., two-pollutant models), which is especially informative when correlations are not high. However, when correlations are high ($r > 0.7$), such as those often encountered for UFP and other traffic-related copollutants, copollutant modeling is less informative. Although the use of single-pollutant models to examine the association between ozone and a health effect or outcome are informative, ideally studies should also include copollutant analyses. Copollutant confounding is evaluated on an individual study basis considering the extent of correlations observed between the copollutant and ozone, and relationships observed with ozone and health effects in copollutant models.
Other Potential Confounding Factors[d]
Controlled Human Exposure:
Preference is given to studies using experimental and control groups that are matched for individual level characteristics (e.g., race/ethnicity, sex, body weight, smoking history, age) and time varying factors (e.g., seasonal and diurnal patterns).
Animal Toxicology:
Preference is given to studies using experimental and control groups that are matched for individual level characteristics (e.g., strain, sex, body weight, litter size, food and water consumption) and time varying factors (e.g., seasonal and diurnal patterns).
Epidemiology:
Factors are considered to be potential confounders if demonstrated in the scientific literature to be related to health effects and correlated with ozone. Not accounting for confounders can produce artifactual associations; thus, studies that statistically adjust for multiple factors or control for them in the study design are emphasized. Less weight is placed on studies that adjust for factors that mediate the relationship between ozone and health effects, which can bias results toward the null. Confounders vary according to study design, exposure duration, and health effect and may include, but are not limited to the following: • Short-term exposure studies: Meteorology, day of week, season, medication use, allergen exposure, and long-term temporal trends. • Long-term exposure studies: Socioeconomic status, race, age, medication use, smoking status, stress, noise, and occupational exposures.
Statistical Methodology
Controlled Human Exposure:
Statistical methods should be clearly described and appropriate for the study design and research question (e.g., correction for multiple comparisons). Generally, statistical significance is used to evaluate the findings of controlled human exposure studies. However, consistent trends are also informative. Detection of statistical significance is influenced by a variety of factors including, but not limited to, the size of the study, exposure and outcome measurement error, and statistical model specifications. Sample size is not a criterion for exclusion; ideally, the sample size should provide adequate power to detect hypothesized effects (e.g., sample sizes less than three are considered less informative). Because statistical tests have limitations, consideration is given to both trends in data and reproducibility of results.

Table Annex 5-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Animal Toxicology:
Statistical methods should be clearly described and appropriate for the study design and research question (e.g., correction for multiple comparisons). Generally, statistical significance is used to evaluate the findings of animal toxicological studies. However, consistent trends are also informative. Detection of statistical significance is influenced by a variety of factors including, but not limited to, the size of the study, exposure and outcome measurement error, and statistical model specifications. Sample size is not a criterion for exclusion; ideally, the sample size should provide adequate power to detect hypothesized effects (e.g., sample sizes less than three are considered less informative). Because statistical tests have limitations, consideration is given to both trends in data and reproducibility of results.
Epidemiology:
Multivariable regression models that include potential confounding factors are emphasized. However, multipollutant models (more than two pollutants) are considered to produce too much uncertainty due to copollutant collinearity to be informative. Models with interaction terms aid in the evaluation of potential confounding as well as effect modification. Sensitivity analyses with alternate specifications for potential confounding inform the stability of findings and aid in judgments of the strength of inference from results. In the case of multiple comparisons, consistency in the pattern of association can increase confidence that associations were not found by chance alone. Statistical methods that are appropriate for the power of the study carry greater weight. For example, categorical analyses with small sample sizes can be prone to bias results toward or away from the null. Statistical tests such as *t*-tests and chi-squared tests are not considered sensitive enough for adequate inferences regarding ozone-health effect associations. For all methods, the effect estimate and precision of the estimate (i.e., width of 95% CI) are important considerations rather than statistical significance.

[a]U.S. EPA (2008).

[b]Murgia et al. (2014); Weakley et al. (2013); Yang et al. (2011); Heckbert et al. (2004); Barr et al. (2002); Muhajarine et al. (1997); Toren et al. (1993).

[c]Burney et al. (1989).

5.4 References

ADA (American Diabetes Association). (2014). Diagnosis and classification of diabetes mellitus. Diabetes Care 37 Suppl 1: S81-S90. http://dx.doi.org/10.2337/dc14-S081.

Akcilar, R; Akcer, S; Simsek, H; Akcilar, A; Bayat, Z; Genc, O. (2015). The effect of ozone on blood pressure in DOCA-salt-induced hypertensive rats. International Journal of Clinical and Experimental Medicine 8: 12783-12791.

Alberti, KG; Eckel, RH; Grundy, SM; Zimmet, PZ; Cleeman, JI; Donato, KA; Fruchart, JC; James, WP; Loria, CM; Smith, SC. (2009). Harmonizing the metabolic syndrome: A joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 120: 1640-1645. http://dx.doi.org/10.1161/CIRCULATIONAHA.109.192644.

Atwal, OS; Wilson, T. (1974). Parathyroid gland changes following ozone inhalation: a morphologic study. Arch Environ Occup Health 28: 91-100.

Barr, RG; Herbstman, J; Speizer, FE; Camargo, CA, Jr. (2002). Validation of self-reported chronic obstructive pulmonary disease in a cohort study of nurses. Am J Epidemiol 155: 965-971. http://dx.doi.org/10.1093/aje/155.10.965.

Bass, V; Gordon, CJ; Jarema, KA; Macphail, RC; Cascio, WE; Phillips, PM; Ledbetter, AD; Schladweiler, MC; Andrews, D; Miller, D; Doerfler, DL; Kodavanti, UP. (2013). Ozone induces glucose intolerance and systemic metabolic effects in young and aged brown Norway rats. Toxicol Appl Pharmacol 273: 551-560. http://dx.doi.org/10.1016/j.taap.2013.09.029.

Boron, W; Boulpaep, E. (2017). Medical physiology. In Medical Physiology (3rd ed.). Philadelphia, PA: Elsevier.

Burney, PG; Laitinen, LA; Perdrizet, S; Huckauf, H; Tattersfield, AE; Chinn, S; Poisson, N; Heeren, A; Britton, JR; Jones, T. (1989). Validity and repeatability of the IUATLD (1984) Bronchial Symptoms Questionnaire: an international comparison. Eur Respir J 2: 940-945.

Chaker, L; Ligthart, S; Korevaar, TI; Hofman, A; Franco, OH; Peeters, RP; Dehghan, A. (2016). Thyroid function and risk of type 2 diabetes: a population-based prospective cohort study. BMC Med 14(150): 1-8. http://dx.doi.org/10.1186/s12916-016-0693-4.

Chen, BY; Chen, CH; Chuang, YC; Kim, H; Honda, Y; Chiang, HC; Guo, YL. (2016a). Schoolchildren's antioxidation genotypes are susceptible factors for reduced lung function and airway inflammation caused by air pollution. Environ Res 149: 145-150. http://dx.doi.org/10.1016/j.envres.2016.05.007.

Chen, Z; Salam, MT; Toledo-Corral, C; Watanabe, RM; Xiang, AH; Buchanan, TA; Habre, R; Bastain, TM; Lurmann, F; Wilson, JP; Trigo, E; Gilliland, FD. (2016b). Ambient air pollutants have adverse effects on insulin and glucose homeostasis in Mexican Americans. Diabetes Care 39: 547-554. http://dx.doi.org/10.2337/dc15-1795.

Chuang, KJ; Yan, YH; Cheng, TJ. (2010). Effect of air pollution on blood pressure, blood lipids, and blood sugar: A population-based approach. J Occup Environ Med 52: 258-262. http://dx.doi.org/10.1097/JOM.0b013e3181ceff7a.

Chuang, KJ; Yan, YH; Chiu, SY; Cheng, TJ. (2011). Long-term air pollution exposure and risk factors for cardiovascular diseases among the elderly in Taiwan. Occup Environ Med 68: 64-68. http://dx.doi.org/10.1136/oem.2009.052704.

Clemons, GK; Garcia, JF. (1980). Changes in thyroid function after short-term ozone exposure in rats. J Environ Pathol Toxicol 4: 359-369.

Crouse, DL; Peters, PA; Hystad, P; Brook, JR; van Donkelaar, A; Martin, RV; Villeneuve, PJ; Jerrett, M; Goldberg, MS; Pope, CA; Brauer, M; Brook, RD; Robichaud, A; Menard, R; Burnett, RT. (2015). Ambient PM 2.5, O 3, and NO 2 exposures and associations with mortality over 16 years of follow-up in the Canadian Census Health and Environment Cohort (CanCHEC). Environ Health Perspect 123: 1180-1186. http://dx.doi.org/10.1289/ehp.1409276.

Dales, RE; Cakmak, S; Vidal, CB; Rubio, MA. (2012). Air pollution and hospitalization for acute complications of diabetes in Chile. Environ Int 46: 1-5. http://dx.doi.org/10.1016/j.envint.2012.05.002.

Dong, G; Qian, Z; Liu, MM; Wang, D; Ren, W; Flick, LH; Fu, J; Wang, J; Chen, W; Simckes, M; Trevathan, E. (2014). Ambient air pollution and the prevalence of obesity in chinese children: The seven northeastern cities study. Obesity (Silver Spring) 22: 795-800. http://dx.doi.org/10.1002/oby.20198.

Dorado-Martinez, C; Parades-Carbajal, C; Mascher, D; Borgonio-Perez, G; Rivas-Arancibia, S. (2001). Effects of different ozone doses on memory, motor activity and lipid peroxidation levels, in rats. Int J Neurosci 108: 149-161. http://dx.doi.org/10.3109/00207450108986511.

Farraj, AK; Hazari, MS; Winsett, DW; Kulukulualani, A; Carll, AP; Haykal-Coates, N; Lamb, CM; Lappi, E; Terrell, D; Cascio, WE; Costa, DL. (2012). Overt and latent cardiac effects of ozone inhalation in rats: evidence for autonomic modulation and increased myocardial vulnerability. Environ Health Perspect 120: 348-354. http://dx.doi.org/10.1289/ehp.1104244.

Farraj, AK; Malik, F; Haykal-Coates, N; Walsh, L; Winsett, D; Terrell, D; Thompson, LC; Cascio, WE; Hazari, MS. (2016). Morning NO2 exposure sensitizes hypertensive rats to the cardiovascular effects of same day O3 exposure in the afternoon. Inhal Toxicol 28: 170-179. http://dx.doi.org/10.3109/08958378.2016.1148088.

Gackière, F; Saliba, L; Baude, A; Bosler, O; Strube, C. (2011). Ozone inhalation activates stress-responsive regions of the CNS. J Neurochem 117: 961-972. http://dx.doi.org/10.1111/j.1471-4159.2011.07267.x.

Gordon, CJ; Jarema, KA; Lehmann, J. R.; Ledbetter, AD; Schladweiler, MC; Schmid, JE; Ward, WO; Kodavanti, UP; Nyska, A; Macphail, RC. (2013). Susceptibility of adult and senescent Brown Norway rats to repeated ozone exposure: an assessment of behavior, serum biochemistry and cardiopulmonary function. Inhal Toxicol 25: 141-159. http://dx.doi.org/10.3109/08958378.2013.764946.

Gordon, CJ; Johnstone, AF; Aydin, C; Phillips, PM; Macphail, RC; Kodavanti, UP; Ledbetter, AD; Jarema, KA. (2014). Episodic ozone exposure in adult and senescent Brown Norway rats: acute and delayed effect on heart rate, core temperature and motor activity. Inhal Toxicol 26: 380-390. http://dx.doi.org/10.3109/08958378.2014.905659.

Gordon, CJ; Phillips, PM; Johnstone, AFM; Beasley, TE; Ledbetter, AD; Schladweiler, MC; Snow, SJ; Kodavanti, UP. (2016). Effect of high-fructose and high-fat diets on pulmonary sensitivity, motor activity, and body composition of brown Norway rats exposed to ozone. Inhal Toxicol 28: 203-215. http://dx.doi.org/10.3109/08958378.2015.1134730.

Gordon, CJ; Phillips, PM; Johnstone, AFM; Schmid, J; Schladweiler, MC; Ledbetter, A; Snow, SJ; Kodavanti, UP. (2017a). Effects of maternal high-fat diet and sedentary lifestyle on susceptibility of adult offspring to ozone exposure in rats. Inhal Toxicol 29: 239-254. http://dx.doi.org/10.1080/08958378.2017.1342719.

Gordon, CJ; Phillips, PM; Ledbetter, A; Snow, SJ; Schladweiler, MC; Johnstone, AF; Kodavanti, UP. (2017b). Active vs. sedentary lifestyle from weaning to adulthood and susceptibility to ozone in rats. Am J Physiol Lung Cell Mol Physiol 312: L100-L109. http://dx.doi.org/10.1152/ajplung.00415.2016.

Heckbert, SR; Kooperberg, C; Safford, MM; Psaty, BM; Hsia, J; McTiernan, A; Gaziano, JM; Frishman, WH; Curb, JD. (2004). Comparison of self-report, hospital discharge codes, and adjudication of cardiovascular events in the Women's Health Initiative. Am J Epidemiol 160: 1152-1158. http://dx.doi.org/10.1093/aje/kwh314.

Henriquez, A; House, J; Miller, DB; Snow, SJ; Fisher, A; Ren, H; Schladweiler, MC; Ledbetter, AD; Wright, F; Kodavanti, UP. (2017a). Adrenal-derived stress hormones modulate ozone-induced lung injury and inflammation. Toxicol Appl Pharmacol 329: 249-258. http://dx.doi.org/10.1016/j.taap.2017.06.009.

Henriquez, AR; Snow, SJ; Schladweiler, MC; Miller, CN; Dye, JA; Ledbetter, AD; Richards, JE; Hargrove, MM; Williams, WC; Kodavanti, UP. (2018). Beta-2 adrenergic and glucocorticoid receptor agonists modulate ozone-induced pulmonary protein leakage and inflammation in healthy and adrenalectomized rats. Toxicol Sci 166: 288-305. http://dx.doi.org/10.1093/toxsci/kfy198.

Henriquez, AR; Snow, SJ; Schladweiler, MC; Miller, CN; Dye, JA; Ledbetter, AD; Richards, JE; Mauge-Lewis, K; Mcgee, MA; Kodavanti, UP. (2017b). Adrenergic and glucocorticoid receptor antagonists reduce ozone-induced lung injury and inflammation. Toxicol Appl Pharmacol 339: 161-171. http://dx.doi.org/10.1016/j.taap.2017.12.006.

Jerrett, M; Brook, R; White, LF; Burnett, RT; Yu, J; Su, J; Seto, E; Marshall, J; Palmer, JR; Rosenberg, L; Coogan, PF. (2017). Ambient ozone and incident diabetes: A prospective analysis in a large cohort of African American women. Environ Int 102: 42-47. http://dx.doi.org/10.1016/j.envint.2016.12.011.

Johnston, RA; Theman, TA; Lu, FL; Terry, RD; Williams, ES; Shore, SA. (2008). Diet-induced obesity causes innate airway hyperresponsiveness to methacholine and enhances ozone-induced pulmonary inflammation. J Appl Physiol (1985) 104: 1727-1735. http://dx.doi.org/10.1152/japplphysiol.00075.2008.

Kilkenny, C; Browne, WJ; Cuthill, IC; Emerson, M; Altman, DG. (2010). Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research [Review]. PLoS Biol 8: e1000412. http://dx.doi.org/10.1371/journal.pbio.1000412.

Kim, JH; Hong, YC. (2012). GSTM1, GSTT1, and GSTP1 polymorphisms and associations between air pollutants and markers of insulin resistance in elderly Koreans. Environ Health Perspect 120: 1378-1384. http://dx.doi.org/10.1289/ehp.1104406.

Klimisch, HJ; Andreae, M; Tillmann, U. (1997). A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regul Toxicol Pharmacol 25: 1-5. http://dx.doi.org/10.1006/rtph.1996.1076.

Kodavanti, UP. (2015). Air pollution and insulin resistance: do all roads lead to Rome? [Comment]. Diabetes 64: 712-714. http://dx.doi.org/10.2337/db14-1682.

Li, M; Qian, Z; Vaughn, M; Boutwell, B; Ward, P; Lu, T, ao; Lin, S; Zhao, Y; Zeng, XW; Liu, RQ; Qin, XD; Zhu, Y; Chen, W; Dong, GH. (2015). Sex-specific difference of the association between ambient air pollution and the prevalence of obesity in Chinese adults from a high pollution range area: 33 Communities Chinese Health Study. Atmos Environ 117: 227-233. http://dx.doi.org/10.1016/j.atmosenv.2015.07.029.

Li, W; Dorans, KS; Wilker, EH; Rice, MB; Kloog, I; Schwartz, JD; Koutrakis, P; Coull, BA; Gold, DR; Meigs, JB; Fox, CS; Mittleman, MA. (2017). Ambient air pollution, adipokines, and glucose homeostasis: The Framingham Heart Study. Environ Int 111: 14-22. http://dx.doi.org/10.1016/j.envint.2017.11.010.

Malmqvist, E; Elding Larsson, H; Jönsson, I; Rignell-Hydbom, A; Ivarsson, SA; Tinnerberg, H; Stroh, E; Rittner, R; Jakobsson, K; Swietlicki, E; Rylander, L. (2015). Maternal exposure to air pollution and type 1 diabetes - Accounting for genetic factors. Environ Res 140: 268-274. http://dx.doi.org/10.1016/j.envres.2015.03.024.

Mathews, JA; Kasahara, DI; Cho, Y; Bell, LN; Gunst, PR; Karoly, ED; Shore, SA. (2017a). Effect of acute ozone exposure on the lung metabolomes of obese and lean mice. PLoS ONE 12: e0181017. http://dx.doi.org/10.1371/journal.pone.0181017.

Mathews, JA; Krishnamoorthy, N; Kasahara, DI; Cho, Y; Wurmbrand, AP; Ribeiro, L; Smith, D; Umetsu, D; Levy, BD; Shore, SA. (2017b). IL-33 drives augmented responses to ozone in obese mice. Environ Health Perspect 125: 246-253. http://dx.doi.org/10.1289/EHP272.

Mathews, JA; Krishnamoorthy, N; Kasahara, DI; Hutchinson, J; Cho, Y; Brand, JD; Williams, AS; Wurmbrand, AP; Ribeiro, L; Cuttitta, F; Sunday, ME; Levy, BD; Shore, SA. (2018). Augmented responses to ozone in obese mice require IL-17A and gastrin-releasing peptide. Am J Respir Cell Mol Biol 58: 341-351. http://dx.doi.org/10.1165/rcmb.2017-0071OC.

Matthews, DR; Hosker, JP; Rudenski, AS; Naylor, BA; Treacher, DF; Turner, RC. (1985). Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412-419. http://dx.doi.org/10.1007/BF00280883.

Mautz, WJ; Bufalino, C. (1989). Breathing pattern and metabolic rate responses of rats exposed to ozone. Respir Physiol Neurobiol 76: 69-77.

Miller, DB; Ghio, AJ; Karoly, ED; Bell, LN; Snow, SJ; Madden, MC; Soukup, J; Cascio, WE; Gilmour, MI; Kodavanti, UP. (2016a). Ozone exposure increases circulating stress hormones and lipid metabolites in humans. Am J Respir Crit Care Med 193: 1382-1391. http://dx.doi.org/10.1164/rccm.201508-1599OC.

Miller, DB; Karoly, ED; Jones, JC; Ward, WO; Vallanat, BD; Andrews, DL; Schladweiler, MC; Snow, SJ; Bass, VL; Richards, JE; Ghio, AJ; Cascio, WE; Ledbetter, AD; Kodavanti, UP. (2015). Inhaled ozone (O3)-induces changes in serum metabolomic and liver transcriptomic profiles in rats. Toxicol Appl Pharmacol 286: 65-79. http://dx.doi.org/10.1016/j.taap.2015.03.025.

Miller, DB; Snow, SJ; Henriquez, A; Schladweiler, MC; Ledbetter, AD; Richards, JE; Andrews, DL; Kodavanti, UP. (2016b). Systemic metabolic derangement, pulmonary effects, and insulin insufficiency following subchronic ozone exposure in rats. Toxicol Appl Pharmacol 306: 47-57. http://dx.doi.org/10.1016/j.taap.2016.06.027.

Miller, DB; Snow, SJ; Schladweiler, MC; Richards, JE; Ghio, AJ; Ledbetter, AD; Kodavanti, UP. (2016c). Acute ozone-induced pulmonary and systemic metabolic effects are diminished in adrenalectomized rats. Toxicol Sci 150: 312-322. http://dx.doi.org/10.1093/toxsci/kfv331.

Muhajarine, N; Mustard, C; Roos, LL; Young, TK; Gelskey, DE. (1997). Comparison of survey and physician claims data for detecting hypertension. J Clin Epidemiol 50: 711-718. http://dx.doi.org/10.1016/S0895-4356(97)00019-X.

Mumaw, CL; Levesque, S; Mcgraw, C; Robertson, S; Lucas, S; Stafflinger, JE; Campen, MJ; Hall, P; Norenberg, JP; Anderson, T; Lund, AK; Mcdonald, JD; Ottens, AK; Block, ML. (2016). Microglial priming through the lung-brain axis: the role of air pollution-induced circulating factors. FASEB J 30: 1880-1891. http://dx.doi.org/10.1096/fj.201500047.

Murgia, N; Brisman, J; Claesson, A; Muzi, G; Olin, AC; Torén, K. (2014). Validity of a questionnaire-based diagnosis of chronic obstructive pulmonary disease in a general population-based study. BMC Pulm Med 14: 49. http://dx.doi.org/10.1186/1471-2466-14-49.

Ramot, Y; Kodavanti, UP; Kissling, GE; Ledbetter, AD; Nyska, A. (2015). Clinical and pathological manifestations of cardiovascular disease in rat models: the influence of acute ozone exposure. Inhal Toxicol 27: 26-38. http://dx.doi.org/10.3109/08958378.2014.954168.

Renzi, M; Cerza, F; Gariazzo, C; Agabiti, N; Cascini, S; Di Domenicantonio, R; Davoli, M; Forastiere, F; Cesaroni, G. (2017). Air pollution and occurrence of type 2 diabetes in a large cohort study. Environ Int 112: 68-76. http://dx.doi.org/10.1016/j.envint.2017.12.007.

Rooney, AA; Boyles, AL; Wolfe, MS; Bucher, JR; Thayer, KA. (2014). Systematic review and evidence integration for literature-based environmental health science assessments. Environ Health Perspect 122: 711-718. http://dx.doi.org/10.1289/ehp.1307972.

Shore, SA. (2007). Obesity and asthma: Lessons from animal models [Review]. J Appl Physiol (1985) 102: 516-528. http://dx.doi.org/10.1152/japplphysiol.00847.2006.

Shore, SA; Lang, JE; Kasahara, DI; Lu, FL; Verbout, NG; Si, H; Williams, ES; Terry, RD; Lee, A; Johnston, RA. (2009). Pulmonary responses to subacute ozone exposure in obese vs. lean mice. J Appl Physiol (1985) 107: 1445-1452. http://dx.doi.org/10.1152/japplphysiol.00456.2009.

Snow, SJ; Henriquez, AR; Costa, DL; Kodavanti, UP. (2018). Neuroendocrine regulation of air pollution health effects: emerging insights. Toxicol Sci 164: 9-20. http://dx.doi.org/10.1093/toxsci/kfy129.

Sun, L; Liu, C; Xu, X; Ying, Z; Maiseyeu, A; Wang, A; Allen, K; Lewandowski, RP; Bramble, LA; Morishita, M; Wagner, JG; Dvonch, JT; Sun, Z; Yan, X; Brook, RD; Rajagopalan, S; Harkema, JR; Sun, Q; Fan, Z. (2013). Ambient fine particulate matter and ozone exposures induce inflammation in epicardial and perirenal adipose tissues in rats fed a high fructose diet. Part Fibre Toxicol 10: 43. http://dx.doi.org/10.1186/1743-8977-10-43.

Theis, WS; Andringa, KK; Millender-Swain, T; Dickinson, DA; Postlethwait, EM; Bailey, SM. (2014). Ozone inhalation modifies the rat liver proteome. 2: 52-60. http://dx.doi.org/doi:10.1016/j.redox.2013.11.006.

Thomson, EM; Pal, S; Guénette, J; Wade, MG; Atlas, E; Holloway, AC; Williams, A; Vincent, R. (2016). Ozone inhalation provokes glucocorticoid-dependent and -independent effects on inflammatory and metabolic pathways. Toxicol Sci 152: 17-28. http://dx.doi.org/10.1093/toxsci/kfw061.

Thomson, EM; Pilon, S; Guénette, J; Williams, A; Holloway, AC. (2018). Ozone modifies the metabolic and endocrine response to glucose: Reproduction of effects with the stress hormone corticosterone. Toxicol Appl Pharmacol 342: 31-38. http://dx.doi.org/10.1016/j.taap.2018.01.020.

Thomson, EM; Vladisavljevic, D; Mohottalage, S; Kumarathasan, P; Vincent, R. (2013). Mapping acute systemic effects of inhaled particulate matter and ozone: multiorgan gene expression and glucocorticoid activity. Toxicol Sci 135: 169-181. http://dx.doi.org/10.1093/toxsci/kft137.

Toren, K; Brisman, J; Jarvholm, B. (1993). Asthma and asthma-like symptoms in adults assessed by questionnaires: A literature review [Review]. Chest 104: 600-608. http://dx.doi.org/10.1378/chest.104.2.600.

Turner, MC; Jerrett, M; Pope, A, III; Krewski, D; Gapstur, SM; Diver, WR; Beckerman, BS; Marshall, JD; Su, J; Crouse, DL; Burnett, RT. (2016). Long-term ozone exposure and mortality in a large prospective study. Am J Respir Crit Care Med 193: 1134-1142. http://dx.doi.org/10.1164/rccm.201508-1633OC.

U.S. EPA (U.S. Environmental Protection Agency). (1991). Guidelines for developmental toxicity risk assessment (pp. 1-71). (EPA/600/FR-91/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=23162.

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

U.S. EPA (U.S. Environmental Protection Agency). (1996b). Guidelines for reproductive toxicity risk assessment (pp. 1-143). (EPA/630/R-96/009). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/sites/production/files/2014-11/documents/guidelines_repro_toxicity.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (1998). Guidelines for neurotoxicity risk assessment [EPA Report] (pp. 1-89). (EPA/630/R-95/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/risk/guidelines-neurotoxicity-risk-assessment.

U.S. EPA (U.S. Environmental Protection Agency). (2005). Guidelines for carcinogen risk assessment [EPA Report] (pp. 1-166). (EPA/630/P-03/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/sites/production/files/2013-09/documents/cancer_guidelines_final_3-25-05.pdf.

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

U.S. EPA (U.S. Environmental Protection Agency). (2008). Integrated science assessment for sulfur oxides: Health criteria [EPA Report]. (EPA/600/R-08/047F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment-RTP. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=198843.

U.S. EPA (U.S. Environmental Protection Agency). (2013a). Integrated science assessment for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-10/076F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment-RTP Division. http://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=247492.

U.S. EPA (U.S. Environmental Protection Agency). (2013b). Toxicological review of trimethylbenzenes (CASRN 25551-13-7, 95-63-6, 526-73-8, and 108-67-8) in support of summary information on the Integrated Risk Information System (IRIS): revised external review draft [EPA Report]. (EPA/635/R13/171a). Washington, D.C.: U.S. Environmental Protection Agency, National Center for Environmental Assessment. http://yosemite.epa.gov/sab/SABPRODUCT.NSF/b5d8a1ce9b07293485257375007012b7/ee1e280e77586de985257b65005d37e7!OpenDocument.

U.S. EPA (U.S. Environmental Protection Agency). (2015). Preamble to the integrated science assessments [EPA Report]. (EPA/600/R-15/067). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, RTP Division. https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=310244.

Uchiyama, I; Simomura, Y; Yokoyama, E. (1986). Effects of acute exposure to ozone on heart rate and blood pressure of the conscious rat. Environ Res 41: 529-537.

Uchiyama, I; Yokoyama, E. (1989). Effects of short- and long-term exposure to ozone on heart rate and blood pressure of emphysematous rats. Environ Res 48: 76-86. http://dx.doi.org/10.1016/S0013-9351(89)80087-8.

Vella, RE; Pillon, NJ; Zarrouki, B; Croze, ML; Koppe, L; Guichardant, M; Pesenti, S; Chauvin, MA; Rieusset, J; Géloën, A; Soulage, CO. (2014). Ozone exposure triggers insulin resistance through muscle c-Jun N-terminal Kinases (JNKs) activation. Diabetes 64: 1011-1024. http://dx.doi.org/10.2337/db13-1181.

von Elm, E; Altman, DG; Egger, M; Pocock, SJ; Gøtzsche, PC; Vandenbroucke, JP. (2007). The strengthening the reporting of observational studies in epidemiology (strobe) statement: guidelines for reporting observational studies [Review]. PLoS Med 4: e296. http://dx.doi.org/10.1371/journal.pmed.0040296.

Wagner, JG; Allen, K; Yang, HY; Nan, B; Morishita, M; Mukherjee, B; Dvonch, JT; Spino, C; Fink, GD; Rajagopalan, S; Sun, Q; Brook, RD; Harkema, JR. (2014). Cardiovascular depression in rats exposed to inhaled particulate matter and ozone: effects of diet-induced metabolic syndrome. Environ Health Perspect 122: 27-33. http://dx.doi.org/10.1289/ehp.1307085.

Wallace, TM; Levy, JC; Matthews, DR. (2004). Use and abuse of HOMA modeling [Review]. Diabetes Care 27: 1487-1495. http://dx.doi.org/10.2337/diacare.27.6.1487.

Ward, WO; Kodavanti, UP. (2015). Pulmonary transcriptional response to ozone in healthy and cardiovascular compromised rat models. Inhal Toxicol 27: 93-104. http://dx.doi.org/10.3109/08958378.2014.954173.

Weakley, J; Webber, MP; Ye, F; Zeig-Owens, R; Cohen, HW; Hall, CB; Kelly, K; Prezant, DJ. (2013). Agreement between obstructive airways disease diagnoses from self-report questionnaires and medical records. Prev Med 57: 38-42. http://dx.doi.org/10.1016/j.ypmed.2013.04.001.

White, LF; Jerrett, M; Yu, J; Marshall, JD; Rosenberg, L; Coogan, PF. (2016). Ambient air pollution and 16-year weight change in African-American women. Am J Prev Med 51: e99e105. http://dx.doi.org/10.1016/j.amepre.2016.03.007.

Yang, BY; Qian, ZM; Li, S; Fan, S; Chen, G; Syberg, KM; Xian, H; Wang, SQ; Ma, H; Chen, DH; Yang, M; Liu, KK; Zeng, XW; Hu, LW; Guo, Y; Dong, GH. (2018). Long-term exposure to ambient air pollution (including PM1) and metabolic syndrome: The 33 Communities Chinese Health Study (33CCHS). Environ Res 164: 204-211. http://dx.doi.org/10.1016/j.envres.2018.02.029.

Yang, CL; To, T; Foty, RG; Stieb, DM; Dell, SD. (2011). Verifying a questionnaire diagnosis of asthma in children using health claims data. BMC Pulm Med 11. http://dx.doi.org/10.1186/1471-2466-11-52.

Ying, Z; Allen, K; Zhong, J; Chen, M; Williams, KM; Wagner, JG; Lewandowski, R; Sun, Q; Rajagopalan, S; Harkema, JR. (2016). Subacute inhalation exposure to ozone induces systemic inflammation but not insulin resistance in a diabetic mouse model. Inhal Toxicol 28: 155-163. http://dx.doi.org/10.3109/08958378.2016.1146808.

Zellner, LC; Brundage, KM; Hunter, DD; Dey, RD. (2011). Early postnatal ozone exposure alters rat nodose and jugular sensory neuron development. Toxicol Environ Chem 93: 2055-2071. http://dx.doi.org/10.1080/02772248.2011.610882.

Zhong, J; Allen, K; Rao, X; Ying, Z; Braunstein, Z; Kankanala, SR; Xia, C; Wang, X; Bramble, LA; Wagner, JG; Lewandowski, R; Sun, Q; Harkema, JR; Rajagopalan, S. (2016). Repeated ozone exposure exacerbates insulin resistance and activates innate immune response in genetically susceptible mice. Inhal Toxicol 28: 383-392. http://dx.doi.org/10.1080/08958378.2016.1179373.

APPENDIX 6 HEALTH EFFECTS—MORTALITY

Summary of Causality Determinations for Short- and Long-Term Ozone Exposure and Total (Nonaccidental) Mortality

This Appendix characterizes the scientific evidence that supports causality determinations for short- and long-term ozone exposure and total mortality. The types of studies evaluated within this Appendix are consistent with the overall scope of the ISA as detailed in the Preface. In assessing the overall evidence, strengths and limitations of individual studies were evaluated based on scientific considerations detailed in the Annex for Appendix 6. More details on the causal framework used to reach these conclusions are included in the Preamble to the ISA (U.S. EPA, 2015, 2013a).

Exposure Duration	Causality Determination
Short-term exposure	Suggestive of, but not sufficient to infer, a causal relationship
Long-term exposure	Suggestive of, but not sufficient to infer, a causal relationship

6.1 Short-Term Ozone Exposure and Mortality

6.1.1 Introduction

The 2013 Integrated Science Assessment for Ozone and Related Photochemical Oxidants (2013 Ozone ISA) concluded there is *likely to be a causal relationship* between short-term ozone exposure and total mortality (U.S. EPA, 2013a), which built upon the evidence presented in the 2006 Ozone Air Quality Criteria Document [AQCD; (U.S. EPA, 2006)]. This conclusion was supported by a number of multicity and multicontinent epidemiologic studies that provided evidence of consistent, positive associations between short-term ozone exposure and mortality in all-year and summer/warm season analyses and across different averaging times (i.e., 1-hour max, 8-hour max, and 24-hour avg), which further confirmed the positive associations reported in multicity studies, single-city studies, and meta-analyses evaluated in previous assessments. The multicity and multicontinent studies evaluated in the 2013 Ozone ISA also addressed key uncertainties and limitations that remained in the evidence base for short-term ozone exposure and mortality upon the completion of the 2006 Ozone AQCD. As summarized below, these studies further informed the relationship between short-term ozone exposure and cause-specific mortality, the potential confounding effects of copollutants and season, spatial

heterogeneity in ozone-mortality risk estimates, the timing of mortality effects, and the shape of the concentration-response (C-R) relationship.

Epidemiologic studies evaluated in the 2013 Ozone ISA expanded upon the evaluation of associations between short-term ozone exposure and cause-specific mortality through multicity studies, which previously was limited to primarily single-city studies. These studies provided evidence of generally consistent, positive associations with both cardiovascular and respiratory mortality in all-year and summer/warm season analyses. The strong and consistent evidence within and across scientific disciplines for respiratory morbidity provided coherence and biological plausibility for respiratory mortality. However, the morbidity evidence supporting cardiovascular mortality was more limited. Although controlled human exposure and animal toxicological studies provided initial evidence supporting a biologically plausible mechanism by which short-term ozone exposure could lead to cardiovascular mortality, there was inconsistency in results between experimental and epidemiologic studies. Specifically, epidemiologic studies did not consistently demonstrate positive associations with other apical cardiovascular effects, such as hospital admissions and emergency department visits.

In the previous ISA, the evaluation of potential confounding of the ozone-mortality relationship in epidemiologic studies focused on assessing both model specification (e.g., control for temporal/seasonal trends) and the influence of copollutants on ozone-mortality associations. An examination of modeling methods indicated that the extent of smoothing used to control for temporal/seasonal trends (i.e., numbers of degrees of freedom used in time splines) can influence the magnitude of associations observed. More detailed analyses of potential copollutant confounding focused on not only PM size fractions, but also $PM_{2.5}$ components, and reported that although associations were attenuated in copollutant models with PM in some instances, overall associations remained positive. However, the assessment of potential copollutant confounding was complicated by the variability in the correlation between PM and ozone across regions and the small number of days with both ozone and PM data due to the PM sampling schedule (i.e., every 3rd or 6th day).

Multicity studies also provided evidence of the geographic pattern of spatial heterogeneity (i.e., regional and city-to-city) in ozone-mortality risk estimates, with associations largest in magnitude in the northeastern U.S. A few studies examined whether specific factors, both time-invariant and time-variant, explained this observed heterogeneity. Examination of the time-invariant factors showed some evidence that individual- and community-level factors may contribute to spatial heterogeneity of ozone-mortality associations, including but not limited to, unemployment rate, prevalence of air conditioning, and indicators of socioeconomic status (SES). Additionally, there was initial evidence that the time-variant factor of daily temperature modifies ozone effects on mortality, specifically high temperatures, may increase the risk of ozone-related mortality.

Lastly, the multicity and multicontinent studies evaluated in the 2013 Ozone ISA provided a more thorough assessment of the timing of mortality effects after ozone exposure and the C-R relationship. Across studies there was evidence that the strongest ozone-mortality association, in terms of magnitude

and precision, occurs within the first few days after exposure, within the range of 0−3 days. Additionally, examination of the C-R relationship between short-term ozone exposure and mortality supported a linear relationship with no evidence of a threshold below which effects do not occur.

Building off the evidence detailed in the 2013 Ozone ISA, the following sections provide a brief, integrated evaluation of recent evidence for short-term ozone exposure and mortality. Specifically, the sections focus on assessing the degree to which newly available studies further characterize the relationship between short-term ozone exposure and mortality, and the continued evaluation of previously identified uncertainties and limitations in the evidence base. The 2013 Ozone ISA informed a series of uncertainties and limitations, specifically: the relationship between short-term ozone exposure and cause-specific mortality, the potential confounding effects of copollutants and season, heterogeneity in ozone-mortality risk estimates, the timing of mortality effects, and the shape of the concentration-response (C-R) relationship. Recent studies, however, support and in some cases further address the uncertainties and limitations in the evidence base examined in those earlier studies. While the evidence in this section will focus on epidemiologic studies, the overall conclusions will draw on the morbidity evidence presented for different health endpoints across the scientific disciplines (i.e., animal toxicological, epidemiologic, and controlled human exposure studies) to assess coherence between the morbidity and mortality evidence and inform biological plausibility for ozone-related mortality.

6.1.1.1 Population, Exposure, Comparison, Outcome, and Study Design (PECOS) Tool

The scope of this section is defined by a scoping tool that generally describes the relevant Population, Exposure, Comparison, Outcome, and Study Design (PECOS). The PECOS tool defines the parameters and provides a framework to help identify the relevant literature to inform the draft 2019 Ozone ISA. Because the 2013 Ozone ISA concluded there is *likely to be a causal relationship* between short-term ozone exposure and total mortality, the studies evaluated are more limited in scope and targeted towards study locations that are most informative in addressing the policy-relevant considerations forming the basis of this section. Therefore, the studies evaluated and subsequently discussed within this section were included if they satisfied all of the components of the following PECOS tool:

- Population: Any U.S. or Canadian population, including populations or lifestages that might be at increased risk
- Exposure: Short-term exposure (on the order of 1 to several days) to ambient concentrations of ozone
- Comparison: Per unit increase (in ppb)
- Outcome: Mortality
- Study Design: Epidemiologic studies consisting of case-crossover or time-series studies

6.1.2 Biological Plausibility

The preceding appendices characterized evidence to evaluate the biological plausibility by which short-term ozone exposure may lead to the morbidity effects that are the most common causes of total (nonaccidental) mortality, specifically respiratory (Appendix 3) and cardiovascular (Appendix 4) morbidity, which comprise ~9 and ~33%, respectively, of total mortality (NHLBI, 2017). This evidence is derived from animal toxicological, controlled human exposure, and epidemiologic studies. Appendix 3 characterized the strong evidence by which ozone exposure could plausibly progress from initial events to endpoints relevant to the respiratory system, including increases in respiratory emergency department (ED) visits and hospital admissions for chronic obstructive pulmonary disease (COPD) and asthma. Appendix 4 outlined the available evidence for plausible mechanisms by which ozone exposure could progress from initial events to endpoints relevant to the cardiovascular system and to population outcomes, such as ED visits and hospital admissions due to cardiovascular disease, particularly ischemic heart disease and congestive heart failure. Collectively, the progression demonstrated in the available evidence for respiratory morbidity supports potential biological pathways by which short-term ozone exposures could result in mortality; the evidence, however, for cardiovascular morbidity is more limited due to the inconsistency in results between experimental and epidemiologic studies.

6.1.3 Total (Nonaccidental) Mortality

Relatively few recent studies have been conducted within the U.S. and Canada that examined the relationship between short-term ozone exposure and total (nonaccidental) mortality since the completion of the 2013 Ozone ISA. Although these recent multicity studies conducted new analyses that further characterize the association between short-term ozone exposure and mortality, most relied on population and air quality data from previously conducted studies (e.g., the National Morbidity, Mortality, and Air Pollution Study [NMMAPS]), with only Di et al. (2017a) using more recent air quality data (i.e., since 2000). Additionally, many of the recent studies continued to use the traditional approach of assigning ozone exposures using ozone concentrations measured at a single monitor or the average of ozone concentrations from multiple monitors within some defined geographic location. Of the studies evaluated, Madrigano et al. (2015) and Di et al. (2017a) used novel exposure assignment methods that allowed for the inclusion of populations residing in more diverse geographic locations (i.e., not limited to major urban centers) through kriging (i.e., spatial interpolation) or the use of multiple sources of air quality data (i.e., land use, chemical transport modeling, and satellite observations). All of the studies evaluated continue to show evidence of consistent, positive associations between short-term ozone exposure and mortality, primarily within the first few days after exposure (i.e., lag 0−2 days), as well as evidence of spatial heterogeneity in risk estimates (Liu et al., 2016). Liu et al. (2016) as depicted in Figure 6-1. Additional study details can be found in Table 6-3. Specifically, recent studies focusing on total (nonaccidental) mortality indicate the following:

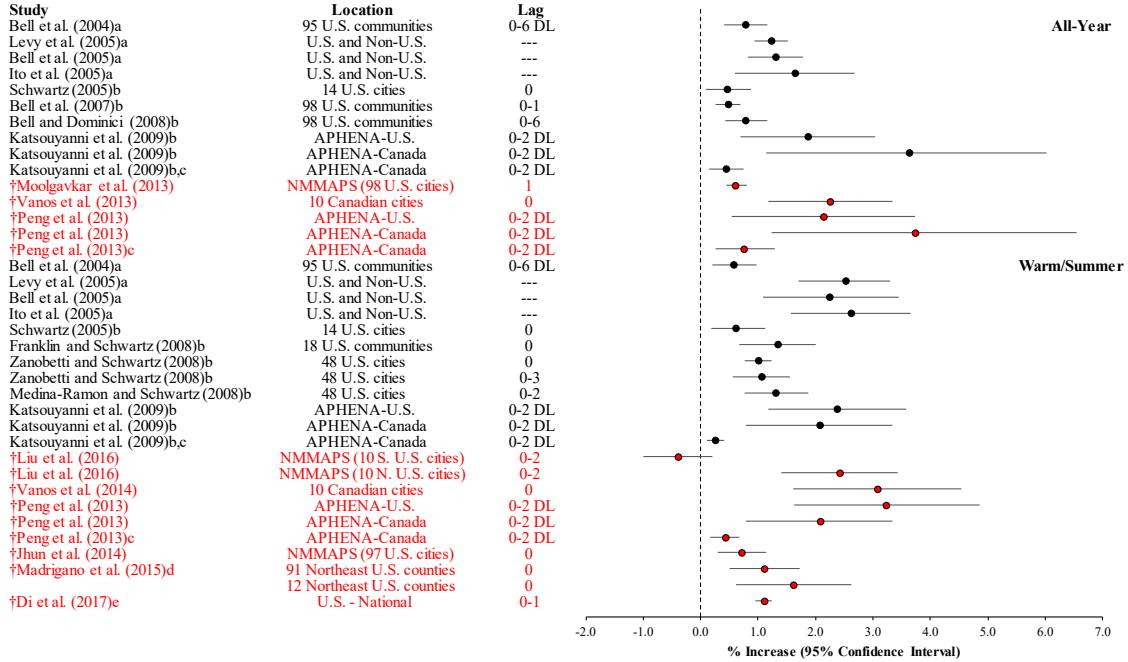
- The strongest recent evidence comes from a study by Di et al. (2017a), who evaluated more recent air quality data (i.e., 2000−2012) and analyzed the largest study population, with over 22 million case days included in the case-crossover analysis. Using a well validated hybrid exposure model, the authors reported a 1.1% increase in all-cause mortality (95% CI: 0.96, 1.24)[1] at lag 0−1 for a 20-ppb increase in 8-hour max ozone concentrations in a single-pollutant model. When limiting ozone data to days where 8-hour max ozone concentrations were less than 60 ppb, there continued to be evidence of a positive association with all-cause mortality in copollutant models with $PM_{2.5}$ (1.16% [95% CI: 0.92, 1.40]; lag 0−1).

- A recent study by Madrigano et al. (2015) provides additional evidence for a positive association between short-term ozone exposure and total mortality and characterizes the variation in the association across urban and nonurban areas. The authors examined older air quality data (i.e., 1988−1999) and used kriging to spatially interpolate ozone concentrations using available monitoring data in 12 counties to examine associations between short-term ozone exposure and total mortality across 91 northeastern U.S. counties. The authors examined associations in both urban (≥1,000 persons/mile^2) and nonurban (<1,000 persons/mile^2) counties. The authors reported positive associations when using both observed and interpolated ozone concentrations (Figure 6-1); they reported evidence of associations that are larger in magnitude for nonurban counties (1.47% [95% CI: 0.38, 2.54], lag 0 for 20-ppb increase in 8-hour max ozone concentrations) than for urban counties (0.90% [95% CI: 0.16, 1.67], lag 0). Although the magnitude of the association is larger for nonurban areas, confidence intervals are larger as well due to larger uncertainty from interpolating ozone concentrations from the fewer monitors located in nonurban areas (Appendix 2—Section 2.3.2.1).

- Multiple recent studies that relied on data from NMMAPS spanning the years 1987−2000 also provide evidence of positive associations between short-term ozone exposure and mortality, but the studies vary by the number of cities, lags, exposure metrics, and seasons examined (Liu et al., 2016; Jhun et al., 2014; Moolgavkar et al., 2013). Additionally, the study by Liu et al. (2016) provided evidence of spatial heterogeneity in ozone-mortality associations in an analysis focusing on 10 northern and 10 southern U.S. cities (see Section 6.1.5.4).

- Peng et al. (2013) provides additional evidence of positive associations for short-term ozone exposure and mortality using 1987−1996 air quality data from NMMAPS (50 U.S. cities all-year data; 36 U.S. cities summer only) as well as data from 12 Canadian cities as part of the Air Pollution and Health: A European and North American Approach (APHENA) study. Using a conservative modeling approach that consisted of penalized splines and 8 degrees of freedom per year (df/yr) to account for temporal trends, positive associations were observed in both all-year and summer season analyses, with evidence of associations that are larger in magnitude in the summer in the U.S. when using the NMMAPS data (Figure 6-1).

[1] Results standardized to a 15-ppb increase in 24-hour avg, 20-ppb increase in 8-hour daily max, or 25-ppb increase in 1-hour daily max ozone concentrations.



DL = distributed lag.

Note: † and red text = recent multicity studies, black = U.S. and Canadian multicity studies and meta-analyses evaluated in the 2006 Ozone AQCD and 2013 Ozone ISA. Results standardized to a 15-ppb increase in 24-hour avg, 20-ppb increase in 8-hour max, or 25-ppb increase in 1-hour max ozone concentrations.

[a]Multicity studies and meta-analyses from the 2006 Ozone AQCD. Bell et al. (2005), Ito et al. (2005) and Levy et al. (2005) used a range of lag days in the meta-analysis: Lag 0, 1, 2, or average 0−1 or 1−2; Single-day lags from 0−3; and Lag 0 and 1−2.

[b]Multicity studies from the 2013 Ozone ISA.

[c]Risk estimates from APHENA-Canada standardized to an approximate IQR of 5.1 ppb for a 1-hour max increase in ozone concentrations as detailed in the 2013 Ozone ISA.

[d]The 91-counties analysis used interpolated ozone concentrations, while the 12-counties analysis used observed ozone concentrations.

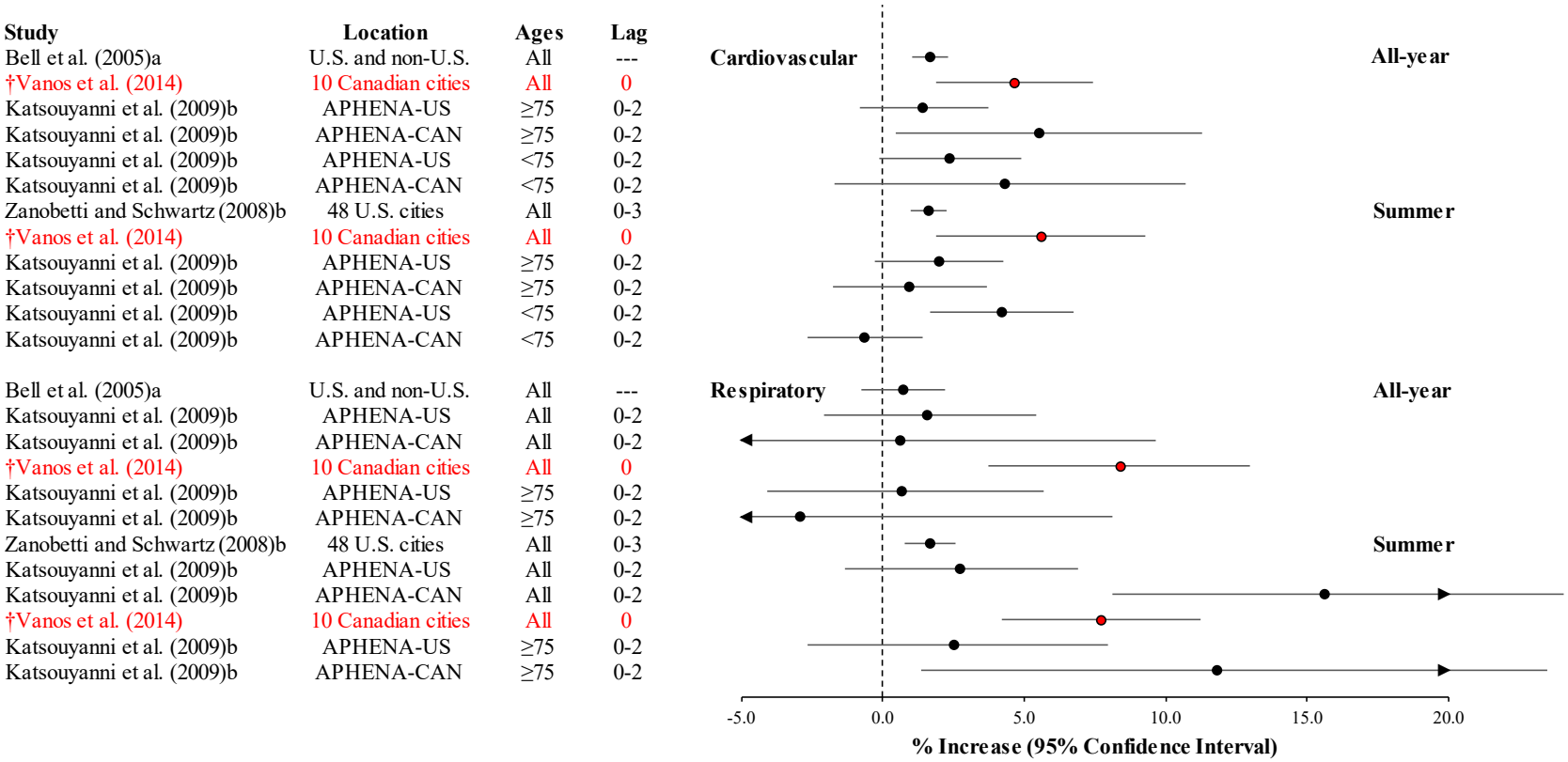
[e]Examined ages 65 and older and all-cause mortality.

Figure 6-1 Summary of associations for short-term ozone exposure and total (nonaccidental) mortality from recent multicity U.S. and Canadian studies, and studies evaluated in previous ozone assessments.

6.1.4 Cause-Specific Mortality

The majority of evidence examining cause-specific mortality consists of studies evaluated in the 2013 Ozone ISA, which reported primarily consistent, positive associations for both cardiovascular and respiratory mortality in all-year and summer/warm season analyses. Recent studies have not extensively examined the relationship between short-term ozone exposure and cause-specific mortality, but both multi- and single-city studies continue to support positive associations, particularly with cardiovascular mortality. Epidemiologic studies evaluated in the 2013 Ozone ISA and recent multicity and single-city studies that examined cause-specific mortality are characterized in Table 6-4 and Table 6-5. In summary:

- Of the recent multicity studies evaluated, only Vanos et al. (2014) in a study of 10 Canadian cities examined cause-specific mortality and reported positive associations with both cardiovascular and respiratory mortality in all-year and summer season analyses. These results are consistent with the multicity studies and meta-analyses evaluated in the 2013 Ozone ISA (Figure 6-2).
- A few single-city studies also examined short-term ozone exposure and cause-specific mortality, with Klemm et al. (2011) examining both respiratory and cardiovascular mortality and Sacks et al. (2012) focusing on cardiovascular mortality in the context of examining the influence of model specification (i.e., control for seasonal/temporal trends, and weather covariates). Sacks et al. (2012) reported evidence of positive associations for cardiovascular mortality ranging from 1.30% (95% CI: −2.1, 4.9) to 2.20% (95% CI: −1.8, 6.4) at lag 0−1 days for a 20-ppb increase in 8-hour max ozone concentrations, specifically for those statistical models that more aggressively controlled for temperature (i.e., using multiple temperature terms or a term for apparent temperature versus including only one temperature term). In a study conducted in Atlanta, GA, Klemm et al. (2011) included 7.5 more years of data than in Klemm and Mason (2000) and Klemm et al. (2004), and reported evidence of a positive association with cardiovascular morality (0.69% [95% CI: −2.28, 3.75]) at lag 0−1 days for a 20-ppb increase in 8-hour max ozone concentrations in all-year analyses. However, the authors found no evidence of an association with respiratory mortality (−0.44% [95% CI: −6.06, 5.51]), contradicting the consistent primarily positive associations reported in multicity studies (Figure 6-2).



Note: † and red text = recent multicity studies, black = U.S. and Canadian multicity studies and meta-analyses evaluated in the 2006 Ozone AQCD and 2013 Ozone ISA. Results standardized to a 15-ppb increase in 24-hour age, 20-ppb increase in 8-hour max, or 25-ppb increase in 1-hour max ozone concentrations.

aMulticity studies and meta-analyses from the 2006 Ozone AQCD. Bell et al. (2005) used a range of lag days in the meta-analysis: Lag 0, 1, 2, or average 0−1 or 1−2.

bMulticity studies from the 2013 Ozone ISA.

Figure 6-2 Summary of associations for short-term ozone exposure and cause-specific mortality from recent multicity U.S. and Canadian studies, and studies evaluated in previous ozone assessments.

6.1.5 Effect Modification of the Ozone-Mortality Relationship

Multicity studies evaluated in the 2013 Ozone ISA reported evidence of spatial heterogeneity, both regional as well as city-to-city, in the magnitude of ozone-mortality risk estimates. To assess what may account for this heterogeneity, studies often examined factors that may modify the ozone-mortality relationship. Studies that conducted such analyses in the 2013 Ozone ISA provided initial evidence that a number of individual- and population-level factors (e.g., race, unemployment rate) may explain this heterogeneity. In addition to examining individual- and population-level factors recent studies also explored specific weather conditions (i.e., season, temperature, and weather patterns) that may modify the ozone-mortality relationship and potentially contribute to these observed differences in risk estimates.

6.1.5.1 Lifestage

Few recent studies have conducted extensive analyses to examine whether specific individual- and population-level factors modify the ozone-mortality relationship. Across studies, there is some evidence of increased risk of ozone-related mortality in older adults (i.e., >65 years of age), particularly as age increases, with more limited evidence for other factors, which is reflected in the following studies:

- In analyses of potential modifiers of the ozone-mortality relationship, Di et al. (2017a) reported slightly elevated risks in females compared to males, as well as for medicaid eligible versus noneligible participants. However, the most pronounced difference across the factors examined was for increasing age, with the risk of mortality attributed to short-term ozone exposure almost double for people 75−84 and ≥85 years of age compared to people ≤69 years of age.
- Vanos et al. (2013) also reported some evidence of increased risk in older individuals, with the risk being greater in individuals 75−84 years of age compared to the other age ranges examined (i.e., ≤64, 65−74, and ≥85; quantitative results not presented).
- Madrigano et al. (2015) examined modification of the ozone-mortality association by county characteristics. The authors reported evidence of increased risk in counties with a large percent of the population over the age of 65 years, which provides some support for the results of Di et al. (2017a) and Vanos et al. (2013). Additionally, Madrigano et al. (2015) reported no evidence of increased risk as the percent of families in poverty or population density increased.

6.1.5.2 Pre-existing Diseases

A limited number of studies evaluated in the 2013 Ozone ISA provided some evidence that pre-existing cardiovascular diseases, such as atrial fibrillation and atherosclerosis, may increase the risk of ozone-related mortality. Recent single-city studies conducted in Montreal, Canada by Goldberg et al. (2013) and Buteau et al. (2018) further examined the role of pre-existing cardiovascular diseases in the relationship between short-term ozone exposure and mortality in individuals ≥65 years of age. Consistent

with the few studies evaluated in the 2013 Ozone ISA, recent studies indicate that some pre-existing cardiovascular disease may increase the risk of ozone-related mortality:

- When examining a distributed lag nonlinear model (DLNM) for 0−2 days, Goldberg et al. (2013) reported little evidence of an association (i.e., positive, but with wide confidence intervals) when focusing on individuals with a diagnosis of any cardiovascular disease 1 year prior to death. Specifically, positive associations were reported in all-year analyses for pre-existing congestive heart failure (2.99% [95% CI: −1.95, 8.17]) and any type of cancer (3.57% [95% CI: 0.16, 7.10]), with associations that are larger in magnitude and more precise in warm seasons analyses for acute coronary artery disease (7.78% [95% CI: 2.43, 13.41]), hypertension (3.70% [95% CI: −0.08, 7.63]), and cerebrovascular disease (4.93% [95% CI: −0.04, 10.16]) for a 15-ppb increase in 24-hour avg ozone concentrations. The authors found no evidence of an association for a number of other pre-existing cardiovascular diseases including diabetes and atrial fibrillation, which was previously found to be associated with an increased risk of ozone-related mortality.
- While Goldberg et al. (2013) examined a number of pre-existing cardiovascular diseases, Buteau et al. (2018) only focused on individuals with pre-existing congestive heart failure with an emphasis on examining associations across five different exposure assignment approaches (i.e., inverse-distance weighting [IDW], back extrapolation method based on land use regression [LUR], Bayesian maximum entropy [BME] model, nearest monitor, and average across all monitors) using two distinct study designs, case-crossover and nested case-control, each of which addresses different questions. In the case-crossover analysis, which focuses on examining why a person died on a particular day rather than on other days within the same month, the authors reported no evidence of an ozone-mortality association across each of the exposure methods used. However, in the nested case-control analysis, where the authors examined why a person died on this day while others did not, there was evidence of positive associations at lag 0−3 DLNM for 20-ppb in 8-hour avg ozone concentrations for the nearest station (6.84% [95% CI: 0.31, 13.79]), IDW (22.69% [95% CI: −3.12, 55.30]), and back extrapolated LUR methods (8.97% [95% CI: 3.67, 14.70]).

6.1.5.3 Season

As detailed in Appendix 1 Section 1.5, ozone concentrations are generally higher in the summer or warm months due to the atmospheric conditions that lead to ozone formation. Therefore, because of the seasonal patterns in ozone concentrations, as well as many locations, particularly within the U.S., only monitoring ozone during the summer or warm months, many of the epidemiologic studies tend to focus on summer or warm season analyses. However, some studies conduct all-year analyses based on areas that monitor ozone all year, with a subset of these studies then examining whether the magnitude of the ozone-mortality association varies either across seasons or in the summer/warm season compared to the entire year. Studies evaluated in the 2013 Ozone ISA, reported evidence of positive ozone-mortality associations in all-year analyses that tended to be larger in magnitude during the warm or summer months. Recent studies that conducted all-year as well as seasonal analyses reported associations in the warm or summer months that were similar or larger in magnitude to those in all-year analyses. Specifically, recent studies indicate:

- In analyses examining ozone-mortality associations across the four seasons, associations were largest in magnitude during the spring and/or summer depending on the mortality outcome examined [i.e., total or cause-specific; (Liu et al., 2016; Vanos et al., 2014)]. However, in Liu et al. (2016), this pattern of associations differed between northern and southern U.S. communities, with only northern U.S. communities having larger associations during the spring and summer. This pattern of associations could be due to the differences in long-term mean temperatures between locations or air conditioning (AC) prevalence (see Section 6.1.5.4).
- Peng et al. (2013) and Goldberg et al. (2013) compared ozone-mortality associations in all-year and broad seasonal analyses (i.e., warm/summer and cold season). In the U.S., Peng et al. (2013) reported a 3.23% (95% CI: 1.63, 4.85) increase in mortality in warm season analyses for a distributed lag (DL) of 0−2 days for a 25-ppb increase in 1-hour max ozone concentrations compared to a 2.13% (95% CI: 0.54, 3.73) increase in an all-year analysis. The pattern of associations observed in Peng et al. (2013) was consistent with Goldberg et al. (2013) in Montreal, Canada across each of the pre-existing cardiovascular disease outcomes examined. However, when examining the Canadian cohort, Peng et al. (2013) did not report associations that are larger in magnitude in the summer season (2.08%) compared with the all-year (3.73%) analysis.

6.1.5.4 Temperature

Ozone formation is tangentially linked to temperature because the periods of greatest solar radiation, a prerequisite for producing ozone (see Appendix 1), occur during months of the year when temperatures are highest. Given this tangential linkage, it has been hypothesized that temperature may modify the relationship between short-term ozone exposure and mortality. Recent studies conducted analyses either focusing on long-term average temperature, daily temperature, or the joint effect of ozone and temperature with the aim of elucidating the role of temperature on the ozone-mortality relationship. These studies indicate that ozone-mortality associations are larger in magnitude in locations with lower long-term average temperature, there is evidence of increased risk of ozone-related mortality at higher daily temperatures, and evidence of a synergistic effect between ozone and temperature at higher ozone concentrations, which is more extensively detailed below:

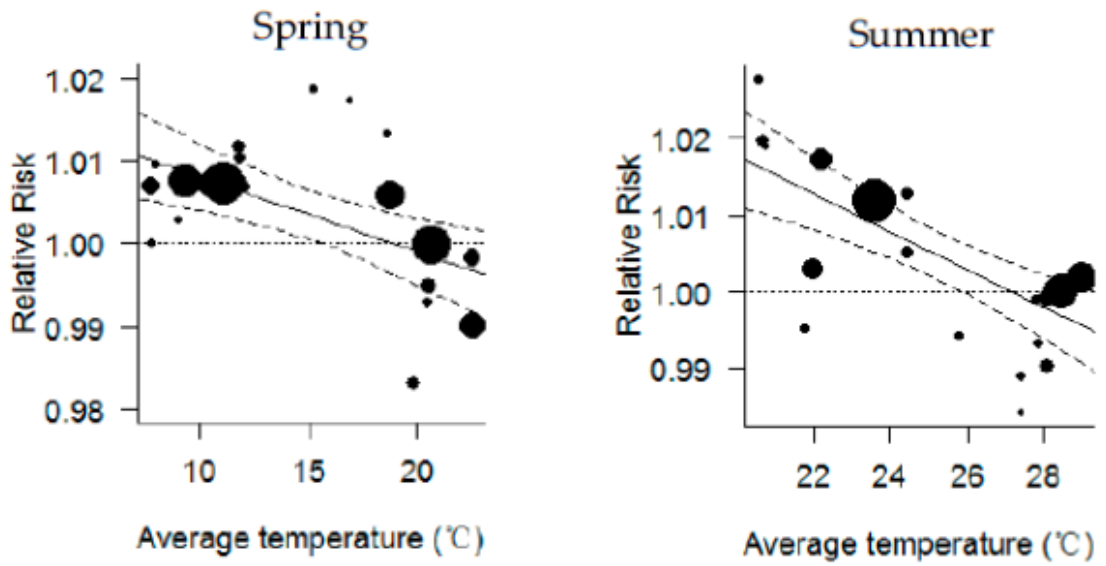
- Both Peng et al. (2013) and Liu et al. (2016) examined whether ozone-mortality associations are modified by long-term average temperature. When examining the distribution of mean temperature across cities, Peng et al. (2013) reported no evidence of ozone-mortality risk estimates increasing as temperatures increased from the 25th to the 75th percentile in the U.S. data set. The results of Peng et al. (2013) in the U.S. cities analysis are supported by Liu et al. (2016). As depicted in Figure 6-3, when examining average temperature, positive associations were only observed in those cities with lower average temperatures.
- When examining the Canadian data set Peng et al. (2013) found that ozone-mortality risk estimates were slightly elevated when moving from the 25th percentile (1.75% increase) to the 75th percentile (2.20% increase) of the mean temperature distribution for a 25-ppb increase in 1-hour max ozone concentrations at lag 0−1. The results of the U.S. analyses by Peng et al. (2013) and Liu et al. (2016), which showed no evidence that mortality risk increased across the temperature distribution, could help explain the positive associations across the temperature distribution in the Canadian analysis because mean temperatures are lower across Canadian cities.

Additionally, the pattern of associations observed in the Canadian analysis could be a reflection of long-term average temperature being a surrogate for AC prevalence as detailed in the 2013 Ozone ISA (U.S. EPA, 2013a).

- Instead of examining long-term average temperatures, Jhun et al. (2014) examined whether average daily temperature modified the ozone-mortality relationship depending on where the temperature fell along the distribution of mean temperatures across the study duration. In three separate analyses, where low and high temperatures were defined as the 25th and 75th percentile, 10th and 90th percentile, and 5th and 95th percentile, the authors reported evidence of a U-shaped curve. Across these analyses, the ozone-mortality risk was highest at low temperatures when using the 25th/75th percentile cutoff, but highest when high temperatures were defined as above the 90th and 95th percentiles. However, the higher ozone-mortality risk estimates at high temperatures were found to be attenuated when examining risks across the distribution of AC prevalence, specifically above the 75th percentile.

- While previous studies focused on whether ozone-mortality risk estimates were modified by long-term average temperature or daily temperature, Wilson et al. (2014) conducted an analysis examining the joint effects of ozone and temperature on mortality. The authors used a spatial monotone surface model to examine the ozone-temperature interaction for the same 95 U.S. cities from NMMAPS detailed in (Bell et al., 2004). This approach allows for the examination of the interaction between ozone and temperature by evaluating mortality risk at different temperature ranges for the same ozone concentration. In analyses focusing on April−October using 1-hour max ozone concentrations at lag 0 and mean temperature, the authors reported evidence of a synergistic effect of temperature on mortality risk at higher ozone concentrations (Figure 6-4). When comparing results across the three different models examined, additive linear, additive nonlinear, and the monotone spatial risk surface model, the percent increase (for an increase from the median of ozone concentrations and temperature to the 95th percentile) in the national estimate was found to vary by 3.06, 3.54, and 3.98%, respectively. These results provide evidence of nonlinearity in the relationship between ozone concentrations and temperature on mortality risk.

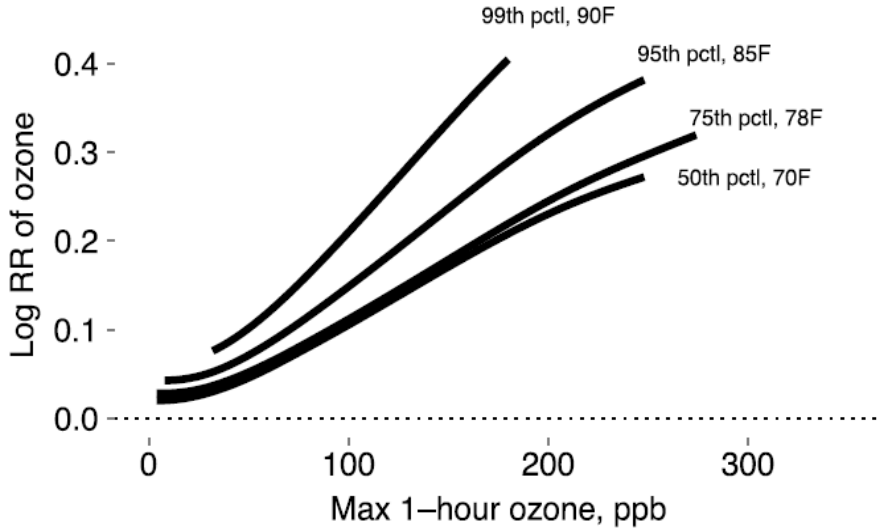
- In regional analyses, Wilson et al. (2014) reported results similar to Liu et al. (2016) by observing a larger degree of interaction in northern U.S. cities where there is a larger difference between temperatures on high temperature and moderate temperature days.



Note: Dot size represents the central estimate and 95% confidence intervals for each individual city, while the solid line and dotted lines represent the central estimate and 95% confidence intervals from the metaregression.

Source: Permission pending, adapted from Liu et al. (2016).

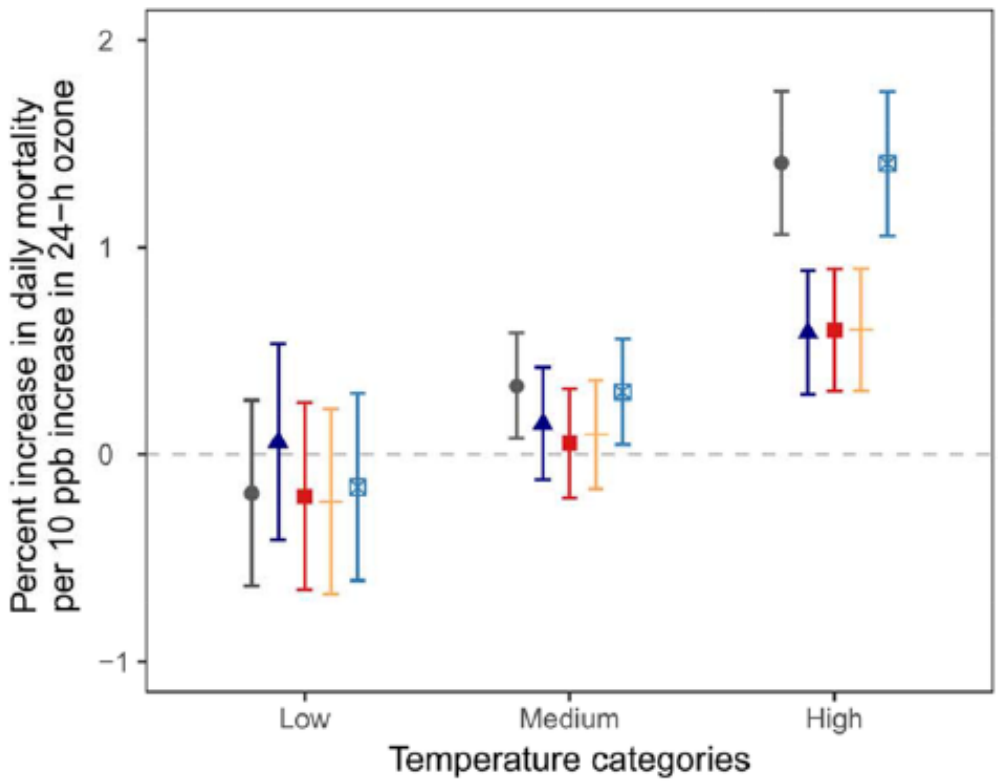
Figure 6-3 Results of a metaregression analysis in Liu et al. (2016) indicating larger ozone-mortality risk estimates in cities with lower average temperatures in the spring and summer seasons.



Source: Permission pending Wilson et al. (2014).

Figure 6-4 Mean log relative risk (RR) for mortality from 95 U.S. cities from the National Morbidity, Mortality, and Air Pollution Study (NMMAPS) at 4 percentiles of temperature (50th, 75th, 95th, 99th).

- Similar to Wilson et al. (2014), Chen et al. (2018) examined a bivariate response surface of ozone and temperature to capture the joint effect on daily mortality in 86 U.S. cities from NMMAPS. The authors observed that temperature positively modified the ozone-mortality relationship. In addition to examining a bivariate response surface, Chen et al. (2018) also examined temperature-stratified ozone-mortality associations across the distribution of temperatures within each city. Overall, the authors reported evidence of modification of the ozone-mortality association at high temperatures (i.e., >75th percentile; Figure 6-5). However, as noted in Section 6.1.6.2, the authors also reported evidence of potential residual confounding when examining temperature-stratified ozone-mortality associations.



Note: Low = <25th percentile; Medium = 25th−75th percentile; High = >75th percentile. Gray and circle = categorical term without adjustment for smooth terms of temperature; purple and triangle = categorical term, plus distributed lag nonlinear model (DLNM) for two different B-splines; red and square = categorical term, plus separate natural splines for low and high temperatures; yellow and dash = categorical term, plus natural spline for high temperatures; blue and square with an x = categorical term, plus natural spline for low temperatures.

Source: Permission pending from Chen et al. (2018).

Figure 6-5 Temperature-stratified ozone-mortality associations from 86 U.S. cities within the National Morbidity, Mortality, and Air Pollution Study (NMMAPS) using different approaches to control for nonlinearity in temperature effects.

6.1.5.5 Weather Patterns

While the majority of studies to date focus on whether season or temperature modify the ozone-mortality relationship, a series of recent studies (Vanos et al., 2015; Vanos et al., 2014; Vanos et al., 2013) conducted in multiple cities in Canada examined the role of specific weather patterns (i.e., synoptic weather categories). The weather categories examined included dry moderate (DM), dry polar (DP), dry tropical (DT), moist moderate (MM), moist polar (MP), moist tropical (MT), and a transitional category (TR), representing the shift from one weather category to another. Although each of the aforementioned studies conducted analyses that differed by lag days, mortality outcome, and years

examined, they all showed some evidence of positive associations for short-term ozone exposure and mortality, as well as cause-specific mortality, across each of the synoptic weather categories examined. When examining individual weather categories, the highest risk was reported with the DT and MT weather categories, which were the weather categories found to encompass the most extreme pollution episodes, as detailed in Vanos et al. (2015).

6.1.6 Potential Confounding of the Ozone-Mortality Relationship

The assessment of potential copollutant confounding in the 2013 Ozone ISA revolved around evaluating studies that focused primarily on $PM_{2.5}$ and PM_{10}. These studies reported that ozone-mortality risk estimates were relatively unchanged in copollutant models, but they had difficulty assessing this evidence due to the regional variability in ozone-PM correlations and the every-3rd or 6th-day PM sampling schedule. Studies evaluated in the 2013 Ozone ISA also examined the impact of controlling for seasonality, with the most extensive analysis conducted within the APHENA study (Katsouyanni et al., 2009), which demonstrated that model misspecification can occur when not enough degrees of freedom (df) are applied to control for the opposing seasonal trends between ozone and mortality. Recent studies that examined whether copollutant exposures and temporal/seasonal trends confound the ozone-mortality relationship provide evidence that continues to support that ozone-mortality associations are relatively unchanged in copollutant models and relatively consistent across a range of df that properly account for temporal/seasonal trends. Additionally, a few recent studies conducted analyses aimed at informing whether the potential confounding effects of temperature have been adequately controlled for when examining ozone-mortality associations.

6.1.6.1 Potential Copollutant Confounding

Recent studies that examined potential copollutant confounding focused primarily on particulate matter, either $PM_{2.5}$ or PM_{10}, with an additional study examining NO_2. The results of these studies are consistent with studies evaluated in the 2013 Ozone ISA that demonstrated that ozone-mortality associations are relatively unchanged in copollutant models with PM as detailed below:

- Using more recent air quality data, Di et al. (2017a) reported that ozone-mortality risk estimates were relatively unchanged in copollutant models with $PM_{2.5}$ (ozone = 1.10 [95% CI: 0.96, 1.24], ozone + $PM_{2.5}$ = 1.02% [95% CI: 0.82, 1.22]; lag 0−1 for a 20-ppb increase in 8-hour max ozone concentrations).
- The remaining multicity U.S. studies that examined potential copollutant confounding by particulate matter focused on PM_{10}. Both Moolgavkar et al. (2013) and Peng et al. (2013) examined potential copollutant confounding using NMMAPS data and provided evidence that associations were slightly attenuated, but remained positive with wider confidence intervals in copollutant models with PM_{10} [Moolgavkar et al. (2013), lag 0−1: ozone = 0.60% (95% CI: 0.44, 0.80), ozone + PM_{10} = 0.33% (95% CI: −0.7, 0.72) for a 15-ppb increase in 24-hour avg ozone

concentrations; Peng et al. (2013), lag 1: ozone = 0.89% (95% CI: 0.00, 1.73), ozone + PM_{10} = 0.64% (95% CI: −0.88, 2.18) for a 25-ppb increase in 1-hour max ozone concentrations]. The increase in the widths of the confidence intervals observed in these studies is consistent with a decrease in precision due to the limited data available to conduct copollutant analyses due to the PM sampling schedule.

- Chen et al. (2018) also used NMMAPS data for 86 U.S. cities to examine the relationship between short-term ozone exposure and mortality and evaluated copollutant models in analyses that were stratified by temperature within each city (i.e., <25th percentile, 25th−75th percentile, >75th percentile). In copollutant models with PM_{10} and NO_2, the authors reported evidence of associations being attenuated, but remaining positive in the high-temperature category (quantitative results not presented). There was limited to no evidence of positive associations in single or copollutant analyses in the low and medium temperature ranges.
- The results of copollutant models from the aforementioned U.S.-based multicity studies are consistent with the single-city analysis conducted in Madrigano et al. (2015) based on the one city (New Haven, CT) that had both PM_{10} and ozone data during the study duration (lag 0: ozone = 5.14% [95% CI: 1.57, 8.85], ozone + PM_{10} = 5.04% [95% CI: 1.38, 8.81] for a 20-ppb increase in 8-hour max ozone concentrations).
- Peng et al. (2013) also examined potential copollutant confounding using data from Canada and reported that results were relatively unchanged when adjusting for PM_{10} (ozone = 2.78% [95% CI: 1.38, 4.14], ozone + PM_{10} = 2.38% [95% CI: −0.88, 6.02]), consistent with the U.S. analysis.

6.1.6.2 Potential Confounding by Temporal/Seasonal Trends and Weather

Recent studies examined the influence of alternative approaches to control for the potential confounding effects of temporal/seasonal trends and weather on the association between short-term ozone exposure and mortality through their systematic evaluations of various statistical models or by varying the parameters of specific covariates included in statistical models (e.g., weather covariates examined, degrees of freedom per year to control for temporal/seasonal trends). Analyses of temporal/seasonal trends support the conclusions of the 2013 Ozone ISA, which demonstrated that not properly accounting for temporal/seasonal trends can result in model misspecification. Additionally, recent studies provide new information indicating that not properly accounting for the potential confounding effects of temperature may result in residual confounding of the ozone-mortality relationship. Specifically, recent studies found the following:

- In all-year analyses, Peng et al. (2013) reported relatively consistent positive associations when examining 3, 8, and 12 df/year using both natural and penalized splines in Canada; however, in the U.S. there was evidence that less than 3 df/year does not properly account for temporal/seasonal trends. While Peng et al. (2013) focused on all-year analyses, Liu et al. (2016) examined the use of 5−9 df/year to account for temporal trends in seasonal analyses for both southern and northern U.S. communities. Results were relatively unchanged for all seasons, except the winter where there was some evidence that the magnitude of the association increased at df/year greater than 7. This observation was more prominent in the southern communities, but a similar pattern was also observed in the northern communities. This indicates a potential subseasonal trend not present for other seasons that requires additional control when focusing on season-specific analyses.

- The summer season results of Liu et al. (2016) are consistent with the warm season analysis conducted by Madrigano et al. (2015) in the 12-county analysis using observed ozone concentrations. Associations of similar magnitude and precision were observed when using either 4 or 7 df/year.
- While the aforementioned studies focused on assessing the control for temporal/seasonal trends, Di et al. (2017a) examined whether the appropriate df were instituted to control for meteorological factors in the statistical model. The authors did not report any evidence that the magnitude of ozone-mortality risk estimates changed when increasing the df for meteorological variables (i.e., temperature and dew point temperature) from 6 to 9.

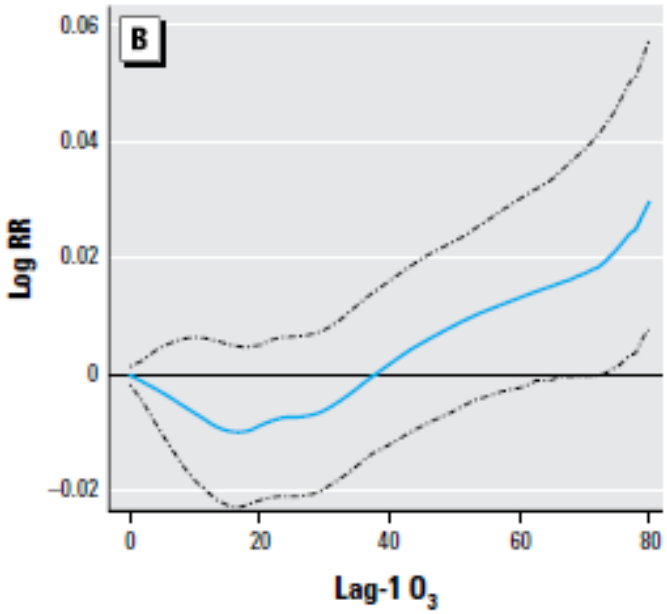
While the studies detailed above focused primarily on examining how changing the df for temporal/seasonal trends or temperature influenced ozone-mortality associations, additional studies conducted systematic evaluations of the relationship between alternative model specifications and ozone-mortality risk estimates and provided new information on the potential for residual confounding:

- Sacks et al. (2012) examined whether similar results were observed across the different statistical models used in multicity studies using a common data set. The authors observed variability in the ozone-cardiovascular mortality relationship corresponding to differing levels of adjustment for temperature. Specifically, those statistical models that more thoroughly controlled for temperature, such as by including multiple temperature terms or a term for apparent temperature, were found to have larger risk estimates (1.3 to 2.2% for a 20-ppb increase in 8-hour max ozone concentrations) compared with those models that included only one temperature term for a single lag day (−1.6 to 0.5%).
- In examining the ozone-mortality relationship at different temperatures, Chen et al. (2018) employed multiple methods to explore whether hot or cold temperatures confounded temperature-stratified ozone-mortality associations. There was evidence of residual confounding and the overestimation of ozone-mortality risk estimates, specifically at high temperatures, which was attributed to not adequately controlling for heat effects.

6.1.7 Shape of the Concentration-Response (C-R) Relationship

Studies included in the 2013 Ozone ISA conducted a variety of statistical analyses to characterize the shape of the concentration-response (C-R) relationship between short-term ozone exposure and mortality and did not observe any evidence of a threshold or deviations from linearity within the range of ozone concentrations observed within the U.S. However, it is important to note that the examination of the ozone-mortality C-R relationship is complicated by previously identified city-to-city and regional heterogeneity in ozone-mortality risk estimates (U.S. EPA, 2013a). Recent studies continue to provide evidence of a linear C-R relationship with no evidence of a threshold below which mortality effects do not occur along the distribution of ozone concentrations observed within the U.S. as described below:

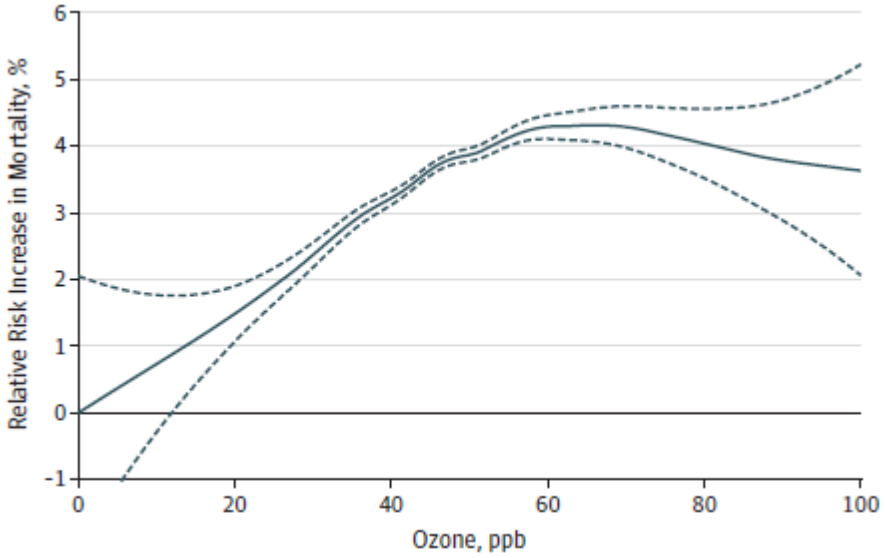
- Moolgavkar et al. (2013) reported evidence of a linear relationship down to concentrations of 60 ppb, with less certainty in the shape of the curve below 60 ppb when examining lag 1, 24-hour avg ozone concentrations in a flexible model using 6 df (Figure 6-6).



Source: Permission pending, Moolgavkar et al. (2013).

Figure 6-6 Flexible concentration-response relationship for short-term ozone exposure and mortality at lag 1 for 24-hour avg ozone concentrations adjusted by size of the bootstrap sample (size of the bootstrap (*d*) = 4).

- While Moolgavkar et al. (2013) focused on 24-hour avg ozone concentrations, Di et al. (2017a) examined the ozone-mortality C-R relationship in a copollutant model with $PM_{2.5}$ using 8-hour max ozone concentrations. In models using penalized splines for both ozone and $PM_{2.5}$, the authors reported evidence of a linear, no-threshold relationship with less certainty at concentrations below approximately 30 ppb (Figure 6-7).



Source: Permission pending, Di et al. (2017a).

Figure 6-7 Percent increase in mortality for ozone in a two-pollutant model with $PM_{2.5}$ using penalized splines for both pollutants at lag 0−1 days in the warm season (April−September).

- While Moolgavkar et al. (2013) and Di et al. (2017a) focused specifically on the shape of the C-R relationship, Peng et al. (2013) examined whether there was evidence of a threshold below which mortality effects are not observed. In a threshold analysis using 1-hour max ozone concentrations where threshold values were set at 5 ppb increments from 0−75 ppb, there was no evidence of a threshold in any of the data sets examined in the APHENA study, including data from the U.S. and Canada.

6.1.8 Summary and Causality Determination

This section describes the evaluation of evidence for total (nonaccidental) mortality based on the scientific considerations detailed in the Annex for Appendix 6, with respect to the causality determination for short-term ozone exposures using the framework described in Table II of the Preamble to the ISAs (U.S. EPA, 2015). The key evidence, as it relates to the causal framework, is summarized in Table 6-1. Recent multicity studies conducted in the U.S. and Canada continue to provide evidence of consistent, positive associations between short-term ozone exposure and total mortality in both all-year and summer/warm season analyses across different averaging times (i.e., 1-hour max, 8-hour max, 8-hour avg, and 24-hour avg; Figure 6-1). The limited assessment of cause-specific mortality by Vanos et al. (2014) is consistent with the pattern of positive associations reported for studies evaluated in the 2013 Ozone ISA (Figure 6-2). However, the experimental evidence, specifically from controlled human

exposure studies, is not consistent with the studies evaluated in the 2013 Ozone ISA. This contributes additional uncertainty for a biologically plausible mechanism by which short-term ozone exposure could lead to cardiovascular mortality. Lastly, most of the recent studies examined associations between short-term ozone exposure and mortality using ozone data prior to the year 2000, with only Di et al. (2017a) focusing on more recent ozone concentrations.

Recent studies continue to assess the influence of potential confounders on the ozone-mortality relationship including copollutants, temporal/seasonal trends, and weather covariates; overall, these studies report that associations remain relatively unchanged across the different approaches used to control for each confounder. The assessment of potential copollutant confounding was examined within the NMMAPS data set by multiple studies (Chen et al., 2018; Moolgavkar et al., 2013; Peng et al., 2013), all of which reported that ozone-mortality associations remained positive in copollutant models with PM_{10} or NO_2. These results were further supported in a large national analysis of Medicare participants (i.e., >65 years of age) in which ozone-mortality associations were similar in magnitude in single pollutant models and copollutant models with PM_{10} (Di et al., 2017a), as well as for an individual city within the study conducted by Madrigano et al. (2015). Importantly, the issues surrounding the assessment of potential copollutant confounding detailed in the 2013 Ozone ISA persists, specifically within studies that relied on NMMAPS data, due to the every-3rd and 6th-day PM sampling.

Additional analyses building off the extensive examination of model specification by Katsouyanni et al. (2009) within APHENA demonstrate that not instituting enough df per year to account for temporal/seasonal trends may underestimate ozone-mortality risk estimates (Peng et al., 2013). However, there is also preliminary indication that some seasons may require additional df when examining seasonal associations [i.e., winter; (Liu et al., 2016)], but additional exploration is warranted because many locations do not monitor ozone outside of the warm/summer season. A limited assessment of model specification with respect to individual weather covariates indicates that increasing the df does not affect ozone-mortality associations (Peng et al., 2013), but not properly accounting for the effect of temperature on mortality may overestimate (Chen et al., 2018) or underestimate (Sacks et al., 2012) ozone-mortality associations.

The effect modification of the ozone-mortality relationship was assessed through studies focusing on both individual and population-level factors, as well as weather-related conditions. There is some evidence of increased risk of ozone-related mortality in older individuals (i.e., >65 years of age), particularly in individuals 75−84 years of age (Di et al., 2017a; Vanos et al., 2013). An assessment of pre-existing disease, which was limited to studies conducted in Montreal, Canada, reported evidence of increased risk in individuals with CHF, and more limited evidence for other cardiovascular-related diseases, including acute coronary artery disease, hypertension, and cerebrovascular disease (Buteau et al., 2018; Goldberg et al., 2013).

While there continues to be some evidence of differential ozone-mortality associations by season (Section 6.1.5.3), the most extensive analyses conducted by recent studies examined whether temperature

modifies the ozone-mortality association. Analyses focusing on temperature, indicate that locations with lower long-term average temperature have higher ozone-mortality risk estimates (Liu et al., 2016; Peng et al., 2013), which is also reflected by the observed difference in risk estimates between northern and southern U.S. cities in Liu et al. (2016). However, long-term average temperature may be a surrogate for air conditioning prevalence. Additionally, studies that examined either the joint effects of ozone and temperature on mortality (Chen et al., 2018; Wilson et al., 2014) or temperature-stratified ozone-mortality associations (Chen et al., 2018; Jhun et al., 2014) provided evidence of ozone-mortality associations that are larger in magnitude at higher temperatures.

Recent multicity studies continue to support a linear a C-R relationship with no evidence of a threshold between short-term ozone exposure and mortality over the range of ozone concentrations typically observed in the U.S. Studies that used different statistical approaches and ozone averaging times (i.e., 24-hour avg and 8-hour max) provide evidence of a linear C-R relationship, with less certainty in the shape of the curve at lower concentrations (i.e., 40 ppb for 24-hour avg (Moolgavkar et al., 2013) and 30 ppb for 8-hour max (Di et al., 2017a)). An examination of whether a threshold exists in the ozone-mortality C-R relationship provided no evidence of a concentration below which mortality effects do not occur when examining 5 $\mu g/m^3$ increments across the range of 1-hour max concentrations reported in the U.S. and Canadian cities included in APHENA (Peng et al., 2013). Collectively, these results continue to support the conclusion of the 2006 Ozone AQCD that "if a population threshold level exists in ozone health effects, it is likely near the lower limit of ambient ozone concentrations in the U.S."

Building on upon the 2013 Ozone ISA, there remains strong evidence for respiratory effects due to short-term ozone exposure (Appendix 3) that is consistent within and across disciplines, and provides coherence and biological plausibility for the positive respiratory mortality associations reported across epidemiologic studies. Although there remains evidence for ozone-induced cardiovascular mortality the preliminary evidence presented in the 2013 Ozone ISA from controlled human exposure and animal toxicological studies that provided a biologically plausible mechanism for ozone-induced cardiovascular mortality is inconsistent with a larger number of recent controlled human exposure studies that do not provide evidence of cardiovascular effects in response to short-term ozone exposure. The limited experimental evidence in combination with the lack of coherence between experimental and epidemiologic studies of cardiovascular morbidity that do not demonstrate consistent evidence of ozone-induced cardiovascular effects complicates the evidence for a biological pathway of events leading to cardiovascular mortality (Appendix 4).

Overall, the recent multicity studies conducted in the U.S. and Canada provide additional support for the consistent, positive associations reported across multicity studies evaluated in the 2006 Ozone AQCD and 2013 Ozone ISA. These results are supported by studies that further examined uncertainties in the ozone-mortality relationship, such as potential confounding by copollutants and other variables, modification by temperature, and the C-R relationship and whether a threshold exists. Although there continues to be strong evidence from studies of respiratory morbidity to support respiratory mortality,

there remains relatively limited biological plausibility and coherence within and across disciplines to support the relatively strong evidence for cardiovascular mortality. Collectively, evidence is suggestive of, but not sufficient to infer, a causal relationship exists between short-term ozone exposure and total mortality.

Table 6-1 Summary of evidence that is suggestive of, but not sufficient to infer, a causal relationship between short-term ozone exposure and total mortality.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Consistent epidemiologic evidence from multiple, high-quality studies at relevant ozone concentrations	Recent multicity studies conducted in the U.S. and Canada continue to support the consistent positive associations between short-term ozone exposure and total mortality in both all-year and warm/summer season analyses.	Di et al. (2017a) Figure 6-1 Section 6.1.3	Mean concentrations across studies: 24-h avg: 14.5−48.7 8-h max/avg: 15.1−62.8 1-h max: 6.7−60.0
Epidemiologic evidence from copollutant models provides some support for an independent ozone association	The few recent multicity studies that examined potential copollutant confounding provide evidence supporting that ozone-mortality risk estimates are relatively unchanged or slightly attenuated, but remain positive, in copollutant models with $PM_{2.5}$, PM_{10}, and NO_2. Studies that reported correlations between ozone and $PM_{2.5}$ or PM_{10} were generally low (<0.40).	Moolgavkar et al. (2013) Peng et al. (2013) Chen et al. (2018) Di et al. (2017a) Section 6.1.5.1	
Epidemiologic evidence continues to support a linear C-R relationship with no evidence of a threshold	Studies continue to provide evidence of a linear C-R relationship with no evidence of a threshold. There is less certainty in the shape of the C-R relationship at the lower end of concentrations observed in the U.S.	Moolgavkar et al. (2013) Di et al. (2017a) Peng et al. (2013) Section 6.1.5.3	24-h avg >40 ppb 8-h max >30 ppb

Table 6-1 (Continued): Summary of evidence that is suggestive of, but not sufficient to infer, a causal relationship between short-term ozone exposure and total mortality.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Limited biological plausibility from studies of cardiovascular morbidity	Animal toxicological and controlled human exposure studies do not provide consistent evidence of potential biological pathways. Additionally, there is a lack of coherence with epidemiologic studies of cardiovascular morbidity to support more overt effects, such as cardiovascular mortality.	Appendix 4	
Biological plausibility from studies of respiratory morbidity	Strong evidence for respiratory effects due to short-term ozone exposure, such as asthma exacerbation, are consistent across disciplines and support potential biological pathways for respiratory mortality.	Appendix 3	
Uncertainty regarding geographic heterogeneity in ozone-mortality associations	Recent studies indicate latitude and temperature may account for some of the observed heterogeneity, but more extensive evaluations have not been conducted.	Section 6.1.3 Section 6.1.5.4	

C-R = concentration-response; NO_2 = nitrogen dioxide; $PM_{2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 2.5 μm; PM_{10} = particulate matter with a nominal aerodynamic diameter less than or equal to 10 μm; ppb = parts per billion.

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to the causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where the full body of evidence is described.

[c]Describes the ozone concentrations with which the evidence is substantiated.

6.2 Long-Term Ozone Exposure and Mortality

6.2.1 Introduction

A limited number of epidemiologic studies have assessed the relationship between long-term ozone exposure and mortality in adults. The 2006 Ozone AQCD concluded that an insufficient amount of evidence existed "to suggest a causal relationship between chronic ozone exposure and increased risk for mortality in humans" (U.S. EPA, 2006). Noting limited support for an association with long-term ozone exposure and total mortality, and inconsistent associations for cardiopulmonary mortality from the ACS and Harvard Six Cities studies, the 2013 Ozone ISA concluded that the evidence was suggestive of a causal relationship between long-term ozone exposure and total mortality (U.S. EPA, 2013a). The strongest evidence for an association between long-term ozone exposure and mortality was derived from associations with respiratory mortality reported by Jerrett et al. (2009) that remained robust after adjusting for $PM_{2.5}$ concentrations and an analysis that reported associations of ambient ozone concentrations and total mortality among populations with pre-existing disease in the Medicare Cohort (Zanobetti and Schwartz, 2011).

The following section provides a brief, integrated evaluation of evidence for long-term ozone exposure and mortality presented in the previous NAAQS review with evidence that is newly available for this review. This section focuses on assessing the degree to which newly available studies further characterize the relationship between long-term ozone exposure and mortality. For example, areas of research that inform differences in the exposure window used to evaluate long-term exposures and mortality or comparisons of statistical techniques will be highlighted. Studies that address the variability in the associations observed across ozone epidemiologic studies due to exposure error and the use of different exposure assessment techniques will be emphasized. Another important consideration will be characterizing the shape of the C-R relationship across the full concentration range observed in epidemiologic studies. The evidence in this section will focus on epidemiologic studies because experimental studies of long-term exposure and mortality are generally not conducted. However, this section will draw from the morbidity evidence presented for different health endpoints across the scientific disciplines (i.e., animal toxicological, epidemiologic, and controlled human exposure studies) to support the associations observed for cause-specific mortality.

6.2.1.1 Population, Exposure, Comparison, Outcome, and Study Design (PECOS) Tool

The scope of this section is defined by a scoping tool that generally defines the relevant Population, Exposure, Comparison, Outcome, and Study Design (PECOS). The PECOS tool defines the parameters and provides a framework to help identify the relevant evidence in the literature to inform the ISA. Because the 2013 Ozone ISA concluded that *evidence existed to suggest a causal relationship* between long-term ozone exposure and total mortality the studies evaluated are less limited in scope and not targeted towards specific study locations, as reflected in the PECOS tool. The studies evaluated and subsequently discussed within this section were identified using the following PECOS tool:

- Population: Any population, including populations or lifestages that might be at increased risk
- Exposure: Long-term ambient concentration of ozone
- Comparison: Per unit increase (in ppb)
- Outcome: Change in risk (incidence/prevalence) of mortality
- Study Design: Epidemiologic cohort studies; time-series, case-crossover, and cross-sectional studies with appropriate timing of exposure for the health endpoint of interest

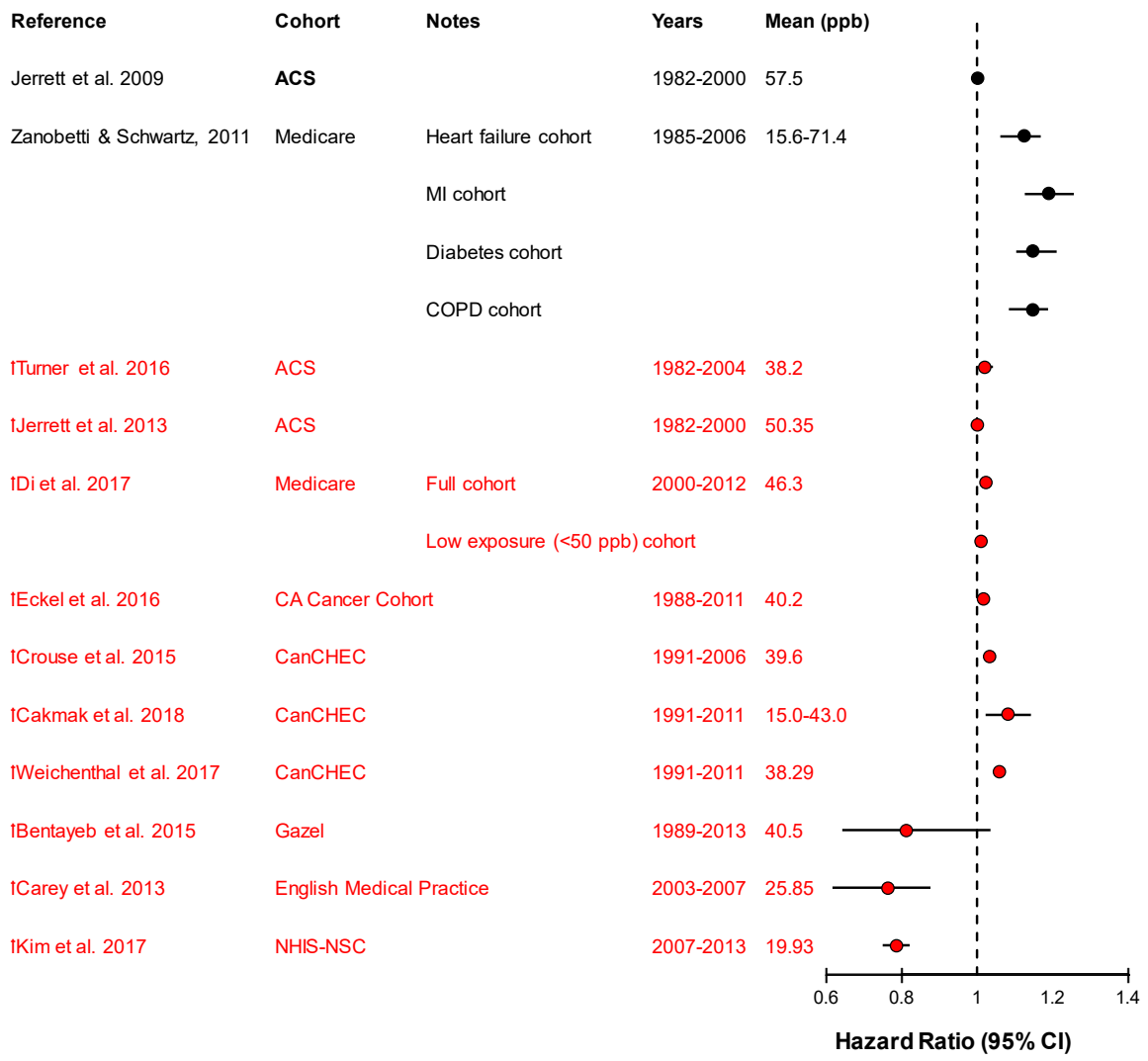
6.2.2 Biological Plausibility

The preceding appendices characterized evidence related to evaluating the biological plausibility by which long-term ozone exposure may lead to the morbidity effects that are the most common causes of total (nonaccidental) mortality, specifically cardiovascular and respiratory morbidity and metabolic disease (Appendix 4, Appendix 3, and Appendix 5, respectively). Respiratory and cardiovascular morbidity comprise ~9 and ~33%, respectively, of total mortality (NHLBI, 2017). This evidence is derived from animal toxicological, controlled human exposure, and epidemiologic studies. Appendix 3 characterizes the available evidence by which inhalation exposure to ozone could progress from initial events to endpoints relevant to the respiratory system and to population outcomes such as exacerbation of COPD. Appendix 4 outlines the available evidence for plausible mechanisms by which inhalation exposure to ozone could progress from initial events to endpoints relevant to the cardiovascular system and to population outcomes such as IHD, stroke, and atherosclerosis. Appendix 5 outlines the available evidence for plausible mechanisms by which inhalation exposure to ozone could progress from initial events (e.g., pulmonary inflammation, autonomic nervous system activation) to intermediate endpoints (e.g., insulin resistance, increased blood glucose and lipids) and result in population outcomes such as metabolic disease and diabetes. Collectively, the progression demonstrated in the available evidence for respiratory morbidity and metabolic disease supports potential biological pathways by which long-term ozone exposures could result in mortality; however, for cardiovascular morbidity, the evidence is more limited due to the few studies that provide generally inconsistent results between experimental and epidemiologic studies.

6.2.3 Total (Nonaccidental) Mortality

When considering the entire body of evidence, there is limited support for an association with long-term ozone exposure and total mortality. Recent studies use fixed-site monitors and models (e.g., CMAQ, dispersion models) measure, estimate, or predict ozone concentrations for use in assigning long-term ozone exposure in epidemiologic studies. There are also hybrid methods that combine two or more fixed-site, mode, and/or satellite-based techniques (Appendix 2 Section 2.3). Generally, epidemiologic studies of long-term ozone exposure and total mortality use the 8-hour daily max ozone metric, though there are instances in some that use the 24-hour avg [e.g., Sesé et al. (2017)], or the 1-hour daily max [e.g., Jerrett et al. (2009)]. The exposure metric used in each study is recorded in the Evidence Inventory tables (Section 6.3.2) for each study when that information was reported by study authors. The strongest evidence comes from analyses of the Medicare cohort data, including a study observing positive associations among different cohorts with pre-existing disease (Zanobetti and Schwartz, 2011) included in the 2013 Ozone ISA, and a recent analysis of more than 61 million individuals in the Medicare cohort (Di et al., 2017b). Results from other recent studies are less consistent, with some U.S. and Canadian cohorts reporting modest positive associations between long-term ozone exposure and total mortality, while other recent studies conducted in the U.S, Europe, and Asia report null or negative associations. The differences in the way exposure to ozone was assessed do not explain the heterogeneity in the observed associations. The results from studies evaluating long-term ozone exposure and total mortality are presented in Figure 6-8. These studies are characterized in Table 6-6. Overall, there is some evidence that long-term ozone exposure is associated with total mortality, especially among individuals with pre-existing disease, but the evidence is not consistent across studies. Specifically:

- The strongest evidence for an association between long-term ozone exposure and total mortality comes from an analysis among four subcohorts with pre-existing disease from the Medicare cohort (Zanobetti and Schwartz, 2011), demonstrating positive associations among those with pre-existing heart failure, MI, diabetes, or COPD. A recent analysis of the entire Medicare cohort, including over 61 million older adults, observed positive associations between long-term ozone exposure and total mortality, even when limited to areas in the U.S. where the predicted annual average ozone concentrations were less than 50 ppb (Di et al., 2017b).
- Several recent analyses of the CanCHEC cohort in Canada provide additional evidence of a modest positive association [consistent in magnitude with the association reported by Di et al. (2017b)] between long-term ozone exposure and total mortality (Cakmak et al., 2017; Weichenthal et al., 2017; Cakmak et al., 2016; Crouse et al., 2015).
- A recent study conducted in California among a cohort of individuals with cancer observed a positive association between long-term ozone exposure and total mortality (Eckel et al., 2016).
- Results from the ACS cohort provide little evidence for an association between long-term ozone exposure and total mortality (Turner et al., 2016; Jerrett et al., 2013; Jerrett et al., 2009).
- Several studies conducted outside of North America report negative associations between long-term ozone exposure and total mortality, specifically in France (Bentayeb et al., 2015), the U.K. (Carey et al., 2013), and South Korea (Kim et al., 2017).



ACS = American Cancer Society; CanCHEC = Canadian Census Health and Environment Cohort; NHIS-NSC = National Health Insurance Service—National Sample Cohort.

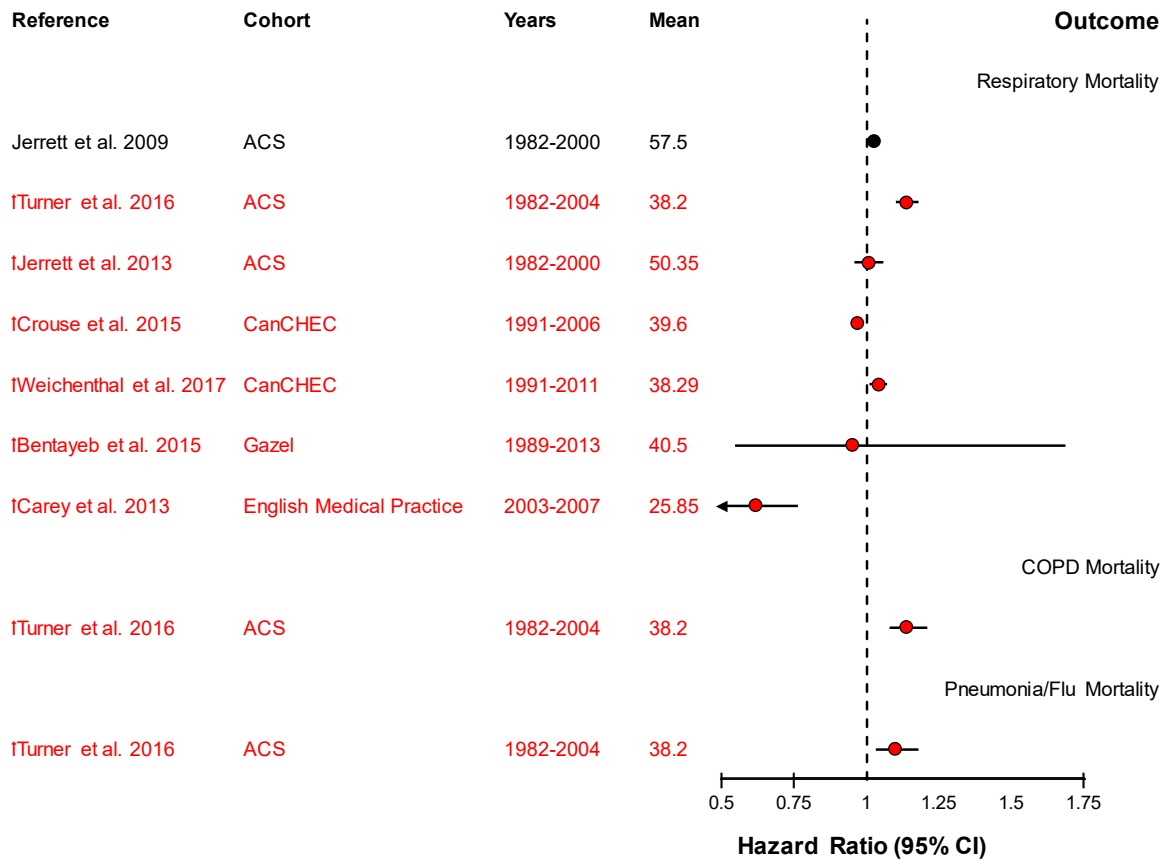
Note: †Studies published since the 2013 Ozone ISA. Associations are presented per 10 ppb increase in pollutant concentration. Circles represent point estimates; horizontal lines represent 95% confidence intervals for ozone. Black text and circles represent evidence included in the 2013 Ozone ISA; red text and circles represent recent evidence not considered in previous ISAs or AQCDs.

Figure 6-8 Associations between long-term exposure to ozone and total (nonaccidental) mortality in recent cohort studies.

6.2.3.1 Respiratory Mortality

When considering the entire body of evidence, there is limited support for an association with long-term ozone exposure and respiratory mortality. Recent studies use both fixed-site monitors and models (e.g., CMAQ, dispersion models) to measure or estimate ozone concentrations for use in assigning long-term ozone exposure in epidemiologic studies (Appendix 2, Section 2.3). The strongest evidence comes from analyses of the ACS cohort data, including studies observing positive associations between long-term ozone exposure and respiratory mortality (Jerrett et al., 2009), and a recent analysis of respiratory, COPD, and pneumonia mortality (Turner et al., 2016). Results from other recent studies are less consistent, with analyses of U.S., Canadian, and European cohorts reporting inconsistent associations between long-term ozone exposure and respiratory mortality. The differences in the way exposure to ozone was assessed do not explain the heterogeneity in the observed associations. The results from studies evaluating long-term ozone exposure and respiratory mortality are presented in Figure 6-9. These studies are characterized in Table 6-7. Overall, there is some evidence that long-term ozone exposure is associated with respiratory mortality, but the evidence is not consistent across studies. Specifically:

- The strongest evidence for an association between long-term ozone exposure and respiratory mortality comes from nationwide analyses of the ACS cohort, demonstrating positive associations with respiratory mortality (Turner et al., 2016; Jerrett et al., 2009) and COPD, and pneumonia/flu (Turner et al., 2016). In contrast, Jerrett et al. (2013) reported a null association between long-term ozone exposure and respiratory mortality in an analysis of the ACS cohort limited to participants from California.
- Several recent analyses of the CanCHEC cohort in Canada provide inconsistent evidence for an association between long-term ozone exposure and respiratory mortality, with one reporting a positive association (Weichenthal et al., 2017) and the other reporting a negative association (Crouse et al., 2015). Cohort studies conducted in France (Bentayeb et al., 2015) and the U.K. (Carey et al., 2013) also report negative associations between long-term ozone exposure and respiratory mortality.



ACS = American Cancer Society; CanCHEC = Canadian Census Health and Environment Cohort.

Note: †Studies published since the 2013 Ozone ISA. Associations are presented per 10 ppb increase in pollutant concentration. Circles represent point estimates; horizontal lines represent 95% confidence intervals for ozone. Black text and circles represent evidence included in the 2013 Ozone ISA; red text and circles represent recent evidence not considered in previous ISAs or AQCDs.

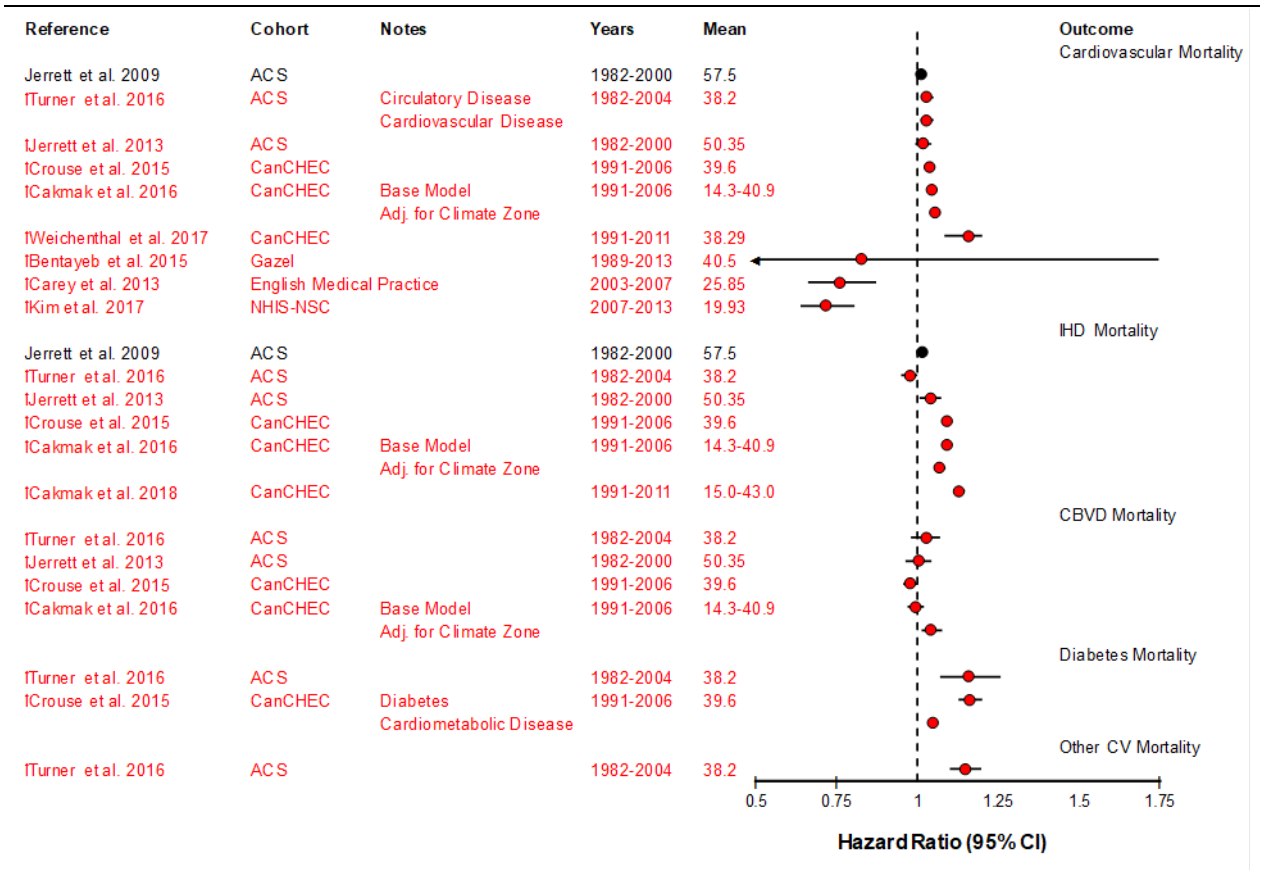
Figure 6-9 Associations between long-term exposure to ozone and respiratory mortality in recent cohort studies.

6.2.3.2 Cardiovascular Mortality

Recent cohort studies extend the body of evidence for the relationship between long-term ozone exposure and cardiovascular mortality. The 2013 Ozone ISA noted inconsistent evidence for cardiopulmonary mortality, and there was limited evidence for the association between long-term ozone exposure and cardiovascular mortality based on an analysis of the ACS cohort (Jerrett et al., 2009). Recent analyses from the ACS cohort in the U.S. and the CanCHEC cohort in Canada provide consistent evidence for positive associations between long-term ozone exposure and cardiovascular and IHD mortality, as well as mortality due to diabetes or cardiometabolic diseases. Associations with mortality due to cerebrovascular disease (e.g., stroke) were less consistent, and generally closer to the null value. Other recent studies conducted in the Europe and Asia report null or negative associations. Similar to total

mortality, the differences in the way exposure to ozone was assessed do not explain the heterogeneity in the observed associations for cardiovascular mortality. The results from studies evaluating long-term ozone exposure and cardiovascular mortality are presented in Figure 6-10. These studies are characterized in Table 6-8 and Table 6-9. Overall, there is increased evidence that long-term ozone exposure is associated with cardiovascular mortality compared to the evidence included in the 2013 Ozone ISA. Specifically:

- The strongest evidence for an association between long-term ozone exposure and cardiovascular mortality comes from nationwide analyses of the ACS cohort, demonstrating positive associations with cardiovascular mortality (Turner et al., 2016; Jerrett et al., 2013; Jerrett et al., 2009), IHD mortality (Jerrett et al., 2013), cerebrovascular disease mortality (Turner et al., 2016), and mortality due to dysrhythmia and heart failure (Turner et al., 2016).
- Several recent analyses of the CanCHEC cohort in Canada provide consistent evidence for a positive association between long-term ozone exposure and cardiovascular and IHD mortality (Cakmak et al., 2017; Cakmak et al., 2016; Crouse et al., 2015).
- Cohort studies conducted in France (Bentayeb et al., 2015), the U.K. (Carey et al., 2013), and South Korea (Kim et al., 2017) report negative associations between long-term ozone exposure and respiratory mortality.
- Several recent studies conducted in the U.S. and Canada provide limited and inconsistent evidence for an association between long-term ozone exposure and mortality due to cerebrovascular disease (Figure 6-10).
- A limited body of evidence demonstrates positive associations between long-term ozone exposure and mortality from diabetes and cardiometabolic diseases (Turner et al., 2016; Crouse et al., 2015).



ACS = American Cancer Society; CanCHEC = Canadian Census Health and Environment Cohort; CBVD = cerebrovascular disease; CV = cardiovascular; IHD = ischemic heart disease; NHIS-NSC = National Health Insurance Service—National Sample Cohort.

Note: †Studies published since the 2013 Ozone ISA. Associations are presented per 10 ppb increase in pollutant concentration. Circles represent point estimates; horizontal lines represent 95% confidence intervals for ozone. Black text and circles represent evidence included in the 2013 Ozone ISA; red text and circles represent recent evidence not considered in previous ISAs or AQCDs.

Figure 6-10 Associations between long-term exposure to ozone and cardiovascular mortality in recent cohort studies.

6.2.3.3 Studies of Life Expectancy

A recent study adds to the body of evidence on the relationship between long-term ozone exposure and mortality by examining temporal trends in ozone concentrations and changes in life expectancy, testing the hypothesis that populations living in areas with higher ozone concentrations have lower life expectancies. Li et al. (2016) reported the mean life expectancy for males and females in the U.S. from 2002 to 2008 at the county level, separating counties into three classes based on average ozone concentrations: Class 1: 36.4 ppb (28.1−39.8); Class 2: 43.3 ppb (39.4, 46.2); and Class 3: 48.8 ppb

(45.7−54.5). Nationwide, ozone concentrations reduced an average of 0.15 ppb during the study period. After adjustment for $PM_{2.5}$ concentrations, life expectancy decreased by 0.2 and 0.6 years for males in Class 2 and Class 3 counties (respectively, compared to counties in Class 1) and by 0.3 and 0.6 years for females in Class 2 and Class 3 counties (respectively, compared to counties in Class 1). When the study authors evaluated the association for all counties on a continuous scale, they observed a 0.25 (0.19, 0.30) year decrease in life expectancy for males and 0.21 (0.17, 0.25) year decrease in life expectancy for females for every 5 ppb increase in average ozone concentration.

6.2.4 Effect Modification of the Ozone-Mortality Relationship

6.2.4.1 Pre-existing Disease

Individuals with certain pre-existing diseases may be considered at greater risk of an air pollution-related health effect because they are likely in a compromised biological state that can vary depending on the disease and severity. The 2013 Ozone ISA concluded that there was adequate evidence for increased ozone-related health effects among individuals with asthma (U.S. EPA, 2013a). The results of controlled human exposure studies, as well as epidemiologic and animal toxicological studies, contributed to this evidence. Specifically, the evidence from controlled human exposure studies provided support for larger decrements in FEV_1 and greater inflammatory responses to ozone in individuals with asthma than in healthy individuals without a history of asthma. Studies of short-term ozone exposure and mortality provided limited evidence for stronger associations among individuals with pre-existing cardiovascular disease or diabetes. When evaluating long-term ozone exposure and mortality, Zanobetti and Schwartz (2011) observed positive associations with total mortality among individuals in the Medicare cohort with a recent hospital admission for heart failure, MI, diabetes, or COPD, although the authors did not provide quantitative results for a comparison population with no recent hospital admissions in their analysis.

A limited number of recent studies provides some evidence that individuals with pre-existing diseases may be at greater risk of mortality associated with long-term ozone exposure. These studies focus on specific diseases of varying severity (e.g., acute respiratory distress syndrome, pulmonary fibrosis, ovarian cancer). In contrast, an analysis of the ACS cohort observed stronger associations between long-term ozone exposure and respiratory or cardiovascular mortality among individuals with no pre-existing disease. Specifically:

- The strongest evidence that individuals with pre-existing disease might be at greater risk of total mortality associated with long-term ozone exposure continues to come from a study of four disease cohorts (i.e., individuals with a recent hospital admission related to heart failure, MI, diabetes, or COPD) among members of the Medicare cohort that observed positive and

statistically significant associations for each of the disease cohorts (Zanobetti and Schwartz, 2011) (Table 6-6). A recent study of the ACS cohort reported contrasting results, with stronger associations among populations with no pre-existing respiratory or cardiovascular disease and respiratory or cardiovascular mortality, respectively.

- In addition, several studies reported positive associations between long-term ozone exposure and total or cancer-specific mortality among those already diagnosed with ovarian cancer (Vieira et al., 2017) or respiratory cancer [i.e., cancers of the nose, nasal cavity and middle ear, larynx, lung and bronchus, pleura and trachea, mediastinum, and other organs; (Xu et al., 2013)].
- Positive associations were observed between long-term ozone exposure and in-hospital mortality among patients with acute respiratory distress syndrome (Rush et al., 2017), but not with total mortality among individuals with idiopathic pulmonary fibrosis (Sesé et al., 2017).

6.2.4.2 Lifestage

The 1996 and 2006 Ozone AQCDs identified children, especially those with asthma, and older adults as at-risk populations (U.S. EPA, 2006, 1996a). In addition, the 2013 Ozone ISA concluded that there was adequate evidence to conclude that children and older adults are at increased risk of ozone-related health effects (U.S. EPA, 2013a). Collectively, the majority of evidence for older adults has come from studies of short-term ozone exposure and mortality, with little evidence contributed by studies of long-term ozone exposure. A limited number of recent studies of long-term exposure to ozone and mortality have compared associations between different age groups, but do not report consistent evidence that older adults are at increased risk:

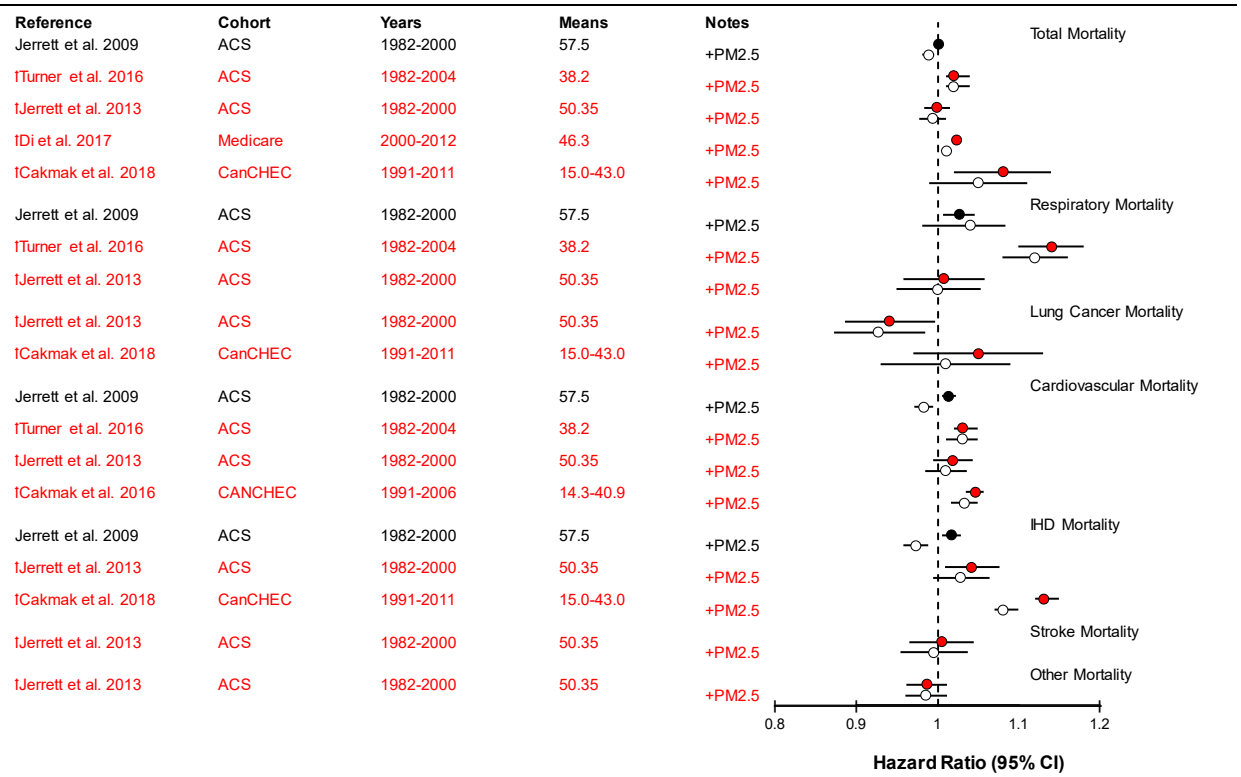
- Turner et al. (2016) observed stronger associations among those less than 65 years old compared to those 65 years and older.
- Results of the CanCHEC cohort observed positive associations between long-term ozone exposure and total mortality that were similar among women aged less than 60, 60−69, and 70−79 years (Crouse et al., 2015). This association was attenuated to null among women aged 80−89 years. For men in CanCHEC cohort, Crouse et al. (2015) observed positive associations between long-term ozone exposure and total mortality among men aged less than 60 and 60−69 years, and these associations were attenuated, but remained positive for men aged 70−79 and 80−89 years.

6.2.5 Potential Copollutant Confounding of the Ozone-Mortality Relationship

The evaluation of potential confounding effects of copollutants on the relationship between long-term ozone exposure and mortality allows for examination of whether ozone risk estimates are changed in copollutant models. Year-round correlations of ozone concentrations with copollutant concentrations can be found in Section 2.5; generally, the strongest positive correlations are with PM_{10} and $PM_{2.5}$, while the strongest negative correlations are observed with CO. Recent studies examined the potential for copollutant confounding by evaluating copollutant models that include $PM_{2.5}$ (Figure 6-11) and NO_2. These recent studies address a previously identified data gap by informing the extent to which

effects associated with long-term ozone exposure are independent of coexposure to correlated copollutants in long-term analyses:

- The 2013 Ozone ISA included the study by Jerrett et al. (2009) that reported associations with respiratory mortality that remained robust after adjustment for $PM_{2.5}$, and associations with cardiovascular mortality that were attenuated, changing from positive to negative, after adjustment for $PM_{2.5}$ concentrations. Recent studies (Figure 6-11)provide generally consistent evidence for associations with ozone that are robust (i.e., relatively unchanged) to adjustment for $PM_{2.5}$ concentrations for total mortality, respiratory mortality, and cardiovascular mortality.
- The correlations between ozone and $PM_{2.5}$ exposures in studies that conducted copollutant analyses were highly variable, ranging from −0.705 to 0.73, and included low (e.g., <0.4), moderate (e.g., 0.4−0.7), and high (e.g., >0.7) correlations (Table 6-6).
- Jerrett et al. (2013) reported copollutant models with ozone and NO_2. The correlation between ozone and NO_2 concentrations was weak ($r = -0.071$), and associations with ozone were robust to inclusion of NO_2 in the model for total, respiratory, and cardiovascular mortality (Figure 6-11).



ACS = American Cancer Society; CanCHEC = Canadian Census Health and Environment Cohort; IHD = ischemic heart disease.

Note: †Studies published since the 2013 Ozone ISA. Associations are presented per 10 ppb increase in pollutant concentration. Circles represent point estimates; horizontal lines represent 95% confidence intervals for ozone. Black text and circles represent evidence included in the 2013 Ozone ISA; red text and circles represent recent evidence not considered in previous ISAs or AQCDs. Closed circles represent effect of ozone in single pollutant models, open circles represent effect of ozone adjusted for $PM_{2.5}$.

Figure 6-11 Associations between long-term exposure to ozone and mortality with and without adjustment for $PM_{2.5}$ concentrations in recent cohort studies.

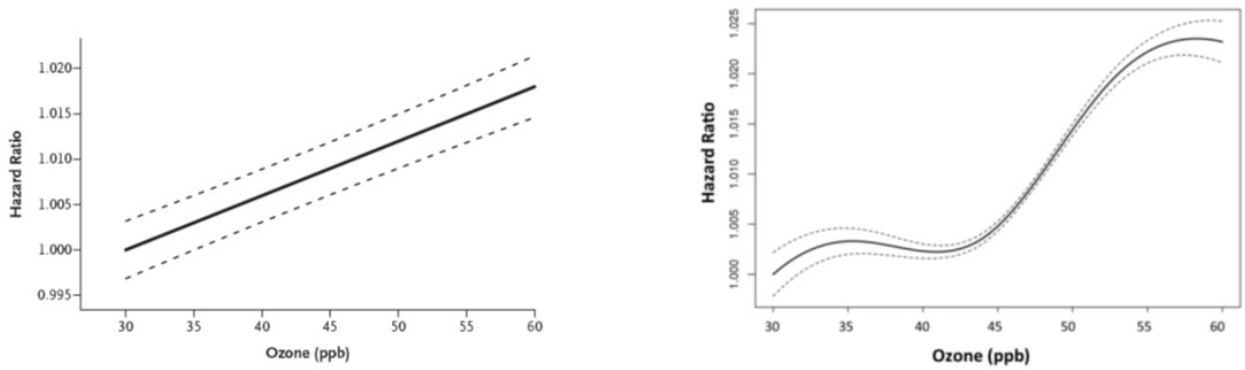
6.2.6 Shape of the Concentration-Response Function

An important consideration in characterizing the ozone-mortality association is whether the concentration-response (C-R) relationship is linear across the full concentration range that is encountered or there are concentration ranges that depart from linearity. The epidemiologic studies included in the 2013 Ozone ISA indicated a "generally linear C-R function with no indication of a threshold" (U.S. EPA, 2013a). With regard to studies of long-term ozone exposure and mortality, a threshold analysis indicated that the linear model was not a better fit to the data ($p > 0.05$) than a threshold representation of the overall ozone-mortality association (Jerrett et al., 2009); however, the authors reported "limited evidence" for an effect threshold at an ozone concentration (seasonal avg of 1-hour max) of 56 ppb ($p = 0.06$).

Visual inspection of this concentration-response function suggests an inflection point just below 60 ppb, which is close to the median concentration across cities (i.e., 57 ppb).

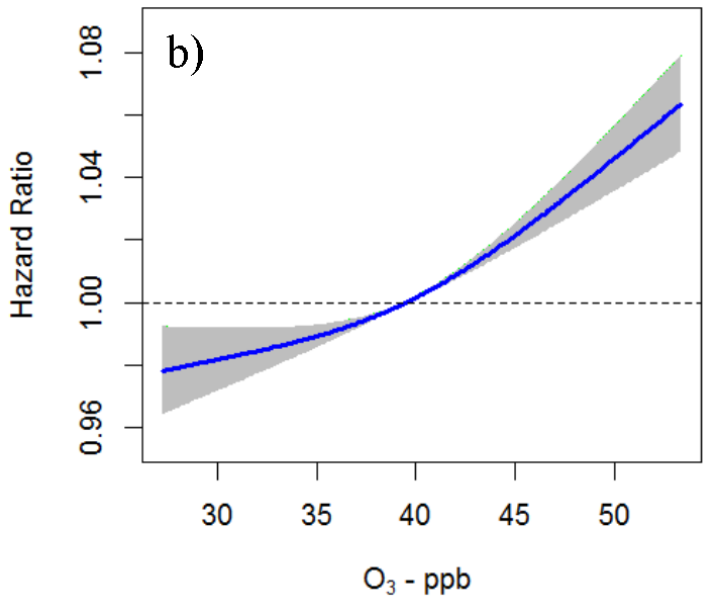
A number of recent studies examined the C-R function between long-term ozone exposure and mortality and observed somewhat inconsistent results. While some studies provide evidence of a generally linear C-R function, others observed a sublinear relationship, indicating larger changes in risk for higher ozone concentrations compared to lower ozone concentrations. Several studies also include threshold analyses, and support the possibility of a threshold near 35 to 40 ppb (8-hour max). Specifically:

- In the U.S. Medicare cohort, Di et al. (2017b) used thin-plate spline regression to evaluate the C-R relationship between long-term ozone exposure and total mortality and observed a generally linear function with no signal of a threshold down to 30 ppb (8-hour max; Figure 6-12, panel A). When Crouse et al. (2015) used restricted cubic spline functions to evaluate the C-R function for long-term ozone exposure and total mortality in the CanCHEC cohort, they observed a sublinear relationship, indicating larger changes in risk for higher ozone concentrations compared with lower ozone concentrations (Figure 6-13).
- Among studies conducting threshold analyses, Di et al. (2017b) reported evidence for a threshold at around 40 ppb (8-hour daily max) based on the minimum AIC value and visual inspection of the C-R function (Figure 6-12, Panel B).
- The C-R relationship between long-term ozone exposure and mortality may differ by the cause of mortality and/or by ozone season. For example, Turner et al. (2016) reported improved model fit for a threshold model compared to a linear model for the association between long-term, year-round ozone exposure and respiratory mortality in the ACS cohort, with evidence of a threshold near 35 ppb (8-hour daily max; Figure 6-14). However, when the data were restricted to warm-season ozone only, the linear model was a better fit than the threshold model.



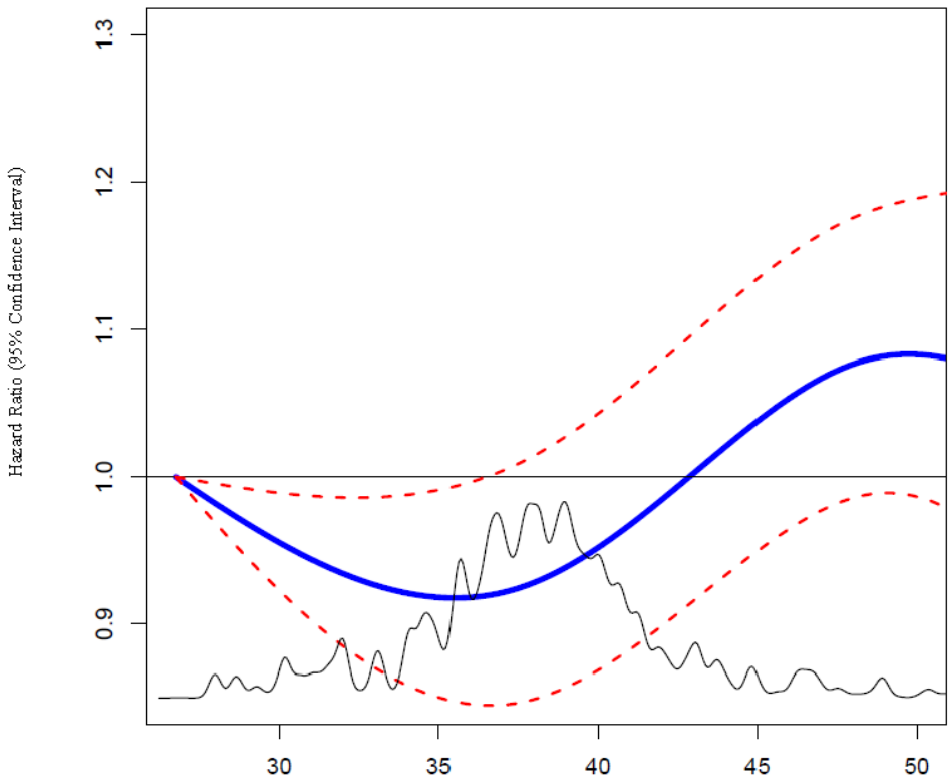
Note: The solid line represents the estimate and the dotted lines represent the 95% confidence interval for the estimate.
Source: Di et al. (2017b)—Permission Pending.

Figure 6-12 The concentration-response relationship estimated with log-linear model with a thin-plate spline (A) and the concentration-response relationship estimated with threshold model (B), indicating the potential for a threshold at 40 ppb (8-hour daily max).



Note: The solid blue line represents the estimate and the gray shaded areas represent the 95% confidence interval for the estimate.
Source: Crouse et al. (2015)—Permission Pending.

Figure 6-13 Concentration-response relationship between ozone concentrations (ppb) and total (nonaccidental) mortality in the CanCHEC cohort (mean 39.6; knots: 30.0, 38.9, 50.7 ppb).



Note: Mean annual 8-hour daily max ozone concentration (ppb), hierarchical Bayesian space-time Model (HBM), U.S., 2002−2004, truncated at 99th percentile. Grey line along abscissa indicates data density.

Note: The solid line represents the estimate and the dotted lines represent the 95% confidence interval for the estimate.

Source: Turner et al. (2016)—Permission Pending.

Figure 6-14 Concentration-response curve for ozone associated with respiratory mortality using a natural spline model with 3 degrees of freedom.

6.2.7 Summary and Causality Determination

This section describes the evaluation of evidence for total (nonaccidental) mortality based on the scientific considerations detailed in the Annex for Appendix 6, with respect to the causality determination for long-term exposures to ozone using the framework described in the Preamble to the ISAs (U.S. EPA, 2015). The key evidence, as it relates to the causal framework, is summarized in Table 6-2. Recent cohort studies provide limited support for the association between long-term ozone exposure and total mortality, with some U.S. and Canadian cohorts reporting modest positive associations between long-term ozone exposure and total mortality, while other recent studies conducted in the U.S, Europe, and Asia report null or negative associations. The strongest evidence for an association between long-term ozone exposure and total mortality continues to come from analyses of the Medicare cohort data included in the 2013 Ozone

ISA. Specifically, Zanobetti and Schwartz (2011) reported associations of ambient ozone concentrations and total mortality among populations with pre-existing disease that remained robust after adjusting for $PM_{2.5}$ concentrations.

Additionally, Jerrett et al. (2009) reported positive associations between long-term ozone exposure and respiratory mortality after adjusting for $PM_{2.5}$ in copollutant models. This evidence is supported by a recent analysis of respiratory, COPD, and pneumonia mortality (Turner et al., 2016). Results from other recent studies are less consistent, with analyses of U.S., Canadian, and European cohorts reporting inconsistent associations between long-term ozone exposure and respiratory mortality.

Whereas the 2013 Ozone ISA noted inconsistent evidence for cardiopulmonary mortality (and limited evidence for cardiovascular mortality, specifically), recent cohort studies extend the body of evidence for the relationship between long-term ozone exposure and cardiovascular mortality. Analysis of the ACS cohort provided limited evidence for the association between long-term ozone exposure and cardiovascular mortality (Jerrett et al., 2009) in the 2013 Ozone ISA. Recent analyses from the ACS cohort in the U.S. and the CanCHEC cohort in Canada provide consistent evidence for positive associations between long-term ozone exposure and cardiovascular and IHD mortality, as well as mortality due to diabetes or cardiometabolic diseases. Associations with mortality due to cerebrovascular disease (e.g., stroke) are less consistent, and generally closer to the null value. Other recent studies conducted in the Europe and Asia report null or negative associations.

Additionally, recent studies that have evaluated copollutant confounding for a limited number of pollutants reduce uncertainties related to potential copollutant confounding by $PM_{2.5}$ and NO_2 (Section 6.2.5) and contribute to the previously limited evidence characterizing the shape of the concentration-response relationship (Section 6.2.6). Recent evidence helps to reduce uncertainties related to potential copollutant confounding by two pollutants of the relationship between long-term ozone exposure and mortality. Multiple studies evaluated $PM_{2.5}$ (Figure 6-9), while fewer evaluated NO_2 in copollutant models, and observed similar hazard ratios for ozone regardless of whether $PM_{2.5}$ or NO_2 were included in the model. This helps to reduce the uncertainty for an independent effect of long-term ozone exposure on mortality.

The body of evidence for total mortality is supported by generally consistent positive associations with cardiovascular mortality, and less so by the somewhat inconsistent evidence for respiratory mortality. There is coherence across the scientific disciplines (i.e., animal toxicology, controlled human exposure studies, and epidemiology) and biological plausibility for ozone-related cardiovascular (Appendix 4) and respiratory (Appendix 3) endpoints, which lend some additional support to the ozone-mortality relationship.

Recent studies use a variety of both fixed-site monitors and models (e.g., CMAQ, dispersion models) to measure or estimate ozone concentrations for use in assigning long-term ozone exposure in epidemiologic studies (Appendix 2, Section 2.3). Overall, the exposure assessment techniques used in

these studies do not help to explain the inconsistent associations observed across studies, although they indicate that the observed effects of long-term ozone exposure on mortality are not overtly influenced by, or a residual of, the exposure assessment technique used in the study.

The number of studies examining the shape of the C-R function for long-term ozone exposure and mortality has substantially increased since the 2013 Ozone ISA. These studies used a number of different statistical techniques to evaluate the shape of the C-R function, including linear models and restricted cubic splines, and generally observed linear, no-threshold relationships down to 35−40 ppb, although the results are not entirely consistent. Some studies observed a sublinear relationship, indicating larger changes in risk for higher ozone concentrations compared with lower ozone concentrations. Several studies also included threshold analyses, and support the possibility of a threshold near 35 to 40 ppb.

Overall, recent epidemiologic studies add to the limited body of evidence that formed the basis of the conclusions of in 2013 Ozone ISA for total mortality. This body of evidence is generally inconsistent, with some U.S. and Canadian cohorts reporting modest positive associations between long-term ozone exposure and total mortality, while other recent studies conducted in the U.S, Europe, and Asia report null or negative associations. The strongest evidence for the association between long-term ozone exposure and total (nonaccidental) mortality continues to come from analyses of patients with pre-existing disease from the Medicare cohort, and recent evidence demonstrating positive associations with cardiovascular mortality. The evidence from the assessment of ozone-related respiratory disease, with more limited evidence from cardiovascular and metabolic morbidity, provides biological plausibility for mortality due to long-term ozone exposures. In conclusion, the inconsistent associations observed across both recent and older cohort and cross-sectional studies conducted in various locations provide limited evidence for an association between long-term ozone exposure and mortality. Collectively, this body of evidence is suggestive of, but not sufficient to infer, a causal relationship between long-term ozone exposure and total mortality.

Table 6-2 Summary of evidence that is suggestive of, but not sufficient to infer, a causal relationship between long-term ozone exposure and total mortality.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Limited and sometimes inconsistent epidemiologic evidence from multiple, high-quality studies at relevant ozone concentrations	Positive associations between long-term ozone exposure and total mortality among those with pre-existing disease in the ACS cohorts, with some additional evidence from recent studies stratifying by disease status.	Zanobetti and Schwartz (2011) Section 6.2.4.1	Mean concentrations across studies: 15.6−71.4 ppb
	Recent analyses from the ACS cohort in the U.S. and the CanCHEC cohort in Canada provide consistent evidence for positive associations between long-term ozone exposure and cardiovascular and IHD mortality, as well as mortality due to diabetes or cardiometabolic diseases	Jerrett et al. (2009); Turner et al. (2016); Jerrett et al. (2013) Crouse et al. (2015); Cakmak et al. (2016); Cakmak et al. (2017) Section 6.2.3.2	Mean concentrations across studies: 14.3−57.5 ppb
	Strong evidence from the ACS cohort demonstrating positive associations between long-term ozone exposure and respiratory mortality. Results from other recent studies are less consistent, with analyses of U.S., Canadian, and European cohorts reporting inconsistent associations.	Jerrett et al. (2009); Turner et al. (2016) Section 6.2.3.1	Mean concentrations across studies: 15.0−57.5
	Some recent U.S. and Canadian cohorts report modest positive associations with total mortality, while other recent studies conducted in the U.S., Europe, and Asia report null or negative associations.	Section 6.2.3	Mean concentrations across studies: 15−71.4 ppb

Table 6-2 (Continued): Summary of evidence that is suggestive of, but not sufficient to infer, a causal relationship between long-term ozone exposure and total mortality.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Epidemiologic evidence from copollutant models provides some support for an independent ozone association	Positive associations observed between long-term ozone exposure and total mortality remain relatively unchanged after adjustment for $PM_{2.5}$, and NO_2. When reported, correlations with copollutants were highly variable (low to high).	Section 6.2.4.1 Figure 6-9	
Limited epidemiologic evidence supports a linear C-R relationship; some evidence for a sublinear C-R relationship	Some studies provide evidence of a generally linear C-R relationship; others observed a sublinear relationship, indicating larger changes in risk for higher compared with lower ozone concentrations.	Section 6.2.6	
Biological plausibility from studies of cardiovascular and respiratory morbidity and metabolic disease	Evidence for respiratory morbidity supports potential biological pathways by which long-term ozone exposures could result in mortality; limited evidence from cardiovascular morbidity and metabolic disease.	Appendix 3 Appendix 4 Appendix 5	

ACS = American Cancer Society; NO_2 = nitrogen dioxide; $PM_{2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 2.5 μm; ppb = parts per billion.

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to the causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where the full body of evidence is described.

[c]Describes the ozone concentrations with which the evidence is substantiated.

6.3 Evidence Inventories—Data Tables to Summarize Study Details

6.3.1 Short-Term Ozone Exposure and Mortality: Data Tables

Table 6-3 Epidemiologic studies of short-term exposure to ozone and total (nonaccidental) mortality.

Study	Study Population	Exposure Assessment	Mean and Upper Percentiles (μg/m^3)	Copollutant Examination	Effect Estimates Percent Increase (95% CI)
Bell et al. (2004) 95 U.S. cities 1987−2000 Times-series study	NMMAPS All ages	Average across all monitors in each city, 10% trimmed mean to correct for yearly averages of each monitor 24-h avg	Mean: 26.0	Correlation (*r*): PM_{10}: −0.38 to 0.63 Copollutant models with: PM_{10}	All-year (lag 0−6 DL) 0.78 (0.40, 1.16) Warm/summer (lag 0−6 DL) 0.58 (0.19, 0.97)
Levy et al. (2005) U.S. and non-U.S. Meta-analysis	U.S. and non-U.S.	24-h avg	NR	Correlation (*r*): NR Copollutant models with: NR	All-year 1.23 (0.94, 1.52) Warm/summer 2.52 (1.70, 3.30)
Bell et al. (2005) U.S. and non-U.S. Meta-analysis	U.S. and non-U.S.	24-h avg	NR	Correlation (*r*): NR Copollutant models with: NR	All-year 1.31 (0.82, 1.77) Warm/summer 2.26 (1.09, 3.45)

Table 6-3 (Continued): Epidemiologic studies of short-term exposure to ozone and total (nonaccidental) mortality.

Study	Study Population	Exposure Assessment	Mean and Upper Percentiles (μg/m^3)	Copollutant Examination	Effect Estimates Percent Increase (95% CI)
Ito et al. (2005) U.S. and non-U.S. Meta-analysis	U.S. and non-U.S.	24-h avg	NR	Correlation (*r*): NR Copollutant models with: NR	All-year 1.65 (0.60, 2.69) Warm/summer 2.61 (1.57, 3.65)
Schwartz (2005) 14 U.S. cities 1986−1993 Case-crossover study	All ages	Average of all monitors in each county 1-h max	Mean: 35.1−60.0 75th: 46.3−69.0	Correlation (*r*): NR Copollutant models with: PM_{10}	All-year (lag 0) 0.47 (0.08, 0.87) Warm/summer (lag 0) 0.63 (0.19, 1.12)
Bell et al. (2007) 98 U.S. communities 1987−2000 Time-series study	NMMAPS All ages	Average across all monitors in each city, 10% trimmed mean to correct for yearly averages of each monitor 24-h avg	Mean: 26.0[a]	Correlation (*r*): $PM_{2.5}$: −0.17 to 0.25; PM_{10}: <0.00 to 0.22 Copollutant models with: $PM_{2.5}$, PM_{10}	All-year (lag 0−1) 0.48 (0.26, 0.69)
Bell and Dominici (2008) 98 U.S. communities 1987−2000 Time-series study	NMMAPS All ages	Average across all monitors in each city; 10% trimmed mean to correct for yearly averages of each monitor 24-h avg	Mean: All-year: 26.8 May–September: 30.0 Maximum: All-year: 37.3 May–September: 47.2	Correlation (*r*): NR Copollutant models with: $PM_{2.5}$, PM_{10}	All-year (lag 0−6) 0.78 (0.42, 1.16)

Table 6-3 (Continued): Epidemiologic studies of short-term exposure to ozone and total (nonaccidental) mortality.

Study	Study Population	Exposure Assessment	Mean and Upper Percentiles ($\mu g/m^3$)	Copollutant Examination	Effect Estimates Percent Increase (95% CI)
Katsouyanni et al. (2009) APHENA 1987−1996 Time-series study	NMMAPS 12 Canadian cities All ages	Exposure assignment approach detailed in original studies and based on all available monitoring data 1-h max	Mean: U.S.: 13.0−38.0 Canada: 6.7−8.4 75th: U.S.: 21.0−52.0 Canada: 8.7−12.5	Correlation (*r*): NR Copollutant models with: PM_{10}	All-year (lag 0−2 DL) U.S.: 1.88 (0.69, 3.03) Canada: 3.63 (1.13, 6.02) Warm/summer (lag 0−2 DL) U.S.: 2.38 (1.18, 3.58) Canada: 2.08 (0.79, 3.33)
Franklin and Schwartz (2008) 18 U.S. communities Time-series study	All ages	Average of all monitors in each county based on the method detailed in Schwartz (2000) 24-h avg	Mean: 21.4−48.7	Correlation (*r*): $PM_{2.5}$: 0.43; SO_4^{2-}: 0.34; OC: 0.50; NO_3^-: 0.24 Copollutant models with: $PM_{2.5}$, SO_4^{2-}, OC, NO_3^-	Warm/summer (lag 0) 1.34 (0.68, 2.00)
Zanobetti and Schwartz (2008a) 48 U.S. cities Case-crossover study	All ages	Average of all monitors in each city 8-h avg	Mean (across seasons): 16.5−47.8 Maximum (across seasons): 40.6−103.0	Correlation (*r*): NR Copollutant models with: NR	Warm/summer (lag 0) 1.00 (0.76, 1.24)
Zanobetti and Schwartz (2008b) 48 U.S. Cities Time-series study	All ages	Average of all monitors in each city 8-h avg	Mean: 15.1−62.8 75th: 19.8−75.4 Maximum: 34.3−146.2	Correlation (*r*): NR Copollutant models with: NR	Warm/summer (lag 0−3) 1.06 (0.56, 1.55)
Medina-Ramón and Schwartz (2008) 48 U.S. cities Case-only study	All ages	Average of all monitors in each county based on the method detailed in Schwartz (2000) 8-h avg	Median: 16.1−58.8	Correlation (*r*): NR Copollutant models with: NR	Warm/summer (lag 0−2) 1.30 (0.76, 1.87)

Table 6-3 (Continued): Epidemiologic studies of short-term exposure to ozone and total (nonaccidental) mortality.

Study	Study Population	Exposure Assessment	Mean and Upper Percentiles (μg/m^3)	Copollutant Examination	Effect Estimates Percent Increase (95% CI)
†Klemm et al. (2011) Atlanta, GA, U.S. 8/1998−12/2007 Time-series study	65+	Data from several monitors. 8-h max	Mean: 35.54 75th: 47.82 Maximum: 109.07	Correlation (*r*): NR Copollutant models with: NR	Lag 0−1: 1.36 (−0.50, 3.25)
†Moolgavkar et al. (2013) 98 U.S. cities 1987−2000 Time-series study	NMMAPS All ages	Average across all monitors in each city; 10% trimmed mean to correct for yearly averages of each monitor 24-h avg	Mean: NR	Correlation (*r*): NR Copollutant models with: PM_{10}	100 df temporal trends (lag 1): 0.60 (0.44, 0.80) 100 df temporal trends with PM_{10} (lag 1): 0.33 (−0.07, 0.72)
†Goldberg et al. (2013) Montreal, Canada 1990−2003 Time-series study	65+	Daily average of each monitor, then average across all monitors 24-h avg	Mean: 16.47 Median: 15.2 75th: 21.4 Maximum: 69.65	Correlation (*r*): $PM_{2.5}$: −0.02; NO_2: −0.23; SO_2: −0.31; Copollutant models with: NR	All-year (0−2 DLNM): −0.37 (−2.30, 1.60) Warm (April−September; 0−2 DLNM): 1.35 (−1.10, 3.87) All-year with CHF (0−2 DLNM): 0.39 (−3.23, 4.15) Warm (April−September) with CHF (0−2 DLNM): 2.99 (−1.95, 8.17) All-year with hypertension (0−2 DLNM): 0.93 (−2.54, 4.53) Warm (April−September) with hypertension (0−2 DLNM): 3.70 (−0.08, 7.63) All-year with cancer (0−2 DLNM): 1.34 (−1.60, 4.35) Warm (April−September) with cancer (0−2 DLNM): 3.57 (0.16, 7.10) All-year with acute CAD (0−2 DLNM): 2.55 (−1.90, 7.19) Warm (April−September) with acute CAD (0−2 DLNM): 7.78 (2.43, 13.41) All-year with cerebrovascular disease (0−2 DLNM): 3.77 (−0.93, 8.70) Warm (April−September) with cerebrovascular disease (0−2 DLNM): 4.93 (−0.04, 10.16)

Table 6-3 (Continued): Epidemiologic studies of short-term exposure to ozone and total (nonaccidental) mortality.

Study	Study Population	Exposure Assessment	Mean and Upper Percentiles (μg/m^3)	Copollutant Examination	Effect Estimates Percent Increase (95% CI)
†Peng et al. (2013) 50 U.S. cities 12 Canadian cities 1987−1996 Time-series study	APHENA All ages	Average of all monitors in each city 1-h max	Mean: NR Median: 6.6−19.4	Correlation (*r*): NR Copollutant models with: PM_{10}	50 U.S. cities All-year (lag 0−2 DL): 2.13 (0.54, 3.73) All-year with PM_{10} (lag 1): 0.64 (−0.88, 2.18) Summer (lag 0−2 DL): 3.23 (1.63, 4.85) 12 Canadian cities All-year (lag 0−2 DL): 3.73 (1.23, 6.54) All-year with PM_{10} (lag 1): 2.38 (−0.88, 6.02) Summer (lag 0−2 DL): 2.08 (0.79, 3.33)
†Vanos et al. (2014) 10 Canadian cities 1981−1999 Time-series study	All ages	Monitor in each city located downtown or at city airports within 27 km of downtown 24-h avg	Mean: 19.3	Correlation (*r*): NR Copollutant models with: NR	All-year (lag 0): NA (NA, NA) Winter (lag 0): 2.70 (−0.04, 5.43) Spring (lag 0): 3.54 (−0.84, 7.90) Summer (lag 0): 3.07 (1.61, 4.53) Fall (lag 0): 1.40 (0.30, 2.49)
†Vanos et al. (2013) 10 Canadian cities 1981−1999 Time-series study	All ages	Air pollution data from NAPS network 24-h avg	Mean: 14.5−23.2	Correlation (*r*): NR Copollutant models with: NR	Overall (lag 0): 2.25 (1.17, 3.33) DM (lag 0): 2.02 (1.48, 2.56) DP (lag 0): 1.32 (0.70, 1.94) DT (lag 0): 4.26 (2.02, 6.56) MM (lag 0): 1.55 (0.86, 2.25) MP (lag 0): 1.94 (0.93, 2.95) MT (lag 0): 3.02 (1.48, 4.64) Transition (lag 0): 1.40 (0.39, 2.33)

Table 6-3 (Continued): Epidemiologic studies of short-term exposure to ozone and total (nonaccidental) mortality.

Study	Study Population	Exposure Assessment	Mean and Upper Percentiles (μg/m^3)	Copollutant Examination	Effect Estimates Percent Increase (95% CI)
†Jhun et al. (2014) 97 U.S. cities 1987−2000 Time-series study	NMMAPS All ages	Average across all monitors in each city; 10% trimmed mean to correct for yearly averages of each monitor 24-h avg	Mean: NR	Correlation (*r*): NR Copollutant models with: NR	Warm season (May−September): linear temp term (lag 0): 0.71 (0.29, 1.14) Warm season (May−September): Categorical temp term (lag 0): 0.81 (0.38, 1.25) Temperature range Warm season (May−September): low temp days (25th percentile; lag 0): 1.08 (0.27, 1.90) Warm season (May−September): moderate temp days (lag 0): 0.59 (−0.04, 1.22) Warm season (May−September): high temp days (75th percentile; lag 0): 0.98 (0.30, 1.64) Warm season (May−September): high temp days (90th percentile; lag 0): 1.25 (0.26, 2.23) Warm season (May−September): high temp days (95th percentile; lag 0): 2.03 (0.66, 3.42)
†Vanos et al. (2015) 12 Canadian cities 1981−2008 Time-series study	All ages	Data from National Air Pollution Surveillance Network database 24-h avg	Mean: 23.04	Correlation (*r*): $PM_{2.5}$: 0.38; NO_2: 0.1; SO_2: 0.05; Copollutant models with: NR	DM (0−6 DLNM): 3.83 (2.75, 4.91) DT (0−6 DLNM): 7.97 (4.09, 11.99) MM (0−6 DLNM): 4.52 (3.26, 5.66) MT (0−6 DLNM): 4.44 (2.80, 6.19) MT+ (0−6 DLNM): 7.84 (2.88, 13.07)
†Liu et al. (2016) 20 U.S. cities (10 northern; 10 southern); 1987−2000 Time-series study	NMMAPS All ages	Average across all monitors in each city; 10% trimmed mean to correct for yearly averages of each monitor 8-h max	Mean: 39.7 75th: 41.2 Maximum: 44.7	Correlation (*r*): NR Copollutant models with: NR	Southern communities Spring (lag 0−2): −0.20 (−1.00, 0.80) Summer (lag 0−2): −0.40 (−1.00, 0.20) Autumn (lag 0−2): 0.60 (−0.60, 2.01) Winter (lag 0−2): 0.60 (−0.60, 1.61) Northern communities Spring (lag 0−2): 1.40 (0.60, 2.41) Summer (lag 0−2): 2.41 (1.40, 3.43) Autumn (lag 0−2): 1.00 (0.20, 2.01) Winter (lag 0−2): −1.99 (−3.17, −1.00)

Table 6-3 (Continued): Epidemiologic studies of short-term exposure to ozone and total (nonaccidental) mortality.

Study	Study Population	Exposure Assessment	Mean and Upper Percentiles (μg/m^3)	Copollutant Examination	Effect Estimates Percent Increase (95% CI)
†Di et al. (2017a) 39,182 zip codes, U.S. 2000−2012 Case-crossover study	Medicare cohort n = 22,433,862 65+	Validated prediction models based on land use, chemical transport modeling, and satellite remote sensing data. 8-h max	Mean: 37.8	Correlation (*r*): NR Copollutant models with: $PM_{2.5}$	Main analysis with $PM_{2.5}$ (lag 0−1): 1.02 (0.82, 1.22) Nearest monitor with $PM_{2.5}$ from 1-km grid model (lag 0−1): 0.70 (0.56, 0.82) Single pollutant (lag 0−1): 1.10 (0.96, 1.24) Limited to days where O_3 <60 ppb w/ $PM_{2.5}$ (lag 0−1): 1.16 (0.92, 1.40)
†Buteau et al. (2018) Montreal, Canada 1991−2002 Case-crossover study Case-control study	n = 63,534 65+ with CHF	Nearest monitoring station 8-h avg	Mean: 28.9 Median: 27.3 75th: 37.5 95th: 57.5 Maximum: 108.8	Correlation (*r*): NR Copollutant models with: NR	Case-crossover Nearest monitoring station (0−3 DLNM): −2.24 (−9.38, 5.31) BME (0−3 DLNM): −5.12 (−16.61, 7.88) BME (0−3 DLNM): 1.38 (−12.25, 16.94) Inverse-distance weighting (0−3 DLNM): 2.90 (−5.87, 12.54) Case-control Inverse-distance weighting (0−3 DLNM): 22.69 (−3.12, 55.30) Back extrapolation from LUR (0−3 DLNM): 4.28 (−5.46, 14.95) Nearest monitoring station (0−3 DLNM): 6.84 (0.31, 13.79) Back extrapolation from LUR (0−3 DLNM): 8.97 (3.67, 14.70)
†Wilson et al. (2014) 95 U.S. cities 1987−2000 Time-series study	NMMAPS All ages	Average across all monitors in each city; 10% trimmed mean to correct for yearly averages of each monitor 1-h max	NR	Correlation (*r*): NR Copollutant models with: NR	April−October (lag 0) Additive linear National: 3.06 (SE = 0.30) Additive nonlinear National: 3.54 (SE = 0.75) Surface National: 3.98 (SE = 0.24)

Table 6-3 (Continued): Epidemiologic studies of short-term exposure to ozone and total (nonaccidental) mortality.

Study	Study Population	Exposure Assessment	Mean and Upper Percentiles (μg/m^3)	Copollutant Examination	Effect Estimates Percent Increase (95% CI)
†Madrigano et al. (2015) 91 northeast U.S. counties 1988−1999 Time-series study	New York: 62 counties New Jersey: 21 counties Connecticut: 8 counties All ages	Analysis of the average across all monitors in each county for 12 counties; kriging for all 91 counties analysis 8-h max	Mean: 12 counties (observed data): 45.6 91 counties (kriging data): 45.7 23 urban counties: 45.6 68 nonurban counties: 45.7 Maximum: 133.5−149	Correlation (*r*): NR Copollutant models with: PM_{10}[b]	April−October (lag 0) 91 U.S. counties (kriging data): 1.10 (0.50, 1.73) 23 U.S. urban counties (kriging data): 0.90 (0.16, 1.67) 68 U.S. nonurban counties (kriging data): 1.47 (0.38, 2.54) 12 U.S. counties (observed data): 1.61 (0.62, 2.62) New Haven, CT (without PM_{10}): 5.14 (1.57, 8.85) New Haven, CT (with PM_{10}): 5.04 (1.38, 8.81)
†Chen et al. (2018) 86 U.S. counties 1987−2000 Time-series study	NMMAPS All ages	Average across all monitors in each city; 10% trimmed mean to correct for yearly averages of each monitor 24-h avg	NR	Correlation (*r*): NR Copollutant models with: PM_{10} and NO_2	Temperature stratification (sTemp:DLNM; lag 0−1) Low temperature (<25th percentile) 0.17 (−0.46, 0.81) Medium temperature (25th−75th percentile) 0.26 (−0.10, 0.62) High temperature (>75th percentile) 0.89 (0.48, 1.28)

APHENA = Air Pollution and Health: A European and North American Approach; CHF = Congestive Heart Failure; CT = Connecticut; NMMAPS = National Morbidity, Mortality, and Air Pollution Study; DL = distributed lag; DLNM = distributed lag nonlinear model; LUR = land use regression; NJ = New Jersey; NR = not reported; SE = standard error; sTemp = smooth temperature term.

Note: † = U.S. and Canadian studies published since the 2013 Ozone ISA.

[a]Study examined all-cause mortality (including accidental deaths).

[b]Copollutant analysis with PM_{10} only conducted in New Haven, CT due to it being the only city that collected PM_{10} data during the study period.

Table 6-4 Epidemiologic studies of short-term exposure to ozone and cardiovascular mortality.

Study	Study Population	Exposure Assessment	Mean and Upper Percentiles (μg/m^3)	Copollutant Examination	Effect Estimates Percent Increase (95% CI)
Bell et al. (2005) Meta-analysis	U.S. and non-U.S.	24-h avg	NR	Correlation (*r*): NR Copollutant models with: NR	All-year: 1.67 (1.02, 2.30)
Katsouyanni et al. (2009) APHENA 1987−1996 Time-series study	NMMAPS 12 Canadian cities All ages	Exposure assignment approach detailed in original studies and based on all available monitoring data 1-h max	Mean: U.S.: 13.−38.4 Canada: 6.7−8.4 75th: U.S.: 21.0−52.0 Canada: 8.7−12.5	Correlation (*r*): NR Copollutant models with: PM_{10}	All-year (lag 0−2; 8 df/yr−NS): ≥75 yr U.S.: 1.43 (−0.83, 3.73) Canada: 5.51 (0.47, 11.26) <75 yr U.S.: 2.38 (−0.10, 4.90) Canada: 4.34 (−1.70, 10.72) Summer (lag 0−2; 8 df/yr−NS): ≥75 yr U.S.: 1.98 (−0.29, 4.29) Canada: 0.93 (−1.75, 3.68) <75 yr U.S.: 4.19 (1.68, 6.74) Canada: −0.64 (−2.67, 1.43)
Zanobetti and Schwartz (2008b) 48 U.S. cities Time-series study	All ages	Average of all monitors in each city 8-h avg	Mean: 15.1−62.8 75th: 19.8−75.4 Maximum: 34.3−146.2	Correlation (*r*): NR Copollutant models with: NR	Summer (lag 0−3) 1.61 (0.96, 2.27)

Table 6 4 (Continued): Epidemiologic studies of short-term exposure to ozone and cardiovascular mortality.

Study	Study Population	Exposure Assessment	Mean and Upper Percentiles (μg/m^3)	Copollutant Examination	Effect Estimates Percent Increase (95% CI)
†Klemm et al. (2011) Atlanta, GA, U.S. 8/1998−12/2007 Time-series study	65+	Data from several monitors. 8-h max	Mean: 35.54 75th: 47.82 Maximum: 109.07	Correlation (*r*): NR Copollutant models with: NR	Lag 0−1: 0.69 (−2.28, 3.75)
†Sacks et al. (2012) Philadelphia, PA, U.S. 5/12/1992−9/30/1995 Time-series study	All ages	Single monitor ~6 km west/southwest of city hall 8-h max	Mean: 36 Median: 33 Maximum: 110	Correlation (*r*): $PM_{2.5}$: 0.43; NO_2: 0.18; SO_2: −0.19; Other: CO: −0.35 Copollutant models with: NR	Harvard (lag 0−1): −1.60 (−5.10, 2.10) California (lag 0−1): 0.20 (−3.40, 3.90) Canada (lag 0−1): 0.50 (−3.10, 4.30) Harvard AT (lag 0−1): 1.30 (−2.10, 4.90) APHEA2 (lag 0−1): 1.70 (−1.80, 5.30) NMMAPS (lag 0−1): 2.20 (−1.80, 6.40)
†Vanos et al. (2014) 10 Canadian cities 1981−1999 Time-series study	All ages	Monitor in each city located downtown or at city airports within 27 km of downtown 24-h avg	Mean: 19.3	Correlation (*r*): NR Copollutant models with: NR	All-year (lag 0): 4.65 (1.86, 7.43) Spring (lag 0): 3.16 (0.25, 6.08) Summer (lag 0): 5.58 (1.94, 9.21) Fall (lag 0): 1.96 (0.13, 3.78) Winter (lag 0): 4.46 (1.55, 7.37)

df = degrees of freedom; NS = natural spline.

Note: † = U.S. and Canadian studies published since the 2013 Ozone ISA.

Table 6-5 Epidemiologic studies of short-term exposure to ozone and respiratory mortality.

Study	Study Population	Exposure Assessment	Mean and Upper Percentiles (μg/m^3)	Copollutant Examination	Effect Estimates Percent Increase (95% CI)
Bell et al. (2005) Meta-analysis	Meta-analysis	24-h avg	NR	Correlation (*r*): NR Copollutant models with: NR	All-year:0.70 (−0.77, 2.21)
Katsouyanni et al. (2009) APHENA 1987−1996 Time-series study	NMMAPS 12 Canadian cities All ages	Exposure assignment approach detailed in original studies and based on all available monitoring data 1-h max	Mean: U.S.: 13.−38.4 Canada: 6.7−8.4 75th: U.S.: 21.0−52.0 Canada: 8.7−12.5	Correlation (*r*): NR Copollutant models with: PM_{10}	All-year (lag 0−2; 8 df/yr−NS): All U.S.: 1.58 (−2.09, 5.41) Canada: 0.64 (−7.60, 9.67) ≥75 yr U.S.: 0.69 (−4.10, 5.66) Canada: −2.91 (−12.56, 8.09) Summer (lag 0−2; 8 df/yr−NS): All U.S.: 2.73 (−1.32, 6.90) Canada: 15.59 (8.09, 24.08) ≥75 yr U.S.: 2.52 (−2.67, 7.94) Canada: 11.79 (1.38, 23.50)
Zanobetti and Schwartz (2008b) 48 U.S. cities Time-series study	All ages	Average of all monitors in each city 8-h avg	Mean: 15.1−62.8 75th: 19.8−75.4 Maximum: 34.3−146.2	Correlation (*r*): NR Copollutant models with: NR	Summer (lag 0−3): 1.67 (0.76, 2.58)

Table 6 5 (Continued): Epidemiologic studies of short-term exposure to ozone and respiratory mortality.

Study	Study Population	Exposure Assessment	Mean and Upper Percentiles (μg/m^3)	Copollutant Examination	Effect Estimates Percent Increase (95% CI)
†Klemm et al. (2011) Atlanta, GA, U.S. 8/1998−12/2007 Time-series study	65+	Data from several monitors. 8-h max	Mean: 35.54 75th: 47.82 Maximum: 109.07	Correlation (*r*): NR Copollutant models with: NR	Lag 0−1: −0.44 (−6.06, 5.51)
†Vanos et al. (2014) 10 Canadian cities, Canada 1981−1999 Time-series study	All ages	Monitor in each city located downtown or at city airports within 27 km of downtown 24-h avg	Mean: 19.3	Correlation (*r*): NR Copollutant models with: NR	Fall (lag 0): 6.04 (3.31, 8.77) Winter (lag 0): 6.23 (1.49, 10.95) Summer (lag 0): 7.71 (4.26, 11.16) All-year (lag 0): 8.36 (3.72, 12.98) Spring (lag 0): 8.64 (2.45, 14.80)

df = degrees of freedom; NS = natural spline.

Note: † = U.S. and Canadian studies published since the 2013 Ozone ISA.

6.3.2 Long-Term Ozone Exposure and Mortality: Data Tables

Table 6-6 Epidemiologic studies of long-term exposure to ozone and total (nonaccidental) mortality.

Study	Study Population	Exposure Assessment	Mean ($\mu g/m^3$)	Copollutant Examination	Effect Estimates HR (95% CI)
Jerrett et al. (2009) Nationwide, U.S. Ozone: 1977−2000 Follow-up: 1982−2000 Cohort study	ACS n = 448,850 30+ yr	Daily maximum of AIRS monitors averaged over each quarter; second and third quarters (April−September) averaged together for each year 1-h max	Median: 57.4	Correlation (*r*): $PM_{2.5}$ 0.64 Copollutant models with: $PM_{2.5}$	Total mortality (96 MSAs): 1.00 (1.00, 1.01) Total mortality (86 MSAs): 1.00 (1.00, 1.01) Total mortality (86 MSAs + $PM_{2.5}$): 0.99 (0.98, 1.00)
Zanobetti and Schwartz (2011) 105 cities, U.S. Ozone: NR Follow-up: 1985−2006 Cohort study	Medicare n = 3,210,511 65+ yr with pre-existing disease	Citywide average from AQS 8-h avg	Mean: 15.6−71.4	Correlation (*r*): NR Copollutant models with: NR	Total mortality (pre-existing heart failure): 1.12 (1.06, 1.17) Total mortality (pre-existing COPD): 1.14 (1.08, 1.19) Total mortality (pre-existing diabetes): 1.14 (1.10, 1.21) Total mortality (pre-existing MI): 1.19 (1.12, 1.25)
†Spencer-Hwang et al. (2011) Nationwide, U.S. Ozone: 1997−2003 Follow-up: 1997−2003 Cohort study	n = 32,239 Kidney transplant recipients	Monthly average of AQS monitors within 50 km of residence and downscaled to zip code using IDW	NR	Correlation (*r*): NR Copollutant models with: PM_{10}	Total mortality: 1.1 (0.99, 1.21) Total mortality + PM_{10}: 1.09 (0.99, 1.21)

Table 6-6 (Continued): Epidemiologic studies of long-term exposure to ozone and total (nonaccidental) mortality.

Study	Study Population	Exposure Assessment	Mean ($\mu g/m^3$)	Copollutant Examination	Effect Estimates HR (95% CI)
†Carey et al. (2013) Nationwide, U.K. Ozone: 2002 Follow-up: 2003−2007 Cohort study	English Medical Practice n = 835,607 Adults, 40−89 yr, from English medical practices	Annual mean estimates from dispersion model for 1-km grid cells linked to nearest residential postal code centroid	Mean: 25.85 Maximum: 31.5	Correlation (*r*): $PM_{2.5}$: −0.39; NO_2: −0.46; SO_2: −0.41; PM_{10}: −0.40 Copollutant models with: NR	Total mortality: 0.76 (0.62, 0.87)
†Jerrett et al. (2013) California, U.S. Ozone: 1988−2002 Follow-up: 1982−2000 Cohort study	ACS n = 73,711 California	Monthly averages calculated from IDW from up to 4 monitors within 50 km of residence	Mean: 50.35 Median: 50.8 75th: 61 90th: 68.56 95th: 74.18 Maximum: 89.33	Correlation (*r*): $PM_{2.5}$: 0.56; NO_2: −0.0071; Copollutant models with: $PM_{2.5}$; NO_2	Total mortality: 1.00 (0.98, 1.01) Total mortality (+ $PM_{2.5}$): 0.99 (0.98, 1.01) Total mortality (+ NO_2): 1.00 (0.99, 1.02)
†Bentayeb et al. (2015) Nationwide, France Ozone: 1989−2008 Follow-up: 1989−2013 Cohort study	Gazel n = 20,327 Adults working at French national electricity and gas company	CHIMERE chemical transport model 8-h max	Mean: 40.5 Median: 48	Correlation (*r*): $PM_{2.5}$: −0.38; NO_2: −0.34; PM_{10}: −0.21 Copollutant models with: NR	Total mortality: 0.81 (0.64, 1.03)
†Crouse et al. (2015) Nationwide, Canada Ozone: 2002−2009 Follow-up: 1991−2006 Cohort study	CanCHEC n = 2,521,525 25+ yr	Model of warm season concentration at 21-km horizontal resolution assigned at postal code 8-h max	Mean: 39.6 Median: 39 75th: 44.2 Maximum: 60	Correlation (*r*): $PM_{2.5}$: 0.73; NO_2: 0.19; Copollutant models with: NR	Total mortality: 1.03 (1.03, 1.04)
†Turner et al. (2016) Nationwide, U.S. Ozone: 2002−2004 Follow-up: 1982−2004 Cohort study	ACS n = 669,046 35+	HBM with inputs from NAMS/SLAMS and CMAQ; downscaler for eastern U.S. 8-h max	Mean: 38.2 Median: 38.1 75th: 40.1 95th: 45 Maximum: 59.3	Correlation (*r*): $PM_{2.5}$: 0.18; NO_2: −0.08; Copollutant models with: $PM_{2.5}$	Total mortality (year-round): 1.02 (1.01, 1.04) Total mortality (year-round; + $PM_{2.5}$): 1.02 (1.01, 1.04)

Table 6-6 (Continued): Epidemiologic studies of long-term exposure to ozone and total (nonaccidental) mortality.

Study	Study Population	Exposure Assessment	Mean (μg/m^3)	Copollutant Examination	Effect Estimates HR (95% CI)
†Eckel et al. (2016) California, U.S. Ozone: 1988−2011 Follow-up: 1988−2011 Cohort study	n = 352,053 California residents with newly diagnosed cancer	Monthly averages calculated from IDW from up to 4 monitors within 50 km of residence 8-h max	Mean: 40.2	Correlation (*r*): $PM_{2.5}$: −0.02; NO_2: −0.01; PM_{10}: 0.36 Copollutant models with: NR	Total mortality: 1.02 (1.02, 1.03)
†Di et al. (2017b) Nationwide, U.S. Ozone: 2000−2012 Follow-up: 2000−2012 Cohort study	Medicare n = 61 million Older adults	Neural network that includes satellite-based measurements, chemical transport model outputs, land-use terms, meteorological data, and observations from 1,877 ozone monitoring stations	Mean: 46.3 95th: 55.86	Correlation (*r*): $PM_{2.5}$: 0.24; Copollutant models with: $PM_{2.5}$	Total mortality (main analysis): 1.01 (1.01, 1.01) Total mortality (single pollutant): 1.02 (1.02, 1.02) Total mortality (low exposure, <50 ppb): 1.01 (1.01, 1.01)
†Weichenthal et al. (2017) Nationwide, Canada Ozone: 2002−2009 Follow-up: 1991−2011 Cohort study	CanCHEC n = 2,448,500 25+ yr	Model of warm season concentration at 21 km horizontal resolution assigned at postal code 8-h max	Mean: 38.29 Median: 38.11 75th: 42.63 95th: 50.51 Maximum: 60.46	Correlation (*r*): NR Copollutant models with: NR	Total mortality: 1.06 (1.05, 1.07)
†Cakmak et al. (2017) Nationwide, Canada Ozone: 2002−2009 Follow-up: 1991−2011 Cohort study	CanCHEC n = 2,291,250 25+ yr	Model of warm season concentration at 21-km horizontal resolution assigned at postal code 8-h max	Mean: 15.0−43.0 Maximum: 46.6−60.6	Correlation (*r*): $PM_{2.5}$: −0.705 Copollutant models with: $PM_{2.5}$	Total mortality: 1.08 (1.02, 1.14) Total mortality (+ $PM_{=}$): 1.05 (0.99, 1.11)

Table 6-6 (Continued): Epidemiologic studies of long-term exposure to ozone and total (nonaccidental) mortality.

Study	Study Population	Exposure Assessment	Mean ($\mu g/m^3$)	Copollutant Examination	Effect Estimates HR (95% CI)
†Kim et al. (2017) Seoul, Korea Ozone: 2007−2013 Follow-up: 2007−2013 Cohort study	NHIS-NSC n = 136,094 18+ yr, no previous history of CVD	27 monitors in Seoul linked to zip code of participant residence	Mean: 19.93 Median: 18.75 75th: 27.08 Maximum: 71.12	Correlation (*r*): $PM_{2.5}$: 0.67; NO_2: 0.68; SO_2: 0.84; CO: 0.55 Copollutant models with: NR	Total mortality: 0.78 (0.75, 0.82)
†Sesé et al. (2017) Nationwide, France Ozone: 2007−2014 Follow-up: 2007−2014 Cohort study	COFI n = 192 Patients with idiopathic pulmonary fibrosis	Measurements from nearest monitor 24-h avg	NR	Correlation (*r*): NR Copollutant models with: NR	Total mortality: 0.79 (0.44, 1.39)

Table 6-7 Epidemiologic studies of long-term exposure to ozone and cardiovascular mortality.

Study	Study Population	Exposure Assessment	Mean (μg/m^3)	Copollutant Examination	Effect Estimates HR (95% CI)
Jerrett et al. (2009) Nationwide, U.S. Ozone: 1977−2000 Follow-up: 1982−2000 Cohort study	ACS n = 448,850 30+ yr	Daily maximum of AIRS monitors averaged over each quarter; second and third quarters (April−September) averaged together for each year 1-h max	Median: 57.4	Correlation (*r*): $PM_{2.5}$ 0.64 Copollutant models with: $PM_{2.5}$	CVD mortality (96 MSAs): 1.01 (1.00, 1.02) CVD mortality (86 MSAs): 1.01 (1.01, 1.02) CVD mortality (86 MSAs + $PM_{2.5}$): 0.98 (0.97, 0.99) IHD mortality (96 MSAs): 1.02 (1.00, 1.03) IHD mortality (86 MSAs): 1.02 (1.01, 1.03) IHD mortality (86 MSAs + $PM_{2.5}$): 0.97 (0.96, 0.99)
†Spencer-Hwang et al. (2011) Nationwide, U.S. Ozone: 1997−2003 Follow-up: 1997−2003 Cohort study	n = 32,239 Kidney transplant recipients	Monthly average of AQS monitors within 50 km of residence and downscaled to zip code using IDW	NR	Correlation (*r*): NR Copollutant models with: PM_{10}	CHD mortality: 1.35 (1.04, 1.77) CHD mortality (+ PM_{10}): 1.34 (1.03, 1.76)
†Carey et al. (2013) Nationwide, U.K. Ozone: 2002 Follow-up: 2003−2007 Cohort study	English medical practice n = 835,607 Adults, 40−89 yr, from English medical practices	Annual mean estimates from dispersion model for 1-km grid cells linked to nearest residential postal code centroid	Mean: 25.85 Maximum: 31.5	Correlation (*r*): $PM_{2.5}$: −0.39; NO_2: −0.46; SO_2: −0.41; PM_{10}: −0.40 Copollutant models with: NR	Circulatory mortality: 0.76 (0.66, 0.87)

Table 6-7 (Continued): Epidemiologic studies of long-term exposure to ozone and cardiovascular mortality.

Study	Study Population	Exposure Assessment	Mean (μg/m^3)	Copollutant Examination	Effect Estimates HR (95% CI)
†Jerrett et al. (2013) California, U.S. Ozone: 1988−2002 Follow-up: 1982−2000 Cohort study	ACS n = 73,711 California	Monthly averages calculated from IDW from up to 4 monitors within 50 km of residence	Mean: 50.35 Median: 50.8 75th: 61 90th: 68.56 95th: 74.18 Maximum: 89.33	Correlation (*r*): $PM_{2.5}$: 0.56; NO_2: −0.0071; Copollutant models with: $PM_{2.5}$; NO_2	Cardiovascular: 1.02 (0.99, 1.04) Cardiovascular (+ $PM_{2.5}$): 1.01 (0.98, 1.04) Cardiovascular (+ NO_2): 1.03 (1.00, 1.05) IHD: 1.04 (1.01, 1.08) IHD (+ $PM_{2.5}$): 1.03 (0.99, 1.06) IHD (+ NO_2): 1.05 (1.02, 1.09) Stroke: 1.00 (0.97, 1.04) Stroke (+ $PM_{2.5}$): 1.00 (0.95, 1.04) Stroke (+ NO_2): 1.01 (0.97, 1.06)
†Bentayeb et al. (2015) Nationwide, France Ozone: 1989−2008 Follow-up: 1989−2013 Cohort study	Gazel n = 20,327 Adults working at French national electricity and gas company	CHIMERE chemical transport model 8-h max	Mean: 40.5 Median: 48	Correlation (*r*): $PM_{2.5}$: −0.38; NO_2: −0.34; PM_{10}: −0.21 Copollutant models with: NR	CVD mortality: 0.83 (0.39, 1.75)
†Crouse et al. (2015) Nationwide, Canada Ozone: 2002−2009 Follow-up: 1991−2006 Cohort study	CanCHEC n = 2,521,525 25+ yr	Model of warm season concentration at 21-km horizontal resolution assigned at postal code 8-h max	Mean: 39.6 Median: 39 75th: 44.2 Maximum: 60	Correlation (*r*): $PM_{2.5}$: 0.73; NO_2: 0.19; Copollutant models with: NR	CVD: 1.04 (1.03, 1.05) Cardiometabolic: 1.05 (1.04, 1.06) IHD: 1.09 (1.08, 1.11) CBVD: 0.98 (0.96, 1.00) Diabetes: 1.16 (1.13, 1.20)
†Turner et al. (2016) Nationwide, U.S. ozone: 2002−2004 Follow-up: 1982−2004 Cohort study	ACS n = 669,046 35+ yr	HBM with inputs from NAMS/SLAMS and CMAQ; downscaler for eastern U.S. 8-h max	Mean: 38.2 Median: 38.1 75th: 40.1 95th: 45 Maximum: 59.3	Correlation (*r*): $PM_{2.5}$: 0.18; NO_2: −0.08; Copollutant models with: $PM_{2.5}$	CVD: 1.03 (1.01, 1.05) IHD: 0.98 (0.95, 1.00) CBVD: 1.03 (0.98, 1.07) Circulatory: 1.03 (1.02, 1.05) Circulatory (+ $PM_{2.5}$): 1.03 (1.01, 1.05) Dysrhythmias, HF, cardiac arrest: 1.15 (1.10, 1.20) Diabetes: 1.16 (1.07, 1.26)

Table 6-7 (Continued): Epidemiologic studies of long-term exposure to ozone and cardiovascular mortality.

Study	Study Population	Exposure Assessment	Mean (μg/m^3)	Copollutant Examination	Effect Estimates HR (95% CI)
†Cakmak et al. (2016) Nationwide, Canada Ozone: 2002−2009 Follow-up: 1991−2006 Cohort study	CANCHEC n = 2,415,505 25+ yr	Model of warm season concentration at 21-km horizontal resolution assigned at postal code 8-h max	Mean: 14.3−40.9 Maximum: 20.1−53	Correlation (*r*): $PM_{2.5}$: −0.67; Copollutant models with: $PM_{2.5}$	CVD (base model): 1.05 (1.03, 1.06) CVD (adjustment for climate zone): 1.06 (1.04, 1.07) CVD (+ $PM_{2.5}$): 1.03 (1.02, 1.05) IHD (base model): 1.09 (1.08, 1.11) IHD (adjustment for climate zone): 1.07 (1.05, 1.09) CBVD (base model): 1.00 (0.97, 1.02) CBVD (adjustment for climate zone): 1.04 (1.01, 1.08)
†Weichenthal et al. (2017) Nationwide, Canada Ozone: 2002−2009 Follow-up: 1991−2011 Cohort study	CanCHEC n = 2,448,500 25+ yr	Model of warm season concentration at 21-km horizontal resolution assigned at postal code 8-h max	Mean: 38.29 Median: 38.11 75th: 42.63 95th: 50.51 Maximum: 60.46	Correlation (*r*): NR Copollutant models with: NR	CVD mortality: 1.16 (1.14, 1.18)
†Cakmak et al. (2017) Nationwide, Canada Ozone: 2002−2009 Follow-up: 1991−2011 Cohort study	CanCHEC n = 2,291,250 25+ yr	Model of warm season concentration at 21-km horizontal resolution assigned at postal code 8-h max	Mean: 15.0−43.0 Maximum: 46.6−60.6	Correlation (*r*): $PM_{2.5}$: −0.705; Copollutant models with: $PM_{2.5}$	IHD: 1.13 (1.12, 1.15) IHD (+ $PM_{2.5}$): 1.08 (1.07, 1.10)
†Kim et al. (2017) Seoul, South Korea Ozone: 2007−2013 Follow-up: 2007−2013 Cohort study	NHIS-NSC n = 136,094 18+ yr, no previous history of CVD	27 monitors in Seoul linked to zip code of participant residence NR	Mean: 19.93 Median: 18.75 75th: 27.08 Maximum: 71.12	Correlation (*r*): $PM_{2.5}$: 0.67; NO_2: 0.68; SO_2: 0.84; CO: 0.55 Copollutant models with: NR	Cardiovascular mortality: 0.72 (0.64, 0.81)

Table 6-8 Epidemiologic studies of long-term exposure to ozone and respiratory mortality.

Study	Study Population	Exposure Assessment	Mean (μg/m^3)	Copollutant Examination	Effect Estimates HR (95% CI)
Jerrett et al. (2009) Nationwide, U.S. Ozone: 1977−2000 Follow-up: 1982−2000 Cohort study	ACS n = 448,850 30+ yr	Daily maximum of AIRS monitors averaged over each quarter; second and third quarters (April−September) averaged together for each year 1-h max	Median: 57.4	Correlation (*r*): $PM_{2.5}$ 0.64 Copollutant models with: $PM_{2.5}$	Resp mortality (96 MSAs): 1.03 (1.01, 1.05) Resp mortality (86 MSAs): 1.03 (1.01, 1.05) Resp mortality (86 MSAs + $PM_{2.5}$): 1.04 (1.01, 1.07)
†Carey et al. (2013) Nationwide, U.K. Ozone: 2002 Follow-up: 2003−2007 Cohort study	English medical practice n = 835,607 Adults, 40−89 yr, from English medical practices	Annual mean estimates from dispersion model for 1-km grid cells linked to nearest residential postal code centroid	Mean: 25.85 Maximum: 31.5	Correlation (*r*): $PM_{2.5}$: −0.39; NO_2: −0.46; SO_2: −0.41; PM_{10}: −0.40 Copollutant models with: NR	Respiratory: 0.62 (0.50, 0.76)
†Jerrett et al. (2013) California, U.S. Ozone: 1988−2002 Follow-up: 1982−2000 Cohort study	ACS n = 73,711 California	Monthly averages calculated from IDW from up to 4 monitors within 50 km of residence	Mean: 50.35 Median: 50.8 75th: 61 90th: 68.56 95th: 74.18 Maximum: 89.33	Correlation (*r*): $PM_{2.5}$: 0.56; NO_2: −0.0071; Copollutant models with: $PM_{2.5}$; NO_2	Respiratory: 1.01 (0.96, 1.06) Respiratory (+ $PM_{2.5}$): 1.00 (0.95, 1.05) Respiratory (+ NO_2): 1.01 (0.96, 1.06)

Table 6-8 (Continued): Epidemiologic studies of long-term exposure to ozone and respiratory mortality.

Study	Study Population	Exposure Assessment	Mean (μg/m^3)	Copollutant Examination	Effect Estimates HR (95% CI)
†Bentayeb et al. (2015) Nationwide, France Ozone: 1989−2008 Follow-up: 1989−2013 Cohort study	Gazel n = 20,327 Adults working at French national electricity and gas company	CHIMERE chemical transport model 8-h max	Mean: 40.5 Median: 48	Correlation (*r*): $PM_{2.5}$: −0.38; NO_2: −0.34; PM_{10}: −0.21 Copollutant models with: NR	Respiratory mortality: 0.95 (0.55, 1.69)
†Crouse et al. (2015) Nationwide, Canada Ozone: 2002−2009 Follow-up: 1991−2006 Cohort study	CanCHEC n = 2,521,525 25+ yr	Model of warm season concentration at 21-km horizontal resolution assigned at postal code 8-h max	Mean: 39.6 Median: 39 75th: 44.2 Maximum: 60	Correlation (*r*): $PM_{2.5}$: 0.73; NO_2: 0.19; Copollutant models with: NR	Respiratory: 0.97 (0.95, 0.99) COPD: 0.97 (0.95, 1.00)
†Turner et al. (2016) Nationwide, U.S. Ozone: 2002−2004 Follow-up: 1982−2004 Cohort study	ACS n = 669,046 35+	HBM with inputs from NAMS/SLAMS and CMAQ; downscaler for eastern U.S. 8-h max	Mean: 38.2 Median: 38.1 75th: 40.1 95th: 45 Maximum: 59.3	Correlation (*r*): $PM_{2.5}$: 0.18; NO_2: −0.08; Copollutant models with: $PM_{2.5}$	Respiratory: 1.14 (1.10, 1.18) Respiratory (+ $PM_{2.5}$): 1.12 (1.08, 1.16) COPD: 1.14 (1.08, 1.21) Pneumonia and flu: 1.10 (1.03, 1.18)
†Weichenthal et al. (2017) Nationwide, Canada Ozone: 2002−2009 Follow-up: 1991−2011 Cohort study	CanCHEC n = 2,448,500 25+ yr	Model of warm season concentration at 21-km horizontal resolution assigned at postal code 8-h max	Mean: 38.29 Median: 38.11 75th: 42.63 95th: 50.51 Maximum: 60.46	Correlation (*r*): NR Copollutant models with: NR	Respiratory mortality: 1.04 (1.01, 1.07)

Table 6-9 Epidemiologic studies of long-term exposure to ozone and other mortality.

Study	Study Population	Exposure Assessment Averaging Time	Mean (μg/m^3)	Copollutant Examination	Effect Estimates HR (95% CI)
†Xu et al. (2013) Los Angeles, CA and Honolulu, HI, U.S. Ozone: 1992−2008 Follow-up: 1992−2008 Cross-sectional study	White respiratory cancer patients	County-level monthly means from U.S. EPA AQS monitors	Mean: NR	Correlation (*r*): NR Copollutant models with: NR	Cancer-specific death: 1.06 (1.04, 1.07) Other cause of death: 1.04 (1.03, 1.06)
†Jerrett et al. (2013) California, U.S. Ozone: 1988−2002 Follow-up: 1982−2000 Cohort study	ACS n = 73,711 California	Monthly averages calculated from IDW from up to four monitors within 50 km of residence	Mean: 50.35 Median: 50.8 75th: 61 90th: 68.56 95th: 74.18 Maximum: 89.33	Correlation (*r*): $PM_{2.5}$: 0.56; NO_2: −0.0071; Copollutant models with: $PM_{2.5}$; NO_2	Other mortality: 0.99 (0.96, 1.01) Other mortality (+ NO_2): 0.99 (0.96, 1.01) Other mortality (+ $PM_{2.5}$): 0.99 (0.96, 1.01)
†Li et al. (2016) 48 states, U.S. Ozone: 2002−2008 Follow-up: 2002−2008 Cross-sectional study	n = 3,109 counties in CONUS County-level rates	Rates of change of county-level ozone concentrations from downscaler CMAQ model 8-h max	Mean: 29.3−64.5	Correlation (*r*): NR Copollutant models with: $PM_{2.5}$	Reduction in life expectancy (males): −0.42 (−0.50, −0.34) Reduction in life expectancy (males): −0.50 (−0.60, −0.38)
†Rush et al. (2017) 30 states, U.S. Ozone: NR Follow-up: 2011 Cohort study	STROBE n = 93,950 Patients in hospital for ARDS	County-level average	NR	Correlation (*r*): NR Copollutant models with: NR	In-hospital mortality (continuous exposure model): 1.07 (1.06, 1.08) In-hospital mortality (15 high ozone cities): 1.11 (1.08, 1.15)

Table 6 9 (Continued: Epidemiologic studies of long-term exposure to ozone and other mortality.

Study	Study Population	Exposure Assessment Averaging Time	Mean (μg/m^3)	Copollutant Examination	Effect Estimates HR (95% CI)
†Vieira et al. (2017) California, U.S. Ozone: 2009−2011 Follow-up: 1996−2006 Cohort study	n = 11,765 Women with ovarian cancer	Daily exceedances over 70 ppb averaged over 3 yr to create value for census tract 8-h max	Median: 3−29 95th: 265−727	Correlation (*r*): NR Copollutant models with: NR	NA

Annex for Appendix 6: Evaluation of Studies on Health Effects of Ozone

This annex describes the approach used in the Integrated Science Assessment (ISA) for Ozone and Related Photochemical Oxidants to evaluate study quality in the available health effects literature. As described in the Preamble to the ISA (U.S. EPA, 2015), causality determinations were informed by the integration of evidence across scientific disciplines (e.g., exposure, animal toxicology, epidemiology) and related outcomes and by judgments of the strength of inference in individual studies. Table Annex 6-1 describes aspects considered in evaluating study quality of controlled human exposure, animal toxicological, and epidemiologic studies. The aspects found in Table Annex 6-1 are consistent with current best practices for reporting or evaluating health science data.1 Additionally, the aspects are compatible with published U.S. EPA guidelines related to cancer, neurotoxicity, reproductive toxicity, and developmental toxicity (U.S. EPA, 2005, 1998, 1996b, 1991).

These aspects were not used as a checklist, and judgments were made without considering the results of a study. The presence or absence of particular features in a study did not necessarily lead to the conclusion that a study was less informative or should be excluded from consideration in the ISA. Further, these aspects were not used as criteria for determining causality in the five-level hierarchy. As described in the Preamble, causality determinations were based on judgments of the overall strengths and limitations of the collective body of available studies and the coherence of evidence across scientific disciplines and related outcomes. Table Annex 6-1 is not intended to be a complete list of aspects that define a study's ability to inform the relationship between ozone and health effects, but it describes the major aspects considered in this ISA to evaluate studies. Where possible, study elements, such as exposure assessment and confounding (i.e., bias due to a relationship with the outcome and correlation with exposures to ozone), are considered specifically for ozone. Thus, judgments on the ability of a study to inform the relationship between an air pollutant and health can vary depending on the specific pollutant being assessed.

1 For example, NTP OHAT approach (Rooney et al., 2014), IRIS Preamble (U.S. EPA, 2013b), ToxRTool (Klimisch et al., 1997), STROBE guidelines (von Elm et al., 2007), and ARRIVE guidelines (Kilkenny et al., 2010).

Table Anne 6-1 Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Study Design
Controlled Human Exposure:
Studies should clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested. Study subjects should be randomly exposed without knowledge of the exposure condition. Preference is given to balanced crossover (repeated measures) or parallel design studies which include control exposures (e.g., to clean filtered air). In crossover studies, a sufficient and specified time between exposure days should be provided to avoid carry over effects from prior exposure days. In parallel design studies, all arms should be matched for individual characteristics, such as age, sex, race, anthropometric properties, and health status. In studies evaluating effects of disease, appropriately matched healthy controls are desired for interpretative purposes.
Animal Toxicology:
Studies should clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested. Studies should include appropriately matched control exposures (e.g., to clean filtered air, time matched). Studies should use methods to limit differences in baseline characteristics of control and exposure groups. Studies should randomize assignment to exposure groups and where possible conceal allocation to research personnel. Groups should be subjected to identical experimental procedures and conditions; animal care including housing, husbandry, etc. should be identical between groups. Blinding of research personnel to study group may not be possible due to animal welfare and experimental considerations; however, differences in the monitoring or handling of animals in all groups by research personnel should be minimized.
Epidemiology:
Inference is stronger for studies that clearly describe the primary and any secondary aims of the study, or specific hypotheses being tested. For short-term exposure, time-series, case-crossover, and panel studies are emphasized over cross-sectional studies because they examine temporal correlations and are less prone to confounding by factors that differ between individuals (e.g., SES, age). Panel studies with scripted exposures, in particular, can contribute to inference because they have consistent, well-defined exposure durations across subjects, measure personal ambient pollutant exposures, and measure outcomes at consistent, well-defined lags after exposures. Studies with large sample sizes and conducted over multiple years are considered to produce more reliable results. Additionally, multicity studies are preferred over single-city studies because they examine associations for large diverse geographic areas using a consistent statistical methodology, avoiding the publication bias often associated with single-city studies.[a] If other quality parameters are equal, multicity studies carry more weight than single-city studies because they tend to have larger sample sizes and lower potential for publication bias. For long-term exposure, inference is considered to be stronger for prospective cohort studies and case-control studies nested within a cohort (e.g., for rare diseases) than cross-sectional, other case-control, or ecologic studies. Cohort studies can better inform the temporality of exposure and effect. Other designs can have uncertainty related to the appropriateness of the control group or validity of inference about individuals from group-level data. Study design limitations can bias health effect associations in either direction.

Table Annex 6-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Study Population/Test Model
Controlled Human Exposure:
In general, the subjects recruited into study groups should be similarly matched for age, sex, race, anthropometric properties, and health status. In studies evaluating effects of specific subject characteristics (e.g., disease, genetic polymorphism, etc.), appropriately matched healthy controls are preferred. Relevant characteristics and health status should be reported for each experimental group. Criteria for including and excluding subjects should be clearly indicated. For the examination of populations with an underlying health condition (e.g., asthma), independent, clinical assessment of the health condition is ideal, but self-report of physician diagnosis generally is considered to be reliable for respiratory and cardiovascular disease outcomes.b The loss or withdrawal of recruited subjects during the course of a study should be reported. Specific rationale for excluding subject(s) from any portion of a protocol should be explained.
Animal Toxicology:
Ideally, studies should report species, strain, substrain, genetic background, age, sex, and weight. Unless data indicate otherwise, all animal species and strains are considered appropriate for evaluating effects of ozone exposure. It is preferred that the authors test for effects in both sexes and multiple lifestages, and report the result for each group separately. All animals used in a study should be accounted for, and rationale for exclusion of animals or data should be specified.
Epidemiology:
There is greater confidence in results for study populations that are recruited from and representative of the target population. Studies with high participation and low dropout over time that is not dependent on exposure or health status are considered to have low potential for selection bias. Clearly specified criteria for including and excluding subjects can aid assessment of selection bias. For populations with an underlying health condition, independent, clinical assessment of the health condition is valuable, but self-report of physician diagnosis generally is considered to be reliable for respiratory and cardiovascular diseases.b Comparisons of groups with and without an underlying health condition are more informative if groups are from the same source population. Selection bias can influence results in either direction or may not affect the validity of results but rather reduce the generalizability of findings to the target population.
Pollutant
Controlled Human Exposure:
The focus is on studies testing ozone exposure.
Animal Toxicology:
The focus is on studies testing ozone exposure.
Epidemiology:
The focus is on studies evaluating ozone exposure.

Table Annex 6-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Exposure Assessment or Assignment
Controlled Human Exposure:
For this assessment, the focus is on studies that use ozone concentrations <0.4 ppm. Studies that use higher exposure concentrations may provide information relevant to biological plausibility, dosimetry, or inter-species variation. Studies should have well-characterized pollutant concentration, temperature, and relative humidity and/or have measures in place to adequately control the exposure conditions. Preference is given to balanced crossover or parallel design studies that include control exposures (e.g., to clean filtered air). Study subjects should be randomly exposed without knowledge of the exposure condition. Method of exposure (e.g., chamber, facemask, etc.) should be specified and activity level of subjects during exposures should be well characterized.
Animal Toxicology:
For this assessment, the focus is on studies that use ozone concentrations <2 ppm. Studies that use higher exposure concentrations may provide information relevant to biological plausibility, dosimetry, or inter-species variation. Studies should characterize pollutant concentration, temperature, and relative humidity and/or have measures in place to adequately control the exposure conditions. The focus is on inhalation exposure. Noninhalation exposure experiments (i.e., intra-tracheal instillation [IT]) are informative for size fractions that cannot penetrate the airway of a study animal and may provide information relevant to biological plausibility and dosimetry. In vitro studies may be included if they provide mechanistic insight or examine similar effects as in vivo studies, but are generally not included. All studies should include exposure control groups (e.g., clean filtered air).
Epidemiology:
Of primary relevance are relationships of health effects with the ambient component of ozone exposure. However, information about ambient exposure rarely is available for individual subjects; most often, inference is based on ambient concentrations. Studies that compare exposure assessment methods are considered to be particularly informative. Inference is stronger when the duration or lag of the exposure metric corresponds with the time course for physiological changes in the outcome (e.g., up to a few days for symptoms) or latency of disease (e.g., several years for cancer). Ambient ozone concentration tends to have low spatial heterogeneity at the urban scale, except near roads where ozone concentration is lower because ozone reacts with nitric oxide emitted from vehicles. For studies involving individuals with near-road or on-road exposures to ozone, in which ambient ozone concentrations are more spatially heterogeneous and relationships between personal exposures and ambient concentrations are potentially more variable, validated methods that capture the extent of variability for the epidemiologic study design (temporal vs. spatial contrasts) and location carry greater weight. Fixed-site measurements, whether averaged across multiple monitors or assigned from the nearest or single available monitor, typically have smaller biases and smaller reductions in precision compared with spatially heterogeneous air pollutants. Concentrations reported from fixed-site measurements can be informative if correlated with personal exposures, closely located to study subjects, highly correlated across monitors within a location, or combined with time-activity information. Atmospheric models may be used for exposure assessment in place of or to supplement ozone measurements in epidemiologic analyses. For example, grid-scale models (e.g., CMAQ) that represent ozone exposure over relatively large spatial scales (e.g., typically greater than 4- × 4-km grid size) often do provide adequate spatial resolution to capture acute ozone peaks that influence short-term health outcomes. Uncertainty in exposure predictions from these models is largely influenced by model formulations and the quality of model input data pertaining to precursor emissions or meteorology, which tends to vary on a study-by-study basis. In studies of short-term exposure, temporal variability of the exposure metric is of primary interest. For long-term exposures, models that capture within-community spatial variation in individual exposure may be given more weight for spatially variable ambient ozone. Given the low spatial variability of ozone at the urban scale, exposure measurement error typically causes health effect estimates to be underestimated for studies of either short-term or long-term exposure. Biases and decreases in the precision of the association (i.e., wider 95% CIs) tend to be small. Even when spatial variability is higher near roads, the reduction in ozone exposure would cause the exposure to be overestimated at a monitor distant from the road or when averaged across a model grid cell, so that health effects would likely be underestimated.

Table Annex 6-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Outcome Assessment/Evaluation
Controlled Human Exposure:
Endpoints should be assessed in the same manner for control and exposure groups (e.g., time after exposure, methods, endpoint evaluator) using valid, reliable methods. Blinding of endpoint evaluators is ideal, especially for qualitative endpoints (e.g., histopathology). For each experiment and each experimental group, including controls, precise details of all procedures carried out should be provided including how, when, and where. Time of the endpoint evaluations is a key consideration that will vary depending on endpoint evaluated. Endpoints should be assessed at time points that are appropriate for the research questions.
Animal Toxicology:
Endpoints should be assessed in the same manner for control and exposure groups (e.g., time after exposure, methods, endpoint evaluator) using valid, reliable methods. Blinding of endpoint evaluators is ideal, especially for qualitative endpoints (e.g., histopathology). For each experiment and each experimental group, including controls, precise details of all procedures carried out should be provided including how, when, and where. Time of the endpoint evaluations is a key consideration that will vary depending on endpoint evaluated. Endpoints should be assessed at time points that are appropriate for the research questions.
Epidemiology:
Inference is stronger when outcomes are assessed or reported without knowledge of exposure status. Knowledge of exposure status could produce artifactual associations. Confidence is greater when outcomes assessed by interview, self-report, clinical examination, or analysis of biological indicators are defined by consistent criteria and collected by validated, reliable methods. Independent, clinical assessment is valuable for outcomes like lung function or incidence of disease, but report of physician diagnosis has shown good reliability.b When examining short-term exposures, evaluation of the evidence focuses on specific lags based on the evidence presented in individual studies. Specifically, the following hierarchy is used in the process of selecting results from individual studies to assess in the context of results across all studies for a specific health effect or outcome: v. Distributed lag models; vi. Average of multiple days (e.g., 0−2); vii. If a priori lag days were used by the study authors these are the effect estimates presented; or viii. If a study focuses on only a series of individual lag days, expert judgment is applied to select the appropriate result to focus on considering the time course for physiologic changes for the health effect or outcome being evaluated. When health effects of long-term exposure are assessed by acute events such as symptoms or hospital admissions, inference is strengthened when results are adjusted for short-term exposure. Validated questionnaires for subjective outcomes such as symptoms are regarded to be reliable,c particularly when collected frequently and not subject to long recall. For biological samples, the stability of the compound of interest and the sensitivity and precision of the analytical method is considered. If not based on knowledge of exposure status, errors in outcome assessment tend to bias results toward the null.
Potential Copollutant Confounding
Controlled Human Exposure:
Exposure should be well characterized to evaluate independent effects of ozone.
Animal Toxicology:
Exposure should be well characterized to evaluate independent effects of ozone.

Table Annex 6-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Epidemiology:
Not accounting for potential copollutant confounding can produce artifactual associations; thus, studies that examine copollutant confounding carry greater weight. The predominant method is copollutant modeling (i.e., two-pollutant models), which is especially informative when correlations are not high. However, when correlations are high ($r > 0.7$), such as those often encountered for UFP and other traffic-related copollutants, copollutant modeling is less informative. Although the use of single-pollutant models to examine the association between ozone and a health effect or outcome are informative, ideally studies should also include copollutant analyses. Copollutant confounding is evaluated on an individual study basis considering the extent of correlations observed between the copollutant and ozone, and relationships observed with ozone and health effects in copollutant models.
Other Potential Confounding Factors[d]
Controlled Human Exposure:
Preference is given to studies using experimental and control groups that are matched for individual level characteristics (e.g., race/ethnicity, sex, body weight, smoking history, age) and time varying factors (e.g., seasonal and diurnal patterns).
Animal Toxicology:
Preference is given to studies using experimental and control groups that are matched for individual level characteristics (e.g., strain, sex, body weight, litter size, food and water consumption) and time varying factors (e.g., seasonal and diurnal patterns).
Epidemiology:
Factors are considered to be potential confounders if demonstrated in the scientific literature to be related to health effects and correlated with ozone. Not accounting for confounders can produce artifactual associations; thus, studies that statistically adjust for multiple factors or control for them in the study design are emphasized. Less weight is placed on studies that adjust for factors that mediate the relationship between ozone and health effects, which can bias results toward the null. Confounders vary according to study design, exposure duration, and health effect and may include, but are not limited to the following: Short-term exposure studies: Meteorology, day of week, season, medication use, allergen exposure, and long-term temporal trends. Long-term exposure studies: Socioeconomic status, race, age, medication use, smoking status, stress, noise, and occupational exposures.
Statistical Methodology
Controlled Human Exposure:
Statistical methods should be clearly described and appropriate for the study design and research question (e.g., correction for multiple comparisons). Generally, statistical significance is used to evaluate the findings of controlled human exposure studies. However, consistent trends are also informative. Detection of statistical significance is influenced by a variety of factors including, but not limited to, the size of the study, exposure and outcome measurement error, and statistical model specifications. Sample size is not a criterion for exclusion; ideally, the sample size should provide adequate power to detect hypothesized effects (e.g., sample sizes less than three are considered less informative). Because statistical tests have limitations, consideration is given to both trends in data and reproducibility of results.

Table Annex 6-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Animal Toxicology:
Statistical methods should be clearly described and appropriate for the study design and research question (e.g., correction for multiple comparisons). Generally, statistical significance is used to evaluate the findings of animal toxicology studies. However, consistent trends are also informative. Detection of statistical significance is influenced by a variety of factors including, but not limited to, the size of the study, exposure and outcome measurement error, and statistical model specifications. Sample size is not a criterion for exclusion; ideally, the sample size should provide adequate power to detect hypothesized effects (e.g., sample sizes less than three are considered less informative). Because statistical tests have limitations, consideration is given to both trends in data and reproducibility of results.
Epidemiology:
Multivariable regression models that include potential confounding factors are emphasized. However, multipollutant models (more than two pollutants) are considered to produce too much uncertainty due to copollutant collinearity to be informative. Models with interaction terms aid in the evaluation of potential confounding as well as effect modification. Sensitivity analyses with alternate specifications for potential confounding inform the stability of findings and aid in judgments of the strength of inference from results. In the case of multiple comparisons, consistency in the pattern of association can increase confidence that associations were not found by chance alone. Statistical methods that are appropriate for the power of the study carry greater weight. For example, categorical analyses with small sample sizes can be prone to bias results toward or away from the null. Statistical tests such as *t*-tests and chi-squared tests are not considered sensitive enough for adequate inferences regarding ozone-health effect associations. For all methods, the effect estimate and precision of the estimate (i.e., width of 95% CI) are important considerations rather than statistical significance.

a(U.S. EPA, 2008).

bMurgia et al. (2014); Weakley et al. (2013); Yang et al. (2011); Heckbert et al. (2004); Barr et al. (2002); Muhajarine et al. (1997); Toren et al. (1993).

cBurney et al. (1989).

dMany factors evaluated as potential confounders can be effect measure modifiers (e.g., season, comorbid health condition) or mediators of health effects related to ozone (comorbid health condition).

6.4 References

Barr, RG; Herbstman, J; Speizer, FE; Camargo, CA, Jr. (2002). Validation of self-reported chronic obstructive pulmonary disease in a cohort study of nurses. Am J Epidemiol 155: 965-971. http://dx.doi.org/10.1093/aje/155.10.965.

Bell, ML; Dominici, F. (2008). Effect modification by community characteristics on the short-term effects of ozone exposure and mortality in 98 US communities. Am J Epidemiol 167: 986-997. http://dx.doi.org/10.1093/aje/kwm396.

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

Bell, ML; Kim, JY; Dominici, F. (2007). Potential confounding of particulate matter on the short-term association between ozone and mortality in multisite time-series studies. Environ Health Perspect 115: 1591-1595. http://dx.doi.org/10.1289/ehp.10108.

Bell, ML; Mcdermott, A; Zeger, SL; Samet, JM; Dominici, F. (2004). Ozone and short-term mortality in 95 US urban communities, 1987-2000. JAMA 292: 2372-2378. http://dx.doi.org/10.1001/jama.292.19.2372.

Bentayeb, M; Wagner, V; Stempfelet, M; Zins, M; Goldberg, M; Pascal, M; Larrieu, S; Beaudeau, P; Cassadou, S; Eilstein, D; Filleul, L; Le Tertre, A; Medina, S; Pascal, L; Prouvost, H; Quénel, P; Zeghnoun, A; Lefranc, A. (2015). Association between long-term exposure to air pollution and mortality in France: A 25-year follow-up study. Environ Int 85: 5-14. http://dx.doi.org/10.1016/j.envint.2015.08.006.

Burney, PG; Laitinen, LA; Perdrizet, S; Huckauf, H; Tattersfield, AE; Chinn, S; Poisson, N; Heeren, A; Britton, JR; Jones, T. (1989). Validity and repeatability of the IUATLD (1984) Bronchial Symptoms Questionnaire: an international comparison. Eur Respir J 2: 940-945.

Buteau, S; Goldberg, MS; Burnett, RT; Gasparrini, A; Valois, MF; Brophy, JM; Crouse, DL; Hatzopoulou, M. (2018). Associations between ambient air pollution and daily mortality in a cohort of congestive heart failure: Case-crossover and nested case-control analyses using a distributed lag nonlinear model. Environ Int. http://dx.doi.org/10.1016/j.envint.2018.01.003.

Cakmak, S; Hebbern, C; Pinault, L; Lavigne, E; Vanos, J; Crouse, DL; Tjepkema, M. (2017). Associations between long-term PM2.5 and ozone exposure and mortality in the Canadian Census Health and Environment Cohort (CANCHEC), by spatial synoptic classification zone. Environ Int 111: 200-211. http://dx.doi.org/10.1016/j.envint.2017.11.030.

Cakmak, S; Hebbern, C; Vanos, J; Crouse, DL; Burnett, R. (2016). Ozone exposure and cardiovascular-related mortality in the Canadian Census Health and Environment Cohort (CANCHEC) by spatial synoptic classification zone. Environ Pollut 214: 589-599. http://dx.doi.org/10.1016/j.envpol.2016.04.067.

Carey, IM; Atkinson, RW; Kent, AJ; van Staa, T; Cook, DG; Anderson, HR. (2013). Mortality associations with long-term exposure to outdoor air pollution in a national English cohort. Am J Respir Crit Care Med 187: 1226-1233. http://dx.doi.org/10.1164/rccm.201210-1758OC.

Chen, K; Wolf, K; Hampel, R; Stafoggia, M; Breitner, S; Cyrys, J; Samoli, E; Andersen, ZJ; Bero-Bedada, G; Bellander, T; Hennig, F; Jacquemin, B; Pekkanen, J; Peters, A; Schneider, A. (2018). Does temperature-confounding control influence the modifying effect of air temperature in ozone-mortality associations? Environmental Epidemiology 2: 1-7. http://dx.doi.org/10.1097/EE9.0000000000000008.

Crouse, DL; Peters, PA; Hystad, P; Brook, JR; van Donkelaar, A; Martin, RV; Villeneuve, PJ; Jerrett, M; Goldberg, MS; Pope, CA; Brauer, M; Brook, RD; Robichaud, A; Menard, R; Burnett, RT. (2015). Ambient PM 2.5, O 3, and NO 2 exposures and associations with mortality over 16 years of follow-up in the Canadian Census Health and Environment Cohort (CanCHEC). Environ Health Perspect 123: 1180-1186. http://dx.doi.org/10.1289/ehp.1409276.

Di, Q; Dai, L; Wang, Y; Zanobetti, A; Choirat, C; Schwartz, JD; Dominici, F. (2017a). Association of short-term exposure to air pollution with mortality in older adults. JAMA 318: 2446-2456. http://dx.doi.org/10.1001/jama.2017.17923.

Di, Q; Wang, Y; Zanobetti, A; Wang, Y; Koutrakis, P; Choirat, C; Dominici, F; Schwartz, JD. (2017b). Air pollution and mortality in the Medicare population. N Engl J Med 376: 2513-2522. http://dx.doi.org/10.1056/NEJMoa1702747.

Eckel, SP; Cockburn, M; Shu, YH; Deng, H; Lurmann, FW; Liu, L; Gilliland, FD. (2016). Air pollution affects lung cancer survival. Thorax 71: 891-898. http://dx.doi.org/10.1136/thoraxjnl-2015-207927.

Franklin, M; Schwartz, J. (2008). The impact of secondary particles on the association between ambient ozone and mortality. Environ Health Perspect 116: 453-458. http://dx.doi.org/10.1289/ehp.10777.

Goldberg, MS; Burnett, RT; Stieb, DM; Brophy, JM; Daskalopoulou, SS; Valois, MF; Brook, JR. (2013). Associations between ambient air pollution and daily mortality among elderly persons in Montreal, Quebec. Sci Total Environ 463-464: 931942. http://dx.doi.org/10.1016/j.scitotenv.2013.06.095.

Heckbert, SR; Kooperberg, C; Safford, MM; Psaty, BM; Hsia, J; McTiernan, A; Gaziano, JM; Frishman, WH; Curb, JD. (2004). Comparison of self-report, hospital discharge codes, and adjudication of cardiovascular events in the Women's Health Initiative. Am J Epidemiol 160: 1152-1158. http://dx.doi.org/10.1093/aje/kwh314.

Ito, K; De Leon, SF; Lippmann, M. (2005). Associations between ozone and daily mortality, analysis and meta-analysis. Epidemiology 16: 446-457. http://dx.doi.org/10.1097/01.ede.0000165821.90114.7f.

Jerrett, M; Burnett, RT; Beckerman, BS; Turner, MC; Krewski, D; Thurston, G; Martin, RV; van Donkelaar, A; Hughes, E; Shi, Y; Gapstur, SM; Thun, MJ; Pope, CA, III. (2013). Spatial analysis of air pollution and mortality in California. Am J Respir Crit Care Med 188: 593-599. http://dx.doi.org/10.1164/rccm.201303-0609OC.

Jerrett, M; Burnett, RT; Pope, CA, III; Ito, K; Thurston, G; Krewski, D; Shi, Y; Calle, E; Thun, M. (2009). Long-term ozone exposure and mortality. N Engl J Med 360: 1085-1095. http://dx.doi.org/10.1056/NEJMoa0803894.

Jhun, I; Fann, N; Zanobetti, A; Hubbell, B. (2014). Effect modification of ozone-related mortality risks by temperature in 97 US cities. Environ Int 73: 128-134. http://dx.doi.org/10.1016/j.envint.2014.07.009.

Katsouyanni, K; Samet, JM; Anderson, HR; Atkinson, R; Le Tertre, A; Medina, S; Samoli, E; Touloumi, G; Burnett, RT; Krewski, D; Ramsay, T; Dominici, F; Peng, RD; Schwartz, J; Zanobetti, A. (2009). Air pollution and health: A European and North American approach (APHENA) (pp. 5-90). (Research Report 142). Boston, MA: Health Effects Institute. http://pubs.healtheffects.org/view.php?id=327.

Kilkenny, C; Browne, WJ; Cuthill, IC; Emerson, M; Altman, DG. (2010). Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research [Review]. PLoS Biol 8: e1000412. http://dx.doi.org/10.1371/journal.pbio.1000412.

Kim, H; Kim, J; Kim, S; Kang, SH; Kim, HJ; Kim, H; Heo, J; Yi, SM; Kim, K; Youn, TJ; Chae, IH. (2017). Cardiovascular effects of long-term exposure to air pollution: a population-based study with 900845person-years of follow-up. J Am Heart Assoc 6. http://dx.doi.org/10.1161/JAHA.117.007170.

Klemm, RJ; Lipfert, FW; Wyzga, RE; Gust, C. (2004). Daily mortality and air pollution in Atlanta: two years of data from ARIES. Inhal Toxicol 16 Suppl 1: 131-141. http://dx.doi.org/10.1080/08958370490443213.

Klemm, RJ; Mason, RM, Jr. (2000). Aerosol Research and Inhalation Epidemiological Study (ARIES): air quality and daily mortality statistical modeling--interim results. J Air Waste Manag Assoc 50: 1433-1439.

Klemm, RJ; Thomas, EL; Wyzga, RE. (2011). The impact of frequency and duration of air quality monitoring: Atlanta, GA, data modeling of air pollution and mortality. J Air Waste Manag Assoc 61: 1281-1291. http://dx.doi.org/10.1080/10473289.2011.617648.

Klimisch, HJ; Andreae, M; Tillmann, U. (1997). A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regul Toxicol Pharmacol 25: 1-5. http://dx.doi.org/10.1006/rtph.1996.1076.

Levy, JI; Chemerynski, SM; Sarnat, JA. (2005). Ozone exposure and mortality, an empiric Bayes metaregression analysis. Epidemiology 16: 458-468. http://dx.doi.org/10.1097/01.ede.0000165820.08301.b3.

Li, C; Balluz, LS; Vaidyanathan, A; Wen, X; Hao, Y; Qualters, J. R. (2016). Long-term exposure to ozone and life expectancy in the United States, 2002 to 2008. Medicine (Baltimore) 95: e2474. http://dx.doi.org/10.1097/MD.0000000000002474.

Liu, T; Zeng, W; Lin, H; Rutherford, S; Xiao, J; Li, X; Li, Z; Qian, Z; Feng, B; Ma, W. (2016). Tempo-spatial variations of ambient ozone-mortality associations in the usa: results from the nmmaps data. Int J Environ Res Public Health 13. http://dx.doi.org/10.3390/ijerph13090851.

Madrigano, J; Jack, D; Anderson, GB; Bell, ML; Kinney, PL. (2015). Temperature, ozone, and mortality in urban and non-urban counties in the northeastern United States. Environ Health 14: 3. http://dx.doi.org/10.1186/1476-069X-14-3.

Medina-Ramón, M; Schwartz, J. (2008). Who is more vulnerable to die from ozone air pollution? Epidemiology 19: 672-679. http://dx.doi.org/10.1097/EDE.0b013e3181773476.

Moolgavkar, SH; Mcclellan, RO; Dewanji, A; Turim, J; Luebeck, EG; Edwards, M. (2013). Time-series analyses of air pollution and mortality in the United States: A subsampling approach. Environ Health Perspect 121: 73-78. http://dx.doi.org/10.1289/ehp.1104507.

Muhajarine, N; Mustard, C; Roos, LL; Young, TK; Gelskey, DE. (1997). Comparison of survey and physician claims data for detecting hypertension. J Clin Epidemiol 50: 711-718. http://dx.doi.org/10.1016/S0895-4356(97)00019-X.

Murgia, N; Brisman, J; Claesson, A; Muzi, G; Olin, AC; Torén, K. (2014). Validity of a questionnaire-based diagnosis of chronic obstructive pulmonary disease in a general population-based study. BMC Pulm Med 14: 49. http://dx.doi.org/10.1186/1471-2466-14-49.

NHLBI (National Institutes of Health, National Heart Lung and Blood Institute). (2017). NHLBI fact book, fiscal year 2012: Disease statistics. Available online at https://www.nhlbi.nih.gov/about/documents/factbook/2012/chapter4 (accessed August 23, 2017).

Peng, RD; Samoli, E; Pham, L; Dominici, F; Touloumi, G; Ramsay, T; Burnett, RT; Krewski, D; Le Tertre, A; Cohen, A; Atkinson, RW; Anderson, HR; Katsouyanni, K; Samet, JM. (2013). Acute effects of ambient ozone on mortality in Europe and North America: results from the APHENA study. Air Qual Atmos Health 6: 445-453. http://dx.doi.org/10.1007/s11869-012-0180-9.

Rooney, AA; Boyles, AL; Wolfe, MS; Bucher, JR; Thayer, KA. (2014). Systematic review and evidence integration for literature-based environmental health science assessments. Environ Health Perspect 122: 711-718. http://dx.doi.org/10.1289/ehp.1307972.

Rush, B; Mcdermid, RC; Celi, LA; Walley, KR; Russell, JA; Boyd, JH. (2017). Association between chronic exposure to air pollution and mortality in the acute respiratory distress syndrome. Environ Pollut 224: 352-356. http://dx.doi.org/10.1016/j.envpol.2017.02.014.

Sacks, JD; Ito, K; Wilson, WE; Neas, LM. (2012). Impact of covariate models on the assessment of the air pollution-mortality association in a single- and multipollutant context. Am J Epidemiol 176: 622-634. http://dx.doi.org/10.1093/aje/kws135.

Schwartz, J. (2000). Assessing confounding, effect modification, and thresholds in the association between ambient particles and daily deaths. Environ Health Perspect 108: 563-568.

Schwartz, J. (2005). How sensitive is the association between ozone and daily deaths to control for temperature? Am J Respir Crit Care Med 171: 627-631. http://dx.doi.org/10.1164/rccm.200407-933OC.

Sesé, L; Nunes, H; Cottin, V; Sanyal, S; Didier, M; Carton, Z; Israel-Biet, D; Crestani, B; Cadranel, J; Wallaert, B; Tazi, A; Maître, B; Prévot, G; Marchand-Adam, S; Guillot-Dudoret, S; Nardi, A; Dury, S; Giraud, V; Gondouin, A; Juvin, K; Borie, R; Wislez, M; Valeyre, D; Annesi-Maesano, I. (2017). Role of atmospheric pollution on the natural history of idiopathic pulmonary fibrosis. Thorax 73: 145-150. http://dx.doi.org/10.1136/thoraxjnl-2017-209967.

Spencer-Hwang, R; Knutsen, SF; Soret, S; Ghamsary, M; Beeson, WL; Oda, K; Shavlik, D; Jaipaul, N. (2011). Ambient air pollutants and risk of fatal coronary heart disease among kidney transplant recipients. Am J Kidney Dis 58: 608-616. http://dx.doi.org/10.1053/j.ajkd.2011.05.017.

Toren, K; Brisman, J; Jarvholm, B. (1993). Asthma and asthma-like symptoms in adults assessed by questionnaires: A literature review [Review]. Chest 104: 600-608. http://dx.doi.org/10.1378/chest.104.2.600.

Turner, MC; Jerrett, M; Pope, A, III; Krewski, D; Gapstur, SM; Diver, WR; Beckerman, BS; Marshall, JD; Su, J; Crouse, DL; Burnett, RT. (2016). Long-term ozone exposure and mortality in a large prospective study. Am J Respir Crit Care Med 193: 1134-1142. http://dx.doi.org/10.1164/rccm.201508-1633OC.

U.S. EPA (U.S. Environmental Protection Agency). (1991). Guidelines for developmental toxicity risk assessment (pp. 1-71). (EPA/600/FR-91/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=23162.

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

U.S. EPA (U.S. Environmental Protection Agency). (1996b). Guidelines for reproductive toxicity risk assessment (pp. 1-143). (EPA/630/R-96/009). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/sites/production/files/2014-11/documents/guidelines_repro_toxicity.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (1998). Guidelines for neurotoxicity risk assessment [EPA Report] (pp. 1-89). (EPA/630/R-95/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/risk/guidelines-neurotoxicity-risk-assessment.

U.S. EPA (U.S. Environmental Protection Agency). (2005). Guidelines for carcinogen risk assessment [EPA Report] (pp. 1-166). (EPA/630/P-03/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/sites/production/files/2013-09/documents/cancer_guidelines_final_3-25-05.pdf.

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

U.S. EPA (U.S. Environmental Protection Agency). (2008). Integrated science assessment for sulfur oxides: Health criteria [EPA Report]. (EPA/600/R-08/047F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment-RTP. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=198843.

U.S. EPA (U.S. Environmental Protection Agency). (2013a). Integrated science assessment for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-10/076F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment-RTP Division. http://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=247492.

U.S. EPA (U.S. Environmental Protection Agency). (2013b). Toxicological review of trimethylbenzenes (CASRN 25551-13-7, 95-63-6, 526-73-8, and 108-67-8) in support of summary information on the Integrated Risk Information System (IRIS): revised external review draft [EPA Report]. (EPA/635/R13/171a). Washington, D.C.: U.S. Environmental Protection Agency, National Center for Environmental Assessment. http://yosemite.epa.gov/sab/SABPRODUCT.NSF/b5d8a1ce9b07293485257375007012b7/ee1e280e77586de985257b65005d37e7!OpenDocument.

U.S. EPA (U.S. Environmental Protection Agency). (2015). Preamble to the integrated science assessments [EPA Report]. (EPA/600/R-15/067). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, RTP Division. https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=310244.

Vanos, JK; Cakmak, S; Bristow, C; Brion, V; Tremblay, N; Martin, SL; Sheridan, SS. (2013). Synoptic weather typing applied to air pollution mortality among the elderly in 10 Canadian cities. Environ Res 126: 66-75. http://dx.doi.org/10.1016/j.envres.2013.08.003.

Vanos, JK; Cakmak, S; Kalkstein, LS; Yagouti, A. (2015). Association of weather and air pollution interactions on daily mortality in 12 Canadian cities. Air Qual Atmos Health 8: 307-320. http://dx.doi.org/10.1007/s11869-014-0266-7.

Vanos, JK; Hebbern, C; Cakmak, S. (2014). Risk assessment for cardiovascular and respiratory mortality due to air pollution and synoptic meteorology in 10 Canadian cities. Environ Pollut 185: 322-332. http://dx.doi.org/10.1016/j.envpol.2013.11.007.

Vieira, VM; Villanueva, C; Chang, J; Ziogas, A; Bristow, RE. (2017). Impact of community disadvantage and air pollution burden on geographic disparities of ovarian cancer survival in California. Environ Res 156: 388-393. http://dx.doi.org/10.1016/j.envres.2017.03.057.

von Elm, E; Altman, DG; Egger, M; Pocock, SJ; Gøtzsche, PC; Vandenbroucke, JP. (2007). The strengthening the reporting of observational studies in epidemiology (strobe) statement: guidelines for reporting observational studies [Review]. PLoS Med 4: e296. http://dx.doi.org/10.1371/journal.pmed.0040296.

Weakley, J; Webber, MP; Ye, F; Zeig-Owens, R; Cohen, HW; Hall, CB; Kelly, K; Prezant, DJ. (2013). Agreement between obstructive airways disease diagnoses from self-report questionnaires and medical records. Prev Med 57: 38-42. http://dx.doi.org/10.1016/j.ypmed.2013.04.001.

Weichenthal, S; Pinault, LL; Burnett, RT. (2017). Impact of oxidant gases on the relationship between outdoor fine particulate air pollution and nonaccidental, cardiovascular, and respiratory mortality. Sci Rep 7: 16401. http://dx.doi.org/10.1038/s41598-017-16770-y.

Wilson, A; Rappold, AG; Neas, LM; Reich, BJ. (2014). Modeling the effect of temperature on ozone-related mortality. Ann Appl Stat 8: 1728-1749. http://dx.doi.org/10.1214/14-AOAS754.

Xu, X; Ha, S; Kan, H; Hu, H; Curbow, BA; Lissaker, CTK. (2013). Health effects of air pollution on length of respiratory cancer survival. BMC Public Health 13: 800. http://dx.doi.org/10.1186/1471-2458-13-800.

Yang, CL; To, T; Foty, RG; Stieb, DM; Dell, SD. (2011). Verifying a questionnaire diagnosis of asthma in children using health claims data. BMC Pulm Med 11. http://dx.doi.org/10.1186/1471-2466-11-52.

Zanobetti, A; Schwartz, J. (2008a). Is there adaptation in the ozone mortality relationship: A multi-city case-crossover analysis. Environ Health 7: 22. http://dx.doi.org/10.1186/1476-069X-7-22.

Zanobetti, A; Schwartz, J. (2008b). Mortality displacement in the association of ozone with mortality: An analysis of 48 cities in the United States. Am J Respir Crit Care Med 177: 184-189. http://dx.doi.org/10.1164/rccm.200706-823OC.

Zanobetti, A; Schwartz, J. (2011). Ozone and survival in four cohorts with potentially predisposing diseases. Am J Respir Crit Care Med 184: 836-841. http://dx.doi.org/10.1164/rccm.201102-0227OC.

APPENDIX 7 HEALTH EFFECTS—OTHER HEALTH ENDPOINTS

Summary of Causality Determinations for Other Health Effects

This Appendix characterizes the scientific evidence that supports causality determinations for short- and long-term ozone exposure and health effects, including Reproductive and Developmental Effects (see Section 7.1), Nervous System Effects (see Section 7.2), and Cancer (see Section 7.3). The types of studies evaluated within this Appendix are consistent with the overall scope of the ISA as detailed in the Preface. In assessing the overall evidence, the strengths and limitations of individual studies were evaluated based on scientific considerations detailed in the Annex for Appendix 7. More details on the causality framework used to reach these conclusions are included in the Preamble to the ISA (U.S. EPA, 2015).

Health Effect	Causality Determination
Short-term exposure	
Nervous system effects	Suggestive of, but not sufficient to infer, a causal relationship
Long-term exposure	
Reproductive and developmental effects	
Male and female reproduction and fertility	Suggestive of, but not sufficient to infer a causal relationship
Pregnancy and birth outcomes	Suggestive of, but not sufficient to infer, a causal relationship
Effects of exposure during developmental periods	Evidence is summarized in Section 7.1.4 but contributes to the causality determinations for relevant organ systems (i.e., Respiratory–Appendix 3, Cardiovascular–Appendix 4, Metabolic–Appendix 5, and Nervous System—Section 7.1.4)
Nervous system effects	Suggestive of, but not sufficient to infer, a causal relationship
Cancer	Inadequate evidence to determine if a causal relationship exists

7.1 Reproductive and Developmental Effects

7.1.1 Introduction, Summary from the 2013 Ozone ISA, and Scope for Current Review

This section evaluates the scientific evidence related to the potential effects of ozone on reproductive outcomes, including (1) male and female reproduction and fertility (Section 7.1.2) and (2) pregnancy and birth outcomes (Section 7.1.3). The effects of exposure during developmental periods (referred to as "developmental effects") are summarized in Section 7.1.4, but are fully evaluated and contribute to causality determinations in the ISA section for the relevant organ system (i.e., respiratory [see Appendix 3], cardiovascular [see Appendix 4], metabolic [see Appendix 5], and nervous system effects [see Section 7.2]). Many studies have been added to the body of literature since the 2013 Ozone ISA (U.S. EPA, 2013a) including epidemiologic studies and short- and long-term animal toxicological and developmental studies. Because the average length of gestation in rodents is 18−24 days, animal toxicological studies investigating the effects of ozone generally are considered short-term exposure periods. For comparison, an epidemiologic study that uses the entire pregnancy as the exposure period is considered to have a long-term exposure period (about 40 weeks, on average). Results from both short- and long-term exposure periods are included in a single section (Section 7.1) and are identified accordingly in the text and tables throughout this section. Well-designed studies that consider sources of bias, including potential confounding by copollutant exposures, are emphasized.

A major issue in studying environmental exposures and reproductive and developmental effects is selecting the relevant exposure period, since the biologically plausible pathways leading to these outcomes and the critical periods of exposure are not completely understood. Thus, multiple exposure periods are evaluated in many epidemiologic studies, including long-term (months to years) exposure periods, such as entire pregnancy, individual trimesters or months of pregnancy, and short-term (days to weeks) exposure periods, such as the days and weeks immediately preceding birth. Thus, the evaluation of biological plausibility for the effects of ozone on reproductive and developmental outcomes will combine short- and long-term exposures. Further, infants and fetal development processes may be particularly sensitive to ozone exposure, and although the physical mechanisms are not always fully understood, the effects from ozone exposure at these critical windows of development may have permanent, lifelong effects.

The 2013 Ozone ISA (U.S. EPA, 2013a) determined that the evidence was suggestive of a causal relationship between exposures to ozone and reproductive and developmental effects. Epidemiologic and toxicological studies provided evidence for an effect of prenatal exposure to ozone on pulmonary structure and function, as well as alterations in placental and pup cytokines, and increased pup airway hyper-reactivity. Also, there was limited toxicological evidence for an effect of prenatal and early life

exposure on central nervous system effects, including laterality, brain morphology, neurobehavioral abnormalities, and sleep aberration. Epidemiologic studies examining the effects of ozone on sperm quality provided limited evidence for decrements in sperm concentration, which was supported by limited toxicological evidence for testicular degeneration associated with ozone exposure. While the collective evidence for many of the birth outcomes examined in the 2013 Ozone ISA was generally inconsistent (including birth defects), there were several well-designed, well-conducted studies that indicated an association between ozone and adverse outcomes. For example, as part of the southern California Children's Health Study, Salam et al. (2005) observed a concentration-response association of decreasing birth weight with increasing ozone concentrations averaged over the entire pregnancy, especially evident at levels above 30-ppb. Similarly, Hansen et al. (2008), using fetal ultrasonic measurements, found a decrease in average fetal size associated with ozone during Days 31−60 of gestation for women living within 2 km of a monitoring site.

The current ISA builds upon findings from the 2013 Ozone ISA but separate causality determinations are made for the male and female fertility and reproduction (see Section 7.1.2), and pregnancy and birth outcomes (see Section 7.1.3), as they are likely to have different etiologies and critical exposure windows over different lifestages. For effects of exposure during developmental periods see Section 7.1.4; however, summaries are included in this section of the ISA, while full descriptions and causality determinations are found in the designated appendix for individual outcomes (i.e., respiratory [see Appendix 3], cardiovascular [see Appendix 4], metabolic [see Appendix 5] and nervous system effects [see Section 7.2].)

7.1.1.1 Population, Exposure, Comparison, Outcome, and Study Design (PECOS) Tool

The scope of this section is defined by a scoping tool that generally describes the relevant Population, Exposure, Comparison, Outcome, and Study Design (PECOS). The PECOS tool defines the parameters and provides a framework to help identify the relevant literature to inform the draft 2019 Ozone ISA. The studies evaluated and subsequently discussed within this section were included if they satisfied all of the components of the following PECOS tool::

- Population: Study population of any animal toxicological study of mammals at any lifestage
- Exposure: Long-term (in the order of months to years) or short-term (hours to less than 4 complete weeks) inhalation exposure to relevant ozone concentrations (i.e., ≤2 ppm)
- Comparison: Appropriate comparison group exposed to a negative control (i.e., clean air or filtered-air control)
- Outcome: Reproductive or developmental effects
- Study Design: In vivo chronic, subchronic or repeated-dose toxicity studies in mammals; reproductive toxicity or immunotoxicity studies; genotoxicity/mutagenicity studies (studies that examine the effects of exposure during developmental periods contribute the causality

determinations in Appendix 3, Appendix 4, and Appendix 5, and Section 7.2.2.5 and are summarized in Section 7.1.4.)

Because the 2013 Ozone ISA concluded that there was evidence to suggest a causal relationship between long-term ozone exposure and reproductive and developmental effects, the studies evaluated are less limited in scope and not targeted towards specific study locations, as reflected in the PECOS tool. The epidemiologic studies evaluated and subsequently discussed within this section were identified using the following PECOS tool:

- Population: Any population, including populations or lifestages that might be at increased risk
- Exposure: Long-term (in the order of months to years) or short-term (hours to less than 4 complete weeks)
- Comparison: Per unit increase (in ppb)
- Outcome: Change in risk (incidence/prevalence) of a reproductive or developmental effect
- Study Design: Epidemiologic studies consisting of cohort and case-control studies; time-series, case-crossover, and cross-sectional studies with appropriate timing of exposure for the health endpoint of interest

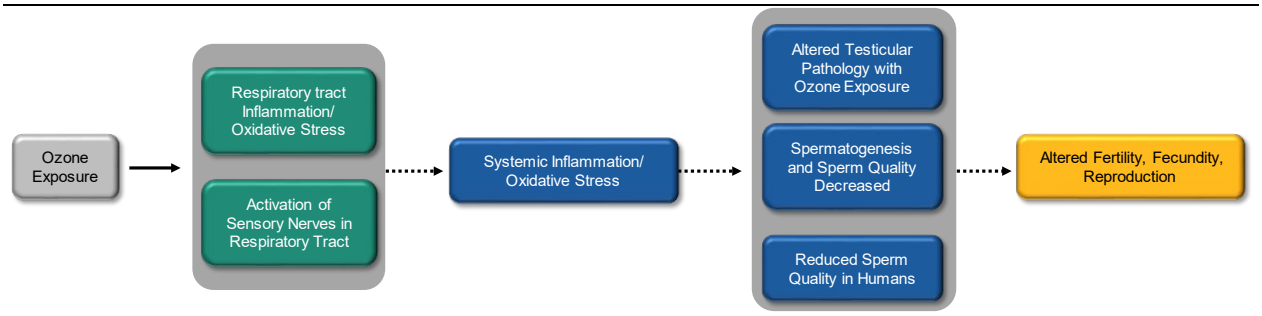
7.1.2 Male and Female Reproduction and Fertility

Reproductive health issues, commonly identified through impaired fecundity (ability to conceive) and fertility (ability to have live born children), affect up to 15% of couples attempting to conceive, with both male and female factors contributing (Thoma et al., 2013). In the U.S., approximately 9% of men aged 18−44 years and 11% of women aged 15−44 years are infertile (Agarwal et al., 2015; Chandra et al., 2013). Reproductive health issues can have negative effects on quality of life and may signal poorer physiological health and increased risk to adverse health outcomes during pregnancy and birth.

7.1.2.1 Biological Plausibility

When considering the available health evidence, there are plausible pathways connecting inhalation of ozone to the apical reproductive and developmental events reported in epidemiologic studies. This section describes biological pathways that potentially underlie reproductive and developmental health effects specific to male and female reproduction and fertility resulting from exposure to ozone. Biological plausibility is graphically depicted via the proposed pathways as a continuum of upstream events, connected by arrows, that may lead to downstream events observed in epidemiologic studies (Figure 7-1). This discussion of "how" exposure to ozone may lead to effects on male and female reproduction and fertility contributes to an understanding of the biological plausibility of epidemiologic results evaluated later.

When considering the available health evidence, there are plausible pathways connecting inhalation of ozone to the apical reproductive effects reported in epidemiologic studies. The biological plausibility for ozone-induced effects on reproduction and fertility is supported by evidence from the 2013 Ozone ISA (U.S. EPA, 2013a) and by new evidence. Once these pathways are initiated, there is evidence from experimental and epidemiologic studies that ozone inhalation may result in a series of physiological responses that could lead to male and female reproductive effects and altered fertility (e.g., fertility, fecundity, reproduction). The evidence for the initial events (Figure 7-1) that could result in effects on fertility and reproduction includes respiratory tract inflammation following the inhalation of ozone. Respiratory tract inflammation can be followed by systemic inflammation [e.g., C-reactive protein (CRP); Lee et al. (2011); see Section 4.2.11]. Ozone exposure may induce inflammatory or other processes in extrapulmonary compartments. Beyond these events, there is also evidence from experimental and epidemiologic studies demonstrating that exposure to ozone could result in a coherent series of physiological responses that provide biological plausibility for the associations reported in epidemiologic and laboratory animal studies, including altered fertility, fecundity, and reproduction.



Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence related to ozone exposure, and the arrows indicate a proposed relationship between those effects. Solid arrows denote evidence of essentiality as provided, for example, by an inhibitor of the pathway or a genetic knockout model used in an experimental study involving ozone exposure. Shading around multiple boxes is used to denote a grouping of these effects. Arrows may connect individual boxes, groupings of boxes, and individual boxes within groupings of boxes. Progression of effects is generally depicted from left to right and color-coded (gray, exposure; green, initial effect; blue, intermediate effect; orange, effect at the population level or a key clinical effect). Here, population level effects generally reflect results of epidemiologic studies. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below.

Figure 7-1 Potential biological pathways for male reproduction and fertility effects following ozone exposure.

As depicted in Figure 7-1, these initial events can give rise to intermediate events, including systemic inflammation from epidemiologic evidence of increased CRP during pregnancy, animal studies of altered sperm quality, altered testicular morphology, aberrant testicular histology, including a depletion of testicular germ cells and a decreased seminiferous tubule epithelial layer, impaired spermatogenesis with focal epithelial cell desquamation to the basement membrane, and presence of giant spermatid cells (Jedlinska-Krakowska et al., 2006b). The 2013 Ozone ISA documented epidemiologic studies that showed decreased sperm quality, and there is recent evidence for sperm-related effects, including

decreased sperm concentration and decreased sperm count with ozone exposure to males with lupus. In studies of female reproduction, epidemiologic studies have shown altered reproductive success with ozone exposure, with the effect differing by timing of ozone exposure. In the animal literature, female reproductive outcomes from the 2013 Ozone ISA showed decreased reproductive success with ozone exposure over most of pregnancy (Gestation Days 9−18); ozone exposure can induce a temporary anorexigenic effect in pregnant dams. Together, these proposed pathways provide biological plausibility for epidemiologic results of reproductive and developmental health effects and will be used to inform a causality determination, which is discussed later in this Appendix.

7.1.2.2 Male Reproduction

7.1.2.2.1 Epidemiologic Evidence of Effects on Male Reproductive Function

Associations between male reproductive health and ozone exposure have been examined through effects on sperm. In the 2013 Ozone ISA, there was limited epidemiologic evidence from few studies for an association between ozone and sperm quality, with associations between reductions in sperm concentration and both short- and long-term ozone exposures. Since then, additional evidence is limited to: a small panel study in Brazilian men with systematic lupus erythematosus that reported decreases in sperm concentration and count with long-term (0−90 days before collection) ozone exposure (Farhat et al., 2016), and a Chinese cohort that observed no evidence of association (Liu et al., 2017). Data from current studies of male reproductive function are extracted and summarized in the evidence inventories (see Table 7-6.)

7.1.2.2.2 Toxicological Evidence of Effects on Male Reproductive Function

There are no recent animal toxicological studies on male reproduction. Evidence from the 2013 Ozone ISA showed decremental effects on testicular morphology demonstrated in a toxicological study with histological evidence of ozone-induced depletion of germ cells in testicular tissue and decreased seminiferous tubule epithelial layer (Jedlinska-Krakowska et al., 2006a). In summary, this study provided toxicological evidence of impaired spermatogenesis with ozone exposure that was attenuated by antioxidant supplements.

7.1.2.2.3 Summary

Overall, there is evidence of impaired spermatogenesis and decreased sperm count and concentration from epidemiologic studies, and decremental effects on testicular morphology and impaired spermatogenesis from toxicological studies with ozone exposures.

7.1.2.3 Female Reproduction

7.1.2.3.1 Epidemiologic Evidence of Effects on Female Reproductive Function

A single study in the 2013 Ozone ISA showed some evidence for increased in vitro fertilization (IVF) success with short-term ozone exposure during ovulation, but long-term exposure during gestation reduced the likelihood of a live birth (Legro et al., 2010). In recent studies, the overall findings are mixed. In a French population undergoing IVF, Carré et al. (2016) observed an increased number of top embryos (i.e., those considered of the best quality) with at least 1 day of high ozone exposure in 30 day periods before ovulation. Another study found no evidence of association with exposure up to 2 months before conception, but did show an improvement in fecundity with ozone exposure post-conception, likely indicating unmeasured confounding (Slama et al., 2013). However, a longitudinal study in 500 U.S. couples reported decreased fecundity with short-term ozone exposure near time of ovulation (Nobles et al., 2018). Data from current studies of female reproductive function are extracted and summarized in Table 7-7.

7.1.2.3.2 Toxicological Evidence of Effects on Female Reproduction

Evidence from the 2013 Ozone ISA showed that, in most toxicological studies, reproductive success appears to be unaffected by ozone exposure. Nonetheless, one study reported that 25% of the BALB/c mouse dams in the highest ozone exposure group (1.2 ppm, short-term exposure GDs 9−18) did not complete a successful pregnancy (Sharkhuu et al., 2011). Ozone administration (continuous 0.4, 0.8 or 1.2 ppm ozone) to CD-1 mouse dams throughout most of the pregnancy (short-term exposure, PNDs 7−17, which excludes the preimplantation period) led to no adverse effects on reproductive success [proportion of successful pregnancies, litter size, sex ratio, frequency of still birth, or neonatal mortality; Bignami et al. (1994)]. There was a statistically nonsignificant increase in pregnancy duration (0.8 and 1.2 ppm ozone). Initially, dam body weight (0.8 and 1.2 ppm ozone), water consumption (0.4, 0.8 and 1.2 ppm ozone), and food consumption (0.4, 0.8 and 1.2 ppm ozone) during pregnancy were decreased with ozone exposure, but these deficits dissipated a week or two after the initial exposure (Bignami et al., 1994). This anorexigenic effect of ozone exposure on the pregnant dam appeared to subside with time; the dams seemed to adapt to the ozone exposure. Some evidence suggests that ozone may affect reproductive success when combined with other chemicals. Kavlock et al. (1979) showed that ozone acted

synergistically with sodium salicylate to increase the rate of pup resorptions after midgestational exposure (1.0 ppm ozone, short-term exposure, GDs 9-12). With ozone exposure, toxicological studies showed reproductive effects to include a transient anorexigenic effect of ozone on gestational weight gain, and a synergistic effect of ozone on salicylate-induced pup resorptions; other fecundity, pregnancy- and gestation-related outcomes appeared unaffected by ozone exposure.

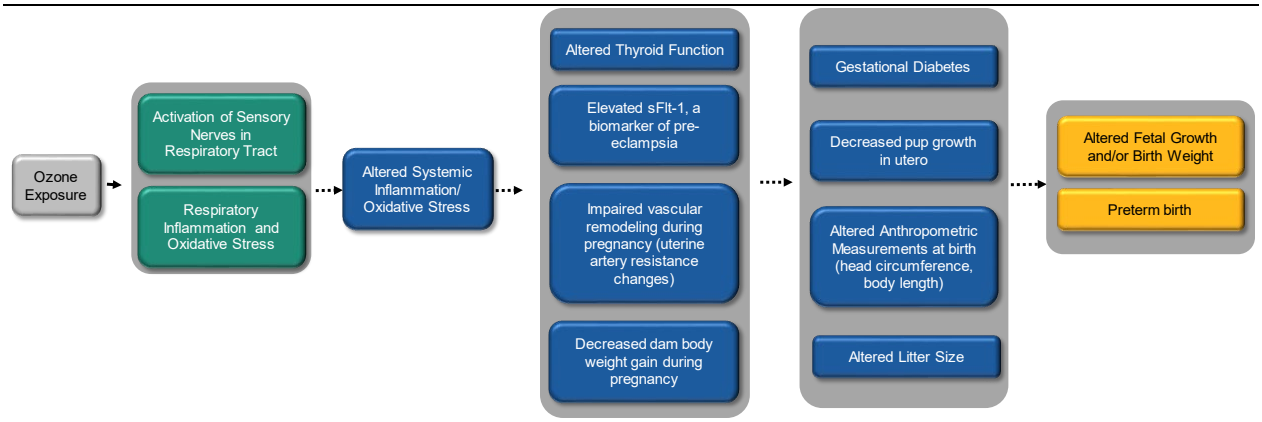
7.1.2.3.3 Summary

In conclusion, results from epidemiologic studies are mixed, with benefits and detriments to female reproductive function with ozone exposures, while toxicological studies show limited evidence of effects on successful completion of pregnancy.

7.1.3 Pregnancy and Birth Outcomes

7.1.3.1 Biological Plausibility

This section describes biological pathways that potentially underlie reproductive and developmental health effects of pregnancy, birth weight, and birth outcomes resulting from exposure to ozone. Figure 7-2 graphically depicts the proposed pathways as a continuum of upstream events, connected by arrows, that may lead to the downstream events observed in epidemiologic studies. This discussion of "how" exposure to ozone may lead to reproductive and developmental health effects contributes to an understanding of the biological plausibility of epidemiologic results evaluated in Section 7.1.3.2 through Section 7.1.3.5.



Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence related to ozone exposure, and the arrows indicate a proposed relationship between those effects. Solid arrows denote evidence of essentiality as provided, for example, by an inhibitor of the pathway or a genetic knockout model used in an experimental study involving ozone exposure. Shading around multiple boxes is used to denote a grouping of these effects. Arrows may connect individual boxes, groupings of boxes, and individual boxes within groupings of boxes. Progression of effects is generally depicted from left to right and color-coded (gray, exposure; green, initial effect; blue, intermediate effect; orange, effect at the population level or a key clinical effect). Here, population level effects generally reflect results of epidemiologic studies. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below.

Figure 7-2 Potential biological pathways for pregnancy and birth outcomes following ozone exposure.

Evidence is accumulating that ozone exposure may affect pregnancy and birth outcomes. The evidence from the 2013 Ozone ISA (U.S. EPA, 2013a) and recent evidence indicates multiple initial events after ozone inhalation contribute to effects on pregnancy and birth outcomes, including systemic inflammation or oxidative stress. Beyond these initial events, there is also evidence from experimental and epidemiologic studies demonstrating that ozone inhalation could result in a coherent series of physiological responses that provide biological plausibility for the associations reported in epidemiologic studies and animal toxicological studies that contribute to the apical endpoint of pregnancy-induced hypertension, altered development, preterm birth, and altered fetal growth or birth weight (Geer et al., 2012; Morello-Frosch et al., 2010; Salam et al., 2005). The initial event of altered systemic oxidative stress is demonstrated in the epidemiologic literature with ozone-dependent increased odds of elevated CRP levels in nonpregnant individuals but CRP was unchanged at GD 5 in ozone exposed pregnant rodents (Miller et al., 2019). Other initial events include activation of sensory nerves in the respiratory tract. In pregnant rodents exposed to ozone peri-implantation at GD 5, circulating serum cytokines are altered including statistically significantly decreased IL-6, IFN-γ, and IL-13 (Miller et al., 2019) at a point when these cytokines may be critical for proper implantation. Serum from these ozone-exposed dams added to trophoblasts in vitro led to impaired trophoblast invasion and migration as well as impaired trophoblast metabolic capacity (Miller et al., 2019). Ozone exposure using this in vitro trophoblast model also caused the trophoblasts to produce increased levels of soluble fms-like tyrosine kinase 1 (sFlt-1), a biomarker of pre-eclampsia (Miller et al., 2019). Further, ozone-dependent reproductive organ-specific

effects, included altered uterine artery vascularity of increased resistance during periods of pregnancy when resistance should be decreasing to accommodate the physiological changes of pregnancy [Miller et al. (2017); Section 7.1.4 and Section 7.1.5]. At a certain point in a normal pregnancy, vascular resistance decreases in the uterine artery, which enhances perfusion of the fetus and placenta. But this pathway is significantly altered in ozone-exposed animals. Evidence from the 2013 ISA showed ozone exposed virgin rodents manifest with impaired thyroid hormone status, decreased T3, T4, and TSH, hormones that are important for pregnancy; thyroid hormone status has not been monitored in gravid animals. Ozone exposed pregnant dams eat less food and gain less weight than control animals (Miller et al., 2019; Miller et al., 2017; Bignami et al., 1994), an effect that may dissipate with time (Bignami et al., 1994) or when ozone exposure ceases (Miller et al., 2017).

7.1.3.2 Maternal Health during Pregnancy

7.1.3.2.1 Epidemiologic Evidence of Effects on Maternal Health during Pregnancy

Studies of maternal health during pregnancy focus on hypertensive disorders of pregnancy, such as preeclampsia and gestational hypertension, and gestational diabetes. Pregnancy-associated hypertension is a leading cause of perinatal and maternal mortality and morbidity. Gestational diabetes may increase the risk of high blood pressure during pregnancy and the occurrence of cesarean delivery; it is also frequently related to later development of type 2 diabetes. Epidemiologic studies related to maternal health during pregnancy were not identified for inclusion in the 2013 Ozone ISA (U.S. EPA, 2013a). Most recent studies in this area investigated associations between ozone exposure and hypertensive disorders of pregnancy, while a limited number examined the development of gestational diabetes.

For hypertensive disorders of pregnancy, study results were mixed, with positive (increased hypertensive disorders associated with increased ozone concentrations) and null associations reported. Studies for gestational diabetes are few, and they reported both null and positive associations depending on timing of exposure.

- There are differences in studies by specific definition of outcomes; some studies examined preeclampsia, some hypertension, and others "hypertensive disorders of pregnancy," which may or may not have included preeclampsia.
- Results for studies of preeclampsia were mixed, with some showing positive associations for 1st-trimester exposures (Lee et al., 2013; Olsson et al., 2013) and others reporting either no evidence of association across different exposure time periods (Mendola et al., 2016b) or positive effects only in some study areas (Wu et al., 2011).
- Studies of "hypertensive disorders of pregnancy" generally reported positive associations (Hu et al., 2016; Michikawa et al., 2015; Mobasher et al., 2013).

- Two studies examining hypertension reported mixed associations with 1st trimester exposure, with Lee et al. (2013) showing positive associations and Xu et al. (2014) showing no evidence of association.
- Increased odds of gestational diabetes were observed for higher ozone exposures during the 1st and 2nd trimesters in a Florida population compared to lower ozone exposures (Hu et al., 2015) and for weekly exposures during the 2nd trimester in a national study (Robledo et al., 2015).
- No evidence of association with gestational diabetes was observed in the national study for ozone exposures 90 days before conception and in the 1st trimester (Robledo et al., 2015).
- The single study of hypertensive disorders of pregnancy examined the potential for copollutant confounding and showed an odds ratio increase (from 1.05 to 1.11) with adjustment for NO_2 (Olsson et al., 2013). In both studies of gestational diabetes, adjustment for copollutants did not change effect estimates (Hu et al., 2015; Robledo et al., 2015), reducing uncertainties that the associations observed with ozone are due to copollutant confounding.

Data from current studies of maternal health during pregnancy are extracted and summarized in the evidence inventories (see Table 7-8 and Table 7-9).

7.1.3.2.2 Toxicological Evidence of Effects on Pregnancy

Studies from the 2013 Ozone ISA demonstrated a transient anorexiogenic effect of ozone on pregnant dam weight gain during pregnancy. Initially, dam body weight (0.8 and 1.2 ppm ozone), water consumption (0.4, 0.8, and 1.2 ppm ozone), and food consumption (0.4, 0.8, and 1.2 ppm ozone) during pregnancy were decreased with ozone exposure but these deficits dissipated a week or two after the initial exposure (Bignami et al., 1994). The anorexigenic effect of ozone exposure on the pregnant dam appears to dissipate with time; the dams seem to adapt to the ozone exposure. Studies from the 2013 Ozone ISA also demonstrated enhanced pulmonary inflammatory response in BALF of pregnant and lactating rodents to ozone exposure (1.0 ppm, 6 hours); there was significantly enhanced sensitivity to ozone-induced pulmonary inflammation during pregnancy, which was maintained during lactation, and disappeared after lactation ceased at weaning (Gunnison et al., 1992). Research since the 2013 Ozone ISA also shows that ozone affects weight gain during pregnancy. Pregnant rats exposed to ozone (0.8 ppm ozone) during the period of implantation (GDs 5-6) showed significantly lower body-weight gain during this period (Miller et al., 2017), demonstrating a similar anorexigenic effect as documented in the 2013 Ozone ISA. Exposure to 0.4 ppm ozone during implantation did not affect dam body weight gain during pregnancy. Miller et al. (2017) also assessed dam blood pressure (GD 15, GD 19, GD 21) and kidney histopathology in near-term ozone exposed dams to evaluate whether ozone exposure might contribute to gestational hypertension/preeclampsia, with data showing null findings. Peri-implantation ozone exposure (1.2 ppm, GD 5) caused increased homeostatic model assessment for insulin resistance (HOMA-IR) and increased area under the curve with the glucose tolerance test in dams immediately after ozone exposure; exposure to 0.4 or 0.8 ppm ozone did not induce these metabolic changes in the dam (Miller et al., 2019). Data from current studies of maternal health during pregnancy are extracted and summarized in the evidence inventories (see Section 7.6.1, Table 7-7.)

7.1.3.2.3 Summary

Evidence for effects on maternal health during pregnancy is mixed, with epidemiologic studies showing limited evidence for effects on hypertensive disorders of pregnancy and gestational diabetes, and toxicological studies showing changes in maternal weight during pregnancy.

7.1.3.3 Fetal Growth, Birth Weight, and Body Length at Birth

Fetal growth is a marker of fetal well-being during pregnancy and an important indicator of future infant and child health. Fetal growth can be difficult to quantify, and growth standards vary by race/ethnicity, infant sex, parity, and maternal size (Zhang et al., 2010). Birth weight is often used as a proxy for fetal growth, either as a continuous measure or below a cutoff (typically 2,500 g) as low birth weight. However birth weight is determined through a mix of factors, including intra-uterine growth and gestational age, among others, so studies of these outcomes will often restrict to term births. Vulnerability to exposures that may affect birth weight could potentially occur throughout pregnancy, as growth may be affected by structural changes in the placenta or the placentation process or through inflammatory processes that restrict nutritional flow to the fetus.

7.1.3.3.1 Epidemiologic Evidence for Fetal Growth, Birth Weight, and Body Length at Birth

In the current review, fetal growth is quantified through small-for-gestational-age measures (typically an infant below the 10th percentile of weight for gestational age accounting for race and sex), continuous birth weight in grams, and dichotomized low birth weight (less than 2,500 g or 5 lbs, 8 oz). In the 2013 Ozone ISA, studies were exclusively of birth weight with only a limited number supporting an association between ozone exposure and lower birth weight. Since then, the number of recent studies has more than doubled, but findings remain largely inconsistent, with studies reporting either lower birth weight or no evidence of association of lower birth weight with ozone across exposure windows, study areas, study designs, and exposure assessment methods. Data from current studies of fetal growth are extracted and summarized in the evidence inventories (see Table 7-10).

- Studies that examined continuous birth weight, including well designed studies [e.g., Vinikoor-Imler et al. (2014); Laurent et al. (2013)], reported primarily that increases in ozone concentrations were associated with decrements in birth weight, although the magnitude of the decrement varied, ranging from −4.61 to −27.27 (per 10 ppb increase in ozone).[1]

[1] All epidemiologic results standardized to a 15-ppb increase in 24-hour avg, 20-ppb increase in 8-hour daily max, 25-ppb increase in 1-hour daily max ozone concentrations, or a 10-ppb increase in seasonal/annual ozone concentrations to facilitate comparability across studies.

- One study using geographically weighted regression indicated variation by spatial characteristics, with lower birth weight associated with higher ozone concentration in less urbanized communities (Tu et al., 2016).
- Some studies of odds of low birth weight (<2,500 g), including well designed studies [e.g., Chen et al. (2017b); Laurent et al. (2016a); Vinikoor-Imler et al. (2014); Laurent et al. (2013)], reported increased odds of low birth weight with increased ozone concentrations; however, those associations are inconsistent across exposure windows.
- In studies with copollutant adjusted models, effect estimates were largely similar to those reported for single-pollutant models for ozone (Smith et al., 2017; Ha et al., 2014; Olsson et al., 2013).

7.1.3.3.2 Toxicological Evidence for Fetal Growth, Birth Weight, and Body Length at Birth

Evidence from the 2013 Ozone ISA showed decreased birth weight in pups whose pregnant dams were exposed to ozone during pregnancy (Sharkhuu et al., 2011; Haro and Paz, 1993), but no effects on the number of pups born. A few studies reported that mice or rats exposed developmentally (gestationally ± lactationally) to ozone had deficits in postnatal body-weight gain (Bignami et al., 1994; Haro and Paz, 1993; Kavlock et al., 1980). Recent animal toxicological evidence also shows that ozone exposure during pregnancy causes decreased fetal weight near term. In summary, animal toxicological models show ozone exposure caused decreased birth weight and decreased postnatal body-weight gain but did not affect litter number.

- There is recent evidence that fetuses whose dams were exposed to ozone (0.8 ppm for both sexes, 0.4 ppm ozone for male fetuses) during the period of implantation (GDs 5-6) weighed significantly less than the air-exposed control pups at GD 21, near the end of pregnancy. There exists a sexual dimorphism with male pups more sensitive to ozone exposure than females. Further examination showed that dams exposed to 0.8 ppm ozone had male and female fetuses with significantly lower lean mass and fat mass compared with control-air dams at GD 21 (Miller et al., 2017).

7.1.3.3.3 Summary

Overall, there is some epidemiologic evidence for the effects of ozone on fetal growth, especially for continuous-term birth weight, a conclusion supported by toxicological evidence in rodents.

7.1.3.4 Preterm Birth

Preterm birth (PTB), delivery that occurs before 37 weeks of completed gestation, is a marker for fetal underdevelopment and is related to subsequent adverse health outcomes (Saigal and Doyle, 2008; IOM, 2007; MacDorman et al., 2007; Gilbert et al., 2003). PTB is characterized by multiple etiologies

(spontaneous, premature rupture of membranes [PROM], or medically induced), which may have either individual or shared mechanistic pathways.

7.1.3.4.1 Epidemiologic Evidence of Preterm Birth

In the 2013 Ozone ISA, short-term exposure to ozone during late pregnancy was consistently not associated with preterm birth. However, associations with long-term exposures were inconsistent across studies, particularly across study locations. Since then, the number of studies examining ozone exposure and preterm birth has doubled. All studies that examined ozone exposures during the 1st or 2nd trimesters reported associations elevated from the null. Effects are more mixed with 3rd trimester and entire-pregnancy exposure, with both positive and null associations present. As in the 2013 Ozone ISA, studies of short-term, near-birth exposures generally reported no evidence of association. Data from current studies of preterm birth are extracted and summarized in the evidence inventories (see Table 7-11).

- One study divided PTB into three categories and looked at 4-week intervals. The study authors observed elevated odds ratios (ORs) for late and moderate PTB (but not severe/very PTB; 20−28 weeks) with exposures during Gestation Weeks 9−12; for 2nd trimester exposures, they observed elevated ORs across preterm birth groups for ozone exposures during Gestation Weeks 17−21, 21−24, and 25−28 (Symanski et al., 2016).
- A single study was conducted on PROM (including both preterm and term births) examining exposures at 0 to 4 hours before delivery and across the entire pregnancy. The association with entire pregnancy exposure was null, however, the associations with short-term, near-birth hourly exposures were all elevated form the null [OR range 1.05 to 1.07; Wallace et al. (2016)].
- Adjustment for copollutants generally moved effect estimates slightly away from the null (Ha et al., 2014; Olsson et al., 2013; Olsson et al., 2012).
- There were no apparent differences in effect estimates based on study location or exposure assessment method used for recent studies.

7.1.3.4.2 Summary

Overall, there is evidence of an association between ozone exposures during early to midpregnancy with preterm birth in epidemiologic studies. However, there are no toxicologic studies specific to preterm birth.

7.1.3.5 Birth Defects

Birth defects are structural and functional abnormalities that can cause physical and intellectual disability and other health problems; they are a leading cause of infant mortality and developmental disability in the U.S. Critical periods for birth defect development are generally known, reducing

uncertainty related to timing of exposure, which is an uncertainty common to other birth and pregnancy outcomes.

7.1.3.5.1 Epidemiologic Evidence of Birth Defects

In the 2013 Ozone ISA, studies of birth defects focused on cardiac and oral defects, showing inconsistent results, perhaps due to variation in study location, study design, and/or analytic methods. In this current review, cardiac defects are the only defect phenotype examined by multiple recent studies. Individual recent studies also report on neurological and limb defects. Data from these studies are extracted and summarized in the evidence inventories (see Table 7-12).

- For cardiac defects, which are themselves a grouping of separate defects, associations are mixed, with both positive and null associations reported across both studies and birth defect types.
- Using the U.S.-based National Birth Defects Prevention Study data, one study reported inverse odds ratios with higher levels of ozone exposure for neurological defects [neural tube defects, anencephaly, and spina bifida; Padula et al. (2013)].
- A Taiwan-based study of birth defects of the limbs—including polydactyly, syndactyly, and limb reduction—observed mixed effect estimates across exposure windows with increasing ozone levels (Lin et al., 2014b).
- In general, when studies look at single and copollutants models, effect estimates were generally similar (Zhang et al., 2016).

7.1.3.5.2 Toxicological Evidence of Birth Defects

Earlier research found eyelid malformation following gestational and postnatal exposure to 0.2 ppm ozone (Veninga, 1967). No recent animal toxicological studies have been conducted on ozone exposure and birth defects since the 2013 Ozone ISA.

7.1.3.5.3 Summary

Findings for ozone-associated birth defects are generally inconsistent in epidemiologic studies, and there are few animal studies on birth defects.

7.1.3.6 Fetal and Infant Mortality

Fetal mortality encompasses spontaneous abortion (fetal deaths occurring before 20 weeks of gestation) and miscarriage/stillbirth (after 20 weeks of completed gestation). Infant mortality is a death occurring in the first year of life. In the 2013 Ozone ISA studies of infant mortality provided no evidence

for an association between ozone exposure and infant mortality. In the current review, studies are primarily of stillbirth, with a U.S.-based study (Ha et al., 2017b) and an Iranian (Dastoorpoor et al., 2017) study including both spontaneous abortion and stillbirth, and another Iranian study examining only spontaneous abortion (Moridi et al., 2014). No studies examined infant mortality, but one examined "late fetal death," that is, less than 24 hours after birth (Arroyo et al., 2016). Findings are inconsistent across both short- and long-term exposure periods. In the studies that examined copollutant models, effect estimates were robust to copollutant inclusion. Data from current studies of fetal and infant mortality are extracted and summarized in the evidence inventories (see Table 7-13).

7.1.3.6.1 Toxicological Evidence of Birth Defects

There are no toxicological studies of fetal and infant mortality.

7.1.3.6.2 Summary

Findings for ozone associated fetal and infant mortality are generally inconsistent across exposure windows in epidemiologic studies, and there are no animal studies.

7.1.4 Effects of Exposure during Developmental Periods

Pregnancy and infancy are periods of rapid development, and exposures occurring during these times may have the potential to have long-lasting effects that do not manifest immediately; the Developmental Origins of Health and Disease (DOHaD) is a theory that early life stressors or environmental exposures can affect later life health outcomes (Heindel et al.). There are sensitive windows of development early in life that have the potential to be reprogrammed and put an individual at increased risk for future health outcomes across lifestages. This theory began nearly 30 years ago with Barker's hypothesis (Barker and Osmond, 1986) that detailed a mismatch between fetal environment (famine) and adult environment (no famine) that was associated with low birth-weight infants that became adults at greater risk for heart disease and cardiovascular mortality. The evidence from the ozone literature indicates that ozone could be an exposure associated with DOHaD.

Researchers have examined several health outcomes in association with ozone exposure during the periods of development, which are summarized below. Studies on the effects of ozone exposure during developmental periods are evaluated with their respective causality determinations in the sections of the ISA for the particular organ system in which the health effect occurs (e.g., respiratory, nervous system, and cardiovascular effects), but are also summarized here with a focus on exposure during developmental periods.

7.1.4.1 Respiratory Development

Several epidemiologic studies conducted in the U.S., Europe, and Asia report no evidence of an association between long-term exposure to ozone during developmental periods (in utero or early life) and asthma (see Appendix 3, Section 3.2.4.1) or allergy (Appendix 3, Section 3.2.4.1). A notable exception is Tétreault et al. (2016) who reported an increase in asthma incidence among children with increasing summertime average ozone concentrations. Experimental animal studies provide support for the effect of long-term exposure to ozone on the development of asthma (Section 3.2.4.1.2) and on lung function development (Section 3.2.4.2.2). Briefly, studies reviewed in the 2013 Ozone ISA demonstrated that cyclic challenge of infant rhesus monkeys to an allergen and ozone during the postnatal period compromised airway growth and development and resulted in changes that favor allergic airway responses and persistent effects on the immune system. Ozone-exposure-induced nasal lesions were demonstrated in infant monkeys, and maternal exposure to ozone during gestation resulted in changes related to immune function and allergic lung disease in the respiratory tract of offspring mice. Recent studies in infant monkeys demonstrated airway smooth-muscle hyperreactivity, an enhanced allergic phenotype, priming of responses to oxidant stress, increased serotonin-positive airway cells, and immunomodulation. Recent studies in rodents demonstrated impaired airway growth and altered airway sensory nerve innervation as a result of postnatal ozone exposure. Another set of recent studies in infant monkeys demonstrated impaired alveolar morphogenesis resulting from postnatal ozone exposure. Injury, inflammation, and oxidative stress were also reported in ozone-exposed neonatal rodents.

7.1.4.2 Neurodevelopment

Effects on laterality, brain morphology, neurobehavioral abnormalities, and sleep aberration were reported in the 2013 Ozone ISA. Evidence relating to neurodevelopmental effects contributes to the causality determination in this ISA as detailed in Section 7.2.2 under long-term exposures. Briefly, there is some epidemiologic evidence to suggest that prenatal or early life exposure to ozone may be associated with autism. The current toxicological data were focused on effects in the peripheral nervous system, showing decreased neuroproliferation (see Section 7.2.2.5.2).

7.1.4.3 Cardiovascular Development

Evidence from studies of ozone exposure and effects on the cardiovascular system that contribute to the causality determination is fully characterized in Appendix 4. Briefly, three studies of exposures during developmental periods, all based in the U.S., reported mixed effects across outcomes studied. One study reported changes in newborn blood pressure with ozone exposure during pregnancy (van Rossem et al., 2015), while another reported no associations with blood pressure in kindergarten or first-grade students (Breton et al., 2016). The kindergarten or first-grade students also showed no changes in carotid

artery intima-media thickness with ozone exposure during pregnancy, while a study of college-aged students reported increased carotid artery intima-media thickness with exposures at 0−5 years of age (Breton et al., 2012). A animal toxicological study in pregnant dams showed altered uterine artery vascularity and resistance during pregnancy (Miller et al., 2017) and is covered in more detail in Section 7.1.3.3.2.

7.1.5 Summary and Causality Determinations

Overall, the evidence is suggestive of, but not sufficient to infer, a causal relationship between ozone exposure and (1) male and female reproduction and fertility, and (2) pregnancy and birth outcomes. Separate conclusions are made for these groups of reproductive effects because they are likely to have different etiologies and critical exposure windows over different lifestages. All available evidence examining the relationship between exposure to ozone and reproductive effects was evaluated using the framework described in the Preamble to the ISAs (U.S. EPA, 2015). As noted previously, studies examining the effect of exposure during developmental periods are summarized in Section 7.1.4 (Table 7-14 and Table 7-17) but contribute to organ-system-specific causality determinations in Appendix 3, Appendix 4, Appendix 5, and Section 7.1.4.

The 2013 Ozone ISA (U.S. EPA, 2013a) concluded that the evidence was suggestive of a causal association between ozone exposure and reproductive and developmental outcomes. The strongest evidence supporting the causality determination from the 2013 Ozone ISA (U.S. EPA, 2013a) came from studies of sperm quality and on continuous birth weight. Current evidence continues to support conclusions for studies of sperm quality and continuous birth weight. There is also new supporting evidence for effects on preterm birth with exposures to ozone, particularly in the first and second trimesters.

Overall the evidence is suggestive of, but not sufficient to infer, a causal relationship between ozone exposure and male and female reproduction and fertility. The key evidence as it relates to the causal framework is summarized in Table 7-1. This determination is supported by evidence across epidemiologic studies, including those from the 2013 Ozone ISA (U.S. EPA, 2013a) of decrements in sperm count and concentration (Farhat et al., 2016; Hansen et al., 2010; Sokol et al., 2006), and by a study showing changes in rodent testicular morphology and spermatogenesis (Jedlinska-Krakowska et al., 2006a). Uncertainties that contribute to the determination include lack of evaluation of copollutant confounding or multiple potential sensitive windows of exposure, and the generally small sample size of studies in human subjects.

Table 7-1 Summary of evidence that is suggestive of, but not sufficient to infer, a causal relationship between ozone exposure and male and female reproduction.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Limited but consistent evidence for male reproduction, effects on sperm	Limited evidence for decrements on sperm count and concentration	Farhat et al. (2016)	~42 ppb
		Sokol et al. (2006)	~21.68 ppb
		Hansen et al. (2010)	~30.8 ppb
	Limited evidence for changes to testicular morphology and spermatogenesis	Jedlinska-Krakowska et al. (2006a)	0.5 ppm
Lack of copollutant models contributes to uncertainty	No epidemiologic studies evaluate potential copollutant confounding using copollutant models		
Limited study sizes	Observed effects are from smaller studies on limited number of individuals	Farhat et al. (2016) Sokol et al. (2006)	
Lack of information of specific timing of exposures	All studies use 0−90 days before sampling exposure window, only one examines smaller periods within this window	Hansen et al. (2010)	

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble to the ISAs (U.S. EPA, 2015).

[b]Describes the key evidence and references supporting or contradicting and contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where the full body of evidence is described.

[c]Describes the ozone concentrations with which the evidence is substantiated.

Overall the evidence is suggestive of, but not sufficient to infer, a causal relationship between ozone exposure and pregnancy and birth outcomes. The key evidence as it relates to the causal framework is summarized in Table 7-2. There are several well-designed, well-conducted studies that indicate an association between ozone and poorer birth outcomes, particularly for outcomes of continuous birth weight and preterm birth. In particular, studies of preterm birth that examine exposures in the first and second trimesters show fairly consistent positive associations (increased ozone exposures associated with increased odds of preterm birth). In addition, some animal toxicological studies demonstrate decreased birth weight and changes in uterine blood flow. Studies of continuous birth weight and preterm birth did not generally adjust for potential copollutant confounding, although studies that did

appeared to show limited impacts. There is also inconsistency across exposure windows for associations with continuous birth weight, and the magnitude of effect estimates varies.

Table 7-2 Summary of evidence that is suggestive of, but not sufficient to infer, a causal relationship between ozone exposure and pregnancy and birth outcomes.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Evidence from multiple epidemiologic studies of continuous birth weight but uncertainties remain	Positive associations from many studies, but variability in timing of exposures and magnitude of effects, and limited assessment of copollutant confounding contribute to uncertainty	Section 7.4.1	Mean concentrations across studies: 4−43 ppb
Evidence from multiple epidemiologic studies and preterm birth but uncertainties remain	Positive associations from many studies that examine exposure windows in the first and second trimesters, but magnitude of effects differ across studies. Copollutant adjustment generally not changing observed effect estimates	Section 7.4.1	Mean concentrations across studies: 16−51 ppb
Limited toxicologic evidence of ozone on fetal growth and birth weight	Decreased pup birth and fetal weights Increased uterine artery blood flow resistance	Haro and Paz (1993) Sharkhuu et al. (2011) Miller et al. (2017)	
Limited assessment of copollutant confounding	Few studies adjust for potential confounding by NO_2 and $PM_{2.5}$	Section 7.4.1	
Lack of information on specific timing of exposures for continuous birth weight	Several potentially sensitive windows are examined, including entire pregnancy, and each trimester, along with others. However, decrements in birth weight are not consistently associated across exposure windows	Section 7.4.1	

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble to the ISAs (U.S. EPA, 2015).

[b]Describes the key evidence and references, supporting or contradicting or contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where full body of evidence is described.

[c]Describes the ozone concentrations with which the evidence is substantiated.

7.2 Nervous System Effects

7.2.1 Short-Term Ozone Exposure

7.2.1.1 Introduction, Summary from the 2013 Ozone ISA, and Scope for Current Review

This section evaluates the scientific evidence related to the potential effects of short-term ozone exposure (i.e., on the order of minutes to weeks) to ozone on the nervous system. The 2013 Ozone ISA (U.S. EPA, 2013a) determined that the available evidence was suggestive of a causal relationship between short-term exposure to ozone and effects on the central nervous system (CNS). This conclusion was based on the "strong" toxicological evidence linking short-term exposure to ozone to effects on the brain and behavior of experimental animals. Specifically, short-term exposure was associated with several effects on CNS structure and function, with several studies indicating the potential for neurodegenerative effects similar to Alzheimer's or Parkinson's diseases in a rat model. Functional deficits in tasks of learning and memory and decreased motor activity were correlated with biochemical and morphological changes in regions that are known to be affected by these diseases, including the hippocampus, striatum, and substantia nigra. A study also reported perturbation of sleeping patterns in rodents. Other CNS regions affected included the olfactory bulb and the frontal/prefrontal cortex. Effects of ozone in the CNS were strongly correlated with increased markers of oxidative stress and inflammation, including lipid peroxidation and microglial activation. There was also limited evidence indicating a role of ozone in modulating neuroendocrine function. Short-term ozone exposure had mixed effects on thyroid hormones, with one study reporting increased serum T3 and another reporting decreases in both T3 and T4. Corticosterone levels were also increased in one study, suggesting a stress response. Epidemiologic studies of short-term exposure to ozone and nervous system effects were lacking in the 2013 Ozone ISA.

The nervous system effects reviewed in this Appendix include brain inflammation and morphology (Section 7.2.1.3); cognitive and behavioral effects, including mood disorders, (Section 7.2.1.4); neuroendocrine effects (Section 7.2.1.5); and hospital admission and emergency department visits (Section 7.2.1.6) for diseases of the nervous system, which are generally defined by International Classification of Diseases (ICD) codes (i.e., ICD-9 codes 290−319 or 320−359 and ICD-10 codes F1-F99 or G00-G99). The subsections below evaluate the scientific evidence relating short-term ozone exposure to nervous system effects. These sections focus on studies published since the completion of the 2013 Ozone ISA. There are a limited number of recent epidemiologic studies examining the effects of short-term ozone exposure on the nervous system. Multiple recent animal toxicological studies support conclusions from the 2013 Ozone ISA (U.S. EPA, 2013a).

7.2.1.1.1 Population, Exposure, Comparison, Outcome, and Study Design (PECOS) Tool

The scope of this section is defined by a scoping tool that generally describes the relevant Population, Exposure, Comparison, Outcome, and Study Design (PECOS). The PECOS tool defines the parameters and provides a framework to help identify the relevant literature to inform the draft 2019 Ozone ISA. The studies evaluated and subsequently discussed within this section were included if they satisfied all of the components of the following PECOS tool:

- Population: Study populations of any controlled human exposure or animal toxicological study of mammals at any lifestage
- Exposure: Short-term (in the order of minutes to weeks) inhalation exposure to relevant ozone concentrations (i.e., ≤0.4 ppm for humans, ≤2 ppm for other mammals)
- Comparison: Human subjects that serve as their own controls with an appropriate washout period or when comparison to a reference population exposed to lower levels is available, or, in toxicological studies of mammals, an appropriate comparison group that is exposed to a negative control (i.e., clean air or filtered air control)
- Outcome: Nervous system effects
- Study Design: Controlled human exposure (i.e., chamber) studies; in vivo acute, subacute or repeated-dose toxicity studies in mammals

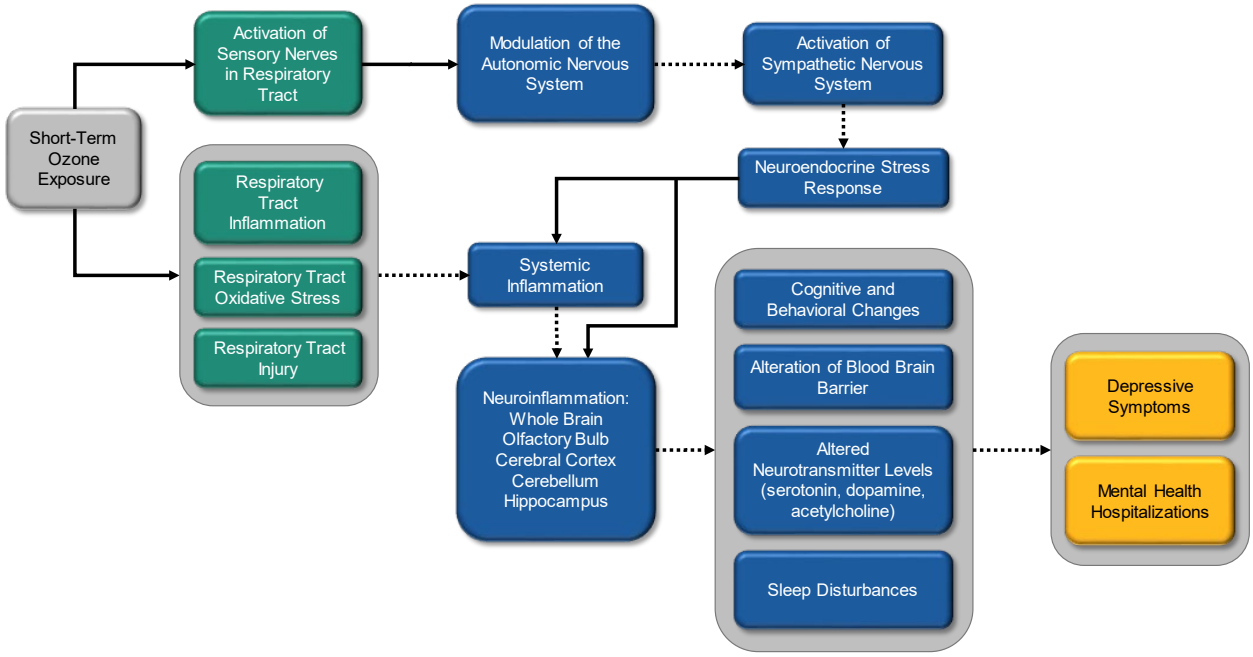
Because the 2013 Ozone ISA concluded that evidence existed to suggest a causal relationship between short-term ozone exposure and nervous system effects, the studies evaluated are less limited in scope and not targeted towards specific study locations, as reflected in the PECOS tool. The epidemiologic studies were evaluated and subsequently discussed using the PECOS tool below:

- Population: Any population, including populations or lifestages that might be at increased risk
- Exposure: Short-term ambient concentration of ozone
- Comparison: Per unit increase (in ppb)
- Outcome: Change in risk (incidence/prevalence) of a nervous system effect
- Study Design: Epidemiologic studies consisting of panel, case-crossover, time-series studies, and case-control studies; cross-sectional studies with appropriate timing of exposure for the health endpoint of interest

7.2.1.2 Biological Plausibility

This section describes biological pathways that potentially underlie nervous system effects resulting from short-term exposure to ozone. Biological plausibility is depicted via the proposed pathways as a continuum of upstream events, connected by arrows, that may lead to downstream events observed in epidemiologic studies (Figure 7-3). This discussion of "how" exposure to ozone may lead to effects on the nervous system contributes to an understanding of the biological plausibility of epidemiologic results

evaluated later. The biological plausibility for ozone-induced effects on the nervous system is supported by evidence from the 2013 Ozone ISA and by recent evidence.



Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence related to ozone exposure, and the arrows indicate a proposed relationship between those effects. Solid arrows denote evidence of essentiality as provided, for example, by an inhibitor of the pathway or a genetic knockout model used in an experimental study involving ozone exposure. Shading around multiple boxes is used to denote a grouping of these effects. Arrows may connect individual boxes, groupings of boxes, and individual boxes within groupings of boxes. Progression of effects is generally depicted from left to right and color-coded (gray, exposure; green, initial effect; blue, intermediate effect; orange, effect at the population level or a key clinical effect). Here, population level effects generally reflect results of epidemiologic studies. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below.

Figure 7-3 Potential biological pathways for nervous system effects following short-term exposure to ozone.

Two primary pathways have been identified by which short-term exposure to ozone is thought to affect the nervous system. In the first pathway, pulmonary inflammation is the initial event that leads to downstream effects on the nervous system, whereas activation of sensory neurons in the lung are the initiating event in the second pathway (Figure 7-3; solid lines). The majority of currently available studies support the first pathway in which proinflammatory responses in the central nervous system are initiated indirectly through respiratory and systemic inflammation. In the lung, ozone reacts with the respiratory epithelial cell lining fluid leading to local and systemic inflammatory responses. The central nervous system is affected when circulating inflammatory cytokines and reactive oxygen species (ROS) present in the bloodstream reach the brain. These can infiltrate the blood-brain barrier or initiate signaling mechanisms that trigger neuroinflammation.

Numerous proinflammatory and oxidative stress responses have been reported in the brain following short-term ozone exposure (see Section 7.2.1.3; Table 7-23). These responses include changes in gene expression, microglial activation, lipid/protein oxidation, and mitochondrial dysfunction in animal models. Although these effects have been observed throughout the brain, the region's most commonly reported to be affected include the hippocampus and cerebral cortex, striatum, and olfactory bulb. Inflammation and oxidative stress in these brain regions are associated with downstream effects, including altered neurotransmitter levels, structural changes to the blood-brain barrier, cognitive and behavioral changes and sleep disturbances. These effects may be drivers of depressive symptoms and mental health hospitalizations (see Section 7.2.1.4 and Section 7.2.1.5; Table 7-24 and Table 7-25). A single study reported accumulation of beta-amyloid proteins, a strong predictor of Alzheimer's disease in humans, in aged mice after a short-term exposure (Tyler et al., 2018); these results are also relevant to long-term exposure and, therefore, will be discussed further in Section 7.2.2.4.

In addition to the inflammation pathway, some data suggest that activation of sensory nerves in the lung is another mechanism by which ozone can elicit nervous system effects. Irritant effects of ozone can modulate autonomic nervous system function and are associated with several cardiovascular and respiratory effects (see Appendix 3). In the lung, vagal nerve stimulation by irritants, including ozone, stimulates the release of acetylcholine which binds to both the M2 and M3 acetylcholine receptors. These two receptors have opposing functions in the airways: M3 receptors stimulate smooth muscle contraction, while M2 receptors inhibit contraction by limiting further release of acetylcholine. M2 receptor activation is also affected by p38 and Jnk/MAPk, which suppress M2 activation and signaling. In guinea pigs, ozone exposure increased airway responsiveness, but these effects were abolished when animals were administered p38 and Jnk/MAPk inhibitors (Verhein et al., 2013), providing direct evidence of the role of ozone in autonomic nervous system modulation (Figure 7-3; solid lines). Activation of pulmonary sensory nerves has also been shown to modulate the sympathetic nervous system triggering the neuroendocrine stress response and wide-ranging effects on the body, including systemic and neuroinflammation (Figure 7-3; solid lines) (Snow et al., 2018; Kodavanti, 2016). Much of the recent research has focused on outcomes related to metabolic function; therefore, this pathway and the potential impacts are discussed in greater detail in Appendix 5. Note that there is some evidence to indicate that these pathways may not be entirely independent of one another.

The proposed pathways described here provide biological plausibility for evidence of cognitive and behavioral effects and sleep disturbances in association with short-term exposure to ozone. These pathways will be used to inform a causality determination.

7.2.1.3 Brain Inflammation and Morphology

7.2.1.3.1 Toxicological Studies

In the 2013 Ozone ISA, short-term ozone exposure resulted in increases in markers of oxidative stress and inflammatory responses. These effects were observed in many regions of the brain, including the olfactory bulbs, striatum, cortex, substantia nigra, and cerebellum and were associated with changes in neuronal morphology, increased apoptosis, and decreased numbers of dopaminergic neurons in the substantia nigra.

Recent studies (see Table 7-23) support the results summarized in the 2013 Ozone ISA, showing increases in inflammatory responses and markers of oxidative stress in various regions of the brain. Most studies evaluated a single concentration of ozone with exposure durations ranging from hours (single exposure) to ≥15 days depending on the study. In studies with multiple time points, the magnitude or severity of effects generally increased with exposure duration. Several studies evaluated both short- and long-term exposures. Cellular markers of oxidative stress were generally seen at the earlier (i.e., short-term) time points; effects on apoptosis/cell counts were primarily observed at the later time points (i.e., long term).

- Increased brain inflammation and oxidative stress was commonly reported following short-term ozone exposure in rodents (Tyler et al., 2018; Mumaw et al., 2016; Mokoena et al., 2015; Rivas-Arancibia et al., 2015; Gómez-Crisóstomo et al., 2014; Gonzalez-Guevara et al., 2014; Pinto-Almazan et al., 2014; Rodríguez-Martínez et al., 2013; Mokoena et al., 2011).
- Inflammation and oxidative stress were associated with increased mitochondrial damage (Rivas-Arancibia et al., 2015; Gómez-Crisóstomo et al., 2014; Rodríguez-Martínez et al., 2013).
- Most of these data were generated in adult male rats (Mokoena et al., 2015; Rivas-Arancibia et al., 2015; Gómez-Crisóstomo et al., 2014; Gonzalez-Guevara et al., 2014; Pinto-Almazan et al., 2014; Rodríguez-Martínez et al., 2013; Mokoena et al., 2011), although Mumaw et al. (2016) did report similar effects in both male and female CD−/− mice (pulmonary immune function impaired).
- Some evidence suggests that aged populations may be more susceptible to ozone-induced inflammation in the brain. One study evaluated the effects of ozone in both adult and aged mice, and although there was a clear main effect of ozone exposure, inflammatory outcomes were more pronounced in the aged animals (Tyler et al., 2018).
- Brain inflammation and oxidative stress were largely observed in the hippocampus (Tyler et al., 2018; Mokoena et al., 2015; Gómez-Crisóstomo et al., 2014; Pinto-Almazan et al., 2014; Rodríguez-Martínez et al., 2013) and cerebral cortex (Tyler et al., 2018; Mumaw et al., 2016; Mokoena et al., 2015; Gonzalez-Guevara et al., 2014; Mokoena et al., 2011), with more limited data for other regions of the brain (Tyler et al., 2018; Rivas-Arancibia et al., 2015).
- There is some evidence in rats to suggest that ozone exposure may affect glial morphology and blood-brain barrier permeability. Changes in glial morphology in the nucleus tractus solitarius were reported following a 24-hour continuous ozone exposure, with treated animals showing increased gial wrapping of synapses. The overall increase in glial coverage was driven by a

decrease in the proportion of synapses with no glial coverage. There were no changes in expression of proteins associated with astrocyte activation (Chounlamountry et al., 2015). In contrast, adult and aged mice exposed to ozone showed effects on blood brain barrier permeability, resulting in increased infiltration of circulatory inflammatory cells and structural changes in the microglia. Notably, this effect was only statistically significant in aged animals. These effects were observed in the cortex, dentate gyrus, hippocampus, and hypothalamus: brain regions that are known to have increased permeability of the blood-brain barrier or high sensitivity of the cells to toxic insult (Tyler et al., 2018).

- The effect of ozone exposure on β-amyloid accumulation and structure was investigated in several studies. Tyler et al. (2018) found that short-term ozone exposure increased β-amyloid formation in aged mice, but several other studies reported no effect in adult rats at the 7 or 15 day time points (Rivas-Arancibia et al., 2017; Fernando Hernandez-Zimbron and Rivas-Arancibia, 2016; Hernandez-Zimbron and Rivas-Arancibia, 2015). β-Amyloid accumulation is strongly associated with Alzheimer's disease in humans; therefore, these data are discussed further in the long-term exposure section (see Section 7.2.2).

7.2.1.4 Cognitive and Behavioral Effects

7.2.1.4.1 Epidemiologic Studies

No epidemiologic studies of short-term ozone exposure and its effects on cognitive and behavioral effects were reviewed in the 2013 Ozone ISA. In a recent study, Lim et al. (2012) examined older adults in South Korea during a 3-year, follow-up study using the Korean Geriatric Depression Scale-Short Form (SGDS-K). An increase in SGDS-K score, indicating increased depressive symptoms, largely driven by emotional symptoms, was associated with 3-day moving avg ozone concentration (see Table 7-18).

7.2.1.4.2 Toxicological Studies

In the 2013 Ozone ISA, short-term exposure to ozone was associated with changes in behavior in rodents, including decreased motor activity, impaired performance on learning and memory tasks, and altered sleep-wake cycles. In general, these effects were more pronounced with increasing exposure durations. Effects on sleep-wake cycles were associated with decreases in acetylcholine levels in the medial preoptic area, a region of the brain that regulates sleep.

Several recent studies (see Table 7-24) have reported cognitive and behavioral changes in rodent models following short-term exposure to ozone. Observed effects on cognition and behavior included increases in depressive-like behaviors in a rodent model of depression and anxiety, decreases in performance on learning and memory tasks, and declines in motor activity. Effects on neurotransmitter levels were also reported.

- One study reported increases in depression and anxiety behaviors in Flinders sensitive line (FSL) rats, a rodent model of depression (Mokoena et al., 2015). In the forced swimming test, ozone enhanced depressive-like behavior as indicated by significantly more time spent immobile and less time attempting to climb to escape the water. Ozone exposure was also found to significantly decrease the time spent in the open arms of the elevated plus maze and in the time spent interacting with a peer in social interaction tests, indicators or increased anxiety.
- Two studies reported deficits in learning and memory following short-term ozone exposure. In the passive avoidance task, male Wistar rats exposed to ozone for ≥15 days showed decreased latency in both the short-term (10 minutes) and long-term (24 hour) tests; these results are indicative of impaired learning/memory function. No effects were observed at the 7-day time point (Pinto-Almazan et al., 2014). Similar results were reported in FSL rats, with exposed animals spending significantly less time exploring a novel object when presented alongside a familiar object. These results suggest that the animals failed to recognize the familiar object (Mokoena et al., 2015).
- Three studies evaluated motor activity following short-term ozone exposure. Of these, two reported statistically significant decreases in motor activity associated with ozone treatment (Gordon et al., 2016; Pinto-Almazan et al., 2014). A similar pattern of behavior was reported by Mokoena et al. (2015): rats exposed to 0.3 ppm ozone showed a slight decrease in total locomotor activity compared to untreated controls; however, this effect was not statistically significant. Notably, ozone-related effects on motor activity were observed in male and female rats exposed to ozone and fed a control diet; however, when animals were fed high-fat or high-fructose diets, the effects of ozone on motor activity were not detected. Diet alone had no effect on motor activity (Gordon et al., 2016) The mechanisms underlying the potentially ozone-mitigating effects of diet remain unclear.
- Changes in cognitive and behavioral function were supported by associated changes in neurotransmitter levels. Exposure ozone for 15 days altered neurotransmitter levels in the brains of FSL rats relative to unexposed controls. Specifically, serotonin levels were reduced in the frontal cortex and hippocampus and norepinephrine levels were reduced in the hippocampus (Mokoena et al., 2015). The neurotransmitter serotonin is believed to play an important role in the pathophysiology of depression, so these data support the increases in depressive-like behaviors described above. Bhoopalan et al. (2013) found decreases in dopamine levels in the striatum after a single ozone exposure, but these effects were not statistically significant.

7.2.1.4.3 Summary

Section 7.2.1 describes and characterizes the epidemiologic and toxicological evidence relating to the effect of short-term ozone exposure on cognition and behavior. There are no epidemiologic studies of cognition or motor-function-related effects. A single epidemiologic study reported an association of short-term ozone exposure with depressive symptoms (Lim et al., 2012). This finding was supported by a toxicological study of FSL rats (Mokoena et al., 2015). In addition, experimental animal studies reported decreased motor activity and impaired learning and memory following short-term exposure to ozone (Gordon et al., 2016; Mokoena et al., 2015; Pinto-Almazan et al., 2014). Some of the behavioral effects in animals are supported by data showing effects on neurotransmitter levels that are associated with these outcomes.

Biological plausibility for the short-term effect of ozone on the nervous system is derived from multiple studies demonstrating that short-term exposure to ozone can to lead to inflammation and oxidative-stress responses in the brain, as well as modulation of the neuroendocrine system.

- Overall, the available evidence pertaining to cognitive and behavioral effects is limited. Increased depressive symptoms were observed in humans and in animals, providing some coherence across scientific disciplines.

7.2.1.5 Neuroendocrine Effects

7.2.1.5.1 Toxicological Evidence

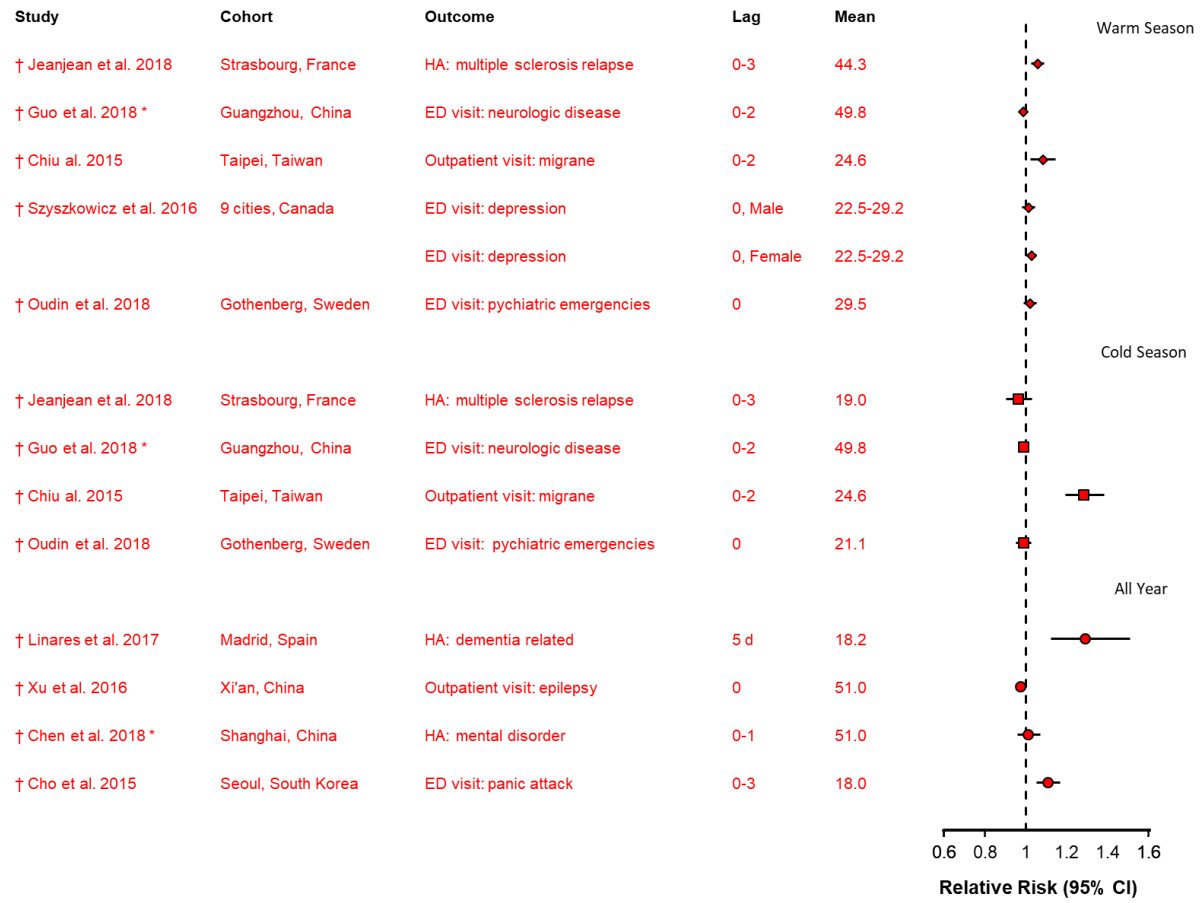
In the 2013 Ozone ISA, two studies provided evidence that ozone alters neuroendocrine function, affecting levels of thyroid hormones and corticosterone following short-term exposure. Since then, several studies have been published investigating the potential effects of ozone on the HPA axis; however, most of the data examine outcomes related to metabolic function and are therefore discussed in detail in Appendix 5.

A recent study (see Table 7-25) evaluated potential neuroendocrine effects of ozone in the nervous system following a short-term ozone exposure in rats (Thomson et al., 2013). A 4-hour exposure induced a transient effect on a wide array of genes involved in antioxidant response, xenobiotic metabolism, inflammation, and endothelial dysfunction. The pattern of gene responses was largely consistent across several organs, including the brain and pituitary, supporting systemic effects of neuroendocrine changes. Notably, the effects observed in the present study were transient, largely disappearing by 24 hour post-exposure; however, chronic exposure could result in prolonged neuroendocrine modulation. As described previously (see Section 7.2.1.2), ozone likely modulates HPA axis function by activating the sensory nerves in the lung and thereby altering autonomic nervous system activity.

7.2.1.6 Hospital Admissions and Emergency Department Visits

There were no studies of hospital admissions, emergency department (ED), or outpatient visits for diseases of the nervous system in the 2012 Ozone ISA. Recent studies (see Table 7-19) examining the association of short-term ozone exposure with hospital admissions, ED visits, or outpatient visits for diseases of the nervous system or mental health are presented in Figure 7-4. Outcomes that are presented on the plot and included in this section generally include hospitalizations for International Classification of Diseases version 9 (ICD-9) codes 290−319 or 320−359 and version 10 (ICD-10) codes F1−F99 or G00−G99. Several of the studies shown in Figure 7-4 are stratified by season, reporting separate associations for the warm and cold seasons.

- Some positive associations with hospitalizations for migraine, dementia, and multiple sclerosis were observed in single studies. Several studies also reported associations of short-term ozone exposure with mental health hospital admissions or ED visits for conditions such as depression and panic attack, but the results were not entirely consistent (Figure 7-4).
- Because hospitalizations or ED visits among those with chronic diseases may be related to comorbid conditions, the extent to which these studies are informative regarding the effect of short-term ozone exposure on nervous system health is uncertain.



Note: Relative risks are standardized to a 15 or 20 ppb increase ozone for 24-hour avg and 8-hour max* metrics, respectively. Lag times reported in days, unless otherwise noted. Diamonds indicate effect estimates for the warm season, squares indicate effect estimates for the cold season and circles indicate year-round effect estimates.

†Studies in red are recent studies.

Figure 7-4 Results of studies of short-term ozone exposure and hospital admissions or emergency department visits for diseases of the nervous system or mental health.

7.2.1.7 Relevant Issues for Interpreting the Epidemiologic Evidence

Evaluations of copollutant confounding and the effect of season were limited to studies of hospital admission, ED visits, or outpatient visits. As discussed in Section 7.2.1.6, the extent to which such studies inform the effect of short-term ozone exposure on nervous system effects is uncertain. Further, the limited evidence did not reveal a clear pattern of association. For example, Chiu and Yang (2015) reported an association with hospitalization for migraine that was larger in the cold season and persisted after adjustment for PM_{10}, SO_2, NO_2, or CO. The inverse association between short-term exposure to ozone and epilepsy outpatient visits observed by Xu et al. (2016) remained after adjustment for NO_2. The small increase in psychiatric ED visits observed by Oudin et al. (2018) was diminished after adjustment for PM_{10} and NO_2. Associations with hospitalization or ED visits for multiple sclerosis or mental health were observed in the warm season (Jeanjean et al., 2018; Oudin et al., 2018; Szyszkowicz et al., 2016) when ozone concentrations are higher.

7.2.1.8 Summary and Causality Determination

The 2013 Ozone ISA (U.S. EPA, 2013a) concluded that the evidence was suggestive of a causal relationship between short-term ozone exposure and nervous system effects. The strongest evidence supporting this causality determination came from experimental animal studies of CNS structure and function. Current evidence continues to support conclusions for related endpoints, including brain inflammation and changes in brain morphology, oxidative stress, and neurotransmitter levels. No epidemiologic studies of short-term ozone exposure and nervous system effects were reviewed in the 2013 Ozone ISA, and the epidemiologic evidence remains limited.

All available evidence examining the relationship between exposure to ozone and nervous system effects was evaluated using the framework described in the Preamble to the ISAs (U.S. EPA, 2015) and summarized in Table 7-3. Most of the recent experimental animal studies indicate that short-term exposure to ozone induces oxidative stress and inflammation in the central nervous system (see Section 7.2.1.3 and Table 7-23). In some cases these effects are associated with changes in brain morphology and effects on neurotransmitters. In some instances, the effects of short-term ozone exposure on the nervous system were exacerbated in aged animals. Adolescent and aged animals showed differences in the patterns of oxidative stress, with young animals showing greater magnitude of effect in the striatum and aged animals showing higher levels in the hippocampus (Tyler et al., 2018).

Epidemiologic studies of effects from short-term ozone exposure were lacking in the previous review. Recent evidence is limited to an association of short-term ozone exposure with depressive symptoms (Lim et al., 2012) and several studies of hospital admissions or ED visits for a range of conditions coded according the International Classification of Disease system as nervous system diseases or mental disorders (e.g., multiple sclerosis, Alzheimer's disease, Parkinson's disease, depression, psychiatric disorders). The findings of Lim et al. (2012) are coherent with experimental animal data

showing depression-like behaviors in rodents (Mokoena et al., 2015). Biological plausibility of these effects is supported by multiple toxicological studies showing inflammation and morphological changes in the brain following short-term ozone exposure (see Section 7.2.1.2). As noted in Section 7.2.1.6, these hospital admission and ED visit studies provide limited information regarding the effect of short-term ozone exposures on the nervous system because the extent to which people are treated for comorbid conditions may not be discernable.

Overall, the evidence is suggestive of, but not sufficient to infer, a causal relationship between short-term exposure to ozone and nervous system effects. This conclusion remains based largely on multiple toxicological studies demonstrating the effect of short-term exposure to ozone on the brain.

Table 7-3 Summary of evidence for a relationship between short-term ozone exposure and nervous system effects that is suggestive of, but not sufficient to infer, a causal relationship.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Limited epidemiologic evidence	An increase in depressive symptoms reported in single study Relevance of studies of hospital admissions, ED visits, and outpatient visits to nervous system effects is uncertain	Lim et al. (2012) Section 7.2.1.6	Mean: 48.1 ppb
Coherence with experimental animal study	Study of FSL rats demonstrates enhanced depressive-like symptoms	Mokoena et al. (2015) Section 7.2.1.4	0.3 ppm
	Single toxicological studies demonstrate effects on motor activity and cognition		0.25−0.8 ppm
Multiple toxicological studies generally support effects on the brain and provide biological plausibility	Multiple studies show brain inflammation and morphological changes following short-term ozone exposure	Section 7.2.1.3	0.25−2 ppm
Epidemiologic evidence from copollutant models lacking	Evaluation of copollutant confounding limited to studies of hospital admissions, ED visits, and outpatient visits, which are subject to limitations	Section 7.2.1.7	

C-R = concentration-response; NO_2 = nitrogen dioxide; ppb = parts per billion; $PM_{2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 2.5 μm; PM_{10} = particulate matter with a nominal aerodynamic diameter less than or equal to 10 μm.

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to the causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where the full body of evidence is described.

[c]Describes the ozone concentrations with which the evidence is substantiated.

7.2.2 Long-Term Ozone Exposure

7.2.2.1 Introduction, Summary from the 2013 Ozone ISA, and Scope for Current Review

This section evaluates the scientific evidence related to the potential effects of long-term exposure to ozone (i.e., on the order of months to years) on the nervous system. The 2013 Ozone ISA (U.S. EPA, 2013a) determined that the evidence was suggestive of, but not sufficient to infer, a causal relationship between exposures to ozone and effects on the central nervous system. The evidence is built on findings from the 2006 Ozone AQCD demonstrating alterations in neurotransmitters, motor activity, memory, and sleep patterns following short-term exposure to ozone, with the addition of studies that demonstrated progressive damage in various regions of the brains of rodents in conjunction with altered behavior following long-term ozone exposure. Specifically, several studies indicating the potential for neurodegenerative effects similar to Alzheimer's or Parkinson's diseases in a rat model were conducted. The evidence from epidemiologic studies of long-term exposure to ozone was limited to a single study reporting cognitive decline in older adults (Chen and Schwartz, 2009).

The nervous system effects reviewed in this Appendix include brain inflammation and morphology (Section 7.2.2.3); effects on cognition, motor activity, and mood (Section 7.2.2.4); and neurodevelopmental effects (Section 7.2.2.5). In addition, issues relevant for interpreting the epidemiologic studies are described in Section 7.2.2.6. The subsections below evaluate the scientific evidence relating long-term ozone exposure to nervous system effects. These sections focus on studies published since the completion of the 2013 Ozone ISA. The body of evidence has grown since the 2013 Ozone ISA. A limited number of recent epidemiologic studies examining nervous system effects are available, with the strongest line of evidence supporting an effect on cognition in adults. Recent experimental animal studies continue to provide coherence for these effects.

7.2.2.1.1 Population, Exposure, Comparison, Outcome, and Study Design (PECOS)

The scope of this section is defined by scoping statements that generally define the relevant PECOS. The PECOS statements define the parameters and provide a framework to help identify the relevant evidence in the literature to inform the ISA. The experimental studies evaluated and subsequently discussed within this section were identified using the PECOS statements below:

- Population: Study population from any animal toxicological study of mammals at any lifestage
- Exposure: Long-term (in the order of months to years) inhalation exposure to relevant ozone concentrations (i.e., ≤2 ppm)

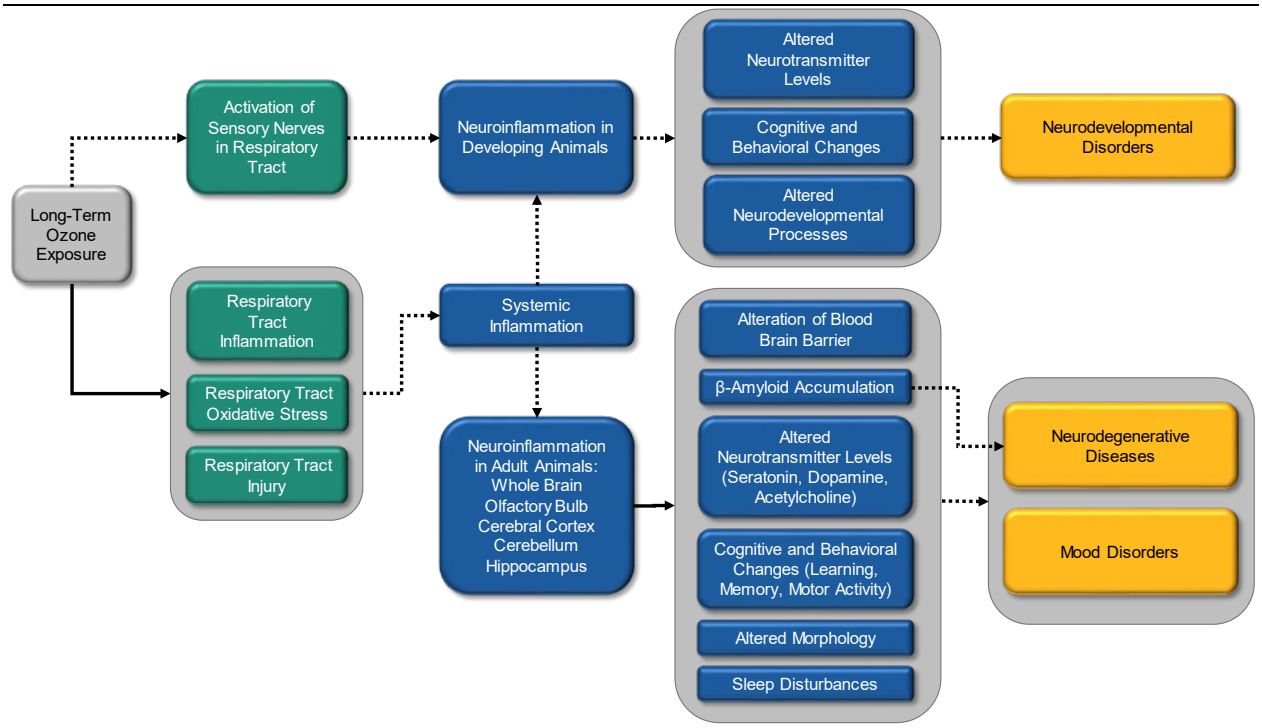
- Comparison: Appropriate comparison group exposed to a negative control (i.e., clean air or filtered-air control)
- Outcome: Nervous system effects
- Study Design: In vivo chronic, subchronic, or repeated-dose toxicity studies in mammals
- Because the 2013 Ozone ISA concluded that there was evidence to suggest a causal relationship between long-term ozone exposure and nervous system effects, the studies evaluated are less limited in scope and not targeted towards specific study locations, as reflected in the PECOS statement. The epidemiologic studies evaluated and discussed within this section were identified using the following PECOS statement:
- Population: Any population, including populations or lifestages that might be at increased risk
- Exposure: Long-term ambient concentration of ozone
- Comparison: Per unit increase (in ppb)
- Outcome: Change in risk (incidence/prevalence) of a nervous system effect
- Study Design: Epidemiologic studies consisting of cohort and case-control studies, time-series, case-crossover, and cross-sectional studies with appropriate timing of exposure for the health endpoint of interest

7.2.2.2 Biological Plausibility

This section describes biological pathways that potentially underlie nervous system effects resulting from long-term and developmental exposure to ozone. Studies that include exposure during the perinatal period are discussed in the long-term exposure section, regardless of the duration of the exposure because of the sensitivity of this lifestage to nervous system effects and potential for long-term health impacts. Biological plausibility is depicted via the proposed pathways as a continuum of upstream events, connected by arrows, that may lead to downstream events observed in epidemiologic studies (Figure 7-5). This discussion of "how" exposure to ozone may lead to effects on the nervous system contributes to an understanding of the biological plausibility of epidemiologic results evaluated later. The biological plausibility for ozone-induced effects on the nervous system is supported by evidence from the 2013 Ozone ISA and by new evidence.

As discussed in the short-term exposure section (see Section 7.2.1.2), inflammation is also expected to be an important mechanism driving nervous system effects following long-term ozone exposure. The first proposed pathway (Figure 7-5) is largely conserved across the short- and long-term exposure durations, however, there is a stronger link to neurodegenerative outcomes in humans following long-term exposures. Briefly, inhaled ozone elicits inflammation releasing inflammatory cytokines and ROS into the bloodstream that trigger systemic inflammation. Proinflammatory markers interact with, and in some cases infiltrate, the blood-brain barrier initiating neuroinflammation, as indicated by altered gene expression, increased apoptosis, lipid/protein oxidation, and microglial activation (see Section 7.2.2.3; Table 7-26). These effects are associated with changes to nervous system function

(e.g., behavior/cognition, sleep disturbances, neurotransmitter levels) and structure (e.g., blood brain barrier, β-amyloid accumulation, morphology) that are associated with neurodegenerative diseases such as Alzheimer's and Parkinson's diseases, and mood disorders.



Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence related to ozone exposure, and the arrows indicate a proposed relationship between those effects. Solid arrows denote evidence of essentiality as provided, for example, by an inhibitor of the pathway or a genetic knockout model used in an experimental study involving ozone exposure. Shading around multiple boxes is used to denote a grouping of these effects. Arrows may connect individual boxes, groupings of boxes, and individual boxes within groupings of boxes. Progression of effects is generally depicted from left to right and color-coded (gray, exposure; green, initial effect; blue, intermediate effect; orange, effect at the population level or a key clinical effect). Here, population level effects generally reflect results of epidemiologic studies. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below.

Figure 7-5 Potential biological pathways for nervous system effects following long-term exposure to ozone.

In the second pathway (Figure 7-5), adverse nervous system effects have also been reported when exposure occurs during development. Inflammation is expected to be a critical pathway for neurodevelopmental effects of ozone on developing offspring, just as it is in adults. In animal models, respiratory and systemic inflammation, either from direct (i.e., inhalation) or indirect (i.e., via the dam) exposure, are expected to elicit neuroinflammation that is associated with altered neurotransmitter levels, cognitive and behavioral changes, and altered development of the peripheral nervous system. Together, these effects may contribute to neurodevelopmental disorders. Neuroinflammation in developing animals

may also be triggered by activation of sensory nerves in the lung, leading to altered neurodevelopment of the nodose and jugular ganglia that transmit sensory information from the lung to the brain (see Section 7.2.2.5; Table 7-28).

The pathway(s) described here provide biological plausibility for evidence of neurodegenerative diseases, mood disorders, and sleep disturbances in adults (see Section 7.2.2.4) and neurodevelopmental disorders in children (see Section 7.2.2.5) in association with long-term exposure to ozone. These pathways will be used to inform a causality determination, which is discussed later in the Appendix.

7.2.2.3 Brain Inflammation and Morphology

7.2.2.3.1 Toxicological Studies

In the 2013 Ozone ISA, long-term ozone exposure elicited similar effects on the brain versus short-term exposure (see Section 7.2.1.3.1), with many studies showing increases of inflammatory and oxidative-stress responses, elevated cell death, and changes in neuronal morphpology in various regions of the brain. In general, the magnitude and severity of the effects was generally increased with longer exposure durations; however, some studies found these effects could be mitigated by coexposure with antioxidants.

As discussed below, the effects of long-term exposure on brain inflammation and morphology were similar to those described in the short-term exposure section (see Section 7.2.1.1); however, the magnitude and severity of the effects were generally increased with longer exposure durations. These effects were observed in multiple brain regions. There is also some evidence to suggest that males may be more susceptible than females to inflammation and oxidative damage. Study details are provided in Table 7-26.

- Multiple studies measured elevated levels of oxidative stress and inflammation in the brains of rats and mice following long-term exposure to ozone (Rodríguez-Martínez et al., 2016; Akhter et al., 2015; Rivas-Arancibia et al., 2015; Gómez-Crisóstomo et al., 2014; Pinto-Almazan et al., 2014; Rodríguez-Martínez et al., 2013; Mokoena et al., 2011). Histological analyses revealed reduced cell counts and increased apoptosis and oxidative damage in several regions of the brain, including the hippocampus (Rodríguez-Martínez et al., 2016; Gómez-Crisóstomo et al., 2014; Pinto-Almazan et al., 2014; Rodríguez-Martínez et al., 2013), frontal cortex (Mokoena et al., 2011), and substantia nigra (Rivas-Arancibia et al., 2015). These regions were also found to have damage to the mitochondria and endoplasmic reticulum (Rivas-Arancibia et al., 2015; Gómez-Crisóstomo et al., 2014; Rodríguez-Martínez et al., 2013).
- Although the majority of the data were generated in male Wistar rats, the study by Akhter et al. (2015) evaluated the oxidative effects of ozone exposure in both males and females using a mouse model of Alzheimer's disease. Ozone-exposed Alzheimer's disease model males exhibited significantly greater apoptosis in the hippocampus relative to the other experimental groups (i.e., wild-type males and females + ozone, Alzheimer's disease model males and

females + filtered air, and Alzheimer's disease females + ozone). Male Alzheimer's disease model mice were found to have significantly lower baseline antioxidant levels than wild-type animals or Alzheimer's disease model females which may make them more susceptible to oxidative stressors (Akhter et al., 2015).

- A series of studies from the same research group reported that long term exposure to ozone affected β-amyloid accumulation and regulation in the brain. These effects are strongly associated with development of Alzheimer's disease. Accumulation of β-amyloid proteins was increased, and alterations in several proteins and genes that regulate β-amyloid formation and degradation were observed in the hippocampus and cortex of male Wistar rats following exposure to 0.25 ppm ozone (Rivas-Arancibia et al., 2017; Fernando Hernandez-Zimbron and Rivas-Arancibia, 2016; Hernandez-Zimbron and Rivas-Arancibia, 2015). In general, these results showed an exposure-dependent trend, with the magnitude of effect increasing with exposure duration. Rivas-Arancibia et al. (2017) also found that ozone exposure induced exposure-dependent changes in the folding of β-amyloid proteins in a manner consistent with those observed in β-amyloid plaques associated with Alzheimer's disease. In some cases, β-amyloid was found to be colocalized with mitochondria (Hernandez-Zimbron and Rivas-Arancibia, 2015) and the endoplasmic reticulum (Fernando Hernandez-Zimbron and Rivas-Arancibia, 2016). In contrast, a single study from a different laboratory did not find an effect of ozone exposure on β-amyloid accumulation in a transgenic Alzheimer's disease mouse model. These animals were intermittently exposed to ozone for 4 months and while all Alzheimer's disease model animals showed β-amyloid accumulation in the hippocampus and cortex, there was no effect of ozone exposure (Akhter et al., 2015).

7.2.2.4 Effects on Cognition, Motor Activity, and Mood

The cognitive and behavioral effects measured in the epidemiologic studies reviewed in this section include scores on the Mini-Mental State Examination (MMSE), which is a questionnaire used to screen for dementia, and performance on neurobehavioral tests of cognitive function. Depression was evaluated using self-reported information on depression diagnosis and use of antidepressant medication. Clinically diagnosed dementia, including Alzheimer's disease and vascular dementia, and Parkinson's disease were also examined in a small number of studies. In a few animal toxicological studies, effects on learning and memory, motor activity, and anxiety were evaluated.

7.2.2.4.1 Epidemiologic Studies

Cognition and Dementia-Related Effects

The 2013 Ozone ISA reported declines on tests of cognitive function measured using Neurobehavioral Evaluation System-2 (NES2), in a cross-sectional analysis of NHANES III (1988−1991) data (Chen and Schwartz, 2009). A small number of recent studies examine the effect of long-term exposure to ozone with performance on neurobehavioral tests (see Table 7-20), Alzheimer's disease, and other forms of dementia (see Table 7-21). Overall, the limited number of epidemiologic studies support

an effect of long-term exposure to ozone on reduced cognitive function, but effect estimates reported in studies of dementia are inconsistent. Examination of copollutants confounding was limited.

- Domain-specific (i.e., executive function) decrements were observed in association with long-term exposure to ozone in a cross-sectional analysis of older adult women in Los Angeles, CA (Gatto et al., 2014). Study participants completed a battery of 14 neurobehavioral tests designed to measure cognitive decline in middle-aged and older adults. Cleary et al. (2018) examined the rate of cognitive decline using the MMSE among subjects followed through U.S. Alzheimer's Disease Centers, reporting an effect of ozone among those who had normal cognition at baseline.
- A small number of studies of Alzheimer's disease or dementia have reported results that vary in direction, magnitude, and precision. Chen et al. (2017c) reported a small (relative to the width of the confidence interval) inverse association with dementia in a population-based cohort study in Ontario, Canada (HR: 0.97; 95% CI: 0.94, 1.00). In this study, information about residential history was linked to modeled ozone concentrations and to registry information on physician-diagnosed dementia (dementia-related ICD codes for hospital admission or three physician claims). A positive association with confirmed Alzheimer's disease, which remained in copollutants models adjusted for CO, NO_2, and SO_2, was observed in a study in Taiwan using the National Health Insurance Research Database (NHIRD) [HR: 1.06; 95% CI: 1.00, 1.12; Jung et al. (2014)]. In a smaller case-control study conducted in Taiwan, relatively large, imprecise associations of long-term exposure to ozone with Alzheimer's disease and vascular dementia were reported [OR: 2.00; 95% CI: 1.14, 3.50 and OR: 2.09; 95% CI: 1.01, 4.33; Wu et al. (2015)].

Motor Function-Related Effects

Parkinson's disease is a nervous system disease that affects movement as well as nonmotor function. It is characterized by loss of dopaminergic neurons in the substantia nigra. There were no epidemiologic studies of Parkinson's disease reviewed in the 2013 Ozone ISA. Recent epidemiologic studies (see Table 7-21) conducted in the U.S. and Taiwan report some positive, although imprecise (i.e., wide confidence intervals), associations. Examination of copollutants confounding was limited.

- Large registry-based prospective studies conducted in Canada and Europe reported associations of ozone exposure with Parkinson's disease. Shin et al. (2018) and Cerza et al. (2018) reported a positive associations (HR: 1.06; 95% CI: 1.02, 1.11 and HR: 1.04; 95% CI: 1.00, 1.11), respectively] with summer average ozone concentrations. The association reported by Cerza et al. (2018) remained after adjustment for NO_2.
- Kirrane et al. (2015) reported an association between prevalent, self-reported doctor-diagnosed Parkinson's disease in farmers in North Carolina (OR: 2.60; 95% CI: 0.94, 7.24, 4-year warm avg) but not in Iowa (OR: 0.46; 95% CI: 0.11, 1.84, 4-year warm-season avg).
- In a nested case-control study of an the National Health Insurance Research Database (NHIRD) of Taiwan, Chen et al. (2017a) reported a positive yet imprecise (i.e., wide confidence intervals) association between Parkinson's disease and long-term exposure to ozone estimated from monitors located in areas where the subjects resided (OR: 1.10; 95% CI: 0.74, 1.48). In contrast, other researchers using the same database but a quantile-based Bayesian maximum entropy spatio-temporal model to characterize long-term exposure, Lee et al. (2016) reported a null

association (OR: 1.00; 95% CI: 0.97, 1.03) comparing the highest quartile of exposure to the lowest quartile (<23.93 ppb).

Mood and Mood Disorders

There were no epidemiologic studies of long-term exposure to ozone and mood disorders in the 2013 Ozone ISA. A prospective cohort study of depression onset among older women enrolled in the Nurses' Health Study (NHS) is currently available for review (Kioumourtzoglou et al., 2017). This study reports an association of long-term exposure to ozone with use of antidepressant medication (HR: 1.08; 95% CI: 1.02, 1.14) but not with self-reported doctor-diagnosed depression (HR: 1.00; 95% CI: 0.92, 1.08; [see Table 7-20]).

7.2.2.4.2 Toxicological Studies

In the 2013 Ozone ISA, toxicological studies showed declines in learning and memory that increased with the exposure duration. Coexposure with antioxidants was found to have a protective effect, suggesting that oxidative damage particularly in regions of the brain that play a role in cognition, may contribute to the observed cognitive decrements. Several recent studies investigated the role of ozone exposure on cognitive and behavioral effects, including changes in learning and memory, motor activity, and anxiety, that are associated with neurodegenerative diseases (see Table 7-27). Neurodegenerative effects of ozone may be driven by increased oxidative stress in inflammatory responses in the central nervous system leading to changes in brain morphology (e.g., increased apoptosis and reduced neuronal cell counts) in regions of the brain associated with Alzheimer's and Parkinson's disease.

- Some evidence in animal models suggests that long-term exposure to ozone impairs learning and memory formation, an important characteristic of Alzheimer's disease. Male Wistar exposed to 0.25 ppm ozone for 30, 60, or 90 days showed decreased latency in both short- (10 minutes) and long-term (24 hour) passive avoidance tests (Pinto-Almazan et al., 2014).
- The effects of ozone exposure on motor activity data were also evaluated, but the results were varied. Most of the studies reported decreased activity in rats (Gordon et al., 2016; Pinto-Almazan et al., 2014; Gordon et al., 2013) and is in agreement with the data included in the 2013 Ozone ISA. In contrast, Gordon et al. (2014) reported a statistically significant increase in motor activity, and Akhter et al. (2015) found no effect in a transgenic model of Alzheimer's disease following ozone exposure. The variability in these results may be attributable to differences in the study designs. Akhter et al. (2015) evaluated effects in mice, including a transgenic model of Alzheimer's disease whereas Gordon et al. (2014) continuously monitored animals' motor activity in the home cage via a subcutaneous radio transmitter.
- Akhter et al. (2015) also found no ozone-mediated effects on behavior in the elevated plus maze, a measure of anxiety, in a mouse model of Alzheimer's disease. No other toxicological data related to mood or mood disorders were available following long-term exposure.

7.2.2.4.3 Summary

The current section describes and characterizes the epidemiologic and toxicological evidence relating to the effect of long-term ozone exposure on cognition, motor activity, and mood.

- Biological plausibility for the long-term effect of ozone on the nervous system is derived from multiple studies demonstrating that long-term exposure to ozone can to lead to inflammation and oxidative stress responses in the brain.
- Limited epidemiologic evidence reports associations with decrements on tests of cognitive function that may be associated with neurodegenerative diseases, such as Alzheimer's and Parkinson's disease. Toxicological studies provide coherence for these findings, but epidemiologic studies of Alzheimer's and Parkinson's disease are not consistent. The animal data do not support an association between long-term ozone exposure and mood disorders. The epidemiologic evidence is limited to a study reporting an association with self-reported depression.

7.2.2.5 Neurodevelopmental Effects

In the 2013 Ozone ISA, discussion of the data on neurodevelopmental effects was split across the short- and long-term exposure sections, however, in the current ISA these data are only reviewed in the long-term exposure section due to the sensitivity of the developing nervous system to toxicants and the potential for long-term impacts. The 2013 Ozone ISA reviewed toxicological evidence for neurodevelopmental effects of prenatal and early life ozone exposure. Exposure that was limited the prenatal period altered gene expression of nerve growth factors, affected regulation of neurotransmitter levels and altered neuroadaptive responses to stress. Social interaction, defensive/submissive behavior, and turning preferences were also affected in animals exposed either prenatally or during both gestation and lactation. Notably, some of these outcomes persisted into adulthood, suggesting early life exposure can have long lasting impacts on neurological function. There were no epidemiologic studies of long-term exposure to ozone and neurodevelopmental outcomes. A recent study by Lin et al. (2014a) examined the effect of prenatal ozone exposure and neurobehavioral outcomes but reported no evidence of an association. The current evidence base also includes several epidemiologic studies of autism spectrum disorder (ASD) and toxicological studies that focus on the peripheral nervous system.

7.2.2.5.1 Epidemiologic Studies

There were no studies of long-term exposure to ozone and autism reviewed in the 2013 Ozone ISA. Several recent studies conducted in the U.S. and Taiwan are currently available (see Table 7-28). Overall, these studies report positive associations, but associations are imprecise (i.e., wide confidence intervals) and are not consistently observed across pregnancy periods. In addition, outcome definitions for autism, which is a heterogenous condition with potentially different etiologies, varied across studies. For

example, Becerra et al. (2013) and Volk et al. (2013) included cases of autistic disorder or full syndrome autism, which are the most severe among the autism spectrum disorders (ASD).

- Becerra et al. (2013) conducted a case-control study of autistic disorder, diagnosed between 3 and 5 years of age, in Los Angeles, CA. Ozone exposure during the entire pregnancy but not trimester-specific exposures was associated with autistic disorder (OR: 1.05; 95% CI: 1.01, 1.10). The effect of ozone remained in copollutant models adjusted for NO_2 estimated using land use regression (LUR), $PM_{2.5}$, or PM_{10}.
- Also in California, among children enrolled in the Childhood Autism Risks from Genetics and the Environment (CHARGE) study, Volk et al. (2013) reported small imprecise (relative the width of the confidence interval) associations of full syndrome autism with ozone concentrations during the 1st year of life, during the entire pregnancy and with trimester-specific ozone concentrations (e.g., OR: 1.05; 95% CI: 0.84, 1.31; entire pregnancy). Scores on cognitive and adaptive scales were not associated with prenatal exposure to ozone among children with ASD among subjects enrolled in the CHARGE cohort (Kerin et al., 2017).
- Additional analyses of the CHARGE cohort reported an interaction between ozone exposure and copy number variation, indicating a larger risk for the joint effect compared to the effect of ozone or duplication burden alone (Kim et al., 2017), but not between ozone exposure and folic acid (Goodrich et al., 2017).
- A cohort study in Taiwan reported an association between long-term ozone exposure and ASD [HR: 1.59; 95% CI: 1.42, 1.78; Jung et al. (2013)]. This association remained after adjusting for CO, NO, and SO_2.

7.2.2.5.2 Toxicological Studies

Two studies from the same research group evaluated neurodevelopmental effects in animal models. Zellner et al. (2011) and Hunter et al. (2011) evaluated the effects of a single 3-hour exposure to 2 ppm ozone during the early postnatal window on lung innervation. As discussed previously, these studies would normally be considered short-term, but due to the sensitivity of the developmental window and increased potential for long-term outcomes, they are discussed below.

- In the first study, ozone exposure on PND 5 resulted in a statistically significant decrease in the total number of neurons and change in the overall pattern of neuroproliferation in the nodose and jugular ganglia. Whereas controls showed a large increase in the average total and substance P-reactive neuron counts on PNDs 15 and 21, neuronal counts generally remained consistent in ozone-treated animals across the four time points (PNDs 10, 15, 21, or 28). Notably, there was high variability among the controls so that the difference for the total neuron count was only statistically significant on PND 21. Although neurons from these ganglia innervate the lung to provide sensory feedback, Zellner et al. (2011) reported no effect of ozone exposure on pulmonary innervation.
- Hunter et al. (2011) studied the effects of ozone on lung NGF levels and sensory innervation. Here, coexposure to NGF on PND 6 and ozone on PND 28 elicited a statistically significant increase in substance P-reactive neurons in the lung and extrapulmonary smooth muscle. Ozone exposure was also found to increase NFG levels in BAL fluid. PND 6 is believed to be a critical

window for neuronal development in the lung, eliciting statistically significant increases in NGF in the short term (24-hour PE) and potentiating the effects of subsequent ozone exposures.

7.2.2.5.3 Summary

Section 7.2.2.5 describes and characterizes the epidemiologic and toxicological evidence relating to the effect of long-term ozone exposure on neurodevelopment. There is some epidemiologic evidence to suggest that prenatal or early life exposure to ozone may increase the risk for autism or autism spectrum disorder. There were no experimental animal studies showing effects in the brain that support the epidemiologic findings on autism. The toxicological evidence was limited to two studies showing effects on the peripheral NS that indicate potential effects on development of sensory neurons in the lung.

7.2.2.6 Relevant Issues for Interpreting the Epidemiologic Evidence

7.2.2.6.1 Potential Copollutant Confounding

Overall, only a few studies considered copollutant confounding in the analysis. For instance, associations observed with autism or ASD persisted after adjustment for CO, NO_2 SO_2 (Jung et al., 2013), $PM_{2.5}$, and PM_{10} (Becerra et al., 2013). The association of ozone with Alzheimer's disease observed by Jung et al. (2014) persisted in copollutant models adjusted for CO, NO_2, and SO_2.

7.2.2.7 Summary and Causality Determination

The strongest evidence supporting the causality determination from the 2013 Ozone ISA (U.S. EPA, 2013a) came from animal toxicological studies demonstrating effects on CNS structure and function, with several studies indicating the potential for neurodegenerative effects similar to Alzheimer's or Parkinson's diseases in a rat model. The body of evidence has grown since the 2013 Ozone ISA. Recent epidemiologic studies examining nervous system effects, including cognitive effects, depression, neurodegenerative disease, and autism, are currently available. Although the epidemiologic evidence remains limited, the strongest line of evidence supports an effect on cognition in adults and recent experimental animal studies continue to provide coherence for these effects. All available evidence examining the relationship between exposure to ozone and reproductive effects was evaluated using the framework described in the Preamble to the ISAs (U.S. EPA, 2015) and summarized in Table 7-4.

Multiple animal recent toxicological studies report increased markers of oxidative stress and inflammation, including lipid peroxidation, microglial activation, and cell death following long-term exposure to ozone. There was some evidence to indicate that aged and young populations may have increased sensitivity to ozone exposure. Functional deficits in tasks of learning and memory and

decreased motor activity were correlated with biochemical and morphological changes in regions that are known to be affected by neurodegenerative diseases, including the hippocampus, striatum, and substantia nigra. Other CNS regions affected include the olfactory bulb and frontal/prefrontal cortex. Epidemiologic studies have reported cognitive decline in association with long-term ozone exposure. Associations with neurodegenerative disease are not entirely consistent, but some positive associations are reported. Epidemiologic studies of Parkinson's disease do not consistently support an association with long-term exposure to ozone, and the findings from toxicological studies of motor function-related effects are mixed, although loss of dopaminergic neurons in the substantia nigra is observed in animals.

Effects on neurotransmitter levels, behavior, and cell signaling were identified in animals that were exposed only during the prenatal period. In some cases these effects persisted into adulthood. Adolescent and aged animals showed differences in the patterns of oxidative stress, with young animals showing higher levels in the striatum and aged animals showing higher levels in the hippocampus. Some epidemiologic studies of autism or ASD reported positive associations, but the biological plausibility of these effects is limited because the toxicological data focused on effects in the peripheral nervous system. A limited number of studies reported copollutant model results. The potential for these effects have been supported by data derived from toxicological studies.

Overall, the evidence is suggestive of, but not sufficient to infer, a causal relationship between long-term ozone exposure and nervous system effects. This is consistent with the conclusion of the 2013 Ozone ISA (U.S. EPA, 2013a). Uncertainties that contribute to the causality determination include the limited number of epidemiologic studies, the lack of consistency across the available studies of Alzheimer's and Parkinson's disease, and the limited evaluation of copollutant confounding. In addition, the evidence supporting the biological plausibility of the associations with autism or ASD in epidemiologic studies is limited.

Table 7-4 Summary of evidence for a relationship between long-term ozone exposure and nervous system effects that is suggestive, but not sufficient to infer, a causal relationship.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Limited epidemiologic evidence is generally consistent for cognitive effects but not for	Associations with reduced cognitive function consistently observed in a small number of epidemiologic studies	Chen and Schwartz (2009) Gatto et al. (2014) Cleary et al. (2018)	

Table 7-4 (Continued): Summary of evidence for a relationship between long-term ozone exposure and nervous system effects that is suggestive, but not sufficient to infer, a causal relationship.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
neurodegenerative disease	Effect estimates reported in studies of Alzheimer's disease or dementia are imprecise and vary in magnitude	Chen et al. (2017c) Jung et al. (2014) Wu et al. (2015)	NR 92.6 ppb NR
	Associations with Parkinson's disease are not consistently observed and generally lack precision (i.e., wide confidence intervals)	Kirrane et al. (2015) Chen et al. (2017a) Lee et al. (2016) Shin et al. (2018)	40.6 NR 26.1 49.8
Coherence across lines of evidence	Toxicological studies provide coherence for neurodegenerative disease in humans, including Alzheimer's and Parkinson's disease	Section 7.2.2.4.2	0.25−1 ppm
Biological plausibility provided by multiple toxicological studies demonstrating effects on the brain	Multiple studies show brain inflammation and morphological changes following short- and long-term ozone exposure	Section 7.2.2.3	0.25−0.8 ppm
Limited number of epidemiologic studies generally report consistent positive associations with autism or ASD	Associations are imprecise (i.e., wide confidence intervals) and are not consistently observed across pregnancy periods	Section 7.2.2.5.1	
Limited evidence of coherence and biological plausibility	Available studies focused on effects in the peripheral nervous system	Section 7.2.2.5.2	2 ppm
Uncertainty regarding the independent effect of ozone exposure	Few studies consider copollutant confounding		

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to the causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where the full body of evidence is described.

[c]Describes the ozone concentrations with which the evidence is substantiated.

7.3 Cancer

7.3.1 Introduction, Summary from the 2013 Ozone ISA, and Scope for Current Review

In the 2013 Ozone ISA (U.S. EPA, 2013a), the available evidence was inadequate to determine whether there was a causal relationship between exposure to ambient ozone and cancer. That review noted that very few epidemiologic and toxicological studies had been published examining ozone as a carcinogen, but that collectively the results of these studies indicated that ozone may contribute to DNA damage. The same conclusions are reached in this review: there continue to be relatively few studies examining the association between ozone and cancer, although some animal toxicological studies have shown indicators of DNA damage in animals. The evidence published since the 2013 Ozone ISA is discussed in greater detail below.

7.3.1.1 Population, Exposure, Comparison, Outcome, and Study Design (PECOS) Tool

The scope of this section is defined by a scoping tool that generally describes the relevant Population, Exposure, Comparison, Outcome, and Study Design (PECOS). The PECOS tool defines the parameters and provides a framework to help identify the relevant literature to inform the draft 2019 Ozone ISA. The studies evaluated and subsequently discussed within this section were included if they satisfied all of the components of the following PECOS tool:

- Population: Study population of any animal toxicological study of mammals at any lifestage
- Exposure: Long-term (in the order of months to years) inhalation exposure to relevant ozone concentrations (i.e., ≤2 ppm)
- Comparison: Appropriate comparison group exposed to a negative control (i.e., clean air or filtered-air control)
- Outcome: Mutagenic, genotoxic, or carcinogenic effects
- Study Design: In vivo chronic, subchronic or repeated-dose toxicity studies in mammals; genotoxicity/mutagenicity studies

Because the 2013 Ozone ISA concluded that evidence was inadequate to determine whether there is a causal relationship between long-term ozone exposure and cancer, the studies evaluated are less limited in scope and not targeted towards specific study locations, as reflected in the PECOS tool. The epidemiologic studies evaluated and discussed in this section were identified using the following PECOS tool:

- Population: Any population, including populations or lifestages that might be at increased risk
- Exposure: Long-term ambient concentration of ozone
- Comparison: Per unit increase (in ppb)
- Outcome: Change in risk (incidence/prevalence) of a cancer effect
- Study Design: Epidemiologic studies consisting of cohort, case-control and cross-sectional studies with appropriate timing of exposure for the health endpoint of interest

7.3.2 Cancer and Related Health Effects

7.3.2.1 Genotoxicity

As noted in the 2013 Ozone ISA, the potential for genotoxic effects relating to ozone exposure was predicted from the radiomimetic properties of ozone. The decomposition of ozone in water produces OH and HO_2 radicals, the same species that are generally considered to be the biologically active products of ionizing radiation. Ozone has been observed to cause degradation of DNA in a number of different models and bacterial strains. The toxic effects of ozone have been generally assumed to be confined to the tissues directly in contact with the gas, such as the respiratory epithelium.

Several epidemiologic studies evaluated in the 2013 Ozone ISA observed positive associations between long-term ozone exposure and DNA damage (i.e., DNA adduct levels, oxidative DNA damage, DNA strand breaks). In addition, there was some evidence of cytogenetic damage (i.e., micronuclei frequency among lymphocytes and buccal cells) after long-term, but not short-term ozone exposure. Such DNA and cytogenic damage may be relevant to mechanisms leading to cancer development and serve as early indicators of an elevated risk of mutagenicity.

Since the 2013 Ozone ISA, a few additional studies have looked at the relationship between ozone exposure and the potential for DNA damage and found inconsistent results:

- Holland et al. (2014) exposed healthy volunteers to filtered air (FA), 100, and 200 ppb ozone and collected blood lymphocytes 24-hours post-exposure. A statistically significant increase in the frequency of micronuclei in binucleated cells was reported with increasing ozone concentrations ($p < 0.05$). However, these authors also reported no appreciable changes in neoplasmic bridges (an indicator of radiation and other types of genotoxic exposure) and no difference in cell proliferation following ozone exposure.
- Finkenwirth et al. (2014) exposed healthy volunteers to FA and ozone, collected lymphocytes and analyzed them for single stranded breaks. No appreciable difference in single stranded breaks were observed at either 30 minutes or 4.5 hours post-exposure.
- In rats, Cestonaro et al. (2017) evaluated exposure to 0.05 ppm ozone from an air purifier for 3 hours or 24 hours per day for 14 or 28 days. The results indicated no significant effects on

indicators of DNA damage such as the frequency of micronuclei in the 3-hour-exposed group (14 or 28 days). In the 24-hour exposure group, there was a statistically significant increase in DNA damage relative to other groups. However, DNA in the tail was less than 1% and not different from control exposure.

- In rat lung tissue, Zhang et al. (2017) reported a significant increase in the most common base lesion 8-oxoG following ozone exposure ($p < 0.05$) and that treatment with the NO-precursor L-arginine reduced the presence of these lesions. Moreover, levels of the base excision repair component OGG1 were significantly decreased following ozone exposure ($p < 0.05$) but treatment with the NO-precursor L-arginine restored these levels.

7.3.2.2 Cancer Incidence, Mortality, and Survival

The 2013 Ozone ISA concluded that the evidence was inadequate to determine whether a causal relationship exists between ambient ozone exposures and cancer. A limited number of epidemiologic and animal toxicological studies of lung cancer mortality among humans and lung tumor incidence among rodents contributed to the evidence informing this conclusion. The reanalysis of the full ACS CPSII cohort reported no association between lung cancer mortality and ozone concentration [HR: 1.00; 95% CI: 0.96, 1.04; Krewski et al. (2009)]. Additionally, no association was observed when the analysis was restricted to the summer months. There was also no association between ozone concentration and lung cancer mortality present in a subanalysis of the cohort in the Los Angeles area. Animal toxicological studies did not demonstrate enhanced lung tumor incidence in male or female rodents. However, there was an increase in the incidence of oviductal carcinoma in mice exposed to 0.5 ppm ozone for 16 weeks (U.S. EPA, 2013a) The implications of this result are unclear because the report lacked statistical information. It was noteworthy that there was no mention of oviductal carcinoma after 32 weeks of exposure, and no oviductal carcinoma was observed after 1 year of exposure.

In contrast, several recent cohort and case-control studies have observed positive associations between long-term ozone exposure and lung cancer incidence or mortality. A single study reported null associations between short-term ozone exposure and lung-cancer mortality. Associations between ozone exposure and other types of cancer were generally null. Specifically:

- A case-control study conducted in Canada (Hystad et al., 2013) and a cohort study conducted in China (Guo et al., 2016) observed positive associations between long-term ozone exposure and lung cancer incidence. Hystad et al. (2013) evaluated both modeled and measured ozone concentrations, while Guo et al. (2016) relied on the exposure assessment hybrid model developed for the Global Burden of Disease study (Brauer et al., 2012). Hystad et al. (2013) observed ORs that were two times higher for squamous cell lung cancer compared with all lung cancers. Guo et al. (2016) reported no differences in effects between men and women, but higher risks for adults aged 65+ years (compared with adults between 30 and 65 years).
- Two U.S.-based cohort studies (Eckel et al., 2016; Xu et al., 2013) reported positive associations between long-term ozone exposure and lung cancer mortality or respiratory cancer mortality among individuals that had already been diagnosed with cancer.

- A number of recent studies conducted in the U.S., Canada, and Europe provided limited and inconsistent evidence for an association between long-term ozone exposure and lung cancer mortality (Cakmak et al., 2017; Turner et al., 2016; Crouse et al., 2015; Carey et al., 2013; Jerrett et al., 2013) (Table 7-30).
- A case-crossover study conducted in Shenyang, China observed null associations between short-term ozone exposure and lung cancer mortality (Xue et al., 2018).
- Studies of childhood leukemia (Badaloni et al., 2013) and breast tissue density, an indicator of breast cancer (Yaghjyan et al., 2017), observed null associations with long-term ozone exposure.

7.3.3 Summary and Causality Determination

In the 2013 Ozone ISA, very few studies were available to assess the relationship between long-term exposure to ozone and carcinogenesis. The few available toxicological and epidemiologic studies suggested that ozone exposure may contribute to DNA damage. However, given the overall lack of studies, the 2013 Ozone ISA concluded that the evidence was inadequate to determine whether a causal relationship existed between ambient ozone exposures and cancer.

The number of studies examining the relationship between ozone exposure and the potential for carcinogenesis reman few. Studies published since the 2013 Ozone ISA provide some additional animal toxicological evidence that ozone exposure can lead to DNA damage. In addition, several but not all recent cohort and case-control studies have observed positive associations between long-term ozone exposure and lung cancer incidence or mortality. Several of the studies evaluating lung cancer mortality were conducted among populations that had already been diagnosed with cancer in a different organ system. Associations between ozone exposure and other types of cancer were generally null. Given the limited evidence base, the lack of an evaluation of copollutant confounding in epidemiologic studies reporting associations, and the evaluation of study populations that had already been diagnosed with cancer in several of the epidemiologic studies, the evidence is not sufficient to draw a conclusion regarding causality (Table 7-5). Thus, **the evidence describing the relationship between exposure to ozone and carcinogenesis remains inadequate to determine if a causal relationship exists**.

Table 7-5 Summary of evidence that is inadequate to determine if a causal relationship exists between long-term ozone exposure and cancer.

Rationale for Causality Determination[a]	Key Evidence[b]	Key References[b]	Ozone Concentrations Associated with Effects[c]
Inconsistent evidence for DNA damage in experimental studies	A limited number of controlled human exposure studies report inconsistent evidence for DNA damage measured in lymphocytes	Holland et al. (2014) Finkenwirth et al. (2014)	100 ppb, 200 ppb
	A limited number of animal toxicological studies report inconsistent evidence for DNA damage measured in lymphocytes	Cestonaro et al. (2017) Zhang et al. (2017)	50 ppb
Some epidemiologic evidence for lung cancer incidence or mortality	A limited number of recent studies observed positive associations between long-term ozone exposure and lung cancer incidence	Hystad et al. (2013)	20.3
		Guo et al. (2016)	56.9
	A limited number of recent studies observed positive associations between long-term ozone exposure and lung cancer or respiratory mortality in study populations already diagnosed with cancer	Eckel et al. (2016)}	28.5
		Xu et al. (2013)	40.2
No epidemiologic evidence for other cancers	A limited number of recent studies observed null associations between long-term ozone exposure and childhood leukemia and breast cancer	Badaloni et al. (2013)	24.2
		Yaghjyan et al. (2017)	36.0
Lack of copollutant models contributes to uncertainty	No epidemiologic studies evaluated potential copollutant confounding using copollutant models		
Limited evidence for biological plausibility	Experimental studies provide inconsistent evidence for DNA damage in humans and laboratory animals		

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble to the ISAs (U.S. EPA, 2015).

[b]Describes the key evidence and references, supporting or contradicting and contributing most heavily to causality determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where full body of evidence is described.

[c]Describes the ozone concentrations with which the evidence is substantiated.

1

7.4 Evidence Inventories—Data Tables to Summarize Reproductive and Developmental Effects Study Details

7.4.1 Epidemiologic Studies

Table 7-6. Epidemiologic studies of exposure to ozone and reproduction—male.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Farhat et al. (2016) São Paulo, Brazil Ozone: 1999−2006 Follow-up: January 2000−January 2006 Panel study	n = 35 Male patients with systematic lupus erythematosus	Monitor 1-h max Other, 90 days before sample collection	Mean: 42 75th: 48 Maximum: 54	Correlation (*r*): NO_2: 0.6; Other: 0.28 Copollutant models with: NR	Total sperm count (Δ million per ejaculate) −74.78 (−136.51, −13.04) Sperm concentration (Δ million per mL) −24.29 (−42.43, −6.15)
†Liu et al. (2017) Wuhan, China Ozone: NR Follow-up: 2013−2015 Cohort study	n = 1,759 men	Monitor Other, 0−90 days before sample collection	Mean: 25−64	Correlation (*r*): NR Copollutant models with: NR	Sperm concentration (Δ million/mL): 0.082 (−0.077, 0.240) Sperm count (Δ million): 0.018 (−0.145, 0.181) Total motility (Δ percentage): 0.082 (−0.068, 0.236) Progressive motility (Δ percentage): 0.068 (−0.086, 0.217) Total motile sperm count (Δ million): 0.041 (−0.113, 0.199)

†Recent studies evaluated since the 2013 Ozone ISA.

Table 7-7 Epidemiologic studies of exposure to ozone and reproduction—female.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Slama et al. (2013) Teplice district, Czech Republic Ozone: NR Follow-up: November 25, 1993−July 31, 1996 Cohort study	n = 1,916 couples	Monitor Other	Mean: NR	Correlation (*r*) (1st mo unprotected intercourse): $PM_{2.5}$: −0.41; NO_2: −0.49; SO_2: −0.69 Copollutant models with: NO_2, $PM_{2.5}$	Fecundity (FR) 30 days before unprotected intercourse: 0.98 (0.81, 1.19) 1st mo unprotected intercourse: 1.12 (0.94, 1.32) Average of 1st mo unprotected intercourse and 30 days previous: 1.08 (0.86, 1.37) 30 days after the end of the 1st mo of unprotected intercourse exposure window (post-outcome): 1.30 (1.08, 1.56)
†Carré et al. (2016) Region NR, France Ozone: 2012−2015 Follow-up: April 2012−December 2015 Cohort study	n = 292 couples Couples undergoing IVF attempts	Monitors Other	NR	Correlation (*r*):NR Copollutant models with: NR	Ovarian response to stimulation and number of top embryos were increased with short- or long-term exposures to high levels of ozone
†Nobles et al. (2018) Michigan and Texas, U.S. Ozone: 2005−2010 Follow-up: 2005−2009 Cohort study	Longitudinal Investigation of Fertility and the Environment (LIFE) Study n = 500 couples Couples had presumed exposure to persistent organic pollutants	Model, modified CMAQ Other	75th (cycle prior to observed cycle): 27.85 90th: 34.2 Maximum: 40.54	Correlation (*r*):NR Copollutant models with: $PM_{2.5}$, NO_X, SO_2, CO	Fecundability (FOR) Cycle prior to observed cycle: 0.93 (0.73, 1.21) Days 1−10 (proliferative phase) of observed cycle: 0.86 (0.62, 1.17) FORs for exposures 5 days before to 10 days after ovulation are generally null or slightly below null (reduced fecundity). Effects for 5 and 1 days before ovulation and day of ovulation are below the null.

†Recent studies evaluated since the 2013 Ozone ISA.

Table 7-8 Epidemiologic studies of exposure to ozone and pregnancy/birth—hypertension disorders.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Lee et al. (2013) Pittsburgh, PA, U.S. Ozone: 1996−2002 Follow-up: 1997−2002 Cohort study	n = 34,702 Magee Obstetric Medical and Infant (MOMI) database	Model, space-time ordinary kriging 1st trimester	Median: 21.7 75th: 30.2 95th: 36.2 Maximum: 46.8	Correlation (*r*): $PM_{2.5}$: 0.5; Other: 0.7 Copollutant models with: NR	Gestational hypertension (OR): 1.07 (0.98, 1.16) Preeclampsia (OR): 1.07 (0.93, 1.23)
†Mobasher et al. (2013) Los Angeles, CA, U.S. Ozone: NR Follow-up: 1996−2008 Case-control study	n = 298 Attended the Los Angeles County + University of Southern California Women's and Children's Hospital	Monitor Other	Mean: 1st trimester: 21.5 2nd trimester: 18.2 3rd trimester: 18.2	Correlation (*r*): 1st trimester $PM_{2.5}$: −0.45; NO_2: −0.72; Other: −0.64 Copollutant models with: NR 2nd trimester $PM_{2.5}$: −0.53; NO_2: −0.74; Other: −0.57 Copollutant models with: NR 3rd trimester $PM_{2.5}$: −0.55; NO_2: −0.78; Other: −0.66 Copollutant models with: NR	Hypertensive disorders of pregnancy (OR) 1st trimester: 0.94 (0.66, 1.32) BMI <30: 0.77 (0.51, 1.19) BMI ≥30: 1.26 (0.58, 2.75) 2nd trimester: 1.61 (1.14, 2.29) BMI <30: 1.67 (1.08, 2.59) BMI ≥30: 1.23 (0.57, 2.63) 3rd trimester: 1.12 (0.80, 1.58) BMI <30: 1.28 (0.84, 1.94) BMI ≥30: 1.02 (0.52, 2.04)
†Olsson et al. (2013) Stockholm, Sweden Ozone: 1997−2006 Follow-up: 1998−2006 Cohort study	n = 120,755 Swedish medical birth registry	Monitor 1st trimester	Mean: 35	Correlation (*r*): NO_2: −0.48 Copollutant models with: NO_2	Preeclampsia (OR) 1.10 (1.04, 1.17) Adjusted for NO_2: 1.23 (1.06, 1.44)

Table 7-8 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—hypertension disorders.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Xu et al. (2014) Florida, U.S. Ozone: 2003−2005 Follow-up: 2004−2005 Cohort study	n = 22,041 birth records	Monitor 24-h avg Other	Mean: 1st trimester: 40 2nd trimester: 41 3rd trimester: 40	Correlation (*r*):NR Copollutant models with: NR	Hypertension (OR) 1st trimester: 1.00 (0.84, 1.19) 2nd trimester: 0.93 (0.80, 1.08) Entire pregnancy: 0.93 (0.63, 1.42)
†Nahidi et al. (2014) Tehran, Iran Ozone: 2010−2011 Follow-up: September 2010−March 2011 Case-control study	n = 65 cases; 130 controls admitted to hospitals in Tehran	Monitor Entire pregnancy	Mean: NR	Correlation (*r*):NR Copollutant models with: NR	Preeclampsia (OR) High vs. low exposure: 1.00 (0.49, 2.03)
†Michikawa et al. (2015) Kyushu-Okinawa District, Japan Ozone: NR Follow-up: 2005−2010 Cohort study	Japan Perinatal Registry Network n = 36,620	Monitor 1st trimester	Mean: 41.3 Median: 40.1 75th: 48	Correlation (*r*): $PM_{2.5}$: 0.12 NO_2: −0.18 SO_2: −0.17 Copollutant models with: NR	Hypertensive disorders of pregnancy (OR) 1st quintile: reference 2nd quintile: 1.24 (1.07, 1.43) 3rd quintile: 1.35 (1.17, 1.56) 4th quintile: 1.26 (1.08, 1.47) 5th quintile: 1.20 (1.01, 1.42)
†Mendola et al. (2016b) U.S. Ozone: NR Follow-up: 2002−2008 Cohort study	Consortium on Safe Labor n = 192,687 women recruited from 12 centers (19 hospitals) across U.S.	Model, modified CMAQ Other	Median: Preconception: 29.7 1st trimester: 29.2 2nd trimester: 29.4 Entire pregnancy: 28.5	Correlation (*r*):NR Copollutant models with: NR	Preeclampsia (OR) Asthma Preconception: 1.02 (0.94, 1.11) 1st trimester: 0.99 (0.91, 1.07) 2nd trimester: 1.00 (0.92, 1.08) Entire pregnancy: 0.97 (0.85, 1.12) No asthma Preconception: 1.01 (0.98, 1.04) 1st trimester: 1.02 (0.99, 1.05) 2nd trimester: 0.97 (0.94, 1.00) Entire pregnancy: 0.94 (0.89, 1.01)

Table 7-8 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—hypertension disorders.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Hu et al. (2016) Florida, U.S. Ozone: NR Follow-up: 2005−2007 Cohort study	n = 655,529 birth records	Model, CMAQ hierarchical Bayesian Other	Mean: 1st trimester: 38.65 2nd trimester: 38.59 Other: 38.63 Median: 1st trimester: 37.91 2nd trimester: 36.95 Other: 5.33	Correlation (*r*):NR Copollutant models with: NR	Hypertensive disorders of pregnancy (each week of 1st two trimesters) 1st trimester: 1.08 (1.06, 1.12) 2nd trimester: 1.06 (1.04, 1.08) 1st and 2nd trimesters: 1.14 (1.10, 1.17) ORs elevated with ozone exposure at each week of pregnancy (1−26)
†Wu et al. (2011) Los Angeles and Orange counties, CA, U.S. Ozone: 1997−2006 Follow-up: NR Cohort study	n = 81,186 hospital-based birth database	Monitor Entire pregnancy	Mean: 36.5	Correlation (*r*): $PM_{2.5}$: −0.61; NO_2: −0.81; Other: −0.74 Copollutant models with: NR	Preeclampsia (OR) Los Angeles: 1.00 (0.74, 1.35) Orange: 1.46 (1.12, 1.90)

†Recent studies evaluated since the 2013 Ozone ISA.

Table 7-9 Epidemiologic studies of exposure to ozone and pregnancy/birth—diabetes.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Robledo et al. (2015) U.S. Ozone: 2001−2008 Follow-up: 2002−2008 Cohort study	Consortium on Safe Labor n = 220,264 12 clinical centers/19 hospitals	Model, modified CMAQ Other	Median: Other: 29.71 1st trimester: 29.21 75th: Other: 35.82 1st trimester: 35.17	Correlation (*r*): $PM_{2.5}$: −0.38; NO_2: −0.39; SO_2: −0.42 Copollutant models with: NR	Gestational diabetes (RR) Preconception, 91 days prior to last menstrual period: 0.94 (0.92, 0.97) 1st trimester: 1.00 (0.98, 1.02)
†Hu et al. (2015) Florida, U.S. Ozone: NR Follow-up: 2004−2005 Cohort study	n = 410,267 birth records	Model, CMAQ hierarchical Bayesian Other	Mean: 1st trimester: 37.22 2nd trimester: 37.54 Entire pregnancy: 37.84 Median: 1st trimester: 36.48 2nd trimester: 36.95 Entire pregnancy: 7.09	Correlation (*r*): $PM_{2.5}$: 0.39 Copollutant models with: NR	Gestational diabetes (OR) 1st trimester: 1.19 (1.14, 1.23) 2nd trimester: 1.25 (1.21, 1.30) Entire pregnancy: 1.39 (1.32, 1.46)

†Recent studies evaluated since the 2013 Ozone ISA.

Table 7-10 Epidemiologic studies of exposure to ozone and pregnancy/birth—fetal growth.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Coneus and Spiess (2012) Germany Ozone: 2002−2007 Follow-up: 2002−2007 Cohort study	German Socioeconomic Panel (SOEP)	Monitor Other	NR	Correlation (*r*): NR Copollutant models with: NR	Reports only betas and statistical significance. No evidence of association between ozone exposures during pregnancy or 1 mo before birth and birth length, fetal growth, or birth weight
†Lee et al. (2013) Pittsburgh, PA, U.S. Ozone: 1996−2002 Follow-up: 1997−2002 Cohort study	n = 34,702 Magee Obstetric Medical and Infant (MOMI) database	Model, space-time ordinary kriging 1st trimester	Median: 21.7 75th: 30.2 95th: 36.2 Maximum: 46.8	Correlation (*r*): $PM_{2.5}$: 0.5; Other: 0.7 Copollutant models with: NR	Small for gestational age (OR): 0.99 (0.91, 1.06)
†Ebisu and Bell (2012) Connecticut, Delaware, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, Virginia, Washington DC, and West Virginia, U.S. Ozone: 1999−2007 Follow-up: 2000−2007 Cohort study	n = 1,207,800 Birth records, term births	Monitor Entire pregnancy	Mean: 23	Correlation (*r*): $PM_{2.5}$: −0.12; NO_2: −0.77; Other: −0.28 Copollutant models with: NR	Low birth weight (percentage change per 7 ppb): −6.3 (−11, −1.3)
†Laurent et al. (2013) Los Angeles and Orange counties CA, U.S. Ozone: NR Follow-up: 1996−2006 Cohort study	n = 70,000 births Hospital-based obstetric database	Monitor Entire pregnancy	Mean: 35.66	Correlation (*r*): $PM_{2.5}$: −0.61; NO_2: −0.81; Other: −0.74 Copollutant models with: NR	Term birth weight (Δ g): −27.27 (−32.02, −22.51) Low birth weight (OR): 1.11 (1.02, 1.21)

Table 7-10 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—fetal growth.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Olsson et al. (2013) Stockholm, Sweden Ozone: 1997−2006 Follow-up: 1998−2006 Cohort study	n = 120,755 Swedish medical birth registry	Monitor 1st trimester	Mean: 35	Correlation (*r*): NO_2: −0.48 Copollutant models with: NO_2	Small for gestational age (OR): 0.98 (0.96, 1.02) Adjusted for NO_2: 0.98 (0.90, 1.06)
†Geer et al. (2012) Texas, U.S. Ozone: NR Follow-up: 1998−2004 Cohort study	n = 1,548,904 Birth records, 40 Texas counties	Monitor Entire pregnancy	Mean: 25.4	Correlation (*r*): NR Copollutant models with: NR	Term birth weight (Δ g): −4.61 (−7.34, −1.88)
†Ritz et al. (2014) New York City, NY, U.S. Ozone: 1993−1996 Follow-up: 1993−1996 Cohort study	Behavior in pregnancy study n = 688	Monitor Other	Mean: 40.2 Maximum: 96.1	Correlation (*r*): NR Copollutant models with: NR	Biparietal diameter (Δ mm) Estimated date of conception to 1st ultrasound date ~0−19 weeks gestation: 0.026 (−0.153, 0.199) 1st to 2nd ultrasound date (~19−29 weeks gestation): 0.041 (−0.104, 0.186) 2nd to 3rd ultrasound date (~29−37 weeks gestation): 0.012 (−0.149, 0.169)
†Laurent et al. (2014) Los Angeles County CA, U.S. Ozone: 2000−2008 Follow-up: 2001−2008 Cohort study	n = 960,945 Birth records, term births	Model, empirical Bayesian kriging based on monitor Entire pregnancy	Mean: 38.95	Correlation (*r*): NR Copollutant models with: NR	Low birth weight (OR): 0.99 (0.98, 1.00)

Table 7-10 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—fetal growth.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Gray et al. (2014) North Carolina, U.S. Ozone: 2001−2006 Follow-up: 2002−2006 Cohort study	n = 457,642 Birth records	CMAQ downscaler Entire pregnancy	Mean: 43.2	Correlation (*r*): NR Copollutant models with: NR	Birth weight (Δ g): −12.54 (−16.10, −8.81) Small for gestational age (OR): 1.76 (1.75, 1.80) Low birth weight (OR): 1.80 (1.75, 1.85)
†Vinikoor-Imler et al. (2014) North Carolina, U.S. Ozone: 2002−2005 Follow-up: 2003−2005 Cohort study	n = 322,981 Birth registry	Model, hierarchical Bayesian model CMAQ and monitor Other	Mean: 40.85 Median: 41.86 75th: 48.86 Maximum: 70.35	Correlation (*r*):NR Copollutant models with: NR	Birth weight (Δ g) 1st trimester: 1.56 (−2.52, 5.64) 2nd trimester: −7.24 (−12.35, −2.13) 3rd trimester: −22.84 (−28.05, −17.95) Low birth weight (RR) 1st trimester: 0.90 (0.85, 0.96) 2nd trimester: 0.87 (0.81, 0.94) 3rd trimester: 1.54 (1.43, 1.66) Small for gestational age (RR): 1st trimester: 1.02 (0.99, 1.04) 2nd trimester: 0.99 (0.96, 1.02) 3rd trimester: 1.09 (1.07, 1.13)
†Ha et al. (2014) Florida, U.S. Ozone: 2003−2005 Follow-up: 2004−2005 Case-control study	n = 423,719 Birth records, singleton live births	Model, CMAQ hierarchical Bayesian Other	Mean: 37.2 Median: 36.5 75th: 41 Maximum: 56.2	Correlation (*r*):NR Copollutant models with: $PM_{2.5}$	Low birth weight (OR) 1st trimester: 0.99 (0.96, 1.03) Adjusted for $PM_{2.5}$: 0.96 (0.89, 1.03) 2nd trimester: 0.97 (0.94, 1.01) Adjusted for $PM_{2.5}$: 1.02 (0.95, 1.09) 3rd trimester: 0.93 (0.89, 0.96) Adjusted for $PM_{2.5}$: 0.95 (0.89, 1.02) Entire pregnancy: 0.91 (0.86, 0.97) Adjusted for $PM_{2.5}$: 0.96 (0.89, 1.04)
†Smith et al. (2015) Texas, U.S. Ozone: NR Follow-up: 2002−2004 Cohort study	n = 565,703 Birth records	Model, CMAQ downscaler	Mean: NR	Correlation (*r*):NR Copollutant models with: NR	"Did not find a statistically significant relationship between 1st-trimester ozone and birth weight" "Negative association between fetal growth and large levels of ozone in the 2nd trimester"

Table 7-10 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—fetal growth.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Brown et al. (2015) New York City, NY, U.S. Ozone: 2001−2006 Follow-up: 2001−2006 Cohort study	n = 421,763 Birth records	Model, CMAQ hierarchical Bayesian Other	Median: 38.77 75th: 42.03 Maximum: 60.35	Correlation (*r*):NR Copollutant models with: NR	Term low birth weight (OR) 1st trimester 10.60−29.51: ref 29.51−39.28: 1.02 (0.96, 1.09) 39.29−47.35: 1.04 (0.98, 1.11) 47.36−66.33: 1.07 (0.99, 1.14) 2nd trimester 11.31−30.11: ref 31.12−40.12: 0.98 (0.92, 1.05) 40.13−47.43: 0.97 (0.91, 1.03) 47.44−65.73: 0.98 (0.92, 1.05) 3rd Trimester 5.08−30.35: ref 30.36−39.57: 0.95 (0.90, 1.02) 39.58−46.74: 0.90 (0.84, 0.96) 46.75−99.69: 0.95 (0.90, 1.02) Entire pregnancy 15.52−35.61: ref 35.62−38.77: 0.88 (0.83, 0.94) 38.78−42.03: 0.86 (0.81, 0.92) 42.04−60.35: 0.98 (0.92, 1.04)
†Capobussi et al. (2016) Como, Italy Ozone: NR Follow-up: 2005−2012 Cohort study	n = 27,128 Birth records	Monitor, within 5 km Entire pregnancy		Correlation (*r*):NR Copollutant models with: NR	Low birth weight (OR): 0.96 (0.85, 1.08) Small for gestational age (OR): 1.00 (0.95, 1.06)
†Lavigne et al. (2016) Ontario, Canada Ozone: 2002−2009 Follow-up: January 1, 2005−March 31, 2012 Cohort study	Better Outcomes Registry & Network Ontario n = 362,800 Singleton live births	Model Entire pregnancy	Mean: 27.8 Median: 28 95th: 33.05	Correlation (*r*): $PM_{2.5}$: −0.14; NO_2: −0.53 Copollutant models with: NR	Term low birth weight (OR): 1.17 (1.09, 1.24) Small for gestational age (OR): 1.24 (1.21, 1.28)

Table 7-10 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—fetal growth.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Yitshak-Sade et al. (2016) Negev, Israel Ozone: NR Follow-up: December 2011−April 2013 Cohort study	n = 959 Bedouin-Arab population in southern Israel, seminomadic	Monitor, IDW Other	Mean: 11	Correlation (*r*): NR Copollutant models with: NR	Birth weight (Δ g) Entire pregnancy: −0.01 (−0.01, 0.00) Last month: −0.03 (−0.06, −0.01) 3rd trimester: −0.11 (−0.18, −0.03)
†Laurent et al. (2016a) California, U.S. Ozone: 2000−2008 Follow-up: 2001−2008 Case-control study	n = 72,632 cases; × five controls Birth records	Model, empirical Bayesian kriging based on monitor Entire pregnancy	Mean: 39.55	Correlation (*r*): NR Copollutant models with: NR	Low birth weight (OR): 1.03 (1.02, 1.05)
†Tu et al. (2016) Atlanta, GA, U.S. Ozone: 2001 Follow-up: 2000 Cohort study	n = 105,818 Term births, birth records	Model, CMAQ downscaler 2001 annual average	Mean: 42.76 Maximum: 48.99	Correlation (*r*):NR Copollutant models with: NR	Ozone was a significant risk factor only in small parts of the state, and variations depend on different socioeconomic status and urbanicity of communities.

Table 7-10 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—fetal growth.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Carvalho et al. (2016) São Paulo, Brazil Ozone: NR Follow-up: April 2011−December 2013 Cohort study	Procriar n = 453 Pregnant women from three prenatal care units	Personal sampler 24-h avg Other—1 day during specified trimester	Mean: 4 Median: 4 Maximum: 7	Correlation (*r*):NR Copollutant models with: NO_2	All outcomes are log-transformed, and all ozone and NO_2 trimester exposures are in the same model Pulsatility index umbilical) (Δ log (index) 1st trimester: 0.07 (−0.13, 0.27) 2nd trimester: 1.56 (1.42, 1.70) 3rd trimester: −1.62 (−1.78, −1.46) Fetal weight (Δ log [g]) 1st trimester: 0.06 (−0.08, 0.18) 2nd trimester: 0.04 (−0.04, 0.12) 3rd trimester: 0.67 (0.57, 0.77) Birth weight (Δ log [g]) 1st trimester: −0.65 (−0.85, −0.45) 2nd trimester: 0.26 (0.14, 0.38) 3rd trimester: 0.25 (0.11, 0.39)
†Arroyo et al. (2016) Madrid, Spain Ozone: 2001−2009 Follow-up: 2001−2009 Time-series study	n = 470 weeks All live singleton births in Madrid	Monitors Other	Mean: 18 Median: NA 75th: NA Maximum: 38	Correlation (*r*): NR Copollutant models with: NR	Low birth weight (RR) Only reported statistically significant results Week 12 of gestation: 1.01 (1.00, 1.02)
†Díaz et al. (2016) Madrid, Spain Ozone: NR Follow-up: 2001−2009 Time-series study	Term births	Monitor Other	Mean: 17 Maximum: 38	Correlation (*r*): NR Copollutant models with: NR	Low birth weight (weekly) Reported only statistically significant effects, no associations reported for ozone

Table 7-10 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—fetal growth.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Michikawa et al. (2017a) Kyushu-Okinawa District, Japan Ozone: NR Follow-up: 2005−2010 Study	Japan Perinatal Registry Network n = 29,177	Monitor Other	Mean: 41.2 Median: 40 75th: 47.8	Correlation (*r*): NR Copollutant models with: NR	Adverse birth weight (small for gestational age and Low birth weight) (OR) Entire pregnancy: 1.06 (0.94, 1.19) 1st trimester: 1.07 (1.01, 1.14) Small for gestational age (OR) Entire pregnancy: 1.06 (0.96, 1.16) 1st trimester: 1.07 (1.01, 1.12)
†Smith et al. (2017) London, U.K. Ozone: NR Follow-up: 2006−2010 Cohort study	n = 540,365 Term births	Model, KCLurban Entire pregnancy	Mean: 16	Correlation (*r*): NR Copollutant models with: NO_2, $PM_{2.5}$	Low birth weight 0.92 (0.86, 0.98) Adjusted NO_2: 0.94 (0.86, 1.02) Adjusted for $PM_{2.5}$: 0.94 (0.88, 1.02) Small for gestational age (OR) 0.98 (0.96, 1.02)
†Fernando Costa Nascimento et al. (2017) São José do Rio Preto, Brazil Ozone: 2011−2013 Follow-up: 2012−2013 Cohort study	n = 8,948 births Birth records, term births singletons no birth defects	Monitor Other	Mean: 28 Median: 28 75th: 30 Maximum: 36	Correlation (*r*): NR Copollutant models with: NR	Low birth weight (OR) Reported elevated ORs for exposures 30, 60, and 90 days before delivery; exposure increment is unclear
†Chen et al. (2017b) Brisbane, Australia Ozone: 2002−2013 Follow-up: July 1 2003−December 31 2013 Cohort study	173,720 birth records	Monitors 24-h avg Other	Mean: 16.82 Median: 16.78 75th: 17.58 Maximum: 22.34	Correlation (*r*): $PM_{2.5}$: 0.27; NO_2: −0.04; SO_2: −0.04; Other: −0.16 Copollutant models with: NR	Low birth weight Entire pregnancy: 2.20 (1.74, 2.75) Adjusted for any copollutant: 1.21 (1.14, 1.29) Trimester effect estimates reported as figures

Table 7-10 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—fetal growth.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Reis et al. (2017) Volta Redonda and Rio de Janeiro, Brazil Ozone: NR Follow-up: 2003−2006 Cohort study	n = 13,660 birth records	Monitor Other	Mean: 30	Correlation (*r*):NR Copollutant models with: NR	Low birth weight Exposure increment not reported, ORs elevated from null reported for 2nd and 3rd trimester exposures but not 1st

†Recent studies evaluated since the 2013 Ozone ISA.

Table 7-11 Epidemiologic studies of exposure to ozone and pregnancy/birth—preterm birth.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Wu et al. (2011) Los Angeles and Orange counties, CA, U.S. Ozone: 1997−2006 Follow-up: NR Cohort study	n = 81,186 Hospital-based birth database	Monitor Entire pregnancy	Mean: 36.5	Correlation (*r*): $PM_{2.5}$: −0.61; NO_2: −0.81; Other: −0.74 Copollutant models with: NR	Very PTB (OR) Los Angeles: 0.94 (0.59, 1.54) Orange: 1.44 (0.72, 2.86) PTB (OR) Los Angeles: 1.00 (0.81, 1.21) Orange: 1.08 (0.86, 1.35)
†Olsson et al. (2012) Stockholm, Sweden Ozone: 1986−1995 Follow-up: 1987−1995 Cohort study	n = 115,588 Swedish medical birth registry	Monitor Other	Mean: 1st trimester: 29 2nd trimester: 29 Other: 30 Median: 1st trimester: 28 2nd trimester: 28 Other: 30	Correlation (*r*): NO_2: 1st trimester: −0.43; 2nd trimester: −0.39; Other: −0.26 Copollutant models with: NO_2	PTB (OR) 1st trimester 1.17 (1.06, 1.28) Adjusted for NO_2: 1.12 (1.00, 1.28) 2nd trimester 1.04 (0.94, 1.14) Adjusted for NO_2: 1.10 (0.96, 1.25) Last week of gestation 1.03 (0.94, 1.12) Adjusted for NO_2: 1.06 (0.94, 1.16) Gestational age (Δ weeks) 1st trimester: −0.12 (−0.16, −0.08) Adjusted for NO_2: −0.10 (−0.16, −0.04) 2nd trimester: −0.06 (−0.10, −0.02) Adjusted for NO_2: −0.14 (−0.20, −0.08) Last week of gestation: −0.06 (−0.09, −0.03) Adjusted for NO_2: −0.06 (−0.09, −0.03)

Table 7-11 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—preterm birth.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Lee et al. (2013) Pittsburgh, PA, U.S. Ozone: 1996−2002 Follow-up: 1997−2002 Cohort study	n = 34,702 Magee Obstetric Medical and Infant (MOMI) database	Model, space-time ordinary kriging 1st trimester	Median: 21.7 75th: 30.2 95th: 36.2 Maximum: 46.8	Correlation (*r*): $PM_{2.5}$: 0.5; Other: 0.7; Copollutant models with: NR	PTB (OR) 1.13 (1.01, 1.27)
†Olsson et al. (2013) Stockholm, Sweden Ozone: 1997−2006 Follow-up: 1998−2006 Cohort study	n = 120,755 Swedish medical birth registry	Monitor 1st trimester	Mean: 35	Correlation (*r*): NO_2: −0.48 Copollutant models with: NO_2	PTB (OR), 1st trimester: 1.08 (1.02, 1.17) Asthmatic mother: 1.17 (1.02, 1.32) Nonasthmatic mother: 1.08 (1.00, 1.17) Adjusted for NO_2: 1.10 (0.98, 1.23) Asthmatic mother: 1.17 (1.00, 1.37) Nonasthmatic mother: 1.10 (0.98, 1.23)
†Warren et al. (2012) Texas, U.S. Ozone: 2002−2004 Follow-up: 2002−2004 Cohort study	n = 32,170 observations Birth records, singleton live birth	Monitor and Model (fused CMAQ) Other		Correlation (*r*):NR Copollutant models with: NR	PTB, results presented as figures Effect estimates elevated from null with exposures in early weeks of pregnancy, and for 1st and 2nd trimester exposures.
†Schifano et al. (2013) Rome, Italy Ozone: 2001−2007 Follow-up: 2001−2010 Cohort study	n = 132,691 births Birth records	Monitor 8-h max	Median: 19 75th: 29 Maximum: 66	Correlation (*r*): NR Copollutant models with: NR	PTB (percentage increase), lag 1−2: 1.01 (0.94, 1.09)

Table 7-11 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—preterm birth.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Gray et al. (2014) North Carolina, U.S. Ozone: 2001−2006 Follow-up: 2002−2006 Cohort study	n = 457,642 Birth records	CMAQ downscaler Entire pregnancy	Mean: 43.2	Correlation (*r*):NR Copollutant models with: NR	PTB (OR): 1.03 (0.98, 1.07)

Table 7-11 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—preterm birth.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Ha et al. (2014) Florida, U.S. Ozone: 2003−2005 Follow-up: 2004−2005 Case-control study	n = 423,719 Birth records, singleton live births	Model, CMAQ hierarchical Bayesian Other	Mean: 1st trimester: 37.2 2nd trimester: 37.6 3rd trimester: 37.4 Entire pregnancy: 37.4 Median: 1st trimester: 36.5 2nd trimester: 37 3rd trimester: 37 Entire pregnancy: 37.9 75th: PTB: 1st trimester: 41 2nd trimester: 41.3 3rd trimester: 41.1 Entire pregnancy: 41 Maximum: PTB: 1st trimester: 56.2 2nd trimester: 57.3 3rd trimester: 69.2 Entire pregnancy: 51.3	Correlation (*r*):NR Copollutant models with: $PM_{2.5}$	Very PTB (OR) 1st trimester: 1.07 (1.02, 1.12) 2nd trimester: 1.09 (1.04, 1.14) 3rd trimester: 0.98 (0.93, 1.04) Entire pregnancy: 1.18 (1.10, 1.27) Adjusted for $PM_{2.5}$ 1st trimester: 1.16 (1.05, 1.28) 2nd trimester: 1.15 (1.05, 1.27) 3rd trimester: 0.97 (0.86, 1.08) Entire pregnancy: 1.25 (1.11, 1.40) PTB (OR) 1st trimester: 1.02 (1.00, 1.03) 2nd trimester: 1.03 (1.01, 1.05) 3rd trimester: 0.99 (0.97, 1.01) Entire pregnancy: 1.04 (1.01, 1.07) Adjusted for $PM_{2.5}$ 1st trimester: 1.06 (1.02, 1.10) 2nd trimester: 1.07 (1.03, 1.11) 3rd trimester: 0.98 (0.95, 1.02) Entire pregnancy: 1.08 (1.03, 1.13)

Table 7-11 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—preterm birth.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Hao et al. (2016) Georgia, U.S. Ozone: 2002−2006 Follow-up: January 1, 2002−February 28, 2006 Cohort study	n = 511,658 births Birth records	Model, CMAQ fused with monitors 8-h max Other	Median: 40.88 75th: 51.14 Maximum: 75.06	Correlation (*r*): $PM_{2.5}$: 0.47; NO_2: −0.19; SO_2: 0.63; Other: 0.7 Copollutant models with: NR	PTB (27−36 weeks, OR) 1st trimester: 1.006 (0.995, 1.017) 2nd trimester: 1.008 (0.997, 1.019) 3rd trimester: 0.992 (0.983, 1.002) Entire pregnancy: 1.012 (0.991, 1.036)
†Lin et al. (2015) Region NR, Taiwan Ozone: 2000−2007 Follow-up: 2001−2007 Case-control study	n = 86,224 cases; 344,896 controls Birth registry	Monitor 8-h max Other	Mean: 1st trimester: 42.74 2nd trimester: 48.43 3rd trimester: 48.98 75th: 1st trimester: 48.22 2nd trimester: 48.43 3rd trimester: 48.98 Maximum: 1st trimester: 77.68 2nd trimester: 77.68 3rd trimester: 86.55	Correlation (*r*): NO_2: −0.05; SO_2: 0.17; Other: 0.53 Copollutant models with: NR	PTB (OR) 1st trimester: 1.03 (1.02, 1.04) 2nd trimester: 1.02 (1.01, 1.02) 3rd trimester: 1.02 (1.01, 1.03)
†Qian et al. (2015) Wuhan, China Ozone: NR Follow-up: August 19, 2010−September 9, 2013 Cohort study	n = 95,911	Monitor Entire pregnancy	Mean: 38 Maximum: 74	Correlation (*r*): $PM_{2.5}$: −0.16; NO_2: −0.12; SO_2: −0.13; Other: −0.12 Copollutant models with: NR	PTB (OR): 2nd trimester: 1.08 (1.04, 1.12) Entire pregnancy: 1.10 (1.04, 1.14) Adjusted for $PM_{2.5}$: 1.08 (1.04, 1.14) Adjusted for NO_2: 1.10 (1.02, 1.17) Adjusted for SO_2: 1.08 (1.02, 1.14)

Table 7-11 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—preterm birth.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Schifano et al. (2016) Rome, Italy and Barcelona, Spain Ozone: NR Follow-up: Rome: 2001−2010 Barcelona: 2007−2012	n = Rome: 78,633 n = Barcelona: 27,255	Monitor 8-h avg Other	Mean: Rome: ~51 Barcelona: ~25	Correlation (*r*): NO_2: Rome: −0.1; Barcelona: −0.36 Other: Rome: 0.17; Barcelona: −0.19 Copollutant models with: NR	Length of gestation (HR) Lag 0−2 Rome: 1.01 (1.00, 1.02) Barcelona: 1.01 (1.00, 1.02)
†Capobussi et al. (2016) Como, Italy Ozone: NR Follow-up: 2005−2012 Cohort study	n = 27,128 Birth records	Monitor, within 5 km Entire pregnancy		Correlation (*r*):NR Copollutant models with: NR	PTB (OR) 0.99 (0.92, 1.06)
†Lavigne et al. (2016) Ontario, Canada Ozone: 2002−2009 Follow-up: January 1, 2005−March 31, 2012 Cohort study	Better Outcomes Registry & Network Ontario n = 362,800 Singleton live births	Model Entire pregnancy	Mean: 27.8 Median: 28 95th: 33.05	Correlation (*r*): $PM_{2.5}$: −0.14; NO_2: −0.53 Copollutant models with: NR	PTB (OR) 1.04 (1.01, 1.08) Asthma: 1.25 (1.07, 1.47) No asthma: 1.04 (1.00, 1.07) Diabetes: 0.89 (0.73, 1.08) No diabetes: 1.07 (1.00, 1.12) Preeclampsia: 1.00 (0.86, 1.16) No preeclampsia: 1.05 (1.01, 1.09)
†Wallace et al. (2016) U.S. Ozone: NR Follow-up: 2002−2008 Cohort study	Consortium on Safe Labor, Air Quality, and Reproductive Health Study n = 223,375 Singleton	Model, modified CMAQ Entire pregnancy	Mean: PROM: 28.5 0 h: 29.3 1 h: 28.9 2 h: 28.8 3 h: 28.8 4 h: 29	Correlation (*r*): NR Copollutant models with: NR	PROM (OR): 1.01 (0.94, 1.09) Lag before delivery 0 h: 1.03 (1.01, 1.05) 1 h: 1.05 (1.03, 1.07) 2 h: 1.05 (1.03, 1.07) 3 h: 1.05 (1.03, 1.07) 4 h: 1.04 (1.02, 1.05) PPROM (OR): 1.08 (0.94, 1.22)

Table 7-11 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—preterm birth.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Mendola et al. (2016a) U.S. Ozone: NR Follow-up: 2002−2008 Cohort study	Consortium on Safe Labor, Air Quality, and Reproductive Health Study n = 223,502 singleton pregnancies Recruited from 12 centers (19 hospitals) across the U.S.	Model, modified CMAQ Other	Mean: 29.65 Median: 37.3	Correlation (*r*): NR Copollutant models with: NR	PTB Elevated ORs with ozone exposure for early PTB (<34 weeks) in mothers without asthma for exposures during Weeks 8−14 and 15−21
†Symanski et al. (2016) Houston, Harris County, TX, U.S. Ozone: NR Follow-up: 2005−2007 Case-control study	n = 10,459 cases; 152,214 singleton births	Monitor Other	Mean: 37.6 Median: 33.7 75th: 47.5 90th: 62.4	Correlation (*r*): NR Copollutant models with: NR	Severe PTB (20−28 weeks) (OR) Weeks 1−4: 0.83 (0.74, 0.94) Weeks 5−8: 0.88 (0.78, 1.01) Weeks 9−12: 0.95 (0.84, 1.09) Weeks 13−16: 1.05 (0.92, 1.19) Weeks 17−20: 1.21 (1.08, 1.36) Entire pregnancy: 1.16 (0.82, 1.62) Moderate PTB (29−32 weeks) (OR) Weeks 1−4: 0.93 (0.85, 1.03) Weeks 5−8: 1.00 (0.89, 1.13) Week 9−12: 1.09 (0.98, 1.22) Weeks 13−16: 1.03 (0.93, 1.15) Weeks 17−20: 1.13 (1.02, 1.25) Weeks 21−24: 1.03 (0.92, 1.16) Weeks 25−28: 1.15 (1.04, 1.27) Entire pregnancy: 1.31 (0.99, 1.74) Late PTB (33−36 weeks) (OR) Weeks 1−4: 0.99 (0.95, 1.02) Weeks 5−8: 1.01 (0.97, 1.05) Weeks 9−12: 1.04 (1.00, 1.08) Weeks 13−16: 1.03 (0.99, 1.07) Weeks 17−20: 1.08 (1.04, 1.12) Weeks 21−24: 1.05 (1.01, 1.09) Weeks 25−28: 1.07 (1.03, 1.10) Weeks 29−32: 1.02 (0.98, 1.05) Entire pregnancy: 1.21 (1.10, 1.32)

Table 7-11 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—preterm birth.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Laurent et al. (2016b) California, U.S. Ozone: 2000−2008 Follow-up: 2001−2008 Case-control study	n = 442,314 cases; × two controls Birth records	Model, empirical Bayesian kriging based on monitor Entire pregnancy	Mean: 39.71	Correlation (*r*): $PM_{2.5}$: −0.24; NO_2: −0.07 Copollutant models with: NR	PTB (OR) 1.08 (1.07, 1.09) Adjusted for $PM_{2.5}$: 1.09 (1.08, 1.10) Adjusted for NO_2: 1.08 (1.07, 1.09)
†Arroyo et al. (2016) Madrid, Spain Ozone: 2001−2009 Follow-up: 2001−2009 Time-series study	n = 470 weeks All live singleton births in Madrid	Monitors Other	Mean: 18 Median: NA 75th: NA Maximum: 38	Correlation (*r*): NR Copollutant models with: NR	PTB (OR) Week 12: 1.02 (1.01, 1.03) Only significant results reported

Table 7-11 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—preterm birth.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Chen et al. (2017b) Brisbane, Australia Ozone: 2002−2013 Follow-up: July 1 2003−Dec 31 2013 Cohort study	173,720 birth records	Monitors 24-h avg	Mean: Entire pregnancy: 16.82 1st trimester: 16.82 2nd trimester: 16.76 3rd trimester: 16.91 Median: Entire pregnancy: 16.78 1st trimester: 16.09 2nd trimester: 16.21 3rd trimester: 16.12 75th: Entire pregnancy: 17.58 1st trimester: 19.02 2nd trimester: 18.67 3rd trimester: 19.1 Maximum: Entire pregnancy: 22.34 1st trimester: 23.55 2nd trimester: 24.35 3rd trimester: 28.21	Correlation (*r*): $PM_{2.5}$: 0.27 NO_2: −0.04; SO_2: −0.04; Other: −0.16 Copollutant models with: NR	PTB (HR) Entire pregnancy: 2.20 (1.85, 2.61) Adjusted for $PM_{2.5}$, NO_2, or SO_2: 1.21 (1.14, 1.29) Elevated HRs for exposures during each trimester reported as figures

Table 7-11 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—preterm birth.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Dastoorpoor et al. (2017) Khuzestan Province, Iran Ozone: March 2008−March 2015 Follow-up: March 2008−March 2015 Time-series study	n = 49,173 births Ahvaz Imam Khomeini Hospital	Monitor 24-h avg	Mean: 32 Median: 24 Maximum: 3,316	Correlation (*r*):NR Copollutant models with: NR	PTB (RR) Cumulative lag 0−14: 0.98 (0.93, 1.03) Lag 0: 1.00 (0.99, 1.01) Lag 1: 1.00 (0.99, 1.01) Lag 2: 1.00 (1.00, 1.01)
†Smith et al. (2015) Texas, U.S Ozone: NR Follow-up: 2002−2004 Cohort study	n = 565,703 Birth records	Model, CMAQ downscaler	Mean: NR	Correlation (*r*):NR Copollutant models with: NR	2nd-trimester ozone exposure increases (low to middle or middle to high) were negatively associated with gestational age in south and east Texas. 1st-trimester ozone was negatively associated with gestational age in southeast Texas

†Recent studies evaluated since the 2013 Ozone ISA.

Table 7-12 Epidemiologic studies of exposure to ozone and pregnancy/birth—birth defects.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Outcomes Examined
†Dadvand et al. (2011) Northeast England Ozone: 1993−2003 Follow-up: Case-control study	Northern Congenital Abnormality Survey n pooled cases = 2,140; controls = 14,256	Monitor Weeks 3−8	Mean: NR	Correlation (*r*):NR Copollutant models with: NR	Congenital malformations of cardiac chambers and connections Congenital malformations of cardiac septa Congenital malformations of pulmonary and tricuspid valves Congenital malformations of aortic and mitral valves Congenital malformations of great arteries and veins Ventricular septal defect Atrial septal defect Congenital pulmonary valve stenosis Tetralogy of Fallot Coarctation of aorta
†Padula et al. (2013) San Joaquin Valley, CA, U.S. Ozone: 1997−2006 Follow-up: October 1997−December 2006 Case-control study	National Birth Defects Prevention Study, CA n = 1,651 subjects	Monitor 8-h max 1st 2 mo of pregnancy	Median: 46.95 75th: 65.65 Maximum: 91.92	Correlation (*r*): $PM_{2.5}$: −0.61; NO_2: −0.35; Other: −0.71 Copollutant models with: NR	Neural tube defects Anencephaly Spina bifida Cleft lip with or without cleft palate Cleft palate only Gastroschisis
†Agay-Shay et al. (2013) Israel Ozone: 1999−2006 Follow-up: 2000−2006 Case-control study	Israel National Birth and Birth Defect Registry	Monitor, within 10 km, inverse distance weighing Weeks 3−8	Mean: 25.1 Median: 26.5 75th: 39.1 Maximum: 128	Correlation (*r*): NR Copollutant models with: NR	Multiple congenital heart defects Atrial and atrial septal defects Isolated ventricular septal defects Patent ductus arteriosus (BW > 2,500 g)

Table 7-12 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—birth defects.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Outcomes Examined
†Vinikoor-Imler et al. (2013) North Carolina, U.S. Ozone: 2002−2005 Follow-up: 2003−2005 Cohort study	n = 322,969 Birth defect surveillance, birth registry	Model, hierarchical Bayesian model CMAQ and monitor Weeks 3−8	Mean: 40.74 Median: 42.15 Maximum: 74.99	Correlation (*r*):NR Copollutant models with: NR	Spina bifida Hydrocephalus Anophthalmia/microphthalmia Congenital cataract Microtia/anotia Transposition of great vessels Tetralogy of Fallot Ventricular septal defect Atrial septal defect Endocardial cushion defect/atrioventricular septal defect Pulmonary valve atresia/stenosis Tricuspid valve atresia/stenosis Aortic valve stenosis Hyperplastic left heart syndrome Coarctation of aorta Cleft palate Cleft lip with or without cleft palate Esophageal atresia/tracheoesophageal fistula Anorectal atresia/stenosis Pyloric stenosis Renal agenesis Obstructive genitourinary defect Hypospadias Deficiency defect—upper limbs Deficiency defect—lower limbs Gastroschisis Omphalocele Diaphragmatic hernia

Table 7-12 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—birth defects.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Outcomes Examined
†Stingone et al. (2014) Arkansas, Iowa, Massachusetts, California, Georgia, New York, North Carolina, Texas, and Utah, U.S. Ozone: 1997−2006 Follow-up: October 1, 1997−December 31, 2006 Case-control study	National Birth Defects Prevention Study n = 3,328 cases; 4,632 controls	Monitor Weeks 2−8	Mean: 42.9 90th: 51.8	Correlation (*r*):NR Copollutant models with: NR	Cardiovascular birth defects Left ventricular outflow tract obstructions Aortic stenosis Coarctation of the aorta Hypoplastic left heart syndrome D-transposition of the great arteries Tetralogy of Fallot Other conotruncals Common truncus Other double-outlet right ventricle with transposition of the great arteries or not (other) Interrupted aortic arch type B or not otherwise specified Conoventricular septal defects Anomalous pulmonary venous return Total anomalous pulmonary venous return Atrioventricular septal defect Right ventricular outflow tract obstructions Pulmonary/tricuspid atresia Pulmonary atresia Tricuspid atresia Pulmonary valve stenosis Ebstein's anomaly Septal defects Perimembranous ventricular septal defects Muscular-muscular ventricular septal defects Atrial septal defect

Table 7-12 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—birth defects.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Outcomes Examined
†Lin et al. (2014b) Taiwan Ozone: 2000−2007 Follow-up: 2001−2007 Case-control study	n = 1,687 cases, 10× controls Birth registry, isolated cases	Monitor Weeks 1−4 Weeks 5−8 Weeks 9−12	Mean: 41	Correlation (*r*): $PM_{2.5}$: 0.52; NO_2: −0.07; SO_2: 0.18; Other: −0.17 Copollutant models with: NR	Limb defects Syndactyly Polydactyly Reduction deformities of limb
†Jurewicz et al. (2014) Poland Ozone: NR Follow-up: NR Cohort study	Environmental factors and male infertility n = 212 men Men attending an infertility clinic for diagnostic purposes	Monitor 90 days before sample collection	Mean: 23 Median: 23 Maximum: 41	Correlation (*r*): $PM_{2.5}$: −0.41; NO_2: −0.44; SO_2: −0.26; Other: −0.43 Copollutant models with: NR	Sperm chromosomal disomy
†Farhi et al. (2014) Israel Ozone: NR Follow-up: 1997−2004 Cohort study	n = 216,730 infants; 207,825 spontaneous conceptions, 8,905 assistive reproductive technology conceptions Birth records	Monitor, kriging 1st trimester	Mean: 1st trimester: 32.4 2nd trimester: 32.7 Entire pregnancy: 32.1 Median: 1st trimester: 32.3 2nd trimester: 32.6 Entire pregnancy: 31.3 75th: 1st trimester: 36.1 2nd trimester: 36.5 Entire pregnancy: 34.6 Maximum: 1st trimester: 54.4 2nd trimester: 54.9 Entire pregnancy: 50	Correlation (*r*):NR Copollutant models with: NR	Total birth defects

Table 7-12 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—birth defects.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Outcomes Examined
†Vinikoor-Imler et al. (2015) Texas, U.S. Ozone: NR Follow-up: 2002−2006 Case-control study	21,060 cases; 1,401,611 controls Birth defect registry	Model, hierarchical Bayesian model CMAQ and monitor 1st trimester	Mean: Texas: 40.3 NBDPS: 37.2 Median: Texas: 40.5 NBDPS: 34.9 75th: Texas: 46.8 NBDPS: 43.4 Maximum: Texas: 65.1 NBDPS: 62.3	Correlation (*r*): NR Copollutant models with: NR	Total birth defects
†Hwang et al. (2015) Taiwan Ozone: NR Follow-up: 2001−2007 Case-control study	n = 1,087 cases; 10,870 controls Birth records	Monitor 1st trimester	Mean: 44.53 Median: 44.14	Correlation (*r*): NR Copollutant models with: NR	Ventricular septal defects Atrial septal defects Patent ductus arteriosus Pulmonary artery and valve Tetralogy of Fallot Transposition of the great arteries Conotuncal defects
†Zhang et al. (2016) Wuhan, China Ozone: 2010−2012 Follow-up: June 10, 2011–June 9, 2013 Cohort study	n = 105,988 births	Central site monitor, nearest 8-h avg 1st trimester	Mean: 37 75th: 54	Correlation (*r*): NO_2: −0.12; SO_2: −0.16; Other: −0.2 Copollutant models with: NO_2, SO_2, CO	Congenital heart defects Ventricular septal defect Tetralogy of Fallot

Table 7-12 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—birth defects.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Outcomes Examined
†Zhou et al. (2016) Arizona, Florida, New York (excluding NYC), and Texas, U.S. Ozone: 2001−2007 Follow-up: 2001−2007 Case-control study	NBDPS n = 7,035 cases; 4,697,523 total live births	Model, CMAQ downscaler 8-h max 5−10 weeks	Mean: All oral clefts: 40.9 Cleft lip with/without palate: 40.9 Cleft palate: 40.7 Median: All oral clefts: 40.3 Cleft lip with/without palate: 40.5 Cleft palate: 40.1 75th: All oral clefts: 48.1 Cleft lip with/without palate: 48 Cleft palate: 48.1 Maximum: All oral clefts: 69 Cleft lip with/without palate: 69 Cleft palate: 64.9	Correlation (*r*):NR Copollutant models with: NR	All oral clefts Cleft palate Cleft lip with/without palate

†Recent studies evaluated since the 2013 Ozone ISA.

Table 7-13 Epidemiologic studies of exposure to ozone and pregnancy/birth—infant and fetal mortality.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Hwang et al. (2011) Taiwan Ozone: NR Follow-up: 2001−2007 Case-control study	n = 9,325 stillbirths; 93,250 controls Birth records	Monitor 1st trimester	Mean: 35.93 Maximum: 61.27	Correlation (*r*): NO_2: −0.31; SO_2: 0.13; Other: 0.62 Copollutant models with: NR	Stillbirth (OR) 1st trimester: 1.01 (0.96, 1.06) 2nd trimester: 0.96 (0.91, 1.01) 3rd trimester: 0.98 (0.93, 1.04) Entire pregnancy: 0.97 (0.91, 1.04)
†Moridi et al. (2014) Tehran, Iran Ozone: NR Follow-up: June 2010−February 2011 Case-control study	n = 148 cases; 148 controls	Monitor 1st trimester	Mean: 22.29−28.88	Correlation (*r*): NR Copollutant models with: NR	Spontaneous abortion before 14 weeks of pregnancy (OR): 2.43 (1.72, 3.42)
†Green et al. (2015) California, U.S. Ozone: NR Follow-up: 1999−2009 Cohort study	n = 13,999 stillbirths; 3,012,270 livebirths Birth records	Monitor 1st trimester	Mean: 48.48 Median: 47.27 75th: 55.52	Correlation (*r*):NR Copollutant models with: NR	Stillbirth (OR) 1st trimester: 1.00 (0.98, 1.02) 2nd trimester: 1.01 (0.99, 1.03) third pregnancy: 1.03 (1.01, 1.05) Entire pregnancy: 1.01 (0.99, 1.04)
†Arroyo et al. (2016) Madrid, Spain Ozone: 2001−2009 Follow-up: 2001−2009 Time-series study	n = 470 weeks All live singleton births in Madrid	Monitors Other	Mean: 18 Median: NA 75th: NA Maximum: 38	Correlation (*r*): $PM_{2.5}$: NR; NO_2: NR; SO_2: NR Copollutant models with: NR	Late fetal death (<24 h) Only reports statistically significant results, examined exposure during each week of pregnancy Week 24: 1.33 (1.32, 1.35)

Table 7-13 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—infant and fetal mortality.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Mendola et al. (2017) U.S. Ozone: NR Follow-up: 2002−2008 Cohort study	Consortium on Safe Labor n = 223,375 singleton pregnancies Recruited from 12 centers (19 hospitals) across the U.S.	Model, modified CMAQ Other	Mean: entire pregnancy: 29.3 1st trimester: 29 Lag-0: 29.9 Lag-1: 30 Lag-2: 30.1 Lag-3: 30.1 Lag-4: 30.1 Lag-5: 30.1 Lag-6: 30 Lag-7: 29.9 Median: entire pregnancy: 28.5 1st trimester: 29.2 75th: entire pregnancy: 32.7 1st trimester: 35.2 95th: entire pregnancy:7.8 1st trimester: 12.3 Lag-0: 17.9 Lag-1: 17.8 Lag-2: 17.7 Lag-3: 17.7 Lag-4: 17.7 Lag-5: 17.7 Lag-6: 17.8 Lag-7: 17.8 Maximum: entire pregnancy: 46.4 1st trimester: 48.7	Correlation (*r*): NR Copollutant models with: NR	Stillbirth (OR) Entire pregnancy: 1.53 (1.06, 2.19) Asthma: 1.29 (0.76, 2.20) No asthma: 1.33 (0.89, 1.97) 1st trimester: 1.14 (1.00, 1.31) Lag 0: 1.07 (0.97, 1.20) Lag 1: 1.07 (0.96, 1.19) Lag 2: 1.13 (1.01, 1.26) Lag 3: 1.11 (1.00, 1.24) Lag 4: 1.10 (0.99, 1.22) Lag 5: 1.18 (1.06, 1.31) Lag 6: 1.15 (1.03, 1.27) Lag 7: 1.12 (1.01, 1.25)

Table 7-13 (Continued): Epidemiologic studies of exposure to ozone and pregnancy/birth—infant and fetal mortality.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Dastoorpoor et al. (2017) Khuzestan Province, Iran Ozone: March 2008−March 2015 Follow-up: March 2008−March 2015 Time-series study	n = 49,173 births Ahvaz Imam Khomeini Hospital	Monitor 24-h avg Other	Mean: 32 Median: 24 Maximum: 3,316	Correlation (*r*): NR Copollutant models with: NR	Spontaneous abortion (OR) Lag 0−14: 0.99 (0.95, 1.03) Lag 0: 1.00 (1.00, 1.01) Lag 1: 1.00 (0.99, 1.00) Lag 2: 1.00 (0.99, 1.00) Stillbirth (OR) Lag 0−14: 0.89 (0.83, 0.96) Lag 0: 0.99 (0.97, 1.01) Lag 1: 0.99 (0.97, 1.00) Lag 2: 0.99 (0.98, 1.00)
†Ha et al. (2017b) Michigan and Texas, U.S. Ozone: 2005−2009 Follow-up: 2005−2009 Cohort study	Longitudinal Investigation of Fertility and the Environment n = 343 Couples trying to get pregnant followed through pregnancy	Model, modified CMAQ 24-h avg Other	Median: 25 75th: 29.5 Maximum: 42.6	Correlation (*r*): $PM_{2.5}$: −0.25; NO_2: −0.42; SO_2: −0.04 Copollutant models with: NR	Pregnancy loss, 1st observed (HR) gestational week of loss (e.g., 5, 6, etc.): 1.00 (0.95, 1.05) Week before loss: 1.05 (0.98, 1.11) Entire pregnancy: 1.15 (1.08, 1.21)
†Yang et al. (2018) Wuhan, China Ozone: NR Follow-up: June 10, 2011−June 9, 2013 Cohort study	n = 95,354	Monitor Entire pregnancy	Mean: 38 Median: 38 75th: 73 Maximum: 76	Correlation (*r*): $PM_{2.5}$: −0.126; NO_2: −0.698; SO_2: −0.468; Other: −0.499 Copollutant models with: NR	Stillbirth (OR): Entire pregnancy: 0.85 (0.71, 1.02) 1st mo: 0.88 (0.35, 2.25) 2nd mo: 1.00 (0.90, 1.10) 3rd mo: 1.14 (0.42, 3.13) 1st trimester: 1.00 (0.88, 1.12) 2nd trimester: 1.02 (0.92, 1.14) 3rd trimester: 0.72 (0.64, 0.81)

†Recent studies evaluated since the 2013 Ozone ISA.

Table 7-14 Epidemiologic studies of exposure to ozone and developmental effects.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Peel et al. (2011) Atlanta, GA, U.S. Ozone: August 1, 1998−December 31, 2002 Follow-up: August 1, 1998−December 31, 2002 Cohort study	n = 4,277 infants Apnea Center of Children's Healthcare of Atlanta at Egleston, children at high risk for cardiorespiratory events	Monitor 8-h max	Mean: 43.9 Median: 39.6 90th: 78 Maximum: 130.8	Correlation (*r*): $PM_{2.5}$: 42; NO_2: 0.45; SO_2: −0.11; Other: 0.48 Copollutant models with: NR	Apnea (OR) Lag 0−1: 1.03 (0.99, 1.07) Bradycardia (OR) Lag 0−1: 1.04 (1.02, 1.06)
†Coneus and Spiess (2012) Nationally representative sample, Germany Ozone: 2002−2007 Follow-up: 2002−2007 Cohort study	German Socioeconomic Panel (SOEP)	Monitor Other	NR	Correlation (*r*): NR Copollutant models with: NR	No correlation between mean Ozone exposure early life and bronchitis, croup syndrome, respiratory disease or other disorders at 2−3 yr of age.
†Breton et al. (2012) Southern California, U.S. Ozone: 1980−2009 Follow-up: 2007−2009 Cohort study	Testing Responses on Youth (TROY) n = 768 College students	Monitors, inverse distance squared weighing, 4 within 50 km 24-h avg	0−5 yr: Mean: 23.1 Maximum: 41.8	Correlation (*r*): $PM_{2.5}$: 0.09; NO_2: 0.09; SO_2: NR; Other: 0.18 Copollutant models with: NR	Carotid artery intima-media thickness (Δ μm), 0−5 yr of age exposure 7.8 (−0.3, 15.9) NO_2 adjusted: 10.0 (1.4, 18.6) PM_{10} adjusted: 8.5 (0.2, 16.9) $PM_{2.5}$ adjusted: 9.1 (0.9, 17.4)
†Volk et al. (2013) California, U.S. Ozone: 1997−2008 Follow-up: 2002−2011 Case-control study	CHARGE n = 524 mother-child pairs	Monitor 8-h max Other		Correlation (*r*): NR Copollutant models with: NR	Autism (OR) 1st trimester: 1.05 (0.97, 1.20) 2nd trimester: 1.02 (0.90, 1.17) 3rd trimester: 1.02 (0.89, 1.15) Entire pregnancy: 1.05 (0.84, 1.31) 1st yr of life: 1.09 (0.82, 1.47)

Table 7-14 (Continued): Epidemiologic studies of exposure to ozone and developmental effects.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Becerra et al. (2013) Los Angeles, CA, U.S. Ozone: 1995−2006 Follow-up: 1998−2009 Case-control study	n = 7,603 cases; 75,782 controls Mothers who gave birth in Los Angeles, CA to children diagnosed with ASD at 3−5 yr old during 1998−2009	Monitors 8-h avg Entire pregnancy	Mean: 36.8 Median: NA 75th: NA	Correlation (*r*): $PM_{2.5}$: −0.47; NO_2: −0.33; SO_2: NR; Other: −0.55 Copollutant models with: NR	Autism (OR) 1.05 (1.01, 1.10) Adjusted for PM_{10}: 1.05 (1.01, 1.10) Adjusted for $PM_{2.5}$: 1.10 (1.05, 1.16) Adjusted for NO: 1.07 (1.03, 1.12) Adjusted for NO_2: 1.07 (1.03, 1.12) >High school: 1.03 (0.99, 1.08) High school: 1.06 (1.01, 1.12) <High school): 1.08 (1.02, 1.14)
†Nishimura et al. (2013) Chicago, IL; Bronx, NY; Houston, TX; San Francisco Bay Area, CA; and Puerto Rico, U.S. Ozone: NR Follow-up: 2006−2011 Case-control study	GALA II and SAGE II n = 2,291 cases; 2,029 controls	Monitor 8-h max 1-h max 1st yr of life	Mean: 8-h: 27.6 1-h: 34.3 Median: 8-h: 27.3 1-h: 33.8 75th: 8-h: 30.9 1-h: 37.5	Correlation (*r*): NR Copollutant models with: NR	Asthma (OR), 1st yr of life 8-h max: 0.90 (0.76, 1.10) 1-h max: 0.94 (0.81, 1.12)

Table 7-14 (Continued): Epidemiologic studies of exposure to ozone and developmental effects.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Fuertes et al. (2013a) Canada, Sweden, Germany Ozone: NR Follow-up: recruitment 1994−1999 Cohort study	Traffic, Asthma, and Genetics (TAG), includes Canadian Asthma Primary Prevention Study (CAPPS), the Study of Asthma, Genes, and the Environment (SAGE), the Children, Allergy, Milieu, Stockholm, Epidemiological (BAMSE) survey, the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study, German Infant Nutritional Intervention (GINIplus) study, and the Lifestyle related factors, Immune System and the development of Allergies in Childhood (LISAplus) study n = 15,299 children Six birth cohorts from Canada and Europe	Model, APMoSPHERE (1 × 1 km) For 2001		Correlation (*r*): $PM_{2.5}$: −0.18; NO_2: −0.25; Other: −0.15 Copollutant models with: NR	Allergic rhinitis (OR) 0.83 (0.59, 1.17) Aeroallergen sensitization (OR) 0.90 (0.66, 1.25)

Table 7-14 (Continued): Epidemiologic studies of exposure to ozone and developmental effects.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†MacIntyre et al. (2014) Europe and Canada Ozone: NR Follow-up: recruitment 1994−1997 Other study	The traffic, asthma and genetics (TAG) study n = 5,115 Metacohort: combination of several cohorts: Canadian Asthma Primary Prevention Study (CAPPS), Study of Asthma, Genes, and the Environment (SAGE), Children, Allergy, Milieu, Stockholm, Epidemiological Survey (BAMSE), German Infant Nutrition Intervention (GINI) study plus environmental and genetic influences on allergy, Influence of Life-Style Factors on the Development of the Immune System and Allergies in East and West Germany plus the influence of traffic emissions and genetics (LISA), Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study	Model for European populations, APMoSPHERE (Air Pollution Modelling for Support to Policy on Health and Environmental Risks in Europe); monitor for Canadian populations using inverse distance weighting of the closest three ambient monitors (within 50 km) 1st yr of life	Mean: 19 Maximum: 28	Correlation (*r*): NO_2: −0.19 Copollutant models with: NR	Current asthma (OR): 0.66 (0.26, 1.61) Ever asthma (OR): 0.74 (0.44, 1.28) Ever wheeze (OR): 0.81 (0.55, 1.21) Ever asthma and current wheeze (OR): 1.02 (0.38, 2.79) Current wheeze (OR): 1.66 (0.77, 3.53)

Table 7-14 (Continued): Epidemiologic studies of exposure to ozone and developmental effects.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Fuertes et al. (2013b) Germany Ozone: NR Follow-up: recruitment 1995−1998 Cohort study	GINIplus and LISAplus n = 6,604 children Birth cohort full term normal weight	APMoSPHERE models, only 2001 Other	Mean: 22 Median: 22 75th: 23 Maximum: 30	Correlation (*r*): NR Copollutant models with: NR	Eyes and nose symptoms (OR): 0.90 (0.63, 1.29) Aeroallergen sensitization (OR): 0.97 (0.68, 1.33) Allergic rhinitis (OR): 1.07 (0.70, 1.64) Asthma (OR): 1.84 (0.93, 3.69)
†Volk et al. (2014) California, U.S. Ozone: 1997−2009 Follow-up: Case-control study	Childhood Autism Risk from Genetics and the Environment Study n = 252 cases; 156 controls	Monitor Entire pregnancy	Mean: NR	Correlation (*r*): NR Copollutant models with: NR	Autism Spectrum Disorder (OR) <41.8 ppb ozone, CG/GG SNP: reference <41.8 ppb ozone, CC SNP: 1.0 (0.59, 1.9) ≥41.8 ppb ozone, CG/GG SNP: 1.2 (0.67, 2.2) ≥41.8 ppb ozone, CC SNP: 0.95 (0.45, 2.2)
†Lin et al. (2014a) Taiwan Ozone: NR Follow-up: October 2003−January 2004, recruitment Cohort study	Taiwan Birth Cohort Pilot Study n = 511 mother-child pairs	Monitor Other	Mean: 31−39	Correlation (*r*): NR Copollutant models with: NR	No associations between ozone exposure at any time period (1st trimester, 2nd and 3rd trimesters, birth−12 mo, or 13−18 mo) and neurodevelopmental scores (gross motor, fine motor, language, and social-personal)
†Orione et al. (2014) São Paulo, Brazil Ozone: NR Follow-up: August 2011−August 2012 Case-control study	n = 20 cases; 56 controls Cases from Pediatric Rheumatology Unit of the Children's Institute	Monitor Entire pregnancy	Mean: NR	Correlation (*r*): NR Copollutant models with: NR	Juvenile dermatomyositis (OR) No association with ozone for entire pregnancy exposures, some inconsistent associations with trimester specific exposures (e.g., elevated OR for middle tertile exposure in 1st trimester)

Table 7-14 (Continued): Epidemiologic studies of exposure to ozone and developmental effects.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†van Rossem et al. (2015) Boston, MA, U.S. Ozone: NR Follow-up: recruited April 1999−July 2002 Cohort study	Project Viva n = 1,131 mother-infant pairs recruited from eight urban and suburban observation offices	Monitor Other	Median: 15−24	Correlation (*r*): NR Copollutant models with: NR	Increases in newborn systolic blood pressure with 1st and 2nd trimester ozone increases (13.0 and 12.8 ppb, respectively), and decreases with 3rd trimester exposure (13.6 ppb). Decreases in blood pressure with exposure lagged from birth up to 90 days.
†Malmqvist et al. (2015) Scania, Sweden Ozone: NR Follow-up: 1999−2013 Cohort study	Skane study (1999−2005), Better Diabetes Diagnosis n = 262 cases; 682 controls	Monitor 1st trimester	Median: 26.5 75th: 30.6	Correlation (*r*): NR Copollutant models with: NR	Type I diabetes 1st trimester <22 ppb: reference 22−26.5 ppb: 1.18 (0.69, 2.04) 26.6−30.6 ppb: 1.26 (0.71, 2.24) >30.6 ppb: 1.52 (0.88, 2.61) 2nd trimester <22 ppb: ref 22−26.5 ppb: 1.36 (0.82, 2.26) 26.6−30.6 ppb: 1.48 (0.86, 2.54) >30.6 ppb: 1.62 (0.99, 2.65) 3rd trimester <22 ppb: ref 22−26.5 ppb: 0.87 (0.52, 1.79) 26.6−30.6 ppb: 1.1 (0.67, 1.79) >30.6 ppb: 0.79 (0.49, 1.27)
†Huang et al. (2015) Taiwan Ozone: 2004−2006 Follow-up: 2005 Cohort study	Taiwan Birth Cohort Study n = 16,686	Monitor, kriging 1st trimester	Mean: 27.9 Median: 27.5 75th: 32.5	Correlation (*r*): NR Copollutant models with: NR	Atopic dermatitis at 6 mo (OR) 1st trimester: 1.05 (0.90, 1.22) 2nd trimester: 1.16 (0.98, 1.37) 3rd trimester: 1.13 (0.94, 1.35) Entire pregnancy: 1.09 (0.92, 1.28) 3 mo post-birth: 1.00 (0.84, 1.20) Entire pregnancy + 3 mo post-birth: 1.13 (0.86, 1.47)

Table 7-14 (Continued): Epidemiologic studies of exposure to ozone and developmental effects.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Breton et al. (2016) California, U.S. Ozone: NR Follow-up: Kindergarten (2002−2003) through age 11 Cohort study	Children's Health Study n = 459 Children enrolled in kindergarten or first grade from public schools	Monitors, IDW2, four within 50 km Other	Mean: NR Median: NA 75th: NA	Correlation (*r*): $PM_{2.5}$: 0.41; NO_2: 0.01; Other: 0.7 Copollutant models with: NR	1st trimester Left CIMT (Δ mm): −0.00 (−0.00, 0.00) Right CIMT: −0.00 (−0.00, 0.00) Systolic BP (Δ mm Hg): −0.14 (−0.53, 0.25) Diastolic BP: −0.15 (−0.43, 0.13) LINE 1 (Δ methylation): −0.20 (−0.32, −0.07) Percentage AluYb8 methylation (OR): 0.94 (0.82, 1.08) 2nd trimester Left CIMT: 0.00 (−0.00, 0.00) Right CIMT: 0.00 (−0.00, 0.00) Systolic BP: 0.05 (−0.33, 0.43) Diastolic BP: −0.04 (−0.32, 0.24) LINE 1: 0.05 (−0.08, 0.18) Percentage AluYb8 methylation: 0.95 (0.83, 1.10) 3rd Trimester Left CIMT: −0.00 (−0.00, 0.00) Right CIMT: −0.00 (−0.00, 0.00) Systolic BP: 0.05 (−0.39, 0.48) Diastolic BP: 0.07 (−0.25, 0.39) LINE 1: 0.15 (0.00, 0.31) Percentage AluYb8 methylation: 1.02 (0.87, 1.19)
†Nishimura et al. (2016) Chicago, IL; Bronx, NY; Houston, TX; San Francisco Bay Area, CA; and Puerto Rico, U.S. Ozone: NR Follow-up: 2006−2011 Cohort study	GALA II n = 1,032 asthma cases	Monitor 8-h max		Correlation (*r*): NR Copollutant models with: NR	Atopic status (OR) 1st year of life: 1.74 (1.23, 2.46)

Table 7-14 (Continued): Epidemiologic studies of exposure to ozone and developmental effects.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Kim et al. (2017) California, U.S. Ozone: 1999−2010 Follow-up: 2002−2011 Case-control study	CHARGE n = 158 cases; 147 controls	Monitor 8-h max		Correlation (*r*): NR Copollutant models with: NR	Autism (OR) Entire pregnancy + 1st two yr of life: 1.34 (0.89, 2.01)
†Conde et al. (2018) São Paulo, Brazil Ozone: NR Follow-up: June 2014−July 2015 Case-control study	n = 30 cSLE patients; 86 healthy controls	1-h max		Correlation (*r*): NR Copollutant models with: NR	Childhood lupus gestational period, and each year to 9 yr Reports significant ORs only. OR elevated from null for 2nd tertile of ozone exposure in 3rd yr of life, but CIs are wide
†Goodrich et al. (2017) California, U.S. Ozone: 1997−2011 Follow-up: 2002−2011 Case-control study	CHARGE n = 346 ASD cases; 260 typical development controls	Monitor 8-h max	Median: 17	Correlation (*r*): $PM_{2.5}$: −0.463; NO_2: −0.425; Other: −0.022 Copollutant models with: NR	Autism spectrum disorder (OR) 1st trimester Low folic acid intake: 1.06 (0.71, 1.58) High folic acid intake: 1.12 (0.75, 1.65) 2nd trimester Low folic acid intake: 1.15 (0.77, 1.71) High folic acid intake: 1.00 (0.67, 1.49) 3rd trimester Low folic acid intake: 1.20 (0.80, 1.82) High folic acid intake: 0.94 (0.64, 1.38)
†França et al. (2018) São Paulo, Brazil Ozone: NR Follow-up: 2013−2014 Case-control study	n = 66 cases; 124 controls Hospital recruits	Monitor Other	Mean: 44	Correlation (*r*): NR Copollutant models with: NR	Juvenile idiopathic arthritis (OR) Examined exposures during each trimester and each year of life to diagnosis. Reported only significant effects, with 2nd tertile (41−44 ppb) of ozone exposure in the 2nd yr of life

Table 7-14 (Continued): Epidemiologic studies of exposure to ozone and developmental effects.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates 95% CI
†Kerin et al. (2017) California, U.S. Ozone: 1998−2008 Follow-up: Cohort study	CHARGE n = 325 ASD cases	Monitor 8-h max	Mean: 37.3	Correlation (*r*): NR Copollutant models with: NR	Neurodevelopmental (Δ score) No evidence of association between prenatal or Yr 1 ozone exposure and any neurodevelopmental score (VABS, MSEL, ADOS CSS)

†Recent studies evaluated since the 2013 Ozone ISA.

Table 7-15 Epidemiologic studies of exposure to ozone and other.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Lee et al. (2011) Pittsburgh, PA, U.S. Ozone: 1996−2001 Follow-up: 1997−2001 Cohort study	Prenatal Exposures and Preeclampsia Prevention Study n = 1,696 women Enrolled in clinics and private practices early in pregnancy	Model, space-time ordinary kriging Other	Mean: 29.9 Median: 30.3 75th: 34.3 95th: 41.6 Maximum: 51.4	Correlation (*r*): $PM_{2.5}$: 0.5; NO_2: 0; SO_2: 0.1; Other: 0.5 Copollutant models with: NR	C-reactive protein (OR, <8 vs. ≥8 ng/mL) Lag Days 0−7 before draw: 1.32 (0.85, 2.04) Lag Days 0−21 before draw: 1.33 (0.74, 2.37) Lag Days 0−28 before draw: 1.07 (0.57, 2.01)
†Lee et al. (2012) Pittsburgh, PA, U.S. Ozone: 1996−2001 Follow-up: 1997−2001 Cohort study	Prenatal Exposures and Preeclampsia Prevention Study n = 1,684 women Enrolled in clinics and private practices early in pregnancy	Model, space-time ordinary kriging 1st trimester	Mean: 22.7 Median: 22.9 75th: 30.1 95th: 35.6 Maximum: 42.7	Correlation (*r*): $PM_{2.5}$: 0.5; NO_2: −0.5; SO_2: −0.6; Other: 0.7 Copollutant models with: NR	Diastolic BP (Δ mm Hg): 0.48 (−0.31, 1.27) Nonsmokers: 0.74 (−0.30, 1.77) Systolic BP (Δ mm Hg): 0.96 (−0.07, 1.99) Nonsmokers: 1.20 (0.69, 3.03)

Table 7-15 (Continued): Epidemiologic studies of exposure to ozone and other.

†Männistö et al. (2015b) U.S. Ozone: 2006 Follow-up: 2006 Cohort study	Consortium on Safe Labor n = 500 Random sample from cohort	Model, modified CMAQ Other	Mean: 41.4 Median: 42.2 Maximum: 68.3	Correlation (*r*): NR Copollutant models with: NR	Per 10% increase in Ozone Diastolic BP (Δ mm Hg) Hourly mean, at BP measurement hour Normotensive: 0.41 (0.07, 0.74) Chronic hypertension: −0.45 (−2.06, 1.16) Pregnancy related hypertension: 0.09 (−0.37, 0.54) Hourly mean, 1 h before BP measurement Normotensive: 0.34 (0.01, 0.66) Chronic hypertension: −0.64 (−2.28, 1.00) Pregnancy related hypertension: 0.05 (−0.35, 0.44) Daily average Normotensive: 0.19 (−0.06, 0.44) Chronic hypertension: −1.02 (−3.41, 1.36) Pregnancy related hypertension: 0.37 (−0.25, 0.99) Systolic BP (Δ mm Hg) Hourly mean, at BP measurement hour Normotensive: 0.27 (−0.15, 0.70) Chronic hypertension: 0.46 (−2.10, 3.02) Pregnancy related hypertension: 0.05 (−0.45, 0.54) Hourly mean, 1 h before BP measurement Normotensive: 0.17 (−0.24, 0.57) Chronic hypertension: −0.01 (−2.65, 2.62) Pregnancy related hypertension: 0.00 (−0.43, 0.43) Daily average Normotensive: 0.15 (−0.16, 0.46) Chronic hypertension: −0.76 (−4.58, 3.07) Pregnancy related hypertension: 0.35 (−0.32, 1.03)

Table 7-15 (Continued): Epidemiologic studies of exposure to ozone and other.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Männistö et al. (2015a) U.S. Ozone: NR Follow-up: 2002−2008 Cohort study	Consortium on Safe Labor n = 223,502 singleton pregnancies Recruited from 12 centers (19 hospitals) across U.S.	Model, modified CMAQ 24-h avg Other	Mean: 29.9 Maximum: 79.8	Correlation (*r*): $PM_{2.5}$: −0.1; NO_2: −0.35; SO_2: −0.18; Other: −0.3 Copollutant models with: NR	Cardiovascular events during labor and delivery. Odds ratios below the null for exposures at lag Days 5, 6, and 7. No association with exposures for lag Days 0 to 4
†Michikawa et al. (2016) Kyushu-Okinawa District, Japan Ozone: NR Follow-up: 2005−2010 Cohort study	Japan Perinatal Registry Network n = 40,573	Monitor Other	Mean: 41.3 Median: 39.9	Correlation (*r*): $PM_{2.5}$: 0.17; NO_2: −0.16; SO_2: −0.09 Copollutant models with: NR	Placenta previa (OR): 0−4 weeks of gestation: 1.08 (1.00, 1.16) 5−12 weeks of gestation: 1.07 (1.00, 1.15) 13−28 weeks of gestation: 0.97 (0.88, 1.08)
†Hettfleisch et al. (2016) São Paulo, Brazil Ozone: NR Follow-up: October 2011−January 2014 Cross-sectional study	n = 229	Personal monitor Other	Mean: 4	Correlation (*r*): NO_2: 0.088 Copollutant models with: NR	No association between placental flow index, placental vascularization index, or placental vascularization flow index with 1 week of ozone exposures
†Michikawa et al. (2017b) Kyushu-Okinawa District, Japan Ozone: NR Follow-up: 2005−2010 Other study	Japan Perinatal Registry Network n = 821 cases	Monitor Other	Mean: 41.1 Median: 40.2 75th: 51.2 90th: 62.6	Correlation (*r*): NR Copollutant models with: NR	No association with placental abruption with daily ozone exposure, lags of 1 to 5 before event

Table 7-15 (Continued): Epidemiologic studies of exposure to ozone and other.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Ha et al. (2017a) U.S. Ozone: NR Follow-up: 2002−2008 Other study	Consortium on Safe Labor n = 680 12 U.S. clinical sites	Model, modified CMAQ 24-h avg Other	Mean: 30.32 75th: 37.85 Maximum: 54.47	Correlation (*r*): NR Copollutant models with: NR	Average of lag Days 0−7 Cardiovascular events at labor and delivery, any (OR): 0.89 (0.72, 1.12) Cardiac arrest (OR): 0.63 (0.45, 0.86) Heart failure (OR): 0.86 (0.47, 1.57) Unspecified event (OR): 1.17 (0.72, 1.93) Stroke (OR): 1.44 (0.80, 2.61) Ischemic heart disease (OR): 2.41 (1.12, 5.23)
†Morokuma et al. (2017) Kyushu-Okinawa District, Japan Ozone: NR Follow-up: 2005−2010 Cohort study	Japan Perinatal Registry Network n = 23,782	Monitor Other	Mean: 1st trimester: 41.3 2nd trimester: 42 3rd trimester: 41.6 Median: 1st trimester: 40.2 2nd trimester: 41 3rd trimester: 40.2 75th: 1st trimester: 47.9 2nd trimester: 48.3 3rd trimester: 50.4	Correlation (*r*): NR Copollutant models with: NR	Fetal heart rate false positives (OR): 0.98 (0.92, 1.05) Fetal heart rate false positives (OR): 1.01 (0.95, 1.08) Fetal heart rate false positives (OR): 1.04 (0.99, 1.10)

†Recent studies evaluated since the 2013 Ozone ISA.

7.4.2 Toxicological Studies

Table 7-16 Study specific details from studies of ozone (O_3) and pregnancy/birth outcomes.

Study	Species (Strain), N, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
†Avdalovic et al. (2012)	Rhesus monkeys (healthy and HDM sensitized) n = 12/group males, 0 females Age: 1 mo	0.5 ppm Ozone: 8 h/day for 5 days followed by 9 days FA HDM: Days 3−5 of exposure and sensitization at Day 14 or Day 28 of exposure	Lung volume, alveolar volume and number, alveolar capillary surface density, mRNA for genes in alveolar growth and angiogenesis pathways (3 or 6 mo of age)
†Gordon et al. (2017a)	Rats (LE); dams fed high fat or control diet for 6 weeks before breeding n = 4 male and 4 female offspring; 10 dams/treatment group Age: PND 161-162 ozone exposure	Offspring 0.8 ppm, 4 h/day for 2 days, offspring exposed at PND 161 and 162, exercise and/or diet challenges	Dam body weight, ventilation, BALF counts, glucose tolerance test (GD 7, dam body weight; PND 162 offspring measurements including BALF, body weight and body composition)
†Miller et al. (2019)	In vitro trophoblast cell culture treated with serum from ozone exposed Long-evans pregnant dams gestation Days 5 and 6 (window of implantation)	LE Dams exposed to 0.4−1.2 ppm for 4 h on gestation Days 5 and 6 (window of implantation)	Serum from control or ozone-exposed dams added to trophoblasts in vitro to evaluate effect on trophoblast invasion and migration as well as trophoblast metabolic capacity

Table 7-16 (Continued): Study specific details from studies of ozone (O_3) and pregnancy/birth outcomes.

Study	Species (Strain), N, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
†Miller et al. (2017)	Rats (LE) n = 10/group adult pregnant females n = 3 females, n = 3 males per treatment group Age: adult	0, 0.4, or 0.8 ppm, 4 h/day for 2 days at implantation (GD 5, GD 6)	BALF (GD 21) Dam blood glucose and serum free fatty acids (GD 21) Fetal growth parameters (body weight, length, percent lean mass, percent fat mass) (GD 21) Dam body weight gain during pregnancy (GDs 5-7) Dam uterine blood flow and resistance (GDs 15, 19, 21) Dam serum inflammatory marker (GD 21)

†Recent studies evaluated since the 2013 Ozone ISA.

Table 7-17 Study specific details from studies of ozone (O_3) and developmental effects.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
†Carey et al. (2011)	Rhesus monkeys n = 4−14	0.5 ppm, acute or chronic ozone exposure; one cycle = 5 consecutive days of ozone for 8 h/day and 9 days of FA (14-day cycle); one group FA, one group one cycle of ozone; one group 11 cycles of ozone beginning at 32−37 days of age, ending at 6 mo of age; animals with one cycle ozone exposure ended at 6 mo of age	Morphological sections of nasal tract (anatomical development); histology of nasal tract (epithelial height, cilia volume, nuclear volume, histological images for necrosis) (24 h PE)
†Chou et al. (2011)	Rhesus monkeys *n = 6/group × 4 groups (n = 24), FA control, HDM (house dust mite), O_3, O_3 + HDM males, 0 females Age: 4−14 weeks of age	0.5 ppm, ozone: one cycle of 14 days = 8 h/day for 5 days + 9 days filtered air; HDM: Days 3−5 of exposure and sensitization at Day 14 or Day 28 of exposure; five cycles of ozone exposure over the time period	Blood (WBC, eosinophils), BAL (total cell number, eosinophils, macrophages, PMNs, lymphocytes; chemotaxis proteins [CCL 11, 24, 26], histology [eosinophil count in airway walls]); CCL in histology staining and CCL mRNA quantified (3 mo of age)
†Hunter et al. (2011)	Rats (F344) n = 4−6*/group males, 0 females Age: PND 6, PND10, PND15, PND21, or PND28	2 ppm, FA or ozone, 3 h, 1 day	NGF protein and mRNA (lung lavage, lung tissue, SP-IR nerve fiber density in extrapulmonary sm muscle) (24 h PE)
†Murphy et al. (2012)	Rhesus monkeys n = *4−6/group males, 0 females Age: 6−12 mo of age	0.5 ppm, 11 cycles. Ozone cycle: 8 h/day for 5 days + 9 days filtered air; HDM: Days 3−5 of exposure	Lung morphology (HE), NK1R protein expression, IL-8 mRNA, oxidative stress challenge to explanted airways (12 mo of age, after end of exposures)

Table 7-17 (Continued): Study specific details from studies of ozone (O_3) and developmental effects.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
†Gabehart et al. (2014)	Mice (BALB/cJ) n = NR Age: PND 3	1 ppm, 3 h/day, 1 day	BAL (cell numbers, neutrophils, albumin), lung histology, electron microscopy of lung, lung gene microarray and pathways analysis (6 or 24 h PE)
†Murphy et al. (2013)	Rhesus monkeys n = *12/group males, 0 females Age: 1, 2, or 6 mo	0.5 ppm Episodic ozone (repeating cycles of ozone for 8 h/day for 5 days followed by 9 days of FA) from 1 to 2 and 6 mo Acute ozone (single 8-h exposure) at 2 or 6 mo	Serotonin patterns in distal and midlevel lung (5HT, 5HTT, and receptors 5HT2aR or 5Ht4R); changes in epithelial thickness (2 or 6 mo of age)
†Gabehart et al. (2015)	Mice (BALB/cJ WT and TLR4-/-) n = 0 males, 3−7/group females Age: neonatal (PND 3), juveniles (2 weeks old), weanlings (3 weeks old), adults (6 weeks old)	1 ppm, 3 h	Whole lung TLR4 expression by age, BAL (neutrophils, antioxidants, albumin leakage, chemokines, mucus production) (Immediately PE)
†Gordon et al. (2017b)	Long-Evans rats n = 8 female pups per treatment group. Age: Sedentary and active rats were housed in cages with or without running wheels from PND 22 to PND 100. During the last week of the study, rats were subjected to the ozone or filtered air.	0.8 ppm, 4 h/day, 2 days	Glucose tolerance testing, BALF immune cells, metabolic function indicators
†Dye et al. (2017)	Rats (LE, S-D, Wistar) n = 7−16 pups Age: exposure on PNDs 14, 21, 28	1 ppm × 2 h, 1 day	Respiratory outcomes, lung antioxidants, redox enzymes

Table 7-17 (Continued): Study specific details from studies of ozone (O_3) and developmental effects.

Study	Species (Strain), n, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
†Miller et al. (2017)	Rats (LE) n = 0 males, 10/group females Age: adult	0, 0.4, or 0.8 ppm, 4 h/day for 2 days (GD 5, GD 6)	BALF (GD 21) Dam blood glucose and serum free fatty acids (GD 21) Dam penh, whole body plethysmography (GDs 5 and 6) Dam blood pressure during pregnancy (GDs 15, 19, 21) Dam minute volume (GD 21) Fetal growth parameters (body weight, length, percent lean mass, percent fat mass) (GD 21) Dam body weight gain during pregnancy (GDs 5-7) Dam kidney histopathology (GD 21) Dam uterine blood flow and resistance (GDs 15, 19, 21) Dam serum inflammatory marker (GD 21)

†Recent studies evaluated since the 2013 Ozone ISA.

7.5 Evidence Inventories—Data Tables to Summarize Nervous System Effects Study Details

7.5.1 Epidemiologic Studies

Table 7-18 Epidemiologic studies of short-term exposure to ozone and effects on cognition, motor activity, and mood.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Lim et al. (2012) Seongbuk-Gu, South Korea Ozone: 2008−2010 Follow-up: August 2008−August 2010 Other study	n = 560 Older adults	Nearest monitor 8-h max	Mean: 48.1 Median: 44 Maximum: 140	Correlation (*r*): $PM_{2.5}$: NR; NO_2: −0.15; SO_2: −0.18; Other: CO: −0.30 Copollutant models with: NR	Factor 3—affective symptoms: 1.07 (0.83, 1.38) Factor 2—somatic symptoms: 1.25 (0.90, 1.74) Factor 1—emotional symptoms: 1.58 (1.16, 2.14)

†Recent studies evaluated since the 2013 Ozone ISA.

Table 7-19 Epidemiologic studies of short-term exposure to ozone and hospital admissions, emergency department, and outpatient visits.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Chiu and Yang (2015) Taipei, Taiwan Ozone: 2006−2011 Follow-up: 2006−2011 Case-crossover study	TNHIP n = 13,676 Random sample of enrollees	6-monitor avg 24-h avg	Mean: 24.6 (all years) Median: 23.77 Maximum: 70.98	Correlation (*r*): NO_2: −0.06; SO_2: 0.07; Other: CO: −0.22 Copollutant models with: yes	Outpatient visit (migraine), ≥23°C: 1.08 (1.02, 1.15) Outpatient visit (migraine), <23°C: 1.28 (1.19, 1.38)
†Xu et al. (2016) Xi'an, China Ozone: 2013−2014 Follow-up: 2013−2014 Time-series study	n = 20,368	13-monitor avg 24-h avg	Mean: 51 Median: 44.37 Maximum: 158.61	Correlation (*r*): $PM_{2.5}$: −0.226; NO_2: −0.165; SO_2: −0.414; Other: −0.454 ozone Copollutant models with: yes—NO_2, SO_2	Epilepsy outpatient visit: 0.98 (0.95, 1.00)
†Linares et al. (2017) Madrid, Spain Ozone: 2001−2009 Follow-up: 2001−2009 Time-series study	HMS n = 1,175	27-monitor avg 24-h avg	Mean: 18.21 Maximum: 45.59	Correlation (*r*):NR Copollutant models with: NR	Dementia-related hospital admission: 1.29 (1.12, 1.51)
†Culqui et al. (2017) Madrid, Spain Ozone: 2001−2009 Follow-up: 2001−2009 Time-series study	HMS n = 1,183	27-monitor avg 24-h avg	Mean: 18.21 Median: 18.21	Correlation (*r*): NR Copollutant models with: NR	Alzheimer's-related hospital admission: (NR, not statistically significant)

Table 7-19 (Continued): Epidemiologic studies of short-term exposure to ozone and hospital admissions, emergency department, and outpatient visits.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Guo et al. (2018) Guangzhou, China Ozone: 2013−2015 Follow-up: 2013−2015 Time-series study		Daily avg of 36 monitors 8-h max	Mean: 49.81 Maximum: 125.89	Correlation (*r*): NR Copollutant models with: NR	ED visit for diseases of neurological system (cold): 0.99 (0.98, 1.00) ED visit for diseases of neurological system (warm): 0.99 (0.97, 1.01)
†Carmona et al. (2018) Madrid, Spain Ozone: 2001−2009 Follow-up: 2001−2009 Time-series study	n = 2,224	Citywide average all monitors 24-h avg	Mean: 18.21 Maximum: 45.59	Correlation (*r*): NR Copollutant models with: NR	ED visit for multiple sclerosis: (NR, not statistically significant)
†Jeanjean et al. (2018) Strasbourg, France Ozone: 2000−2009 Follow-up: 2000−2009 Case-crossover study	EDMUS n = 1,783 Relapse occurrence in registry	ADMS-Urban Air Dispersion model 24-h avg	Mean: 44.29 Median: 42.55 Maximum: 112.71	Correlation (*r*): NO_2: −0.06; Other: −0.21 Copollutant models with: NR	Multiple sclerosis relapse (cold, October−March): 0.96 (0.90, 1.03) Multiple sclerosis relapse (hot, April−September): 1.06 (1.03, 1.09)
†Lee et al. (2017) Seoul, South Korea Ozone: 2002−2013 Follow-up: 2002−2013 Case-crossover study	NHIS-NSC n = 314	27-monitor avg 8-h avg	Mean: 24.2	Correlation (*r*): NR Copollutant models with: NR	Parkinson's disease hospital admission: (NR, figure only: no statistically significant associations)
†Lim et al. (2012) Seongbuk-Gu, South Korea Ozone: 2008−2010 Follow-up: August 2008−August 2010 Other study	n = 560 Older adults	Nearest monitor 8-h max	Mean: 48.1 Median: 44 Maximum: 140	Correlation (*r*): $PM_{2.5}$: NR; NO_2: −0.15; SO_2: −0.18; Other: CO: −0.30 Copollutant models with: NR	Factor 3—affective symptoms: 1.07 (0.83, 1.38) Factor 2—somatic symptoms: 1.25 (0.90, 1.74) Factor 1—emotional symptoms: 1.58 (1.16, 2.14)

Table 7-19 (Continued): Epidemiologic studies of short-term exposure to ozone and hospital admissions, emergency department, and outpatient visits.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Cho et al. (2015) Seoul, South Korea Ozone: 2005−2009 Follow-up: 2005−2009 Time-series study	HIRA n = 2,320	27-monitor avg 24-h avg	Mean: 18 Median: 16 90th: 31	Correlation (*r*): $PM_{2.5}$: NR Copollutant models with: NR	ED visit for panic attack: 1.11 (1.05, 1.17)
†Chen et al. (2018) Shanghai, China Ozone: 2013−2015 Follow-up: 2013−2015 Time-series study	SHIS n = 39,143	10 monitoring stations 8-h max	Mean: 51 Median: 48.96 Maximum: 135.66	Correlation (*r*): $PM_{2.5}$: −0.03; NO_2: −0.21; SO_2: −0.2; Other: −0.22 Copollutant models with: NR	Mental disorder hospital admission: 1.01 (0.96, 1.07) Mental disorder hospital admission: 1.03 (0.95, 1.12)
†Oudin et al. (2018) Gothenburg, Sweden Ozone: NR Follow-up: July 2012−November 2016 Case-crossover study	n = NR	One monitor 24-h avg	Mean: 25.65 Median: 25.6 Maximum: 79.05	Correlation (*r*): NR Copollutant models with: yes	ED visit for psychiatric emergencies (cold, October−March): 0.99 (0.95, 1.03) ED visit for psychiatric emergencies (all year): 1.00 (0.98, 1.03) ED visit for psychiatric emergencies (warm, April−September): 1.02 (0.99, 1.05)

Table 7-19 (Continued): Epidemiologic studies of short-term exposure to ozone and hospital admissions, emergency department, and outpatient visits.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Szyszkowicz et al. (2016) Nine urban areas, Canada Ozone: April 2004−December 2011 Follow-up: April 2004−December 2011 Case-crossover study	NACRS n = 118,602	Daily average of monitors within 3 km of postal code 24-h avg	Mean: 22.5−29.2 Maximum: 60.7−80.0	Correlation (*r*): NR Copollutant models with: NR	ED visit for depression (all year, male): 1.00 (0.98, 1.03) ED visit for depression (warm, April−September, male): 1.01 (0.98, 1.04) ED visit for depression (all year, female): 1.01 (0.99, 1.03) ED visit for depression (warm, April−September, female): 1.03 (1.00, 1.05)

†Recent studies evaluated since the 2013 Ozone ISA.

Table 7-20 Epidemiologic studies of long-term exposure to ozone and cognitive/behavioral effects.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Gatto et al. (2014) Los Angeles, U.S. Ozone: 2000−2006 Follow-up: 2000−2006 Cross-sectional study	BVAIT, WISH, ELITE n = 1,496 3 RCTs	Monitor within 5 km of residence, average of monitors within 100 km (IDW) 8-h max	Mean: NR—geographic variability in concentration shown in Figure 2 Maximum: >25	Correlation (*r*): $PM_{2.5}$: −0.62; NO_2: −0.77; Copollutant models with: NR	Global cognition (>49 ppb vs. ≤34 [reference]): −0.08 (−0.45, 0.28) Semantic memory (>49 ppb vs. ≤34 [reference]): −0.12 (−0.5, 0.26) Verbal learning (34−49 ppb vs. ≤34 [reference]): −0.13 (−0.41, 0.16) Visual processing (34−49 ppb vs. ≤34 [reference]): −0.18 (−0.43, 0.07) Verbal learning (>49 ppb vs. ≤34 [reference]): −0.2 (−0.63, 0.23) Visual processing (>49 ppb vs. ≤34 [reference]): −0.2 (−0.59, 0.18) Executive function (34−49 ppb vs. ≤34 [reference]): −0.23 (−0.68, 0.22) Executive function (>49 ppb vs. ≤34 [reference]): −0.66 (−1.35, 0.03) Visual memory (>49 ppb vs. ≤34 [reference]): 0.01 (−0.42, 0.44) Global cognition (34−49 ppb vs. ≤34 [reference]): 0.05 (−0.19, 0.29) Semantic memory (34−49 ppb vs. ≤34 [reference]): 0.08 (−0.17, 0.33) Visual memory (34−49 ppb vs. ≤34 [reference]): 0.12 (−0.16, 0.4) Logical memory (>49 ppb vs. ≤34 [reference]): 0.24 (−0.21, 0.68) Logical memory (34−49 ppb vs. ≤34 [reference]): 0.31 (0.01, 0.6)

Table 7-20 (Continued): Epidemiologic studies of long-term exposure to ozone and cognitive/behavioral effects.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Cleary et al. (2018) Nation-wide, U.S. Ozone: 2001−2008 Follow-up: 2004−2008	NACC Alzheimer's Disease Center participants	HBM to combine monitored and predicted (CMAQ) concentrations 8-h max	Mean: NR (figure only)	Correlation (*r*): NR Copollutant models with: NR	Cognitive decline on MMSE and CDR-SB with ozone exposure among those with no baseline impairment
†Kioumourtzoglou et al. (2017) 48 continental states, U.S. Ozone: 1996−2008 Follow-up: 1996−2008 Cohort study	NHS n = 41,844 Women	Summer average (May−Sept) of up to five monitors (at least one monitor within 50 km (IDW), at residential address Other	Mean: 31.9	Correlation (*r*): NR Copollutant models with: NR	Depression onset (depression diagnosis): 1.00 (0.92, 1.08) Depression onset (antidepressant or depression): 1.06 (1.00, 1.12) Depression onset (use of antidepressant medication): 1.08 (1.02, 1.14)

†Recent studies evaluated since the 2013 Ozone ISA.

Table 7-21 Epidemiologic studies of long-term exposure to ozone and neurodegenerative diseases.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Jung et al. (2014) National, Taiwan Ozone: 2000−2010 Follow-up: 2000−2010 Case-control study	LHID2000-NHIRD n = 97,627	Annual avg of three nearest monitors within 25 km of grid cell (IDW), assigned to postal code of residence 8-h max	Mean: 92.64 Maximum: 137.65	Correlation (*r*): NO_2: −0.05; SO_2: 0.27; Other: CO: 0.10; PM_{10}: −0.26 Copollutant models with: yes	Alzheimer's disease Baseline ozone concentration: 1.06 (1.00, 1.12) Change in ozone concentration at follow-up minus concentration at baseline: 2.84 (2.67, 3.01)
†Kirrane et al. (2015) North Carolina and Iowa, U.S. Ozone: 2002−2006 Enrollment: 1993−2005 Follow-up: 1997−2010 Case-control study	AHS North Carolina: n = 104 cases; 29,612 controls Iowa: n = 195 cases; 53,024 controls Farmer pesticide applicators and their spouses	Annual, seasonal (April−October) 4-yr avg of daily predictions using measured concentrations and CMAQ 8-h max	Mean: 40.6	Correlation (*r*): $PM_{2.5}$: −0.15 to 0.06, depending on metric Copollutant models with: NR	Parkinson's disease (Iowa 4-yr avg): 0.46 (0.13, 1.69) Parkinson's disease (Iowa warm season average): 0.46 (0.11, 1.84) Parkinson's disease (North Carolina 4-yr avg): 1.49 (0.43, 5.16) Parkinson's disease (North Carolina warm season average): 2.60 (0.94, 7.24)
†Chen et al. (2017a) National, Taiwan Ozone: 2000−2013 Follow-up: 2000−2013 Case-control study	TNHIP-NHIRD n = 249 cases; 497 controls ≤40 yr	Monthly average during follow-up in areas where participants reside	Mean: NR	Correlation (*r*): NR Copollutant models with: NR	Parkinson's disease: 1.10 (0.74, 1.48)

Table 7-21 (Continued): Epidemiologic studies of long-term exposure to ozone and neurodegenerative diseases.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Chen et al. (2017c) Ontario, Canada Ozone: 1994−2013 Follow-up: April 2001−March 2013 Cohort study	ONPHEC n = 2,066,639 55−85 yr at enrollment	5-yr avg estimated using monitor concentrations with physically based air quality prediction model (2002−2009) calibrated using data from 1995−2013	Mean: 45.8	Correlation (*r*): $PM_{2.5}$: 0.38; NO_2: −0.22 Copollutant models with: NR (multipollutant only)	Dementia: 0.97 (0.94, 1.00)
†Wu et al. (2015) Multicity, Taiwan Ozone: 2007−2010 Follow-up: 2007−2010 Case-control study	Hospitals and clinics n = 1,060 cases; 4,240 controls ≤60 yr	Spatiotemporal model, cumulative annual average	Mean: NR	Correlation (*r*): NR Copollutant models with: NR	Alzheimer's disease (20.20−21.56 vs. <20.20 [reference]): 0.60 (0.33, 1.09) Vascular dementia (>20.20−21.56 vs. <20.20 [reference]): 0.62 (0.28, 1.38) Alzheimer's disease (>21.56 vs. <20.20 ppb [reference]): 2.00 (1.14, 3.50) Vascular dementia (>21.56 vs. <20.20 ppb [reference]): 2.09 (1.01, 4.33)
†Lee et al. (2016) National, Taiwan Ozone: 1998−2009 Follow-up: 2007−2009 Case-control study	NHIRD First clinic visit for PD (patients ≥35 yr) n = 11,117 cases; 4-to-1 match	QBME spatio-temporal model	Mean 26.1	Correlation (*r*): SO_2: 0.01; CO: −0.60 Copollutant models with: NR	Parkinson's disease 1.00 (0.93, 1.07)

Table 7-21 (Continued): Epidemiologic studies of long-term exposure to ozone and neurodegenerative diseases.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Shin et al. (2018) Ontario, Canada Follow-up: 2001−2013 1994−2013	ONPHEC Registry record for Parkinson's disease healthcare or medication 55+ yr old n = 38,745 cases (~2.2 million followed)	Summer average, fused-based optimal interpolation of measured and predicted ozone, 21 × 21 km grid 8 h max	Mean: 49.8	Correlation (*r*): NR Copollutant models with: NR	Parkinson's disease 1.06 (1.02, 1.11)
†Cerza et al. (2018) Rome Italy Ozone: 2008 Follow-up: 2008−2013 Cohort study	Regional Health Information System Insurance registry claim for Parkinson's disease 50+ yr n = 1,008,253	Summer average, chemical dispersion model with grid resolution of 1 × 1 km 8-h avg	Mean: 45.5	Correlation (*r*): NR Copollutant models with: NO_2	Parkinson's disease 1.04 (1.00, 1.11)

†Recent studies evaluated since the 2013 Ozone ISA.

Table 7-22 Epidemiologic studies of long-term exposure to ozone and neurodevelopmental effects.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Becerra et al. (2013) Los Angeles County, U.S Ozone: 1995−2006 Follow-up: 1998−2009 Case-control study	DDS registry n = 7,594 cases; 75,635 controls Diagnosis between 36 and 71 mo	Nearest monitor, trimester and whole pregnancy averages 8-h avg	Mean: 36.8	Correlation (*r*): $PM_{2.5}$: −0.47; NO_2: −0.34; Other: CO: −0.55, PM_{10}: −0.17, NO : −0.73 Copollutant models with: yes	Autism disorder: 1.05 (1.01, 1.10)
†Jung et al. (2013) National, Taiwan Ozone: 2000−2010 Follow-up: 2000−2010 Cohort study	LHID2000-NHIRD n = 49,073 ASD	Annual average of three nearest monitors within 25 km of grid cell (IDW), assigned to postal code of residence 8-h max	Mean: 90−120 depending on season	Correlation (*r*): NO_2: 0; SO_2: 0.22; Other: PM_{10}: 0.66 Copollutant models with: yes	ASD: 1.59 (1.42, 1.78)
†Kerin et al. (2017) California, U.S. Ozone: NR Follow-up: Case-control study	CHARGE n = 325 cases born 1999−2007 Diagnosed with ASD between 24 and 60 mo	Pregnancy, Yr 1 average of up to four monitors within 50 km (IDW) or one monitor within 5 km 8-h max	Mean: 37.3	Correlation (*r*): $PM_{2.5}$: −0.21; NO_2: −0.45; Other: PM_{10}: 0.04 Copollutant models with: NR	ADDS-CSS: 0.99 (0.94, 1.05) MSEL: 0.99 (0.88, 1.10) VABS: 1.00 (0.96, 1.04)

Table 7-22 (Continued): Epidemiologic studies of long-term exposure to ozone and neurodevelopmental effects.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Volk et al. (2013) California, U.S. Ozone: 1997−2009 Follow-up: 1997−2008 Case-control study	CHARGE Full-syndrome autism (ADOS and autism diagnostic interview—revised.) n = 534 cases and controls Diagnosed with autism between 24 and 60 mo	1st yr, entire pregnancy, 1st trimester, 2nd trimester, 3rd trimester Average of four closest monitors within 50 km (IDW) or one monitor within 5 km 8-h max	Mean: NR	Correlation (*r*): NR Copollutant model: NR	Autism: 1.05 (0.84, 1.31), entire pregnancy
†Kim et al. (2017) California, U.S. Follow-up: 1999−2008 Ozone: 1997−2009 Case-control study	CHARGE Confirmed autism n = 158 cases; 147 controls Diagnosed with autism between 24 and 60 mo	Pregnancy, 1st yr, 2nd trimester Average of four closest monitors within 50 km (IDW) or one monitor within 5 km 8-h max	Mean: NR	Correlation (*r*): NR Copollutant model: NR	Joint effect of copy number and ozone greater than effect of each ozone or duplication burden alone
†Goodrich et al. (2017) California, U.S. Follow-up: 1999−2008 Ozone: 1997−2009 Case-control study	CHARGE n = 297 confirmed autism; 143 ASD; 326 controls Diagnosed with autism between 24 and 60 mo	1st trimester Average of four closest monitors within 50 km (IDW) or one monitor within 5 km 8-h max	Mean: NR	Correlation (*r*): NR Copollutant model: NR	No interaction between ozone exposure and folic acid intake

Table 7-22 (Continued): Epidemiologic studies of long-term exposure to ozone and neurodevelopmental effects.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Lin et al. (2014a) 11 towns, Taiwan Follow-up: October 2003−January 2004 Cohort study	n = 511 mother-infant pairs, neurodevelopment assessed by parent report	1st, 2nd, 3rd trimester monitor average	Mean: NR	Correlation (*r*): NR Copollutant model: NR	No associations with ozone reported

ADOS-CSS = Autism Diagnostic Observation Schedule derived-Calculated Severity Score; AHS = Agricultural Health Study; ASD = autism spectrum disorder; BVAIT = B-Vitamin Atherosclerosis Intervention Trial; CHARGE = Childhood Autism Risks from Genetics and the Environment; CMAQ = Community Multiscale Air Quality; DDS = Department of Developmental Services; DSM-IV-R = Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision; EDMUS = European Database for Multiple Sclerosis; ELITE = Early Versus Late Intervention Trial with Estradiol; HIRA = Health Insurance Review and Assessment Service; HMS = Hospital Morbidity Survey; LHID2000 = Longitudinal Health Insurance Database 2000; NACRS = National Ambulatory Care Reporting System; NHIRD = National Health Insurance Research Database; NHIS-NSC = National Health Insurance Service-National Sample Cohort; NHS = Nurses' Health Study; ONPHEC = Ontario Population Health and Environment Cohort; SHIS = Shanghai Health Insurance System; TNHIP = Taiwan National Health Insurance Program; WISH = Women's Isoflavone Soy Health.

†Recent studies evaluated since the 2013 Ozone ISA.

7.5.2 Toxicological Studies

Table 7-23 Study-specific details from short-term studies of brain inflammation and morphology.

Study	Population Species (Strain), N, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Chounlamountry et al. (2015)	Rats (Wistar) n = 3−5 males, 0 females Age: 6−7 weeks	2 ppm, 24 h, single exposure	Glial remodeling; markers of astrocyte activation (GFAP, S100b, GLT1, Glyn Syn, ezrin) (immediately PE)
Gómez-Crisóstomo et al. (2014)	Rats (Wistar) n = 12 males, 0 females Age: NR (250−300 g)	0.25 ppm, 4 h/day for 7, 15, 30, 60, or 90 days	Markers of oxidative stress and apoptosis (pFoxO 3a/1a, Mn SOD, Cyclin D2, caspase 3) (24 h PE)
Gonzalez-Guevara et al. (2014)	Rats (Wistar) n = 3 males, 0 females Age: NR (250−300 g)	1 ppm, 1, 3, 6 h (single exposure); 1 h/day or 3 h/day for 5 days	Markers of inflammation (TNF-α, IL-6, NF-κB, GFAP) (immediately PE)
Fernando Hernandez-Zimbron and Rivas-Arancibia (2016)	Rats (Wistar) n = 3−6 males, 0 females Age: NR (250−300 g)	0.25 ppm, 4 h/day for 7, 15, 30, 60, or 90 days	β-Amyloid accumulation in the endoplasmic reticulum (2 h PE)
Hernandez-Zimbron and Rivas-Arancibia (2015)	Rats (Wistar) n = 6 males, 0 females Age: NR (250−300 g)	0.25 ppm, 4 h/day for 7, 15, 30, 60, or 90 days	β-Amyloid accumulation in the endoplasmic reticulum (2 h PE)
Pinto-Almazan et al. (2014)	Rats (Wistar) n = 10 males, 0 females Age: NR (250−300 g)	0.25 ppm, 4 h/day for 7, 15, 30, or 60 days	Markers of oxidative stress (NT, 4-HNE); loss of pyramidal neurons in the hippocampus (PE)
Rivas-Arancibia et al. (2015)	Rats (Wistar) n = 6 males, 0 females Age: NR (250−300 g)	0.25 ppm, 4 h/day for 7, 15, 30, 60, or 90 days	Markers of oxidative stress and inflammation (Iba-1, NF-κB, GFAP, COX-2); mitochondrial dysfunction and cell loss in substantia nigra

Table 7-23 (Continued): Study-specific details from short-term studies of brain inflammation and morphology.

Study	Population Species (Strain), N, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Rivas-Arancibia et al. (2017)	Rats (Wistar) n = 6 males, 0 females Age: NR (250−300 g)	0.25 ppm, 4 h/day for 7, 15, 30, 60, or 90 days	β-Amyloid structure (2 h PE)
Rodríguez-Martínez et al. (2013)	Rats (Wistar) n = 6 males, 0 females Age: NR (250−300 g)	0.25 ppm, 4 h/day for 7, 15, 30, or 60 days	Protein oxidation; antioxidant activity (SOD, GPx); mitochondrial dysfunction and cell damage in hippocampus
Mokoena et al. (2011)	Rats (S-D) n = 7−9 males, 0 females Age: NR (270−310 g)	0.25 or 0.7 ppm, 4 h Or 0.25 ppm, 4 h/day for 30 days	Lipid peroxidation/superoxide formation in frontal cortex (PE)
Mokoena et al. (2015)	Rats (FRL or FSL) n = 8−12 males, 0 females Age: NR (230−250 g)	0.3 ppm, 4 h/day for 15 days	Lipid peroxidation; antioxidant (SOD, CAT) activity (PE)
Mumaw et al. (2016)	Rats (S-D) n = 7−9 males, 0 females Age: 8 weeks	1 ppm, 4 h	Microglial activation; markers of inflammation (TNF-α, IL-1β) (24 h PE)
Tyler et al. (2018)	Mice (C57BL/6) n = 3−11 males, 0 females Age: adult (8−10 week); aged (12−18 mo)	1 ppm, 4 h	Blood-brain barrier permeability/infiltration; microglial activation; β-amyloid accumulation (20 h PE)
Rodríguez-Martínez et al. (2016)	Rats (Wistar) n = 6 males, 0 females Age: NR (250−300 g)	0.25 ppm, 4 h/day for 7, 15, 30, or 60 days	Endoplasmic reticulum dysfunction and cell death in the hippocampus

Table 7-24 Study-specific details from short-term studies of cognitive and behavioral effects.

Study	Population Species (Strain), N, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Bhoopalan et al. (2013)	Rats (S-D) n = 6 males, 0 females Age: 9−10 weeks	0.8 ppm, 3 h, single exposure	Dopamine levels in the striatum (~24 h PE)
Pinto-Almazan et al. (2014)	Rats (Wistar) n = 10 males, 0 females Age: NR (250−300 g)	0.25 ppm, 4 h/day for 7, 15, 30, or 60 days	Passive avoidance, motor activity (2 or 24 h PE)
Mokoena et al. (2015)	Rats (FRL or FSL) n = 8−12 males, 0 females Age: NR (230−250 g)	0.3 ppm, 4 h/day, 15 days	Novel object recognition, motor activity, forced swim test, elevated plus maze (immediately PE)
Gordon et al. (2016)	Rats (BN) n = 9−10 males, 9−10 females Age: adult (~20 week)	0.8 ppm, 5 h/day, 1 day/week, 4 week	Motor activity (measured after 1 week exposure)

Table 7-25 Study-specific details from short-term studies of neuroendocrine effects.

Study	Population Species (Strain), N, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Thomson et al. (2013)	Rats (F344) n = 4−6 males, 0 females Age: NR (200−250 g)	0.4 or 0.8 ppm, 4 h	Expression of genes related to antioxidant response, xenobiotic metabolism, inflammation, and endothelial dysfunction; adrenal hormones levels in serum

Table 7-26 Study-specific details from long-term studies of brain inflammation and morphology.

Study	Population Species (Strain), N, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Akhter et al. (2015)	Mice (C57BL/6 APP+/PS1+ or WT) n = 5−7 males, 5−7 females Age: 6 weeks	0.8 ppm, 16 weeks (eight cycles: 7 h/day for 5 days, 9 days FA only)	Lipid/protein oxidation, antioxidant levels, cell death in the hippocampus
Fernando Hernandez-Zimbron and Rivas-Arancibia (2016)	Rats (Wistar) n = 3− 6 males, 0 females Age: NR (250−300 g)	0.25 ppm, 4 h/day for 7, 15, 30, 60, or 90 days	β-Amyloid in the endoplasmic reticulum (2 h PE); β-amyloid accumulation
Gómez-Crisóstomo et al. (2014)	Rats (Wistar) n = 12 males, 0 females Age: NR (250−300 g)	0.25 ppm, 4 h/day for 7, 15, 30, 60, or 90 days	Markers of oxidative stress and apoptosis (pFoxO 3a/1a, Mn SOD, Cyclin D2, caspase 3) (24 h PE)
Hernandez-Zimbron and Rivas-Arancibia (2015)	Rats (Wistar) n = 6 males, 0 females Age: NR (250−300 g)	0.25 ppm, 4 h/day for 7, 15, 30, 60, or 90 days	β-amyloid accumulation in the endoplasmic reticulum (2 h PE)
Pinto-Almazan et al. (2014)	Rats (Wistar) n = 10 males, 0 females Age: NR (250−300 g)	0.25 ppm, 4 h/day for 7, 15, 30, or 60 days	Lipid/protein oxidation; loss of pyramidal neurons in the hippocampus (PE)
Mokoena et al. (2011)	Rats (S-D) n = 7−9 males, 0 females Age: NR (270−310 g)	0.25 or 0.7 ppm, 4 h or 0.25 ppm, 4 h/day for 30 days	Lipid peroxidation/superoxide formation in frontal cortex (PE)
Rivas-Arancibia et al. (2015)	Rats (Wistar) n = 6 males, 0 females Age: NR (250−300 g)	0.25 ppm, 4 h/day for 7, 15, 30, 60, or 90 days	Markers of oxidative stress and inflammation (Iba-1, NF-κB, GFAP, COX-2); mitochondrial dysfunction and cell loss in substantia nigra
Rivas-Arancibia et al. (2017)	Rats (Wistar) n = 6 males, 0 females Age: NR (250−300 g)	0.25 ppm, 4 h/day for 7, 15, 30, 60, or 90 days	β-Amyloid structure (2 h PE)

Table 7-26 (Continued): Study-specific details from long-term studies of brain inflammation and morphology.

Study	Population Species (Strain), N, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Rodríguez-Martínez et al. (2013)	Rats (Wistar) n = 6 males, 0 females Age: NR (250−300 g)	0.25 ppm, 4 h/day for 7, 15, 30, or 60 days	Protein oxidation; antioxidant activity (SOD, GPx); mitochondrial dysfunction and cell damage in hippocampus
Rodríguez-Martínez et al. (2016)	Rats (Wistar) n = 6 males, 0 females Age: NR (250−300 g)	0.25 ppm, 4 h/day for 7, 15, 30, or 60 days	Endoplasmic reticulum dysfunction and cell death in the hippocampus

Table 7-27 Study-specific details from long-term studies of cognitive and behavioral effects.

Study	Population Species (Strain), N, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Akhter et al. (2015)	Mice (C57BL/6 APP+/PS1+ or WT) n = 5−7 males, 5−7 females Age: 6 weeks	0.8 ppm, 16 w/4 mo (eight cycles: 7 h/day for 5 days, 9 days FA only)	Swim maze, elevated plus maze, motor activity
Pinto-Almazan et al. (2014)	Rats (Wistar) n = 10 males, 0 females Age: NR (250−300 g)	0.25 ppm, 4 h/day for 7, 15, 30, or 60 days	Passive avoidance, motor activity (2 or 24 h PE)
Gordon et al. (2014)	Rats (BN) n = 5−6 males, 0 females Age: Adult (4 mo), senescent (20 mo)	1 ppm, 6 h/day, 2 days/week, 13 week	Motor activity
Gordon et al. (2013)	Rats (BN) n = 7−8 males, 0 females Age: Adult (4 mo), senescent (20 mo)	0.8 ppm, 6 h/day, 1 day/week, 17 week	Motor activity

Table 7-28 Study effects.

Study	Population Species (Strain), N, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Hunter et al. (2011)	Rats (NR) n = 4−5 NR Age: PNDs 6, 10, 15, 21, 28	2 ppm, 3 h	Lung innervation, NGF production
Zellner et al. (2011)	Rats (F344) n = 3, ganglia weight; n = 6−13, nerve cell count, males and females combined Age: PNDs 10, 15, 21, 28	2 ppm, 3 h	Neurodevelopment (nodose and jugular sensory ganglion) (5−23 days PE); lung innervation

7.6 Evidence Inventories—Data Tables to Summarize Cancer Study Details

7.6.1 Epidemiologic Studies

Table 7-29 Epidemiologic studies of long-term exposure to ozone and cancer incidence.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Hystad et al. (2013) Nationwide, Canada Ozone: 1975−1994 Follow-up: 1994−1997 Case-control study	NECSS n = 2,390 cases	Spatio-temporal model; 25 × 25 km; May−September; includes residential history	Mean: 20.3 Maximum: 33.8	Correlation (*r*): $PM_{2.5}$: 0.25; NO_2: 0.11 Copollutant models with: NR	OR for all lung cancers: 1.09 (0.85, 1.37) OR for large cell lung cancer: 0.89 (0.57, 1.38) OR for adenocarcinoma: 1.04 (0.74, 1.44) OR for small cell lung cancer: 1.07 (0.65, 1.75) OR for squamous cell lung cancer: 1.19 (0.82, 1.71)
†Guo et al. (2016) Nationwide, China Ozone: 19,902,005 Follow-up: 1990−2009 Cohort study	n = 368,762 lung cancer cases 30+ yrs old	Hybrid model from Global Burden of Disease	Mean: 56.9 Median: 56.8 75th: 60.5 Maximum: 76.8	Correlation (*r*): NR Copollutant models with: NR	Lung cancer incidence—all: 1.09 (1.08, 1.1) Lung cancer incidence—female: 1.08 (1.07, 1.09) Lung cancer incidence—males: 1.09 (1.08, 1.1) Lung cancer incidence—ages 30−65: 1.08 (1.07, 1.09) Lung cancer incidence—ages 65−75: 1.12 (1.11, 1.13) Lung cancer incidence—ages 75+: 1.1 (1.08, 1.12)

†Recent studies evaluated since the 2013 Ozone ISA.

Table 7-30 Epidemiologic studies of ozone exposure and lung cancer mortality.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Carey et al. (2013) Nationwide, U.K. Ozone: 2002 Follow-up: 2003−2007 Cohort study	English Medical Practice n = 835,607 Age: adults, 40−89 yr, from English medical practices	Annual mean estimates from dispersion model for 1-km grid cells linked to nearest residential postal code centroid	Mean: 25.85 Maximum: 31.5	Correlation (*r*): $PM_{2.5}$: −0.39; NO_2: −0.46; SO_2: −0.41; PM_{10}: −0.40 Copollutant models with: NR	Lung cancer: 0.66 (0.50, 0.87)
†Jerrett et al. (2013) California, U.S. Ozone: 1988−2002 Follow-up: 1982−2000 Cohort study	ACS n = 73,711	Monthly averages calculated from IDW from up to four monitors within 50 km of residence	Mean: 50.35 Median: 50.8 75th: 61 90th: 68.56 95th: 74.18 Maximum: 89.33	Correlation (*r*): $PM_{2.5}$: 0.56; NO_2: −0.0071 Copollutant models with: $PM_{2.5}$; NO_2	Lung cancer: 0.94 (0.89, 1.00) Lung cancer (+ $PM_{2.5}$): 0.93 (0.87, 0.98) Lung cancer (+ NO_2): 0.95 (0.89, 1.01)
†Crouse et al. (2015) Nationwide, Canada Ozone: 2002−2009 Follow-up: 1991−2006 Cohort study	CanCHEC n = 2,521,525 Age: 25+ yr	Model of warm season concentration at 21-km horizontal resolution assigned at postal code 8-h max	Mean: 39.6 Median: 39 75th: 44.2 Maximum: 60	Correlation (*r*): $PM_{2.5}$: 0.73; NO_2: 0.19 Copollutant models with: NR	Lung cancer: 1.01 (0.99, 1.02)
†Turner et al. (2016) Nationwide, U.S. Ozone: 2002−2004 Follow-Up: 1982−2004 Cohort study	ACS n = 669,046 Age: 35+	HBM with inputs from NAMS/SLAMS and CMAQ; downscaler for the eastern U.S. 8-h max	Mean: 38.2 Median: 38.1 75th: 40.1 95th: 45 Maximum: 59.3	Correlation (*r*): $PM_{2.5}$: 0.18; NO_2: −0.08 Copollutant models with: $PM_{2.5}$	Lung cancer: 0.96 (0.91, 1.00)
†Cakmak et al. (2017) Nationwide, Canada Ozone: 2002−2009 Follow-up: 1991−2011 Cohort study	CanCHEC n = 2,291,250 Age: 25+ yr	Model of warm season concentration at 21-km horizontal resolution assigned at postal code 8-h max	Mean: 15.0−43.0 Maximum: 46.6−60.6	Correlation (*r*): $PM_{2.5}$: −0.705 Copollutant models with: $PM_{2.5}$	Lung cancer: 1.05 (0.97, 1.13) Lung cancer (+ $PM_{2.5}$): 1.01 (0.93, 1.09)

Table 7-30 (Continued): Epidemiologic studies of ozone exposure and lung cancer mortality.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Xue et al. (2018) Shenyang, China Ozone: 2013−2015 Follow-up: 2013−2015 Case-crossover study	n = 29,112 lung cancer deaths	Average of six monitors 24-h avg	Mean: 28.5 Median: 26.9 75th: 39.1 Maximum: 90.4	Correlation (*r*): $PM_{2.5}$: 0.25; NO_2: 0.51; SO_2: 0.55; Other: PM_{10}: 0.21; CO: 0.25 Copollutant models with: NR	Lung cancer mortality (lag 0−1): 0.98 (0.81, 1.21) Lung cancer mortality (lag 0−2): 0.98 (0.83, 1.19)
†Eckel et al. (2016) California, U.S. Ozone: 1988−2011 Follow-up: 1988−2011 Cohort study	n = 352,053 California residents with new diagnosis of cancer	Monthly averages calculated from IDW from up to four monitors within 50 km of residence 8-h max	Mean: 40.2	Correlation (*r*): $PM_{2.5}$: −0.02; NO_2: −0.01; Other: PM_{10}: 0.36 Copollutant models with: NR	Lung cancer mortality: 1.03 (1.02, 1.03)

†Recent studies evaluated since the 2013 Ozone ISA.

Table 7-31 Epidemiologic studies of long-term exposure to ozone and other cancer endpoints.

Study	Study Population	Exposure Assessment	Mean (ppb)	Copollutant Examination	Effect Estimates HR (95% CI)
†Badaloni et al. (2013) Nationwide, Italy Ozone: NR Follow-up: children born between 1998−2001 Case-control study	SETIL n = 620 cases Age: children <10 yr	LUR—6-yr mean	Mean: 24.2 Maximum: 50.1	Correlation (*r*): NR Copollutant models with: NR	Q2 vs. Q1 ozone exposure—incident leukemia: 0.88 (0.65, 1.19) Q3 vs. Q1 ozone exposure—incident leukemia: 1.2 (0.87, 1.65) Q4 vs. Q1 ozone exposure—incident leukemia: 1.1 (0.76, 1.59)
†Turner et al. (2017) Nationwide, U.S. Ozone: 2002−2004 Follow-up: 1982−2004 Cohort study	ACS n = 623,048 Age: 30+ yr old	HBM with inputs from NAMS/SLAMS and CMAQ 8-h max	Mean: 38.2 Median: 38.1 75th: 40.1 95th: 44.9 Maximum: 59.3	Correlation (*r*): NO_2: −0.09 Copollutant models with: NR	(Selected results; highest and lowest magnitude results presented) Salivary gland cancer (n = 58): 1.70 (0.87, 3.34) Pharynx cancer (n = 243): 1.16 (0.80, 1.68) Eye cancer (n = 26): 0.67 (0.25, 1.85) Connective tissue cancer (n = 377): 0.84 (0.65, 1.12)
†Yaghjyan et al. (2017) Nationwide, U.S. Ozone: 2001−2008 Follow-up: 2001−2009 Cohort study	BCSC n = 279,967 Age: 40+ yr old women with no history of breast cancer	CMAQ-HBM 8-h max	Median: 36 75th: 37.9	Correlation (*r*): NR Copollutant models with: NR	Q4 vs. Q1 ozone exposure; breast tissue density: 0.8 (0.73, 0.87)

†Recent studies evaluated since the 2013 Ozone ISA.

7.6.2 Toxicological Studies

Table 7-32 Study specific details of ozone exposure and DNA damage.

Study	Population Species (Strain), N, Sex, Age	Exposure Details (Concentration, Duration)	Endpoints Examined
Holland et al. (2014)	Healthy adults n = 11 males, 11 females Age: 18−50 yr	0.10 and 0.20 ppm, 4 h with intermittent exercise	Cell viability and proliferation in culture (blood drawn at 0 and 24 h) Frequencies of micronuclei and nucleoplasmic bridges in blood lymphocytes (blood drawn at 0 and 24 h)
Finkenwirth et al. (2014)	Healthy adults n = 19 in placebo group 18 in ozone group males, 0 females Age: 18−50 yr	0.21 ppm, 2 h	DNA damage in isolated lymphocytes (30 min and 4.5 h PE)
Cestonaro et al. (2017)	Rats (Wistar) n = 12/group males, 0 females Age: 9−10 weeks	0.05 ppm, 24 h/day for 14 or 28 days 0.05 ppm, 3 h/day for 14 and 28 days	DNA in tail/olive tail moment (PE) Micronuclei induction (post-exposure)
Zhang et al. (2017)	Rats (S-D) n = 4 males/group* for each time point, 0 females Age: NR	2 ppm, 30 min and room air for 12 days. Groups also treated with l-argenine and L-NAME.	Production of 8-oxoG/OGG1 during lung injury (baseline 4, 8, and 12 days PE)

PE = post-exposure

Annex for Appendix 7: Evaluation of Studies on Health Effects of Ozone

This annex describes the approach used in the Integrated Science Assessment (ISA) for Ozone and Related Photochemical Oxidants to evaluate study quality in the available health effects literature. As described in the Preamble to the ISA (U.S. EPA, 2015), causality determinations were informed by the integration of evidence across scientific disciplines (e.g., exposure, animal toxicology, epidemiology) and related outcomes and by judgments of the strength of inference in individual studies. Table Annex 6-1 describes aspects considered in evaluating study quality of controlled human exposure, animal toxicological, and epidemiologic studies. The aspects found in Table Annex 6-1 are consistent with current best practices for reporting or evaluating health science data.1 Additionally, the aspects are compatible with published U.S. EPA guidelines related to cancer, neurotoxicity, reproductive toxicity, and developmental toxicity (U.S. EPA, 2005, 1998, 1996, 1991).

These aspects were not used as a checklist, and judgments were made without considering the results of a study. The presence or absence of particular features in a study did not necessarily lead to the conclusion that a study was less informative or should be excluded from consideration in the ISA. Further, these aspects were not used as criteria for determining causality in the five-level hierarchy. As described in the Preamble, causality determinations were based on judgments of the overall strengths and limitations of the collective body of available studies and the coherence of evidence across scientific disciplines and related outcomes. Table Annex 6-1 is not intended to be a complete list of aspects that define a study's ability to inform the relationship between ozone and health effects, but it describes the major aspects considered in this ISA to evaluate studies. Where possible, study elements, such as exposure assessment and confounding (i.e., bias due to a relationship with the outcome and correlation with exposures to ozone), are considered specifically for ozone. Thus, judgments on the ability of a study to inform the relationship between an air pollutant and health can vary depending on the specific pollutant being assessed.

1 For example, NTP OHAT approach (Rooney et al., 2014), IRIS Preamble (U.S. EPA, 2013b), ToxRTool (Klimisch et al., 1997), STROBE guidelines (von Elm et al., 2007), and ARRIVE guidelines (Kilkenny et al., 2010).

Table Annex 7-1 Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Study Design
Controlled Human Exposure:
Studies should clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested. Study subjects should be randomly exposed without knowledge of the exposure condition. Preference is given to balanced crossover (repeated measures) or parallel design studies which include control exposures (e.g., to clean filtered air). In crossover studies, a sufficient and specified time between exposure days should be provided to avoid carry over effects from prior exposure days. In parallel design studies, all arms should be matched for individual characteristics, such as age, sex, race, anthropometric properties, and health status. In studies evaluating effects of disease, appropriately matched healthy controls are desired for interpretative purposes.
Animal Toxicology:
Studies should clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested. Studies should include appropriately matched control exposures (e.g., to clean filtered air, time matched). Studies should use methods to limit differences in baseline characteristics of control and exposure groups. Studies should randomize assignment to exposure groups and where possible conceal allocation to research personnel. Groups should be subjected to identical experimental procedures and conditions; animal care including housing, husbandry, etc. should be identical between groups. Blinding of research personnel to study group may not be possible due to animal welfare and experimental considerations; however, differences in the monitoring or handling of animals in all groups by research personnel should be minimized.
Epidemiology:
Inference is stronger for studies that clearly describe the primary and any secondary aims of the study, or specific hypotheses being tested. For short-term exposure, time-series, case-crossover, and panel studies are emphasized over cross-sectional studies because they examine temporal correlations and are less prone to confounding by factors that differ between individuals (e.g., SES, age). Panel studies with scripted exposures, in particular, can contribute to inference because they have consistent, well-defined exposure durations across subjects, measure personal ambient pollutant exposures, and measure outcomes at consistent, well-defined lags after exposures. Studies with large sample sizes and conducted over multiple years are considered to produce more reliable results. Additionally, multicity studies are preferred over single-city studies because they examine associations for large diverse geographic areas using a consistent statistical methodology, avoiding the publication bias often associated with single-city studies.[a] If other quality parameters are equal, multicity studies carry more weight than single-city studies because they tend to have larger sample sizes and lower potential for publication bias. For long-term exposure, inference is considered to be stronger for prospective cohort studies and case-control studies nested within a cohort (e.g., for rare diseases) than cross-sectional, other case-control, or ecologic studies. Cohort studies can better inform the temporality of exposure and effect. Other designs can have uncertainty related to the appropriateness of the control group or validity of inference about individuals from group-level data. Study design limitations can bias health effect associations in either direction.
Study Population/Test Model
Controlled Human Exposure:
In general, the subjects recruited into study groups should be similarly matched for age, sex, race, anthropometric properties, and health status. In studies evaluating effects of specific subject characteristics (e.g., disease, genetic polymorphism, etc.), appropriately matched healthy controls are preferred. Relevant characteristics and health status should be reported for each experimental group. Criteria for including and excluding subjects should be clearly indicated. For the examination of populations with an underlying health condition (e.g., asthma), independent, clinical assessment of the health condition is ideal, but self-report of physician diagnosis generally is considered to be reliable for respiratory and cardiovascular disease outcomes.b The loss or withdrawal of recruited subjects during the course

of a study should be reported. Specific rationale for excluding subject(s) from any portion of a protocol should be explained.

Animal Toxicology:

Ideally, studies should report species, strain, substrain, genetic background, age, sex, and weight. Unless data indicate otherwise, all animal species and strains are considered appropriate for evaluating effects of ozone exposure. It is preferred that the authors test for effects in both sexes and multiple lifestages, and report the result for each group separately. All animals used in a study should be accounted for, and rationale for exclusion of animals or data should be specified.

Epidemiology:

There is greater confidence in results for study populations that are recruited from and representative of the target population. Studies with high participation and low dropout over time that is not dependent on exposure or health status are considered to have low potential for selection bias. Clearly specified criteria for including and excluding subjects can aid assessment of selection bias. For populations with an underlying health condition, independent, clinical assessment of the health condition is valuable, but self-report of physician diagnosis generally is considered to be reliable for respiratory and cardiovascular diseases.b Comparisons of groups with and without an underlying health condition are more informative if groups are from the same source population. Selection bias can influence results in either direction or may not affect the validity of results but rather reduce the generalizability of findings to the target population.

Pollutant

Controlled Human Exposure:

The focus is on studies testing ozone exposure.

Animal Toxicology:

The focus is on studies testing ozone exposure.

Epidemiology:

The focus is on studies evaluating ozone exposure.

Exposure Assessment or Assignment

Controlled Human Exposure:

For this assessment, the focus is on studies that use ozone concentrations <0.4 ppm. Studies that use higher exposure concentrations may provide information relevant to biological plausibility, dosimetry, or inter-species variation. Studies should have well-characterized pollutant concentration, temperature, and relative humidity and/or have measures in place to adequately control the exposure conditions. Preference is given to balanced crossover or parallel design studies that include control exposures (e.g., to clean filtered air). Study subjects should be randomly exposed without knowledge of the exposure condition. Method of exposure (e.g., chamber, facemask, etc.) should be specified and activity level of subjects during exposures should be well characterized.

Animal Toxicology:

For this assessment, the focus is on studies that use ozone concentrations <2 ppm. Studies that use higher exposure concentrations may provide information relevant to biological plausibility, dosimetry, or inter-species variation. Studies should characterize pollutant concentration, temperature, and relative humidity and/or have measures in place to adequately control the exposure conditions. The focus is on inhalation exposure. Noninhalation exposure experiments (i.e., intra-tracheal instillation [IT]) are informative for size fractions that cannot penetrate the airway of a study animal

and may provide information relevant to biological plausibility and dosimetry. In vitro studies may be included if they provide mechanistic insight or examine similar effects as in vivo studies, but are generally not included. All studies should include exposure control groups (e.g., clean filtered air).

Epidemiology:

Of primary relevance are relationships of health effects with the ambient component of ozone exposure. However, information about ambient exposure rarely is available for individual subjects; most often, inference is based on ambient concentrations. Studies that compare exposure assessment methods are considered to be particularly informative. Inference is stronger when the duration or lag of the exposure metric corresponds with the time course for physiological changes in the outcome (e.g., up to a few days for symptoms) or latency of disease (e.g., several years for cancer).

Ambient ozone concentration tends to have low spatial heterogeneity at the urban scale, except near roads where ozone concentration is lower because ozone reacts with nitric oxide emitted from vehicles. For studies involving individuals with near-road or on-road exposures to ozone, in which ambient ozone concentrations are more spatially heterogeneous and relationships between personal exposures and ambient concentrations are potentially more variable, validated methods that capture the extent of variability for the epidemiologic study design (temporal vs. spatial contrasts) and location carry greater weight.

Fixed-site measurements, whether averaged across multiple monitors or assigned from the nearest or single available monitor, typically have smaller biases and smaller reductions in precision compared with spatially heterogeneous air pollutants. Concentrations reported from fixed-site measurements can be informative if correlated with personal exposures, closely located to study subjects, highly correlated across monitors within a location, or combined with time-activity information.

Atmospheric models may be used for exposure assessment in place of or to supplement ozone measurements in epidemiologic analyses. For example, grid-scale models (e.g., CMAQ) that represent ozone exposure over relatively large spatial scales (e.g., typically greater than 4 × 4-km grid size) often do provide adequate spatial resolution to capture acute ozone peaks that influence short-term health outcomes. Uncertainty in exposure predictions from these models is largely influenced by model formulations and the quality of model input data pertaining to precursor emissions or meteorology, which tends to vary on a study-by-study basis.

In studies of short-term exposure, temporal variability of the exposure metric is of primary interest. For long-term exposures, models that capture within-community spatial variation in individual exposure may be given more weight for spatially variable ambient ozone. Given the low spatial variability of ozone at the urban scale, exposure measurement error typically causes health effect estimates to be underestimated for studies of either short-term or long-term exposure. Biases and decreases in the precision of the association (i.e., wider 95% CIs) tend to be small. Even when spatial variability is higher near roads, the reduction in ozone exposure would cause the exposure to be overestimated at a monitor distant from the road or when averaged across a model grid cell, so that health effects would likely be underestimated.

Outcome Assessment/Evaluation

Controlled Human Exposure:

Endpoints should be assessed in the same manner for control and exposure groups (e.g., time after exposure, methods, endpoint evaluator) using valid, reliable methods. Blinding of endpoint evaluators is ideal, especially for qualitative endpoints (e.g., histopathology). For each experiment and each experimental group, including controls, precise details of all procedures carried out should be provided including how, when, and where. Time of the endpoint evaluations is a key consideration that will vary depending on endpoint evaluated. Endpoints should be assessed at time points that are appropriate for the research questions.

Animal Toxicology:

Endpoints should be assessed in the same manner for control and exposure groups (e.g., time after exposure, methods, endpoint evaluator) using valid, reliable methods. Blinding of endpoint evaluators is ideal, especially for qualitative endpoints (e.g., histopathology). For each experiment and each experimental group, including controls, precise details of all procedures carried out should be provided including how, when, and where. Time of the endpoint evaluations is a key consideration that will vary depending on endpoint evaluated. Endpoints should be assessed at time points that are appropriate for the research questions.

Table Annex 7-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Epidemiology:

Inference is stronger when outcomes are assessed or reported without knowledge of exposure status. Knowledge of exposure status could produce artifactual associations. Confidence is greater when outcomes assessed by interview, self-report, clinical examination, or analysis of biological indicators are defined by consistent criteria and collected by validated, reliable methods. Independent, clinical assessment is valuable for outcomes like lung function or incidence of disease, but report of physician diagnosis has shown good reliability.b When examining short-term exposures, evaluation of the evidence focuses on specific lags based on the evidence presented in individual studies. Specifically, the following hierarchy is used in the process of selecting results from individual studies to assess in the context of results across all studies for a specific health effect or outcome:

ix. Distributed lag models;

x. Average of multiple days (e.g., 0−2);

xi. If a priori lag days were used by the study authors these are the effect estimates presented; or

xii. If a study focuses on only a series of individual lag days, expert judgment is applied to select the appropriate result to focus on considering the time course for physiologic changes for the health effect or outcome being evaluated.

When health effects of long-term exposure are assessed by acute events such as symptoms or hospital admissions, inference is strengthened when results are adjusted for short-term exposure. Validated questionnaires for subjective outcomes such as symptoms are regarded to be reliable,c particularly when collected frequently and not subject to long recall. For biological samples, the stability of the compound of interest and the sensitivity and precision of the analytical method is considered. If not based on knowledge of exposure status, errors in outcome assessment tend to bias results toward the null.

Potential Copollutant Confounding

Controlled Human Exposure:

Exposure should be well characterized to evaluate independent effects of ozone.

Animal Toxicology:

Exposure should be well characterized to evaluate independent effects of ozone.

Epidemiology:

Not accounting for potential copollutant confounding can produce artifactual associations; thus, studies that examine copollutant confounding carry greater weight. The predominant method is copollutant modeling (i.e., two-pollutant models), which is especially informative when correlations are not high. However, when correlations are high ($r > 0.7$), such as those often encountered for UFP and other traffic-related copollutants, copollutant modeling is less informative. Although the use of single-pollutant models to examine the association between ozone and a health effect or outcome are informative, ideally studies should also include copollutant analyses. Copollutant confounding is evaluated on an individual study basis considering the extent of correlations observed between the copollutant and ozone, and relationships observed with ozone and health effects in copollutant models.

Other Potential Confounding Factors[d]

Controlled Human Exposure:

Preference is given to studies using experimental and control groups that are matched for individual level characteristics (e.g., race/ethnicity, sex, body weight, smoking history, age) and time varying factors (e.g., seasonal and diurnal patterns).

Table Annex 7-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Animal Toxicology:
Preference is given to studies using experimental and control groups that are matched for individual level characteristics (e.g., strain, sex, body weight, litter size, food and water consumption) and time varying factors (e.g., seasonal and diurnal patterns).
Epidemiology:
Factors are considered to be potential confounders if demonstrated in the scientific literature to be related to health effects and correlated with ozone. Not accounting for confounders can produce artifactual associations; thus, studies that statistically adjust for multiple factors or control for them in the study design are emphasized. Less weight is placed on studies that adjust for factors that mediate the relationship between ozone and health effects, which can bias results toward the null. Confounders vary according to study design, exposure duration, and health effect and may include, but are not limited to the following: Short-term exposure studies: Meteorology, day of week, season, medication use, allergen exposure, and long-term temporal trends. Long-term exposure studies: Socioeconomic status, race, age, medication use, smoking status, stress, noise, and occupational exposures.
Statistical Methodology
Controlled Human Exposure:
Statistical methods should be clearly described and appropriate for the study design and research question (e.g., correction for multiple comparisons). Generally, statistical significance is used to evaluate the findings of controlled human exposure studies. However, consistent trends are also informative. Detection of statistical significance is influenced by a variety of factors including, but not limited to, the size of the study, exposure and outcome measurement error, and statistical model specifications. Sample size is not a criterion for exclusion; ideally, the sample size should provide adequate power to detect hypothesized effects (e.g., sample sizes less than three are considered less informative). Because statistical tests have limitations, consideration is given to both trends in data and reproducibility of results.
Animal Toxicology:
Statistical methods should be clearly described and appropriate for the study design and research question (e.g., correction for multiple comparisons). Generally, statistical significance is used to evaluate the findings of animal toxicology studies. However, consistent trends are also informative. Detection of statistical significance is influenced by a variety of factors including, but not limited to, the size of the study, exposure and outcome measurement error, and statistical model specifications. Sample size is not a criterion for exclusion; ideally, the sample size should provide adequate power to detect hypothesized effects (e.g., sample sizes less than three are considered less informative). Because statistical tests have limitations, consideration is given to both trends in data and reproducibility of results.

Table Annex 7-1 (Continued): Scientific considerations for evaluating the strength of inference from studies on the health effects of ozone.

Epidemiology:
Multivariable regression models that include potential confounding factors are emphasized. However, multipollutant models (more than two pollutants) are considered to produce too much uncertainty due to copollutant collinearity to be informative. Models with interaction terms aid in the evaluation of potential confounding as well as effect modification. Sensitivity analyses with alternate specifications for potential confounding inform the stability of findings and aid in judgments of the strength of inference from results. In the case of multiple comparisons, consistency in the pattern of association can increase confidence that associations were not found by chance alone. Statistical methods that are appropriate for the power of the study carry greater weight. For example, categorical analyses with small sample sizes can be prone to bias results toward or away from the null. Statistical tests such as *t*-tests and chi-squared tests are not considered sensitive enough for adequate inferences regarding ozone-health effect associations. For all methods, the effect estimate and precision of the estimate (i.e., width of 95% CI) are important considerations rather than statistical significance.

a(U.S. EPA, 2008).

bMurgia et al. (2014); Weakley et al. (2013); Yang et al. (2011); Heckbert et al. (2004); Barr et al. (2002); Muhajarine et al. (1997); Toren et al. (1993).

cBurney et al. (1989).

dMany factors evaluated as potential confounders can be effect measure modifiers (e.g., season, comorbid health condition) or mediators of health effects related to ozone (comorbid health condition).

7.7 References

Agarwal, A; Mulgund, A; Hamada, A; Chyatte, MR. (2015). A unique view on male infertility around the globe. Reprod Biol Endocrinol 13: 37. http://dx.doi.org/10.1186/s12958-015-0032-1.

Agay-Shay, K; Friger, M; Linn, S; Peled, A; Amitai, Y; Peretz, C. (2013). Air pollution and congenital heart defects. Environ Res 124: 28-34. http://dx.doi.org/10.1016/j.envres.2013.03.005.

Akhter, H; Ballinger, C; Liu, N; van Groen, T; Postlethwait, EM; Liu, R. (2015). Cyclic ozone exposure induces gender-dependent neuropathology and memory decline in an animal model of alzheimer's disease. Toxicol Sci 147: 222-234. http://dx.doi.org/10.1093/toxsci/kfv124.

Arroyo, V; Díaz, J; Carmona, R; Ortiz, C; Linares, C. (2016). Impact of air pollution and temperature on adverse birth outcomes: Madrid, 2001-2009. Environ Pollut 218: 1154-1161. http://dx.doi.org/10.1016/j.envpol.2016.08.069.

Avdalovic, MV; Tyler, NK; Putney, L; Nishio, SJ; Quesenberry, S; Singh, PJ; Miller, LA; Schelegle, ES; Plopper, CG; Vu, T; Hyde, DM. (2012). Ozone exposure during the early postnatal period alters the timing and pattern of alveolar growth and development in nonhuman primates. Anat Rec 295: 1707-1716. http://dx.doi.org/10.1002/ar.22545.

Badaloni, C; Ranucci, A; Cesaroni, G; Zanini, G; Vienneau, D; Al-Aidrous, F; De Hoogh, K; Magnani, C; Forastiere, F; Group, SS. (2013). Air pollution and childhood leukaemia: a nationwide case-control study in Italy. Occup Environ Med 70: 876-883. http://dx.doi.org/10.1136/oemed-2013-101604.

Barker, DJ; Osmond, C. (1986). Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet 1: 1077-1081.

Barr, RG; Herbstman, J; Speizer, FE; Camargo, CA, Jr. (2002). Validation of self-reported chronic obstructive pulmonary disease in a cohort study of nurses. Am J Epidemiol 155: 965-971. http://dx.doi.org/10.1093/aje/155.10.965.

Becerra, TA; Wilhelm, M; Olsen, J; Cockburn, M; Ritz, B. (2013). Ambient air pollution and autism in Los Angeles County, California. Environ Health Perspect 121: 380-386. http://dx.doi.org/10.1289/ehp.1205827.

Bhoopalan, V; Han, SG; Shah, MM; Thomas, DM; Bhalla, DK. (2013). Tobacco smoke modulates ozone-induced toxicity in rat lungs and central nervous system. Inhal Toxicol 25: 21-28. http://dx.doi.org/10.3109/08958378.2012.751143.

Bignami, G; Musi, B; Dell'Omo, G; Laviola, G; Alleva, E. (1994). Limited effects of ozone exposure during pregnancy on physical and neurobehavioral development of CD-1 mice. Toxicol Appl Pharmacol 129: 264-271. http://dx.doi.org/10.1006/taap.1994.1251.

Brauer, M; Amann, M; Burnett, RT; Cohen, A; Dentener, F; Ezzati, M; Henderson, SB; Krzyzanowski, M; Martin, RV; Van Dingenen, R; van Donkelaar, A; Thurston, GD. (2012). Exposure assessment for estimation of the global burden of disease attributable to outdoor air pollution. Environ Sci Technol 46: 652-660. http://dx.doi.org/10.1021/es2025752.

Breton, CV; Wang, X; Mack, WJ; Berhane, K; Lopez, M; Islam, TS; Feng, M; Lurmann, F; McConnell, R; Hodis, HN; Künzli, N; Avol, E. (2012). Childhood air pollutant exposure and carotid artery intima-media thickness in young adults. Circulation 126: 1614-1620. http://dx.doi.org/10.1161/CIRCULATIONAHA.112.096164.

Breton, CV; Yao, J; Millstein, J; Gao, L; Siegmund, KD; Mack, W; Whitfield-Maxwell, L; Lurmann, F; Hodis, H; Avol, E; Gilliland, FD. (2016). Prenatal Air Pollution Exposures, DNA Methyl Transferase Genotypes, and Associations with Newborn LINE1 and Alu Methylation and Childhood Blood Pressure and Carotid Intima-Media Thickness in the Children's Health Study. Environ Health Perspect 124: 1905-1912. http://dx.doi.org/10.1289/EHP181.

Brown, JM; Harris, G; Pantea, C; Hwang, SA; Talbot, TO. (2015). Linking air pollution data and adverse birth outcomes: Environmental public health tracking in New York State. J Public Health Manag Pract 21: S68-S74. http://dx.doi.org/10.1097/PHH.0000000000000171.

Burney, PG; Laitinen, LA; Perdrizet, S; Huckauf, H; Tattersfield, AE; Chinn, S; Poisson, N; Heeren, A; Britton, JR; Jones, T. (1989). Validity and repeatability of the IUATLD (1984) Bronchial Symptoms Questionnaire: an international comparison. Eur Respir J 2: 940-945.

Cakmak, S; Hebbern, C; Pinault, L; Lavigne, E; Vanos, J; Crouse, DL; Tjepkema, M. (2017). Associations between long-term PM2.5 and ozone exposure and mortality in the Canadian Census Health and Environment Cohort (CANCHEC), by spatial synoptic classification zone. Environ Int 111: 200-211. http://dx.doi.org/10.1016/j.envint.2017.11.030.

Capobussi, M; Tettamanti, R; Marcolin, L; Piovesan, L; Bronzin, S; Gattoni, ME; Polloni, I; Sabatino, G; Tersalvi, CA; Auxilia, F; Castaldi, S. (2016). Air pollution impact on pregnancy outcomes in Como, Italy. J Occup Environ Med 58: 47-52. http://dx.doi.org/10.1097/JOM.0000000000000630.

Carey, IM; Atkinson, RW; Kent, AJ; van Staa, T; Cook, DG; Anderson, HR. (2013). Mortality associations with long-term exposure to outdoor air pollution in a national English cohort. Am J Respir Crit Care Med 187: 1226-1233. http://dx.doi.org/10.1164/rccm.201210-1758OC.

Carey, SA; Ballinger, CA; Plopper, CG; Mcdonald, RJ; Bartolucci, AA; Postlethwait, EM; Harkema, J. R. (2011). Persistent rhinitis and epithelial remodeling induced by cyclic ozone exposure in the nasal airways of infant monkeys. Am J Physiol Lung Cell Mol Physiol 300: L242-L254. http://dx.doi.org/10.1152/ajplung.00177.2010.

Carmona, R; Linares, C; Recio, A; Ortiz, C; Díaz, J. (2018). Emergency multiple sclerosis hospital admissions attributable to chemical and acoustic pollution: Madrid (Spain), 2001-2009. Sci Total Environ 612: 111-118. http://dx.doi.org/10.1016/j.scitotenv.2017.08.243.

Carré, J; Gatimel, N; Moreau, J; Parinaud, J; Leandri, R. (2016). Influence of air quality on the results of in vitro fertilization attempts: A retrospective study. Eur J Obstet Gynecol Reprod Biol 210: 116-122. http://dx.doi.org/10.1016/j.ejogrb.2016.12.012.

Carvalho, MA; Bernardes, LS; Hettfleisch, K; Pastro, LD; Vieira, SE; Saldiva, SR; Saldiva, PH; Francisco, RP. (2016). Associations of maternal personal exposure to air pollution on fetal weight and fetoplacental Doppler: A prospective cohort study. Reprod Toxicol 62: 9-17. http://dx.doi.org/10.1016/j.reprotox.2016.04.013.

Cerza, F; Renzi, M; Agabiti, N; Marino, C; Gariazzo, C; Davoli, M; Michelozzi, P; Forastiere, F; Cesaroni, G. (2018). Residential exposure to air pollution and incidence of Parkinsons disease in a large metropolitan cohort. Environmental Epidemiology 2: e023. http://dx.doi.org/10.1097/EE9.0000000000000023.

Cestonaro, LV; Marcolan, AM; Rossato-Grando, LG; Anzolin, AP; Goethel, G; Vilani, A; Garcia, SC; Bertol, CD. (2017). Ozone generated by air purifier in low concentrations: friend or foe? Environ Sci Pollut Res Int 24: 22673-22678. http://dx.doi.org/10.1007/s11356-017-9887-3.

Chandra, A; Copen, CE; Stephen, EH. (2013). Infertility and impaired fecundity in the United States, 1982-2010: Data from the National Survey of Family Growth. Atlanta, GA: Centers for Disease Control. http://www.cdc.gov/nchs/data/nhsr/nhsr067.pdf.

Chen, C; Liu, C; Chen, R; Wang, W; Li, W; Kan, H; Fu, C. (2018). Ambient air pollution and daily hospital admissions for mental disorders in Shanghai, China. Sci Total Environ 613-614: 324-330. http://dx.doi.org/10.1016/j.scitotenv.2017.09.098.

Chen, CY; Hung, HJ; Chang, KH; Hsu, CY; Muo, CH; Tsai, CH; Wu, TN. (2017a). Long-term exposure to air pollution and the incidence of Parkinson's disease: A nested case-control study. PLoS ONE 12: e0182834. http://dx.doi.org/10.1371/journal.pone.0182834.

Chen, G; Guo, Y; Abramson, MJ; Williams, G; Li, S. (2017b). Exposure to low concentrations of air pollutants and adverse birth outcomes in Brisbane, Australia, 2003-2013. Sci Total Environ 622-623: 721-726. http://dx.doi.org/10.1016/j.scitotenv.2017.12.050.

Chen, H; Kwong, JC; Copes, R; Hystad, P; van Donkelaar, A; Tu, K; Brook, JR; Goldberg, MS; Martin, RV; Murray, BJ; Wilton, AS; Kopp, A; Burnett, RT. (2017c). Exposure to ambient air pollution and the incidence of dementia: A population-based cohort study. Environ Int 108: 271-277. http://dx.doi.org/10.1016/j.envint.2017.08.020.

Chen, JC; Schwartz, J. (2009). Neurobehavioral effects of ambient air pollution on cognitive performance in US adults. Neurotoxicology 30: 231-239. http://dx.doi.org/10.1016/j.neuro.2008.12.011.

Chiu, H; Yang, C. (2015). Air pollution and daily clinic visits for migraine in a subtropical city: Taipei, Taiwan. J Toxicol Environ Health A 78: 549-558. http://dx.doi.org/10.1080/15287394.2015.983218.

Cho, J; Choi, YJ; Sohn, J; Suh, M; Cho, SK; Ha, KH; Kim, C; Shin, DC. (2015). Ambient ozone concentration and emergency department visits for panic attacks. J Psychiatr Res 62: 130-135. http://dx.doi.org/10.1016/j.jpsychires.2015.01.010.

Chou, DL; Gerriets, JE; Schelegle, ES; Hyde, DM; Miller, LA. (2011). Increased CCL24/eotaxin-2 with postnatal ozone exposure in allergen-sensitized infant monkeys is not associated with recruitment of eosinophils to airway mucosa. Toxicol Appl Pharmacol 257: 309-318. http://dx.doi.org/10.1016/j.taap.2011.09.001.

Chounlamountry, K; Boyer, B; Penalba, V; François-Bellan, AM; Bosler, O; Kessler, JP; Strube, C. (2015). Remodeling of glial coverage of glutamatergic synapses in the rat nucleus tractus solitarii after ozone inhalation. J Neurochem 134: 857-864. http://dx.doi.org/10.1111/jnc.13193.

Cleary, EG; Cifuentes, M; Grinstein, G; Brugge, D; Shea, TB. (2018). Association of low-level ozone with cognitive decline in older adults. J Alzheimers Dis 61: 67-78. http://dx.doi.org/10.3233/JAD-170658.

Conde, PG; Farhat, LC; Braga, ALF; Sallum, AEM; Farhat, SCL; Silva, CA. (2018). Are prematurity and environmental factors determinants for developing childhood-onset systemic lupus erythematosus? Mod Rheumatol 28: 156-160. http://dx.doi.org/10.1080/14397595.2017.1332508.

Coneus, K; Spiess, CK. (2012). Pollution exposure and child health: evidence for infants and toddlers in Germany. J Health Econ 31: 180-196. http://dx.doi.org/10.1016/j.jhealeco.2011.09.006.

Crouse, DL; Peters, PA; Hystad, P; Brook, JR; van Donkelaar, A; Martin, RV; Villeneuve, PJ; Jerrett, M; Goldberg, MS; Pope, CA; Brauer, M; Brook, RD; Robichaud, A; Menard, R; Burnett, RT. (2015). Ambient PM 2.5, O 3, and NO 2 exposures and associations with mortality over 16 years of follow-up in the Canadian Census Health and Environment Cohort (CanCHEC). Environ Health Perspect 123: 1180-1186. http://dx.doi.org/10.1289/ehp.1409276.

Culqui, DR; Linares, C; Ortiz, C; Carmona, R; Díaz, J. (2017). Association between environmental factors and emergency hospital admissions due to Alzheimer's disease in Madrid. Sci Total Environ 592: 451-457. http://dx.doi.org/10.1016/j.scitotenv.2017.03.089.

Dadvand, P; Rankin, J; Rushton, S; Pless-Mulloli, T. (2011). Ambient air pollution and congenital heart disease: A register-based study. Environ Res 111: 435-441. http://dx.doi.org/10.1016/j.envres.2011.01.022.

Dastoorpoor, M; Idani, E; Goudarzi, G; Khanjani, N. (2017). Acute effects of air pollution on spontaneous abortion, premature delivery, and stillbirth in Ahvaz, Iran: a time-series study. Environ Sci Pollut Res Int 25: 5447-5458. http://dx.doi.org/10.1007/s11356-017-0692-9.

Díaz, J; Arroyo, V; Ortiz, C; Carmona, R; Linares, C. (2016). Effect of Environmental Factors on Low Weight in Non-Premature Births: A Time Series Analysis. PLoS ONE 11: e0164741. http://dx.doi.org/10.1371/journal.pone.0164741.

Dye, JA; Gibbs-Flournoy, EA; Richards, JH; Norwood, J; Kraft, K; Hatch, GE. (2017). Neonatal rat age, sex and strain modify acute antioxidant response to ozone. Inhal Toxicol 29: 291-303. http://dx.doi.org/10.1080/08958378.2017.1369602.

Ebisu, K; Bell, ML. (2012). Airborne PM2.5 chemical components and low birth weight in the Northeastern and Mid-Atlantic regions of the United States. Environ Health Perspect 120: 1746-1752. http://dx.doi.org/10.1289/ehp.1104763.

Eckel, SP; Cockburn, M; Shu, YH; Deng, H; Lurmann, FW; Liu, L; Gilliland, FD. (2016). Air pollution affects lung cancer survival. Thorax 71: 891-898. http://dx.doi.org/10.1136/thoraxjnl-2015-207927.

Farhat, J; Farhat, SC; Braga, AL; Cocuzza, M; Borba, EF; Bonfá, E; Silva, CA. (2016). Ozone decreases sperm quality in systemic lupus erythematosus patients. Revista Brasileira de Reumatologia 56: 212-219. http://dx.doi.org/10.1016/j.rbre.2015.08.005.

Farhi, A; Boyko, V; Almagor, J; Benenson, I; Segre, E; Rudich, Y; Stern, E; Lerner-Geva, L. (2014). The possible association between exposure to air pollution and the risk for congenital malformations. Environ Res 135C: 173-180. http://dx.doi.org/10.1016/j.envres.2014.08.024.

Fernando Costa Nascimento, L; Blanco Machin, A; Antonio Almeida Dos Santos, D. (2017). Are there differences in birth weight according to sex and associations with maternal exposure to air pollutants? A cohort study. 135: 347-354. http://dx.doi.org/10.1590/1516-3180.2016.0262100317.

Fernando Hernandez-Zimbron, L; Rivas-Arancibia, S. (2016). Syntaxin 5 overexpression and beta-amyloid 1-42 accumulation in endoplasmic reticulum of hippocampal cells in rat brain induced by ozone exposure. BioMed Res Int 2016: 2125643. http://dx.doi.org/10.1155/2016/2125643.

Finkenwirth, C; Rossbach, B; Schroeder, HC; Muttray, A. (2014). In vivo ozone exposure does not increase DNA single-strand breaks in human peripheral lymphocytes. Hum Exp Toxicol 33: 517-521. http://dx.doi.org/10.1177/0960327113499164.

França, CMP; Sallum, AME; Braga, ALF; Strufaldi, FL; Silva, CAA; Farhat, SCL. (2018). Risk factors associated with juvenile idiopathic arthritis: Exposure to cigarette smoke and air pollution from pregnancy to disease diagnosis. J Rheumatol 45: 248-256. http://dx.doi.org/10.3899/jrheum.161500.

Fuertes, E; Brauer, M; MacIntyre, E; Bauer, M; Bellander, T; von Berg, A; Berdel, D; Brunekreef, B; Chan-Yeung, M; Gehring, U; Herbarth, O; Hoffmann, B; Kerkhof, M; Klümper, C; Koletzko, S; Kozyrskyj, A; Kull, I; Heinrich, J; Melén, E; Pershagen, G; Postma, D; Tiesler, CM; Carlsten, C. (2013a). Childhood allergic rhinitis, traffic-related air pollution, and variability in the GSTP1, TNF, TLR2, and TLR4 genes: Results from the TAG Study. J Allergy Clin Immunol 132: 342-352.e342. http://dx.doi.org/10.1016/j.jaci.2013.03.007.

Fuertes, E; Standl, M; Cyrys, J; Berdel, D; von Berg, A; Bauer, CP; Krämer, U; Sugiri, D; Lehmann, I; Koletzko, S; Carlsten, C; Brauer, M; Heinrich, J. (2013b). A longitudinal analysis of associations between traffic-related air pollution with asthma, allergies and sensitization in the GINIplus and LISAplus birth cohorts. Peer J 1: e193. http://dx.doi.org/10.7717/peerj.193.

Gabehart, K; Correll, KA; Loader, JE; White, CW; Dakhama, A. (2015). The lung response to ozone is determined by age and is partially dependent on toll-Like receptor 4. Respir Res 16: 117. http://dx.doi.org/10.1186/s12931-015-0279-2.

Gabehart, K; Correll, KA; Yang, J; Collins, ML; Loader, JE; Leach, S; White, CW; Dakhama, A. (2014). Transcriptome profiling of the newborn mouse lung response to acute ozone exposure. Toxicol Sci 138: 175-190. http://dx.doi.org/10.1093/toxsci/kft276.

Gatto, NM; Henderson, VW; Hodis, HN; St John, JA; Lurmann, F; Chen, JC; Mack, WJ. (2014). Components of air pollution and cognitive function in middle-aged and older adults in Los Angeles. Neurotoxicology 40: 1-7. http://dx.doi.org/10.1016/j.neuro.2013.09.004.

Geer, LA; Weedon, J; Bell, ML. (2012). Ambient air pollution and term birth weight in Texas from 1998 to 2004. J Air Waste Manag Assoc 62: 1285-1295. http://dx.doi.org/10.1080/10962247.2012.707632.

Gilbert, WM; Nesbitt, TS; Danielsen, B. (2003). The cost of prematurity: Quantification by gestational age and birth weight. Obstet Gynecol 102: 488-492. http://dx.doi.org/10.1016/S0029-7844(03)00617-3.

Gómez-Crisóstomo, NP; Rodríguez Martínez, E; Rivas-Arancibia, S. (2014). Oxidative stress activates the transcription factors FoxO 1a and FoxO 3a in the hippocampus of rats exposed to low doses of ozone. Oxid Med Cell Longev 2014: 805764. http://dx.doi.org/10.1155/2014/805764.

Gonzalez-Guevara, E; Carlos Martinez-Lazcano, J; Custodio, V; Hernandez-Ceron, M; Rubio, C; Paz, C. (2014). Exposure to ozone induces a systemic inflammatory response: possible source of the neurological alterations induced by this gas. Inhal Toxicol 26: 485-491. http://dx.doi.org/10.3109/08958378.2014.922648.

Goodrich, AJ; Volk, HE; Tancredi, DJ; Mcconnell, R; Lurmann, FW; Hansen, RL; Schmidt, RJ. (2017). Joint effects of prenatal air pollutant exposure and maternal folic acid supplementation on risk of autism spectrum disorder. Autism Res 11: 69-80. http://dx.doi.org/10.1002/aur.1885.

Gordon, CJ; Jarema, KA; Lehmann, J. R.; Ledbetter, AD; Schladweiler, MC; Schmid, JE; Ward, WO; Kodavanti, UP; Nyska, A; Macphail, RC. (2013). Susceptibility of adult and senescent Brown Norway rats to repeated ozone exposure: an assessment of behavior, serum biochemistry and cardiopulmonary function. Inhal Toxicol 25: 141-159. http://dx.doi.org/10.3109/08958378.2013.764946.

Gordon, CJ; Johnstone, AF; Aydin, C; Phillips, PM; Macphail, RC; Kodavanti, UP; Ledbetter, AD; Jarema, KA. (2014). Episodic ozone exposure in adult and senescent Brown Norway rats: acute and delayed effect on heart rate, core temperature and motor activity. Inhal Toxicol 26: 380-390. http://dx.doi.org/10.3109/08958378.2014.905659.

Gordon, CJ; Phillips, PM; Johnstone, AFM; Beasley, TE; Ledbetter, AD; Schladweiler, MC; Snow, SJ; Kodavanti, UP. (2016). Effect of high-fructose and high-fat diets on pulmonary sensitivity, motor activity, and body composition of brown Norway rats exposed to ozone. Inhal Toxicol 28: 203-215. http://dx.doi.org/10.3109/08958378.2015.1134730.

Gordon, CJ; Phillips, PM; Johnstone, AFM; Schmid, J; Schladweiler, MC; Ledbetter, A; Snow, SJ; Kodavanti, UP. (2017a). Effects of maternal high-fat diet and sedentary lifestyle on susceptibility of adult offspring to ozone exposure in rats. Inhal Toxicol 29: 239-254. http://dx.doi.org/10.1080/08958378.2017.1342719.

Gordon, CJ; Phillips, PM; Ledbetter, A; Snow, SJ; Schladweiler, MC; Johnstone, AF; Kodavanti, UP. (2017b). Active vs. sedentary lifestyle from weaning to adulthood and susceptibility to ozone in rats. Am J Physiol Lung Cell Mol Physiol 312: L100-L109. http://dx.doi.org/10.1152/ajplung.00415.2016.

Gray, SC; Edwards, SE; Schultz, BD; Miranda, ML. (2014). Assessing the impact of race, social factors and air pollution on birth outcomes: a population-based study. Environ Health 13: 4. http://dx.doi.org/10.1186/1476-069X-13-4.

Green, R; Sarovar, V; Malig, B; Basu, R. (2015). Association of stillbirth with ambient air pollution in a California cohort study. Am J Epidemiol 181: 874-882. http://dx.doi.org/10.1093/aje/kwu460.

Gunnison, AF; Weideman, PA; Sobo, M. (1992). Enhanced inflammatory response to acute ozone exposure in rats during pregnancy and lactation. Toxicol Sci 19: 607-612. http://dx.doi.org/10.1016/0272-0590(92)90100-V.

Guo, P; Feng, W; Zheng, M; Lv, J; Wang, L; Liu, J; Zhang, Y; Luo, G; Zhang, Y; Deng, C; Shi, T; Liu, P; Zhang, L. (2018). Short-term associations of ambient air pollution and cause-specific emergency department visits in Guangzhou, China. Sci Total Environ 613-614: 306-313. http://dx.doi.org/10.1016/j.scitotenv.2017.09.102.

Guo, Y; Zeng, H; Zheng, R; Li, S; Barnett, AG; Zhang, S; Zou, X; Huxley, R; Chen, W; Williams, G. (2016). The association between lung cancer incidence and ambient air pollution in China: A spatiotemporal analysis. Environ Res 144: 60-65. http://dx.doi.org/10.1016/j.envres.2015.11.004.

Ha, S; Hu, H; Roussos-Ross, D; Haidong, K; Roth, J; Xu, X. (2014). The effects of air pollution on adverse birth outcomes. Environ Res 134C: 198-204. http://dx.doi.org/10.1016/j.envres.2014.08.002.

Ha, S; Männistö, T; Liu, D; Sherman, S; Ying, Q; Mendola, P. (2017a). Air pollution and cardiovascular events at labor and delivery: a case-crossover analysis. Ann Epidemiol 27: 377-383. http://dx.doi.org/10.1016/j.annepidem.2017.05.007.

Ha, S; Sundaram, R; Buck Louis, GM; Nobles, C; Seeni, I; Sherman, S; Mendola, P. (2017b). Ambient air pollution and the risk ofpregnancy loss: a prospective cohort study. Fertil Steril 109: 148-153. http://dx.doi.org/10.1016/j.fertnstert.2017.09.037.

Hansen, C; Luben, TJ; Sacks, JD; Olshan, A; Jeffay, S; Strader, L; Perreault, SD. (2010). The effect of ambient air pollution on sperm quality. Environ Health Perspect 118: 203-209. http://dx.doi.org/10.1289/ehp.0901022.

Hansen, CA; Barnett, AG; Pritchard, G. (2008). The effect of ambient air pollution during early pregnancy on fetal ultrasonic measurements during mid-pregnancy. Environ Health Perspect 116: 362-369. http://dx.doi.org/10.1289/ehp.10720.

Hao, H; Chang, HH; Holmes, HA; Mulholland, JA; Klein, M; Darrow, LA; Strickland, MJ. (2016). Air pollution and preterm birth in the U.S. state of Georgia (2002-2006): Associations with concentrations of 11 ambient air pollutants estimated by combining community multiscale air quality model (CMAQ) simulations with stationary monitor measurements. Environ Health Perspect 124: 875-880. http://dx.doi.org/10.1289/ehp.1409651.

Haro, R; Paz, C. (1993). Effects of ozone exposure during pregnancy on ontogeny of sleep in rats. Neurosci Lett 164: 67-70. http://dx.doi.org/10.1016/0304-3940(93)90859-J.

Heckbert, SR; Kooperberg, C; Safford, MM; Psaty, BM; Hsia, J; McTiernan, A; Gaziano, JM; Frishman, WH; Curb, JD. (2004). Comparison of self-report, hospital discharge codes, and adjudication of cardiovascular events in the Women's Health Initiative. Am J Epidemiol 160: 1152-1158. http://dx.doi.org/10.1093/aje/kwh314.

Heindel, JJ; Skalla, LA; Joubert, BR; Dilworth, CH; Gray, KA. (In Press) Review of developmental origins of health and disease publications in environmental epidemiology. Reprod Toxicol. http://dx.doi.org/10.1016/j.reprotox.2016.11.011.

Hernandez-Zimbron, LF; Rivas-Arancibia, S. (2015). Oxidative stress caused by ozone exposure induces beta-amyloid 1-42 overproduction and mitochondrial accumulation by activating the amyloidogenic pathway. Neuroscience 304: 340-348. http://dx.doi.org/10.1016/j.neuroscience.2015.07.011.

Hettfleisch, K; Bernardes, LS; Carvalho, MA; Pastro, LD; Vieira, SE; Saldiva, SR; Saldiva, P; Francisco, RP. (2016). Short-Term Exposure to Urban Air Pollution and Influences on Placental Vascularization Indexes. Environ Health Perspect 125: 753-759. http://dx.doi.org/10.1289/EHP300.

Holland, N; Davé, V; Venkat, S; Wong, H; Donde, A; Balmes, JR; Arjomandi, M. (2014). Ozone inhalation leads to a dose-dependent increase of cytogenetic damage in human lymphocytes. Environ Mol Mutagen 56: 378-387. http://dx.doi.org/10.1002/em.21921.

Hu, H; Ha, S; Henderson, BH; Warner, TD; Roth, J; Kan, H; Xu, X. (2015). Association of atmospheric particulate matter and ozone with gestational diabetes mellitus. Environ Health Perspect 123: 853-859. http://dx.doi.org/10.1289/ehp.1408456.

Hu, H; Ha, S; Xu, X. (2016). Ozone and hypertensive disorders of pregnancy in Florida: Identifying critical windows of exposure. Environ Res 153: 120-125. http://dx.doi.org/10.1016/j.envres.2016.12.002.

Huang, CC; Wen, HJ; Chen, PC; Chiang, TL; Lin, SJ; Guo, YL. (2015). Prenatal air pollutant exposure and occurrence of atopic dermatitis. Br J Dermatol 173: 981-988. http://dx.doi.org/10.1111/bjd.14039.

Hunter, DD; Carrell-Jacks, LA; Batchelor, TP; Dey, RD. (2011). Role of nerve growth factor in ozone-induced neural responses in early postnatal airway development. Am J Respir Cell Mol Biol 45: 359-365. http://dx.doi.org/10.1165/rcmb.2010-0345OC.

Hwang, BF; Lee, YL; Jaakkola, JJ. (2011). Air pollution and stillbirth: A population-based case-control study in Taiwan. Environ Health Perspect 119: 1345-1349. http://dx.doi.org/10.1289/ehp.1003056.

Hwang, BF; Lee, YL; Jaakkola, JJ. (2015). Air Pollution and the Risk of Cardiac Defects: A Population-Based Case-Control Study. Medicine (Baltimore) 94: e1883. http://dx.doi.org/10.1097/MD.0000000000001883.

Hystad, P; Demers, PA; Johnson, KC; Carpiano, RM; Brauer, M. (2013). Long-term residential exposure to air pollution and lung cancer risk. Epidemiology 24: 762-772. http://dx.doi.org/10.1097/EDE.0b013e3182949ae7.

IOM (Institute of Medicine). (2007). Preterm birth: Causes, consequences, and prevention. In Preterm birth: Causes, consequences, and prevention. Washington, DC: The National Academies Press. http://dx.doi.org/10.17226/11622.

Jeanjean, M; Bind, MA; Roux, J; Ongagna, JC; de Sèze, J; Bard, D; Leray, E. (2018). Ozone, NO2and PM10are associated with the occurrence of multiple sclerosis relapses. Evidence from seasonal multi-pollutant analyses. Environ Res 163: 43-52. http://dx.doi.org/10.1016/j.envres.2018.01.040.

Jedlinska-Krakowska, M; Bomba, G; Jakubowski, K; Rotkiewicz, T; Jana, B; Penkowskii, A. (2006a). Impact of oxidative stress and supplementation with vitamins E and C on testes morphology in rats. J Reprod Dev 52: 203-209.

Jedlinska-Krakowska, M; Gizejewski, Z; Dietrich, GJ; Jakubowski, K; Glogowski, J; Penkowski, A. (2006b). The effect of increased ozone concentrations in the air on selected aspects of rat reproduction. Pol J Vet Sci 9: 11-16.

Jerrett, M; Burnett, RT; Beckerman, BS; Turner, MC; Krewski, D; Thurston, G; Martin, RV; van Donkelaar, A; Hughes, E; Shi, Y; Gapstur, SM; Thun, MJ; Pope, CA, III. (2013). Spatial analysis of air pollution and mortality in California. Am J Respir Crit Care Med 188: 593-599. http://dx.doi.org/10.1164/rccm.201303-0609OC.

Jung, CR; Lin, YT; Hwang, BF. (2013). Air pollution and newly diagnostic autism spectrum disorders: a population-based cohort study in taiwan. PLoS ONE 8: e75510. http://dx.doi.org/10.1371/journal.pone.0075510.

Jung, CR; Lin, YT; Hwang, BF. (2014). Ozone, particulate matter, and newly diagnosed alzheimer's disease: A population-based cohort study in Taiwan. J Alzheimers Dis 44: 573-584. http://dx.doi.org/10.3233/JAD-140855.

Jurewicz, J; Radwan, M; Sobala, W; Polańska, K; Radwan, P; Jakubowski, L; Ulańska, A; Hanke, W. (2014). The relationship between exposure to air pollution and sperm disomy. Environ Mol Mutagen 56: 50-59. http://dx.doi.org/10.1002/em.21883.

Kavlock, R; Daston, G; Grabowski, CT. (1979). Studies on the developmental toxicity of ozone. I. Prenatal effects. Toxicol Appl Pharmacol 48: 19-28. http://dx.doi.org/10.1016/S0041-008X(79)80004-6.

Kavlock, RJ; Meyer, E; Grabowski, CT. (1980). Studies on the developmental toxicity of ozone: Postnatal effects. Toxicol Lett 5: 3-9. http://dx.doi.org/10.1016/0378-4274(80)90141-1.

Kerin, T; Volk, H; Li, W; Lurmann, F; Eckel, S; Mcconnell, R; Hertz-Picciotto, I. (2017). Association Between Air Pollution Exposure, Cognitive and Adaptive Function, and ASD Severity Among Children with Autism Spectrum Disorder. J Autism Dev Disord. http://dx.doi.org/10.1007/s10803-017-3304-0.

Kilkenny, C; Browne, WJ; Cuthill, IC; Emerson, M; Altman, DG. (2010). Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research [Review]. PLoS Biol 8: e1000412. http://dx.doi.org/10.1371/journal.pbio.1000412.

Kim, D; Volk, H; Girirajan, S; Pendergrass, S; Hall, MA; Verma, SS; Schmidt, RJ; Hansen, RL; Ghosh, D; Ludena-Rodriguez, Y; Kim, K; Ritchie, MD; Hertz-Picciotto, I; Selleck, SB. (2017). The joint effect of air pollution exposure and copy number variation on risk for autism. Autism Res 10: 1470-1480. http://dx.doi.org/10.1002/aur.1799.

Kioumourtzoglou, MA; Power, MC; Hart, JE; Okereke, OI; Coull, BA; Laden, F; Weisskopf, MG. (2017). The association between air pollution and onset of depression among middle-aged and older women. Am J Epidemiol 185: 801-809. http://dx.doi.org/10.1093/aje/kww163.

Kirrane, EF; Bowman, C; Davis, JA; Hoppin, JA; Blair, A; Chen, H; Patel, MM; Sandler, DP; Tanner, CM; Vinikoor-Imler, L; Ward, MH; Luben, TJ; Kamel, F. (2015). Associations of ozone and PM2.5 concentrations with Parkinson's disease among participants in the agricultural health study. J Occup Environ Med 57: 509-517. http://dx.doi.org/10.1097/JOM.0000000000000451.

Klimisch, HJ; Andreae, M; Tillmann, U. (1997). A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regul Toxicol Pharmacol 25: 1-5. http://dx.doi.org/10.1006/rtph.1996.1076.

Kodavanti, UP. (2016). Stretching the stress boundary: Linking air pollution health effects to a neurohormonal stress response [Review]. Biochim Biophys Acta 1860: 2880-2890. http://dx.doi.org/10.1016/j.bbagen.2016.05.010.

Krewski, D; Jerrett, M; Burnett, RT; Ma, R; Hughes, E; Shi, Y; Turner, MC; Pope, CA, III; Thurston, G; Calle, EE; Thun, MJ; Beckerman, B; Deluca, P; Finkelstein, N; Ito, K; Moore, DK; Newbold, KB; Ramsay, T; Ross, Z; Shin, H; Tempalski, B. (2009). Extended follow-up and spatial analysis of the American Cancer Society study linking particulate air pollution and mortality [HEI]. (HEI Research Report 140). Boston, MA: Health Effects Institute. https://www.healtheffects.org/system/files/Krewski140Statement.pdf.

Laurent, O; Hu, J; Li, L; Cockburn, M; Escobedo, L; Kleeman, MJ; Wu, J. (2014). Sources and contents of air pollution affecting term low birth weight in Los Angeles County, California, 20012008. Environ Res 134: 488-495. http://dx.doi.org/10.1016/j.envres.2014.05.003.

Laurent, O; Hu, J; Li, L; Kleeman, MJ; Bartell, SM; Cockburn, M; Escobedo, L; Wu, J. (2016a). Low birth weight and air pollution in California: Which sources and components drive the risk? Environ Int 92-93: 471-477. http://dx.doi.org/10.1016/j.envint.2016.04.034.

Laurent, O; Hu, J; Li, L; Kleeman, MJ; Bartell, SM; Cockburn, M; Escobedo, L; Wu, J. (2016b). A statewide nested case-control study of preterm birth and air pollution by source and composition: California, 2001-2008. Environ Health Perspect 124: 1479-1486. http://dx.doi.org/10.1289/ehp.1510133.

Laurent, O; Wu, J; Li, L; Chung, J; Bartell, S. (2013). Investigating the association between birth weight and complementary air pollution metrics: a cohort study. Environ Health 12: 18. http://dx.doi.org/10.1186/1476-069X-12-18.

Lavigne, E; Yasseen, AS; Stieb, DM; Hystad, P; van Donkelaar, A; Martin, RV; Brook, JR; Crouse, DL; Burnett, RT; Chen, H; Weichenthal, S; Johnson, M; Villeneuve, PJ; Walker, M. (2016). Ambient air pollution and adverse birth outcomes: Differences by maternal comorbidities. Environ Res 148: 457-466. http://dx.doi.org/10.1016/j.envres.2016.04.026.

Lee, H; Myung, W; Kim, DK; Kim, SE; Kim, CT; Kim, H. (2017). Short-term air pollution exposure aggravates Parkinson's disease in a population-based cohort. Sci Rep 7: 44741. http://dx.doi.org/10.1038/srep44741.

Lee, PC; Liu, LL; Sun, Y; Chen, YA; Liu, CC; Li, CY; Yu, HL; Ritz, B. (2016). Traffic-related air pollution increased the risk of Parkinson's disease in Taiwan: A nationwide study. Environ Int 96: 75-81. http://dx.doi.org/10.1016/j.envint.2016.08.017.

Lee, PC; Roberts, JM; Catov, JM; Talbott, EO; Ritz, B. (2013). First Trimester Exposure to Ambient Air Pollution, Pregnancy Complications and Adverse Birth Outcomes in Allegheny County, PA. Matern Child Health J 17: 545-555. http://dx.doi.org/10.1007/s10995-012-1028-5.

Lee, PC; Talbott, EO; Roberts, JM; Catov, JM; Bilonick, RA; Stone, RA; Sharma, RK; Ritz, B. (2012). Ambient air pollution exposure and blood pressure changes during pregnancy. Environ Res 117: 46-53. http://dx.doi.org/10.1016/j.envres.2012.05.011.

Lee, PC; Talbott, EO; Roberts, JM; Catov, JM; Sharma, RK; Ritz, B. (2011). Particulate air pollution exposure and C-reactive protein during early pregnancy. Epidemiology 22: 524-531. http://dx.doi.org/10.1097/EDE.0b013e31821c6c58.

Legro, RS; Sauer, MV; Mottla, GL; Richter, KS; Li, X; Dodson, WC; Liao, D. (2010). Effect of air quality on assisted human reproduction. Hum Reprod 25: 1317-1324. http://dx.doi.org/10.1093/humrep/deq021.

Lim, YH; Kim, H; Kim, JH; Bae, S; Park, HY; Hong, YC. (2012). Air pollution and symptoms of depression in elderly adults. Environ Health Perspect 120: 1023-1028. http://dx.doi.org/10.1289/ehp.1104100.

Lin, CC; Yang, SK; Lin, KC; Ho, WC; Hsieh, WS; Shu, BC; Chen, PC. (2014a). Multilevel analysis of air pollution and early childhood neurobehavioral development. Int J Environ Res Public Health 11: 6827-6841. http://dx.doi.org/10.3390/ijerph110706827.

Lin, Y, uT; Jung, C; Lee, Y; Hwang, BF. (2015). Associations Between Ozone and Preterm Birth in Women Who Develop Gestational Diabetes. Am J Epidemiol 181: 280-287. http://dx.doi.org/10.1093/aje/kwu264.

Lin, YT; Lee, YL; Jung, CR; Jaakkola, JJ; Hwang, BF. (2014b). Air pollution and limb defects: A matched-pairs case-control study in Taiwan. Environ Res 132: 273-280. http://dx.doi.org/10.1016/j.envres.2014.04.028.

Linares, C; Culqui, D; Carmona, R; Ortiz, C; Díaz, J. (2017). Short-term association between environmental factors and hospital admissions due to dementia in Madrid. Environ Res 152: 214-220. http://dx.doi.org/10.1016/j.envres.2016.10.020.

Liu, Y; Zhou, Y; Ma, J; Bao, W; Li, J; Zhou, T; Cui, X; Peng, Z; Zhang, H; Feng, M; Yuan, Y; Chen, Y; Huang, X; Li, Y; Duan, Y; Shi, T; Jin, L; Wu, L. (2017). Inverse Association between Ambient Sulfur Dioxide Exposure and Semen Quality in Wuhan, China. Environ Sci Technol 51: 12806-12814. http://dx.doi.org/10.1021/acs.est.7b03289.

MacDorman, MF; Callaghan, WM; Mathews, TJ; Hoyert, DL; Kochanek, KD. (2007). Trends in preterm-related infant mortality by race and ethnicity, United States 1999-2004. Int J Health Serv 37: 635-641. http://dx.doi.org/10.2190/HS.37.4.c.

MacIntyre, EA; Brauer, M; Melén, E; Bauer, CP; Bauer, M; Berdel, D; Bergström, A; Brunekreef, B; Chan-Yeung, M; Klümper, C; Fuertes, E; Gehring, U; Gref, A; Heinrich, J; Herbarth, O; Kerkhof, M; Koppelman, GH; Kozyrskyj, AL; Pershagen, G; Postma, DS; Thiering, E; Tiesler, CM; Carlsten, C. (2014). GSTP1 and TNF gene variants and associations between air pollution and incident childhood asthma: The traffic, asthma and genetics (TAG) study. Environ Health Perspect 122: 418-424. http://dx.doi.org/10.1289/ehp.1307459.

Malmqvist, E; Elding Larsson, H; Jönsson, I; Rignell-Hydbom, A; Ivarsson, SA; Tinnerberg, H; Stroh, E; Rittner, R; Jakobsson, K; Swietlicki, E; Rylander, L. (2015). Maternal exposure to air pollution and type 1 diabetes - Accounting for genetic factors. Environ Res 140: 268-274. http://dx.doi.org/10.1016/j.envres.2015.03.024.

Männistö, T; Mendola, P; Grantz, KL; Leishear, K; Sundaram, R; Sherman, S; Ying, Q; Liu, D. (2015a). Acute and recent air pollution exposure and cardiovascular events at labour and delivery. Heart 101: 1491-1498. http://dx.doi.org/10.1136/heartjnl-2014-307366.

Männistö, T; Mendola, P; Liu, D; Leishear, K; Ying, Q, i; Sundaram, R. (2015b). Temporal variation in the acute effects of air pollution on blood pressure measured at admission to labor/delivery. Air Qual Atmos Health 8: 13-28. http://dx.doi.org/10.1007/s11869-014-0268-5.

Mendola, P; Ha, S; Pollack, AZ; Zhu, Y; Seeni, I; Kim, SS; Sherman, S; Liu, D. (2017). Chronic and Acute Ozone Exposure in the Week Prior to Delivery Is Associated with the Risk of Stillbirth. Int J Environ Res Public Health 14. http://dx.doi.org/10.3390/ijerph14070731.

Mendola, P; Wallace, M; Hwang, BS; Liu, D; Robledo, C; Männistö, T; Sundaram, R; Sherman, S; Ying, Q; Grantz, KL. (2016a). Preterm birth and air pollution: Critical windows of exposure for women with asthma. J Allergy Clin Immunol 138: 432-440.e435. http://dx.doi.org/10.1016/j.jaci.2015.12.1309.

Mendola, P; Wallace, M; Liu, D; Robledo, C; Männistö, T; Grantz, KL. (2016b). Air pollution exposure and preeclampsia among US women with and without asthma. Environ Res 148: 248-255. http://dx.doi.org/10.1016/j.envres.2016.04.004.

Michikawa, T; Morokuma, S; Fukushima, K; Kato, K; Nitta, H; Yamazaki, S. (2017a). Maternal exposure to air pollutants during the first trimester and foetal growth in Japanese term infants. Environ Pollut 230: 387-393. http://dx.doi.org/10.1016/j.envpol.2017.06.069.

Michikawa, T; Morokuma, S; Fukushima, K; Ueda, K; Takeuchi, A; Kato, K; Nitta, H. (2015). A register-based study of the association between air pollutants and hypertensive disorders in pregnancy among the Japanese population. Environ Res 142: 644-650. http://dx.doi.org/10.1016/j.envres.2015.08.024.

Michikawa, T; Morokuma, S; Yamazaki, S; Fukushima, K; Kato, K; Nitta, H. (2016). Exposure to air pollutants during the early weeks of pregnancy, and placenta praevia and placenta accreta in the western part of Japan. Environ Int 92-93: 464-470. http://dx.doi.org/10.1016/j.envint.2016.04.037.

Michikawa, T; Morokuma, S; Yamazaki, S; Fukushima, K; Kato, K; Nitta, H. (2017b). Air pollutant exposure within a few days of delivery and placental abruption in Japan. Epidemiology 28: 190-196. http://dx.doi.org/10.1097/EDE.0000000000000605.

Miller, CN; Dye, JA; Ledbetter, AD; Schladweiler, MC; Richards, JH; Snow, SJ; Wood, CE; Henriquez, AR; Thompson, LC; Farraj, AK; Hazari, MS; Kodavanti, UP. (2017). Uterine artery flow and offspring growth in long-evans rats following maternal exposure to ozone during implantation. Environ Health Perspect 125: 127005. http://dx.doi.org/10.1289/EHP2019.

Miller, CN; Stewart, EJ; Snow, SJ; Williams, WC; Richards, JH; Thompson, LC; Schladweiler, MC; Farraj, AK; Kodavanti, UP; Dye, JA. (2019). Ozone Exposure During Implantation Increases Serum Bioactivity in HTR-8/SVneo Trophoblasts. Toxicol Sci 168: 535-550. http://dx.doi.org/10.1093/toxsci/kfz003.

Mobasher, Z; Salam, MT; Goodwin, TM; Lurmann, F; Ingles, SA; Wilson, ML. (2013). Associations between ambient air pollution and hypertensive disorders of pregnancy. Environ Res 123: 9-16. http://dx.doi.org/10.1016/j.envres.2013.01.006.

Mokoena, ML; Brink, CB; Harvey, BH; Oliver, DW. (2011). Appraisal of ozone as biologically active molecule and experimental tool in biomedical sciences. Med Chem Res 20: 1687-1695. http://dx.doi.org/10.1007/s00044-010-9493-0.

Mokoena, ML; Harvey, BH; Viljoen, F; Ellis, SM; Brink, CB. (2015). Ozone exposure of Flinders Sensitive Line rats is a rodent translational model of neurobiological oxidative stress with relevance for depression and antidepressant response. Psychopharmacology 232: 2921-2938. http://dx.doi.org/10.1007/s00213-015-3928-8.

Morello-Frosch, R; Jesdale, BM; Sadd, JL; Pastor, M. (2010). Ambient air pollution exposure and full-term birth weight in California. Environ Health 9: 44. http://dx.doi.org/10.1186/1476-069X-9-44.

Moridi, M; Ziaei, S; Kazemnejad, A. (2014). Exposure to ambient air pollutants and spontaneous abortion. J Obstet Gynaecol Res 40: 743-748. http://dx.doi.org/10.1111/jog.12231.

Morokuma, S; Michikawa, T; Yamazaki, S; Nitta, H; Kato, K. (2017). Association between exposure to air pollution during pregnancy and false positives in fetal heart rate monitoring. Sci Rep 7: 12421. http://dx.doi.org/10.1038/s41598-017-12663-2.

Muhajarine, N; Mustard, C; Roos, LL; Young, TK; Gelskey, DE. (1997). Comparison of survey and physician claims data for detecting hypertension. J Clin Epidemiol 50: 711-718. http://dx.doi.org/10.1016/S0895-4356(97)00019-X.

Mumaw, CL; Levesque, S; Mcgraw, C; Robertson, S; Lucas, S; Stafflinger, JE; Campen, MJ; Hall, P; Norenberg, JP; Anderson, T; Lund, AK; Mcdonald, JD; Ottens, AK; Block, ML. (2016). Microglial priming through the lung-brain axis: the role of air pollution-induced circulating factors. FASEB J 30: 1880-1891. http://dx.doi.org/10.1096/fj.201500047.

Murgia, N; Brisman, J; Claesson, A; Muzi, G; Olin, AC; Torén, K. (2014). Validity of a questionnaire-based diagnosis of chronic obstructive pulmonary disease in a general population-based study. BMC Pulm Med 14: 49. http://dx.doi.org/10.1186/1471-2466-14-49.

Murphy, SR; Schelegle, ES; Edwards, PC; Miller, LA; Hyde, DM; Van Winkle, LS. (2012). Postnatal exposure history and airways oxidant stress responses in airway explants. Am J Respir Cell Mol Biol 47: 815-823. http://dx.doi.org/10.1165/rcmb.2012-0110OC.

Murphy, SR; Schelegle, ES; Miller, LA; Hyde, DM; Van Winkle, LS. (2013). Ozone exposure alters serotonin and serotonin receptor expression in the developing lung. Toxicol Sci 134: 168-179. http://dx.doi.org/10.1093/toxsci/kft090.

Nahidi, F; Gholami, R; Rashidi, Y; Majd, HA. (2014). Relationship between air pollution and pre-eclampsia in pregnant women: a case-control study. East Mediterr Health J 19: S60-S66.

Nishimura, KK; Galanter, JM; Roth, LA; Oh, SS; Thakur, N; Nguyen, EA; Thyne, S; Farber, HJ; Serebrisky, D; Kumar, R; Brigino-Buenaventura, E; Davis, A; Lenoir, MA; Meade, K; Rodriguez-Cintron, W; Avila, PC; Borrell, LN; Bibbins-Domingo, K; Rodriguez-Santana, J. R.; Sen, S; Lurmann, F; Balmes, J. R.; Burchard, EG. (2013). Early-life air pollution and asthma risk in minority children: The GALA II and SAGE II studies. Am J Respir Crit Care Med 188: 309-318. http://dx.doi.org/10.1164/rccm.201302-0264OC.

Nishimura, KK; Iwanaga, K; Oh, SS; Pino-Yanes, M; Eng, C; Keswani, A; Roth, LA; Nguyen, EA; Thyne, SM; Farber, HJ; Serebrisky, D; Meade, K; Lenoir, MA; Rodriguez-Cintron, W; Borrell, LN; Bibbins-Domingo, K; Lurmann, F; Sen, S; Rodriguez-Santana, J. R.; Brigino-Buenaventura, E; Avila, PC; Balmes, J. R.; Kumar, R; Burchard, EG. (2016). Early-life ozone exposure associated with asthma without sensitization in Latino children [Letter]. J Allergy Clin Immunol 138: 1703-1706.e1701. http://dx.doi.org/10.1016/j.jaci.2016.03.058.

Nobles, CJ; Schisterman, EF; Ha, S; Buck Louis, GM; Sherman, S; Mendola, P. (2018). Time-varying cycle average and daily variation in ambient air pollution and fecundability. Hum Reprod 33: 166-176. http://dx.doi.org/10.1093/humrep/dex341.

Olsson, D; Ekström, M; Forsberg, B. (2012). Temporal variation in air pollution concentrations and preterm birth-a population based epidemiological study. Int J Environ Res Public Health 9: 272-285. http://dx.doi.org/10.3390/ijerph9010272.

Olsson, D; Mogren, I; Forsberg, B. (2013). Air pollution exposure in early pregnancy and adverse pregnancy outcomes: a register-based cohort study. BMJ Open 3. http://dx.doi.org/10.1136/bmjopen-2012-001955.

Orione, MA; Silva, CA; Sallum, AM; Campos, LM; Omori, CH; Braga, AL; Farhat, SC. (2014). Risk factors for juvenile dermatomyositis: Exposure to tobacco and air pollutants during pregnancy. Arthritis Care and Research 66: 1571-1575. http://dx.doi.org/10.1002/acr.22358.

Oudin, A; Åström, DO; Asplund, P; Steingrimsson, S; Szabo, Z; Carlsen, HK. (2018). The association between daily concentrations of air pollution and visits to a psychiatric emergency unit: a case-crossover study. Environ Health 17: 4. http://dx.doi.org/10.1186/s12940-017-0348-8.

Padula, AM; Tager, IB; Carmichael, SL; Hammond, SK; Lurmann, F; Shaw, GM. (2013). The association of ambient air pollution and traffic exposures with selected congenital anomalies in the San Joaquin Valley of California. Am J Epidemiol 177: 1074-1085. http://dx.doi.org/10.1093/aje/kws367.

Peel, JL; Klein, M; Flanders, WD; Mulholland, JA; Freed, G; Tolbert, PE. (2011). Ambient air pollution and apnea and bradycardia in high-risk infants on home monitors. Environ Health Perspect 119: 1321-1327. http://dx.doi.org/10.1289/ehp.1002739.

Pinto-Almazan, R; Rivas-Arancibia, S; Farfan-Garcia, ED; Rodriguez-Martinez, E; Guerra-Araiza, C. (2014). Neuroprotective effects of tibolone against oxidative stress induced by ozone exposure. Rev Neurol 58: 441-449.

Qian, Z; Liang, S; Yang, S; Trevathan, E; Huang, Z; Yang, R; Wang, J; Hu, K; Zhang, Y; Vaughn, M; Shen, L; Liu, W; Li, P; Ward, P; Yang, L; Zhang, W; Chen, W; Dong, G; Zheng, T; Xu, S; Zhang, B. (2015). Ambient air pollution and preterm birth: A prospective birth cohort study in Wuhan, China. Int J Hyg Environ Health 219: 195-203. http://dx.doi.org/10.1016/j.ijheh.2015.11.003.

Reis, MMD; Guimarães, MT; Braga, ALF; Martins, LC; Pereira, LAA. (2017). Air pollution and low birth weight in an industrialized city in southeastern Brazil, 2003-2006. Brazilian Journal of Epidemiology 20: 189-199. http://dx.doi.org/10.1590/1980-5497201700020001.

Ritz, B; Qiu, J; Lee, PC; Lurmann, F; Penfold, B; Erin Weiss, R; Mcconnell, R; Arora, C; Hobel, C; Wilhelm, M. (2014). Prenatal air pollution exposure and ultrasound measures of fetal growth in Los Angeles, California. Environ Res 130: 7-13. http://dx.doi.org/10.1016/j.envres.2014.01.006.

Rivas-Arancibia, S; Hernandez Zimbron, LF; Rodriguez-Martinez, E; Maldonado, PD; Borgonio Perez, G; Sepulveda-Parada, M. (2015). Oxidative stress-dependent changes in immune responses and cell death in the substantia nigra after ozone exposure in rat. Front Aging Neurosci 7: 65. http://dx.doi.org/10.3389/fnagi.7015.00065.

Rivas-Arancibia, S; Rodríguez-Martínez, E; Badillo-Ramírez, I; López-González, U; Saniger, JM. (2017). Structural changes of amyloid beta in hippocampus of rats exposed to ozone: A Raman spectroscopy study. Frontiers in Molecular Neuroscience 10: 137. http://dx.doi.org/10.3389/fnmol.2017.00137.

Robledo, CA; Mendola, P; Yeung, E; Männistö, T; Sundaram, R; Liu, D; Ying, Q; Sherman, S; Grantz, KL. (2015). Preconception and early pregnancy air pollution exposures and risk of gestational diabetes mellitus. Environ Res 137: 316-322. http://dx.doi.org/10.1016/j.envres.2014.12.020.

Rodríguez-Martínez, E; Martínez, F; Espinosa-García, MT; Maldonado, P; Rivas-Arancibia, S. (2013). Mitochondrial dysfunction in the hippocampus of rats caused by chronic oxidative stress. Neuroscience 252: 384-395. http://dx.doi.org/10.1016/j.neuroscience.2013.08.018.

Rodríguez-Martínez, E; Nava-Ruiz, C; Escamilla-Chimal, E; Borgonio-Perez, G; Rivas-Arancibia, S. (2016). The Effect of Chronic Ozone Exposure on the Activation of Endoplasmic Reticulum Stress and Apoptosis in Rat Hippocampus. Front Aging Neurosci 8: 245. http://dx.doi.org/10.3389/fnagi.2016.00245.

Rooney, AA; Boyles, AL; Wolfe, MS; Bucher, JR; Thayer, KA. (2014). Systematic review and evidence integration for literature-based environmental health science assessments. Environ Health Perspect 122: 711-718. http://dx.doi.org/10.1289/ehp.1307972.

Saigal, S; Doyle, LW. (2008). An overview of mortality and sequelae of preterm birth from infancy to adulthood. Lancet 371: 261-269. http://dx.doi.org/10.1016/S0140-6736(08)60136-1.

Salam, MT; Millstein, J; Li, YF; Lurmann, FW; Margolis, HG; Gilliland, FD. (2005). Birth outcomes and prenatal exposure to ozone, carbon monoxide, and particulate matter: Results from the Children's Health Study. Environ Health Perspect 113: 1638-1644. http://dx.doi.org/10.1289/ehp.8111.

Schifano, P; Asta, F; Dadvand, P; Davoli, M; Basagana, X; Michelozzi, P. (2016). Heat and air pollution exposure as triggers of delivery: A survival analysis of population-based pregnancy cohorts in Rome and Barcelona. Environ Int 88: 153-159. http://dx.doi.org/10.1016/j.envint.2015.12.013.

Schifano, P; Lallo, A; Asta, F; De Sario, M; Davoli, M; Michelozzi, P. (2013). Effect of ambient temperature and air pollutants on the risk of preterm birth, Rome 2001-2010. Environ Int 61: 77-87. http://dx.doi.org/10.1016/j.envint.2013.09.005.

Sharkhuu, T; Doerfler, DL; Copeland, C; Luebke, RW; Gilmour, MI. (2011). Effect of maternal exposure to ozone on reproductive outcome and immune, inflammatory, and allergic responses in the offspring. J Immunotoxicol 8: 183-194. http://dx.doi.org/10.3109/1547691X.2011.568978.

Shin, S; Burnett, RT; Kwong, JC; Hystad, P; van Donkelaar, A; Brook, JR; Copes, R; Tu, K; Goldberg, MS; Villeneuve, PJ; Martin, RV; Murray, BJ; Wilton, AS; Kopp, A; Chen, H. (2018). Effects of ambient air pollution on incident Parkinson's disease in Ontario, 2001 to 2013: a population-based cohort study. Int J Epidemiol 47: 2038-2048. http://dx.doi.org/10.1093/ije/dyy172.

Slama, R; Bottagisi, S; Solansky, I; Lepeule, J; Giorgis-Allemand, L; Sram, R. (2013). Short-term impact of atmospheric pollution on fecundability. Epidemiology 24: 871-879. http://dx.doi.org/10.1097/EDE.0b013e3182a702c5.

Smith, LB; Reich, BJ; Herring, AH; Langlois, PH; Fuentes, M. (2015). Multilevel quantile function modeling with application to birth outcomes. Biometrics 71: 508-519. http://dx.doi.org/10.1111/biom.12294.

Smith, RB; Fecht, D; Gulliver, J; Beevers, SD; Dajnak, D; Blangiardo, M; Ghosh, RE; Hansell, AL; Kelly, FJ; Anderson, HR; Toledano, MB. (2017). Impact of London's road traffic air and noise pollution on birth weight: retrospective population based cohort study. BMJ 359: j5299. http://dx.doi.org/10.1136/bmj.j5299.

Snow, SJ; Henriquez, AR; Costa, DL; Kodavanti, UP. (2018). Neuroendocrine regulation of air pollution health effects: emerging insights. Toxicol Sci 164: 9-20. http://dx.doi.org/10.1093/toxsci/kfy129.

Sokol, RZ; Kraft, P; Fowler, IM; Mamet, R; Kim, E; Berhane, KT. (2006). Exposure to environmental ozone alters semen quality. Environ Health Perspect 114: 360-365. http://dx.doi.org/10.1289/ehp.8232.

Stingone, JA; Luben, TJ; Daniels, JL; Fuentes, M; Richardson, DB; Aylsworth, AS; Herring, AH; Anderka, M; Botto, L; Correa, A; Gilboa, SM; Langlois, PH; Mosley, B; Shaw, GM; Siffel, C; Olshan, AF. (2014). Maternal exposure to criteria air pollutants and congenital heart defects in offspring: Results from the national birth defects prevention study. Environ Health Perspect 122: 863-872. http://dx.doi.org/10.1289/ehp.1307289.

Symanski, E; Mchugh, MK; Zhang, X; Craft, ES; Lai, D. (2016). Evaluating narrow windows of maternal exposure to ozone and preterm birth in a large urban area in Southeast Texas. J Expo Sci Environ Epidemiol 26: 167-172. http://dx.doi.org/10.1038/jes.2015.32.

Szyszkowicz, M; Kousha, T; Kingsbury, M; Colman, I. (2016). Air Pollution and Emergency Department Visits for Depression: A Multicity Case-Crossover Study. Environ Health Insights 10: 155-161. http://dx.doi.org/10.4137/EHI.S40493.

Tétreault, LF; Doucet, M; Gamache, P; Fournier, M; Brand, A; Kosatsky, T; Smargiassi, A. (2016). Childhood exposure to ambient air pollutants and the onset of asthma: an administrative cohort study in Québec. Environ Health Perspect 124: 1276-1282. http://dx.doi.org/10.1289/ehp.1509838.

Thoma, ME; Mclain, AC; Louis, JF; King, RB; Trumble, AC; Sundaram, R; Buck Louis, GM. (2013). Prevalence of infertility in the United States as estimated by the current duration approach and a traditional constructed approach. Fertil Steril 99: 1324-1331.e1321. http://dx.doi.org/10.1016/j.fertnstert.2012.11.037.

Thomson, EM; Vladisavljevic, D; Mohottalage, S; Kumarathasan, P; Vincent, R. (2013). Mapping acute systemic effects of inhaled particulate matter and ozone: multiorgan gene expression and glucocorticoid activity. Toxicol Sci 135: 169-181. http://dx.doi.org/10.1093/toxsci/kft137.

Toren, K; Brisman, J; Jarvholm, B. (1993). Asthma and asthma-like symptoms in adults assessed by questionnaires: A literature review [Review]. Chest 104: 600-608. http://dx.doi.org/10.1378/chest.104.2.600.

Tu, J; Tu, W; Tedders, SH. (2016). Spatial variations in the associations of term birth weight with ambient air pollution in Georgia, USA. Environ Int 92-93: 146-156. http://dx.doi.org/10.1016/j.envint.2016.04.005.

Turner, MC; Jerrett, M; Pope, A, III; Krewski, D; Gapstur, SM; Diver, WR; Beckerman, BS; Marshall, JD; Su, J; Crouse, DL; Burnett, RT. (2016). Long-term ozone exposure and mortality in a large prospective study. Am J Respir Crit Care Med 193: 1134-1142. http://dx.doi.org/10.1164/rccm.201508-1633OC.

Turner, MC; Krewski, D; Diver, WR; Pope, CA; Burnett, RT; Jerrett, M; Marshall, JD; Gapstur, SM. (2017). Ambient air pollution and cancer mortality in the Cancer Prevention Study II. Environ Health Perspect 125: 087013. http://dx.doi.org/10.1289/EHP1249.

Tyler, CR; Noor, S; Young, T; Rivero, V; Sanchez, B; Lucas, S; Caldwell, KK; Milligan, ED; Campen, MJ. (2018). Aging Exacerbates Neuroinflammatory Outcomes Induced by Acute Ozone Exposure. Toxicol Sci. http://dx.doi.org/10.1093/toxsci/kfy014.

U.S. EPA (U.S. Environmental Protection Agency). (1991). Guidelines for developmental toxicity risk assessment (pp. 1-71). (EPA/600/FR-91/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=23162.

U.S. EPA (U.S. Environmental Protection Agency). (1996). Guidelines for reproductive toxicity risk assessment (pp. 1-143). (EPA/630/R-96/009). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/sites/production/files/2014-11/documents/guidelines_repro_toxicity.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (1998). Guidelines for neurotoxicity risk assessment [EPA Report] (pp. 1-89). (EPA/630/R-95/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/risk/guidelines-neurotoxicity-risk-assessment.

U.S. EPA (U.S. Environmental Protection Agency). (2005). Guidelines for carcinogen risk assessment [EPA Report] (pp. 1-166). (EPA/630/P-03/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/sites/production/files/2013-09/documents/cancer_guidelines_final_3-25-05.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2008). Integrated science assessment for sulfur oxides: Health criteria [EPA Report]. (EPA/600/R-08/047F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment-RTP. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=198843.

U.S. EPA (U.S. Environmental Protection Agency). (2013a). Integrated science assessment for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-10/076F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment-RTP Division. http://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=247492.

U.S. EPA (U.S. Environmental Protection Agency). (2013b). Toxicological review of trimethylbenzenes (CASRN 25551-13-7, 95-63-6, 526-73-8, and 108-67-8) in support of summary information on the Integrated Risk Information System (IRIS): revised external review draft [EPA Report]. (EPA/635/R13/171a). Washington, D.C.: U.S. Environmental Protection Agency, National Center for Environmental Assessment. http://yosemite.epa.gov/sab/SABPRODUCT.NSF/b5d8a1ce9b0729348525737500701 2b7/ee1e280e77586de985257b65005d37e7!OpenDocument.

U.S. EPA (U.S. Environmental Protection Agency). (2015). Preamble to the integrated science assessments [EPA Report]. (EPA/600/R-15/067). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, RTP Division. https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=310244.

van Rossem, L; Rifas-Shiman, SL; Melly, SJ; Kloog, I; Luttmann-Gibson, H; Zanobetti, A; Coull, BA; Schwartz, JD; Mittleman, MA; Oken, E; Gillman, MW; Koutrakis, P; Gold, DR. (2015). Prenatal air pollution exposure and newborn blood pressure. Environ Health Perspect 123: 353-359. http://dx.doi.org/10.1289/ehp.1307419.

Veninga, TS. (1967). Toxicity of ozone in comparison with ionizing radiation. Strahlentherapie 134: 469-477.

Verhein, KC; Salituro, FG; Ledeboer, MW; Fryer, AD; Jacoby, DB. (2013). Dual p38/JNK mitogen activated protein kinase inhibitors prevent ozone-induced airway hyperreactivity in guinea pigs. PLoS ONE 8: e75351. http://dx.doi.org/10.1371/journal.pone.0075351.

Vinikoor-Imler, LC; Davis, JA; Meyer, RE; Luben, TJ. (2013). Early prenatal exposure to air pollution and its associations with birth defects in a state-wide birth cohort from North Carolina. Birth Defects Res A Clin Mol Teratol 97: 696-701. http://dx.doi.org/10.1002/bdra.23159.

Vinikoor-Imler, LC; Davis, JA; Meyer, RE; Messer, LC; Luben, TJ. (2014). Associations between prenatal exposure to air pollution, small for gestational age, and term low birthweight in a state-wide birth cohort. Environ Res 132: 132-139. http://dx.doi.org/10.1016/j.envres.2014.03.040.

Vinikoor-Imler, LC; Stewart, TG; Luben, TJ; Davis, JA; Langlois, PH. (2015). An exploratory analysis of the relationship between ambient ozone and particulate matter concentrations during early pregnancy and selected birth defects in Texas. Environ Pollut 202: 1-6. http://dx.doi.org/10.1016/j.envpol.2015.03.001.

Volk, HE; Kerin, T; Lurmann, F; Hertz-Picciotto, I; McConnell, R; Campbell, DB. (2014). Autism spectrum disorder: Interaction of air pollution with the MET receptor tyrosine kinase gene. Epidemiology 25: 44-47. http://dx.doi.org/10.1097/EDE.0000000000000030.

Volk, HE; Lurmann, F; Penfold, B; Hertz-Picciotto, I; McConnell, R. (2013). Traffic-related air pollution, particulate matter, and autism. Arch Gen Psychiatry 70: 71-77. http://dx.doi.org/10.1001/jamapsychiatry.2013.266.

von Elm, E; Altman, DG; Egger, M; Pocock, SJ; Gøtzsche, PC; Vandenbroucke, JP. (2007). The strengthening the reporting of observational studies in epidemiology (strobe) statement: guidelines for reporting observational studies [Review]. PLoS Med 4: e296. http://dx.doi.org/10.1371/journal.pmed.0040296.

Wallace, ME; Grantz, KL; Liu, D; Zhu, Y; Kim, SS; Mendola, P. (2016). Exposure to ambient air pollution and premature rupture of membranes. Am J Epidemiol 183: 1114-1121. http://dx.doi.org/10.1093/aje/kwv284.

Warren, J; Fuentes, M; Herring, A; Langlois, P. (2012). Spatial-temporal modeling of the association between air pollution exposure and preterm birth: Identifying critical windows of exposure. Biometrics 68: 1157-1167. http://dx.doi.org/10.1111/j.1541-0420.2012.01774.x.

Weakley, J; Webber, MP; Ye, F; Zeig-Owens, R; Cohen, HW; Hall, CB; Kelly, K; Prezant, DJ. (2013). Agreement between obstructive airways disease diagnoses from self-report questionnaires and medical records. Prev Med 57: 38-42. http://dx.doi.org/10.1016/j.ypmed.2013.04.001.

Wu, J; Wilhelm, M; Chung, J; Ritz, B. (2011). Comparing exposure assessment methods for traffic-related air pollution in an adverse pregnancy outcome study. Environ Res 111: 685-692. http://dx.doi.org/10.1016/j.envres.2011.03.008.

Wu, YC; Lin, YC; Yu, HL; Chen, JH; Chen, TF; Sun, Y; Wen, LL; Yip, PK; Chu, YM; Chen, YC. (2015). Association between air pollutants and dementia risk in the elderly. 1: 220-228. http://dx.doi.org/10.1016/j.dadm.2014.11.015.

Xu, C; Fan, YN; Kan, HD; Chen, RJ; Liu, JH; Li, YF; Zhang, Y; Ji, AL; Cai, TJ. (2016). The Novel Relationship between Urban Air Pollution and Epilepsy: A Time Series Study. PLoS ONE 11: e0161992. http://dx.doi.org/10.1371/journal.pone.0161992.

Xu, X; Ha, S; Kan, H; Hu, H; Curbow, BA; Lissaker, CTK. (2013). Health effects of air pollution on length of respiratory cancer survival. BMC Public Health 13: 800. http://dx.doi.org/10.1186/1471-2458-13-800.

Xu, X; Hu, H; Ha, S; Roth, J. (2014). Ambient air pollution and hypertensive disorder of pregnancy. J Epidemiol Community Health 68: 13-20. http://dx.doi.org/10.1136/jech-2013-202902.

Xue, X; Chen, J; Sun, B; Zhou, B; Li, X. (2018). Temporal trends in respiratory mortality and short-term effects of air pollutants in Shenyang, China. Environ Sci Pollut Res Int 25: 11468-11479. http://dx.doi.org/10.1007/s11356-018-1270-5.

Yaghjyan, L; Arao, R; Brokamp, C; O'Meara, ES; Sprague, BL; Ghita, G; Ryan, P. (2017). Association between air pollution and mammographic breast density in the Breast Cancer Surveilance Consortium. Breast Cancer Research 19: 36. http://dx.doi.org/10.1186/s13058-017-0828-3.

Yang, CL; To, T; Foty, RG; Stieb, DM; Dell, SD. (2011). Verifying a questionnaire diagnosis of asthma in children using health claims data. BMC Pulm Med 11. http://dx.doi.org/10.1186/1471-2466-11-52.

Yang, S; Tan, Y; Mei, H; Wang, F; Li, N; Zhao, J; Zhang, Y; Qian, Z; Chang, JJ; Syberg, KM; Peng, A; Mei, H; Zhang, D; Zhang, Y; Xu, S; Li, Y; Zheng, T; Zhang, B. (2018). Ambient air pollution the risk of stillbirth: A prospective birth cohort study in Wuhan, China. Int J Hyg Environ Health 221: 502-509. http://dx.doi.org/10.1016/j.ijheh.2018.01.014.

Yitshak-Sade, M; Novack, L; Landau, D; Kloog, I; Sarov, B; Hershkovitz, R; Karakis, I. (2016). Relationship of ambient air pollutants and hazardous household factors with birth weight among Bedouin-Arabs. Chemosphere 160: 314-322. http://dx.doi.org/10.1016/j.chemosphere.2016.06.104.

Zellner, LC; Brundage, KM; Hunter, DD; Dey, RD. (2011). Early postnatal ozone exposure alters rat nodose and jugular sensory neuron development. Toxicol Environ Chem 93: 2055-2071. http://dx.doi.org/10.1080/02772248.2011.610882.

Zhang, B; Zhao, J; Yang, R; Qian, Z; Liang, S; Bassig, BA; Zhang, Y; Hu, K; Xu, S; Dong, G; Zheng, T; Yang, S. (2016). Ozone and other air pollutants and the risk of congenital heart defects. Sci Rep 6: 34852. http://dx.doi.org/10.1038/srep34852.

Zhang, J; Merialdi, M; Platt, LD; Kramer, MS. (2010). Defining normal and abnormal fetal growth: Promises and challenges [Review]. Am J Obstet Gynecol 202: 522-528. http://dx.doi.org/10.1016/j.ajog.2009.10.889.

Zhang, S; Li, J; Li, Y; Liu, Y; Guo, H; Xu, X. (2017). Nitric oxide synthase activity correlates with OGG1 in ozone-induced lung injury animal models. Front Physiol 8: 249. http://dx.doi.org/10.3389/fphys.2017.00249.

Zhou, Y; Gilboa, SM; Herdt, ML; Lupo, PJ; Flanders, WD; Liu, Y; Shin, M; Canfield, MA; Kirby, RS. (2016). Maternal exposure to ozone and PM2.5 and the prevalence of orofacial clefts in four U.S. states. Environ Res 153: 35-40. http://dx.doi.org/10.1016/j.envres.2016.11.007..

APPENDIX 8 ECOLOGICAL EFFECTS

Summary of Causality Determinations for Ecological Effects

This Appendix characterizes the scientific evidence that supports causality determinations for ozone exposure and ecological effects. More details on the causal framework used to reach these conclusions are included in the Preamble to the ISA (U.S. EPA, 2015). Causality determinations that are new or revised since the last review are indicated with an asterisk.

Vegetation and Ecosystem Effects	Causality Determination
Visible foliar injury	Causal
Reduced vegetation growth	Causal
Reduced plant reproduction	Causal*
Increased tree mortality	Likely to be causal*
Reduced yield and quality of agricultural crops	Causal
Alteration of herbivore growth and reproduction	Likely to be causal*
Alteration of plant-insect signaling	Likely to be causal*
Reduced productivity in terrestrial ecosystems	Causal
Reduced carbon sequestration in terrestrial ecosystems	Likely to be causal
Alteration of belowground biogeochemical cycles	Causal
Alteration of terrestrial community composition	Causal*
Alteration of ecosystem water cycling	Likely to be causal

8.1 Introduction

This Appendix evaluates the relevant scientific information on ecological effects as part of the review of the air quality criteria for ozone and other photochemical oxidants and to help form the scientific foundation for the review of the secondary National Ambient Air Quality Standard (NAAQS) for ozone. It serves as a concise update to Chapter 9 of the 2013 Ozone ISA (U.S. EPA, 2013) and Appendix 9 of the 2006 Ozone Air Quality Criteria Document [AQCD; U.S. EPA (2006)]. Numerous studies on the effects of ozone on vegetation and ecosystems were reviewed in the 2013 Ozone ISA. The

document concluded that responses to ozone exposure occur across a broad array of spatial scales and ecological endpoints (summarized here in Figure 8-1 and Table 8-1). The majority of evidence for ecological effects has been for vegetation. Effects at the individual plant level can result in broad ecosystem-level changes, such as productivity, carbon storage, water cycling, nutrient cycling, and community composition. Figure 8-1 shows ozone's major ecological effects at multiple levels of biological organization from the biochemical and subleaf level up to its effects on ecosystem services, which are the benefits that ecosystems provide people, either directly or indirectly (Costanza et al., 2017). The focus of the current ISA and literature evaluated therein are those effects observed at the individual, organism level of biological organization and higher (e.g., population, community, ecosystem, etc.).

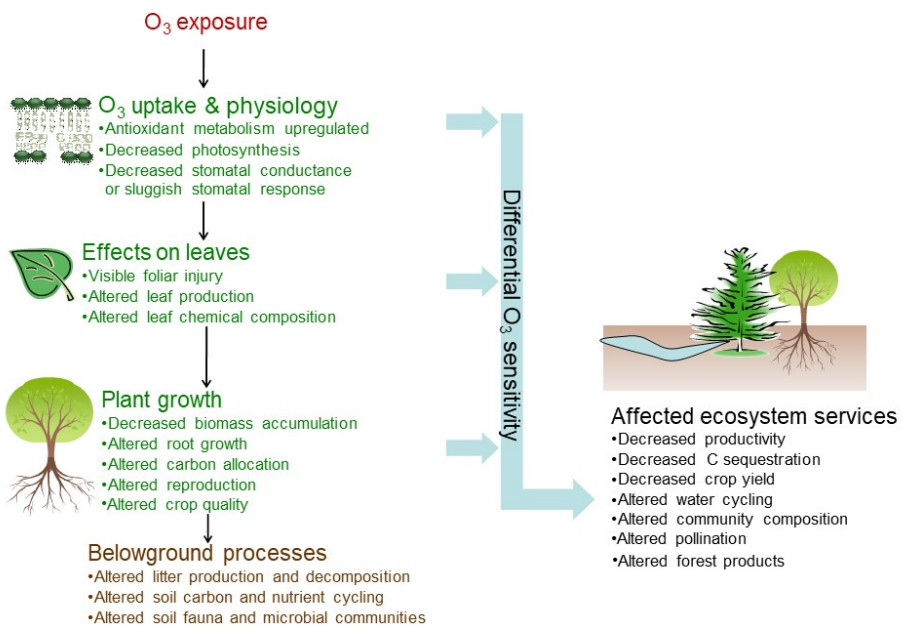


Figure 8-1 Illustrative diagram of ozone effects in plants and ecosystems adapted from the 2013 Ozone ISA.

8.1.1 Scope

The causality determinations for ecological effects of ozone from the 2013 Ozone ISA (Table 8-1) inform the scope of the current review. The causality determinations are generally organized according to biological scale of organization ranging from the individual organism-level to ecosystem-level processes. As described in the Preamble to the ISA (U.S. EPA, 2015), the U.S. EPA uses

a structured causality framework to provide a consistent and transparent basis for classifying the weight of available evidence for health and welfare[1] effects according to a five-level hierarchy: (1) causal relationship; (2) likely to be a causal relationship; (3) suggestive, but not sufficient, to infer a causal relationship; (4) inadequate to infer a causal relationship; and (5) not likely to be a causal relationship.

Table 8-1 Summary of ozone causality determinations for effects on vegetation and ecosystems in the 2013 Ozone ISA.

Vegetation and Ecosystem Effects	Causality Determination from 2013 Ozone ISA
Visible foliar injury	Causal
Reduced vegetation growth	Causal
Reduced productivity in terrestrial ecosystems	Causal
Reduced carbon sequestration in terrestrial ecosystems	Likely to be causal
Reduced yield and quality of agricultural crops	Causal
Alteration of terrestrial ecosystem water cycling	Likely to be causal
Alteration of belowground biogeochemical cycles	Causal
Alteration of terrestrial community composition	Likely to be causal

The current ISA has adopted the use of the Population, Exposure, Comparison, Outcome, and Study Design (PECOS) tool to further define the scope of the current review by conveying the criteria for inclusion or exclusion of studies (cite IRP; Table 8-2). The units of study as defined in the PECOS for ecological effects of ozone are the individual organism, species, population (in the sense of a group of individuals of the same species), community, or ecosystem. All studies included in the ISA were conducted at concentrations occurring in the environment or experimental ozone concentrations within an order of magnitude of recent concentrations observed in the U.S. (as described in Appendix 1). The level of causality determination (from the five-tier framework) in the 2013 Ozone ISA informed how the PECOS was designed to scope the review. For ecological endpoints for which the 2013 Ozone ISA concluded that the evidence was sufficient to infer a causal relationship (i.e., foliar injury, vegetation growth, ecosystem productivity, yield and quality of agricultural crops, belowground biogeochemical cycling), the current review only evaluates studies conducted in North America (Table 8-2). There were

[1] The Clean Air Act definition of welfare includes, but is not limited to, effects on soils, water, wildlife, vegetation, visibility, weather, and climate, as well as effects on man-made materials, economic values, and personal comfort and well-being.

no geographic constraints for all the other endpoints evaluated (terrestrial water cycling; carbon sequestration; terrestrial community composition; plant reproduction, phenology, and survival; insects and other wildlife; and plant-animal signaling). In the PECOS for ecological effects, relevant study designs include laboratory, greenhouse, field, gradient, open top chamber (OTC), Free-Air Carbon Dioxide Enrichment (FACE), and modeling studies.

Table 8-2 Population, exposure, comparison, outcome, and study design (PECOS) tool for ozone effects on vegetation and ecosystems.

Ecological Endpoint	Population, Exposure, Comparison, Outcome, and Study Design (PECOS) Tool
Visible foliar injury, vegetation growth, yield/quality of agricultural crops, productivity, belowground biogeochemical cycling	**Population:** For any species, an individual, population (in the sense of a group of individuals of the same species), community, or ecosystem in North America **Exposure:** Concentrations occurring in the environment or experimental ozone concentrations within an order of magnitude of recent concentrations (as described in Appendix 1) **Comparison:** Relevant control sites, treatments, or parameters **Outcome:** Visible foliar injury, alteration of vegetative growth, yield/quality of agricultural crops, productivity, belowground biogeochemical cycles **Study Design:** Laboratory, greenhouse, OTC, FACE, field, gradient, or modeling studies
Terrestrial water cycling; carbon sequestration; terrestrial community composition; plant reproduction, phenology, or mortality; insects, other wildlife, plant-animal signaling	**Population:** For any species, an individual, population (in the sense of a group of individuals of the same species), community, or ecosystem in any continent[a] **Exposure:** Concentrations occurring in the environment or experimental ozone concentrations within an order of magnitude of recent concentrations (as described in Appendix 1) **Comparison:** Relevant control sites, treatments, or parameters **Outcome:** Alteration of: terrestrial water cycling; carbon sequestration; terrestrial community composition; plant reproduction, phenology, mortality; growth reproduction and survival of insects and other wildlife; plant-animal signaling **Study Design:** Laboratory, greenhouse, OTC, FACE, field, gradient, or modeling studies

Comparator = change in endpoint observed by unit increase in concentration of ozone in the same or in a control population; exposure = environmental variable to which population is exposed; outcome = measurable endpoint resulting from exposure; population = unit of study; study design = laboratory, field, gradient, open top chamber (OTC), Free-Air Carbon Dioxide Enrichment (FACE), greenhouse, and modeling studies.

[a]In cases where a comprehensive list of affected species was available, nonagricultural North American species were separated out from the larger data sets and the evidence was evaluated (e.g., foliar injury, biomass).

Notes: This definition of population is for the purpose of applying PECOS to ecology. Ecological populations are defined as a group of individuals of the same species.

Exposure methodologies included in the PECOS and used to evaluate the ecological effects of ozone are discussed in Section 8.1.2. This discussion is followed by a description of the biological pathways and mechanisms by which ozone exposure may lead to effects at higher levels of biological organization (Section 8.1.3). Effects of ozone exposure on major endpoints are discussed in separate sections and include the following: visible foliar injury (Section 8.2), plant growth and biomass (Section 8.3); plant reproduction, phenology, and mortality (Section 8.4); and reduced crop yield and quality (Section 8.5). Ecological effects of ozone extend to plant-associated fauna, primarily insect herbivores (Section 8.6). Plant-insect interactions can be altered via ozone's effect on volatile plant signaling compounds (Section 8.7). This is followed by a discussion of changes in ecosystem structure and function in response to ozone, including reduced primary production and carbon sequestration (Section 8.8), altered belowground processes (Section 8.9), shifts in terrestrial community composition (Section 8.10), and altered water cycling (Section 8.11). Modifying factors that may exacerbate or negate the effects of ozone are reviewed in Section 8.12. Finally, indices of ozone exposure and dose modeling are discussed in Section 8.13. For each of the endpoint categories, key findings and conclusions from the 2013 Ozone ISA are briefly summarized followed by new evidence. Important older studies may be discussed to reinforce key concepts and conclusions.

8.1.2 Assessing Ecological Response to Ozone

This section reviews the methodologies of experimental studies and definitions of ecologically relevant ozone exposure metrics that are discussed in the rest of the Appendix.

8.1.2.1 Experimental Exposure Methodologies

A variety of methods for studying plant response to ozone exposures have been developed over the last several decades. Most of the current methodologies were discussed in detail in the 1996 Ozone AQCD (U.S. EPA, 1996), 2006 Ozone AQCD (U.S. EPA, 2006), and the 2013 Ozone ISA (U.S. EPA, 2013). Exposure methodologies such as greenhouse studies, continuous stirred tank reactors (CSTRs), open top chambers (OTCs), and free-air fumigation are applied for assessing ozone effects on individual plants and ecosystems. Free-air carbon dioxide/ozone enrichment (FACE) systems are a more natural way of estimating ozone effects on aboveground and belowground processes. Other methods include the use of ambient ozone gradients across the landscape and of multivariate statistical methods to control for other environmental variables across space and time.

8.1.2.1.1 Indoor, Controlled Environment, and Greenhouse Chambers

The earliest experimental investigations of the effects of ozone on plants used simple glass or plastic-covered chambers, often located within greenhouses, into which a flow of ozone-enriched air or oxygen could be passed to provide the exposure. The types, shapes, styles, materials of construction, and locations of these chambers have been numerous. Hogsett et al. (1987a) summarized the construction and performance of the more elaborate and better instrumented chambers since the 1960s, including those installed in greenhouses (with or without some control of temperature and light intensity).

One greenhouse chamber approach that continues to yield useful information on the relationships of ozone uptake to both physiological and growth effects employs continuous stirred tank reactors (CSTRs) first described by Heck et al. (1978). Although originally developed to permit mass-balance studies of ozone flux to plants, these use of these reactors has recently expanded to include short-term physiological and growth studies of ozone × CO_2 interactions (Grantz et al., 2016; Grantz et al., 2012; Loats and Rebbeck, 1999; Reinert et al., 1997; Rao et al., 1995; Reinert and Ho, 1995; Heagle et al., 1994), and validation of visible foliar injury on a variety of plant species (Kline et al., 2009; Orendovici et al., 2003). In many cases, supplementary lighting and temperature control of the surrounding structure have been used to control or modify the environmental conditions (Heagle et al., 1994).

Many investigations have used commercially available controlled-environment chambers and walk-in rooms adapted to permit the introduction of a flow of ozone into the controlled air volume (also called phytotrons). Like greenhouse chambers, these chambers have temperature and light control and can be used to study interactions with other pollutants.

8.1.2.1.2 Field Chambers

In general, field chamber studies are dominated by the use of various versions of the open top chamber (OTC) design, first described by Heagle et al. (1973) and Mandl et al. (1973). The OTC method continues to be widely used in the U.S. and Europe for exposing plants to varying levels of ozone. Chambers are generally ~3 m in diameter with 2.5-m-high walls. Hogsett et al. (1987b) described in detail many of the various modifications to the original OTC designs that have appeared subsequently; these have included the use of larger chambers for exposing small trees (Kats et al., 1985) or grapevines (Mandl et al., 1989) and the addition of a conical baffle at the top to improve ventilation (Kats et al., 1976), a frustum at the top to reduce ambient air incursions, and a plastic rain-cap to exclude precipitation (Hogsett et al., 1985). All versions of OTCs discharge air via ports in annular ducting or interiorly perforated double-layered walls at the base of the chambers to provide turbulent mixing and the upward mass flow of air.

Chambered systems, including OTCs, have several advantages. For instance, they can provide a range of treatment levels including clean air (charcoal-filtered [CF]) control, ambient air control, and

several above ambient concentrations for ozone experiments. Depending on experimental intent, a replicated, clean-air control treatment is an essential component in many experimental designs. The OTC can provide a consistent, definable exposure because of the constant wind speed and delivery systems. Statistically robust concentration-response (C-R) functions can be developed using such systems to evaluate the implications of various alternative air quality scenarios on vegetation response. Nonetheless, there are several characteristics of the OTC design and operation that can lead to exposures that might differ from those experienced by plants in the field. First, the OTC plants are subjected to constant air flow turbulence, which, by lowering the boundary layer resistance to diffusion, may result in increased uptake. This may lead to an overestimation of effects relative to areas with less turbulence (Krupa et al., 1995; Legge et al., 1995). Research has also found that OTCs may slightly change the vapor-pressure deficit (VPD) in a way that may decrease the uptake of ozone into leaves (Piikki et al., 2008). As with all methods that expose vegetation to modified ozone concentrations in chambers, OTCs create internal environments that differ from ambient air. This so-called "chamber effect" refers to the modification of microclimatic variables, including reduced and uneven light intensity, uneven rainfall, constant wind speed, reduced dew formation, and increased air temperatures (Fuhrer, 1994; Manning and Krupa, 1992). However, in at least one case where canopy resistance was quantified in OTCs and in the field, it was determined that gaseous pollutant exposure to crops in OTCs was similar to that which would have occurred at the same concentration in the field (Unsworth et al., 1984a, b). Because of the standardized methodology and protocols used in National Crop Loss Assessment Network (NCLAN) and similar programs, the database are generally assumed to be internally consistent.

While it is clear that OTCs can alter some aspects of the microenvironment and plant growth, it is important to establish whether these differences affect the relative response of a plant to ozone. As noted in the 1996 Ozone AQCD, evidence from several comparative studies of OTCs and other exposure systems suggested that responses were essentially the same regardless of the exposure system used and that chamber effects did not significantly affect response. In studies that included exposure to ambient concentrations of ozone in both OTCs, and open-air, chamberless control plots, responses in the OTCs were the same as in open-air plots (see Section 9.26 of the 2013 Ozone ISA).

Other types of field chambers such as the "terracosm" (Lee et al., 2009) and the recirculating Outdoor Plant Environment Chambers [OPECs; Flowers et al. (2007)] have been used less frequently in recent years. See the 2013 Ozone ISA for more details (U.S. EPA, 2013).

8.1.2.1.3 Free-Air Carbon Dioxide/Ozone Enrichment (FACE) and Plume-Type Systems

Plume systems are chamberless exposure facilities in which the atmosphere surrounding plants in the field is modified by the injection of pollutant gas into the air above or surrounding them. This is typically accomplished by releasing the pollutant gas from tubing with multiple orifices spaced to permit diffusion and turbulence, so as to establish relatively homogeneous conditions as the individual plumes disperse and mix with the ambient air (Ormrod et al., 1988).

The most common plume system used in the U.S. is a modification of the free-air carbon dioxide/ozone enrichment (FACE) system (Miglietta et al., 2001; Hendrey et al., 1999; Hendrey and Kimball, 1994). Although originally designed to provide chamberless field facilities for studying the effects of CO_2, FACE systems have been adapted to include the dispensing of ozone (Karnosky et al., 1999 Morgan, 2004, 72764 Morgan, 2004, 72764). This method has been employed in Illinois (SoyFACE) to study soybeans [*Glycine max;*((Morgan et al., 2004; Rogers et al., 2004)] and in Wisconsin (Aspen FACE) to study quaking aspen (*Populus tremuloides*), birch (*Betula papyrifera*), and maple [*Acer saccharum*; (Karnosky et al., 1999)]. Volk et al. (2003) described a similar system for exposing grasslands that uses 7-m-diameter plots. Other FACE systems have been used in Finland (Saviranta et al., 2010; Oksanen, 2003), China (Feng et al., 2015), and Japan (Hoshika et al., 2012b).

The Aspen FACE system in the U.S. discharges the pollutant gas (ozone and/or CO_2) through orifices spaced along an annular ring (or torus) or at different heights on a ring of vertical pipes. In general, these systems allow for two ozone levels (local ambient and elevated). Computer-controlled feedback from the monitoring of gas concentration regulates the feed rate of enriched air to the dispersion pipes. Feedback of wind speed and directional information ensures that the discharges only occur upwind of the treatment plots, and that discharge is restricted or closed down during periods of low wind speed or calm conditions. The diameter of the arrays and their height (25−30 m) in some FACE systems require large throughputs of enriched air per plot, particularly in forest tree systems. The cost of the throughputs tends to limit the number of enrichment treatments, although Hendrey et al. (1999) argued that the cost on an enriched volume basis is comparable to that of chamber systems. In a similar system, the SoyFACE uses an octagon (21 m in diameter) of horizontal pipes that releases ozone to provide a constant elevated ozone concentration above the concurrent local ozone concentration. Ozone release is maintained at approximately 10 cm above the top of the crop canopy throughout the growing season by raising the horizontal pipes as the crop grows taller (Morgan et al., 2004; Miglietta et al., 2001). Research conducted at the SoyFACE facility in Illinois (to study soybeans) and the Aspen FACE system in Wisconsin (to study responses in broadleaf forest), have contributed a substantial body of evidence in characterizing ozone effects at multiple scales. Aspen FACE (in operation from 1998 to 2011) enabled long-term characterization of ozone effects in mixed forest communities.

A different FACE-type facility has been developed for the Kranzberg Ozone Fumigation Experiment (KROFEX) in Germany beginning in 2000 (Nunn et al., 2002; Werner and Fabian, 2002). The experiment was designed to study the effects of ozone on mature stands of beech (*Fagus sylvatica*) and spruce (*Picea abies*) trees in a system that functions independently of wind direction. The enrichment of a large volume of the ambient air within the canopy takes place via orifices in vertical tubes suspended from a horizontal grid supported above the canopy.

Although plume systems make virtually none of the modifications to the physical environment that are inevitable with chambers, their successful use depends on selecting the appropriate numbers, sizes, and orientations of the discharge orifices to avoid “hot-spots” resulting from the direct impingement

of jets of pollutant-enriched air on plant foliage (Werner and Fabian, 2002). Because mixing is unassisted and completely dependent on wind turbulence and diffusion, local gradients are inevitable, especially in large-scale systems. FACE systems have provisions for shutting down under low wind speed or calm conditions and for an experimental area that is usually defined within a generous border in order to strive for homogeneity of the exposure concentrations within the treatment area. They are also dependent on continuous computer-controlled feedback of the ozone concentrations in the mixed treated air and of the meteorological conditions. FACE and other plume systems are also unable to reduce ozone levels below local ambient conditions.

8.1.2.1.4 Ambient Gradients

The occurrence of ambient ozone concentration gradients in the U.S. hold potential for the examination of plant responses over multiple levels of exposure. However, few such gradients can be found that meet the rigorous statistical requirements for comparable site characteristics such as soil type, temperature, rainfall, radiation, and aspect (Manning and Krupa, 1992); although with small plants, soil variability can be avoided by placing plants in large pots. The use of soil monoliths transported to various locations along natural ozone gradients is another possible approach to overcome differences in soils; however, this approach is also limited to small plants.

Studies in the 1970s used the natural gradients occurring in southern California to assess yield losses of alfalfa and tomato (Oshima et al., 1977; Oshima et al., 1976). A transect study of the impact of ozone on the growth of white clover and barley in the U.K. was confounded by differences in the concurrent gradients of SO_2 and NO_2 pollution (Ashmore et al., 1988). Studies of forest tree species in national parks in the eastern U.S. (Winner et al., 1989) revealed increasing gradients of ozone and visible foliar injury with increased elevation.

Several studies have used the San Bernardino Mountains Gradient Study in southern California to study the effects of ozone and N deposition on forests dominated by ponderosa and Jeffrey pine (Jones and Paine, 2006; Arbaugh et al., 2003; Grulke, 1999; U.S. EPA, 1977). However, it is difficult to separate the effects of N and ozone in some instances in these studies (Arbaugh et al., 2003). An ozone gradient in Wisconsin has been used to study foliar injury in a series of quaking aspen clones (*Populus tremuloides*) differing in ozone sensitivity (Maňkovská et al., 2005; Karnosky et al., 1999). Also in the Midwest, an east-west ozone gradient around southern Lake Michigan was used to look at growth and visible foliar injury in black cherry (*P. serotina*) and common milkweed (*Asclepias syriaca*) (Bennett et al., 2006).

Studies have been published that have used natural gradients to study a variety of endpoints and species. For example, Gregg et al. (2003) studied cottonwood (*Populus deltoides*) saplings grown in an urban to rural gradient of ozone by using seven locations in the New York City area. The secondary nature of the reactions of ozone formation and NO_X titration reactions within the city center resulted in significantly higher cumulative ozone exposures in more rural sites. Potential modifying factors such as

other pollutants, soil composition, moisture, or temperature were either controlled or accounted for in the analysis.

8.1.2.2 Definitions of Exposure Metrics and Indices

Exposure indices are metrics that quantify exposure as it relates to measured plant damage (e.g., reduced growth). The details of these metrics are discussed in Section 8.13.1. In the over 60 years of research, many forms of exposure metrics have been used, including 7-, 12-, and 24-hour averages. The current secondary standard form of the 4th highest 8-hour max avg over 3 years is rarely reported in the vegetation research.

The most useful metrics in vegetation research have been differentially weighted hourly concentrations that are cumulative during the growth of plants. The 2013 Ozone ISA primarily discussed SUM06, AOTx, and W126 exposure metrics. Below are the definitions of the three cumulative index forms:

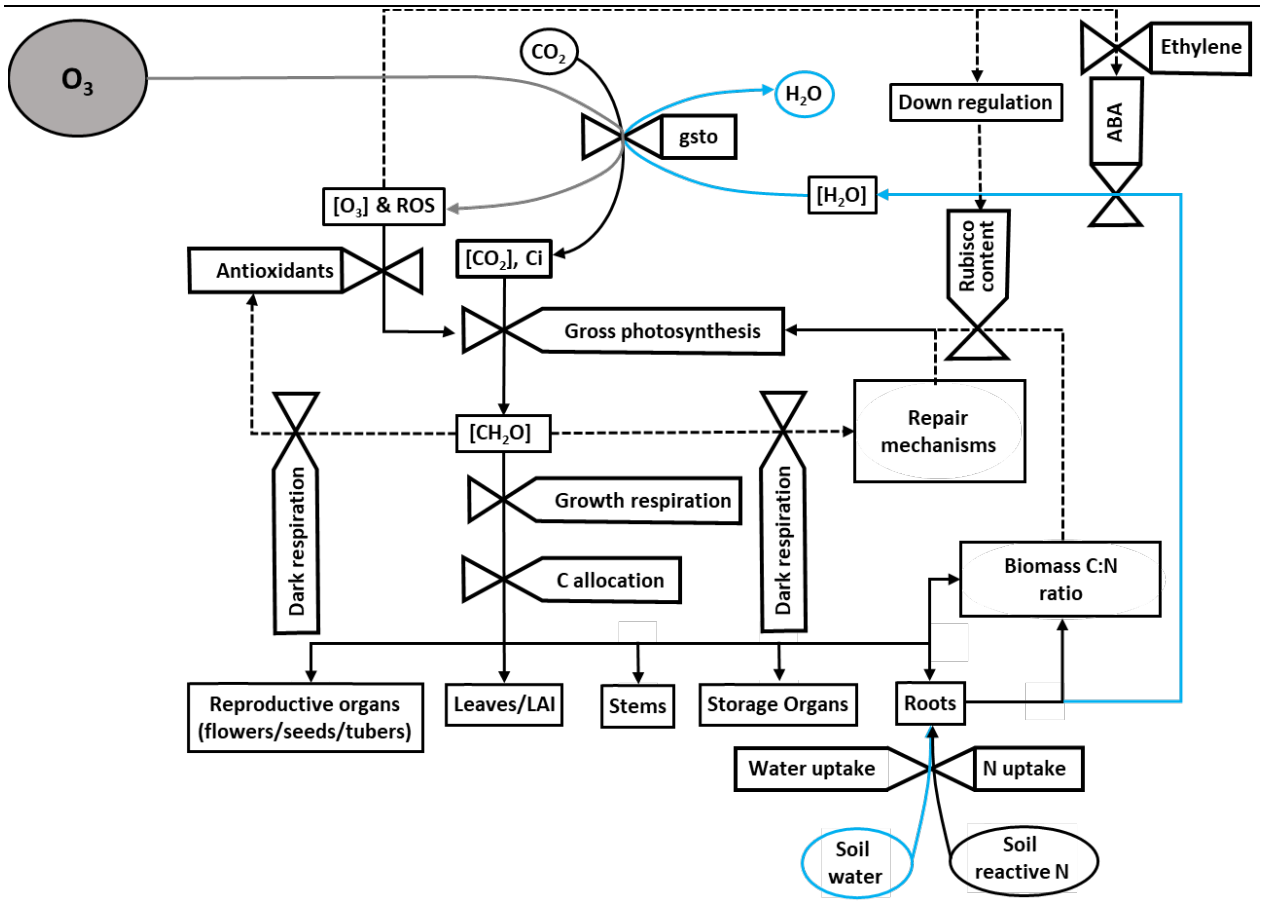
- *SUM06:* Sum of all hourly ozone concentrations greater than or equal to 0.06 ppm observed during a specified daily and seasonal time window.
- *AOTx:* Sum of the differences between hourly ozone concentrations greater than a specified threshold during a specified daily and seasonal time window. For example, AOT40 is sum of the differences between hourly concentrations above 0.04 ppm during a specified period.
- *W126:* Sigmoidally weighted sum of all hourly ozone concentrations observed during a specified daily and seasonal time window (Lefohn et al., 1988; Lefohn and Runeckles, 1987). The sigmoidal weighting of hourly ozone concentration is given in the equation below, where C is the hourly ozone concentration in ppm:

$$w_c = \frac{1}{1 + 4403e^{-126C}}$$

Equation 8-1

8.1.3 Mechanisms Governing Vegetation Response to Ozone

The ecological effects of ozone are observed across multiple levels of biological organization, starting at the subcellular and cellular level, then to individual organisms, and finally to ecosystem-level processes. The 2013 Ozone ISA summarized in detail the mechanisms for ozone's effects at the leaf level (Section 9.3 of 2013 Ozone ISA). Figure 8-2 summarizes current scientific understanding of effects of ozone on plant physiology at the biochemical and leaf level. These effects lead to changes in photosynthesis and carbon allocation to different plant carbon pools. Carbon allocation links ozone effects at the subleaf and leaf level to changes at larger scales.



ABA = abscisic acid; C = carbon; CH_2O = carbohydrate; Ci = intra-cellular carbon dioxide; CO_2 = carbon dioxide; gsto = leaf stomatal openings; H_2O = water; LAI = leaf area index; N = nitrogen; ROS = reactive oxygen species.

Note: Flow of matter (carbon, water, ozone, and nitrogen) are indicated by solid arrows, relationships between processes are indicated by broken lines. Ozone is in gray and water is in blue. Circles indicate compounds outside the plant tissue.

Source: Permission pending, adapted from Emberson et al. (2018).

Figure 8-2 Schematic representation of the cellular and metabolic effects of ozone on vegetation.

As seen in Figure 8-2, ozone ("O_3"; represented in gray) enters the plant through leaf stomatal openings ("gsto") during gas exchange, although some reproductive tissues are also directly affected by ozone exposure (see Section 8.4). Ozone and its derivatives, referred to as reactive oxygen species ("ROS"), are phytotoxic sources of oxidative stress in plants [Section 9.3.2 in U.S. EPA (2013)]. They may be partially detoxified by "antioxidants" [Section 9.3.4 in U.S. EPA (2013)]; however, any remaining effective ozone flux causes damage to photosynthetic machinery and results in declines in "gross photosynthesis." Ozone flux into the leaf may also cause "downregulation" of RuBisCO (the enzyme responsible for carbon fixation), which also results in declines in gross photosynthesis [Section 9.3.5 in U.S. EPA (2013)]. Ozone exposure may cause elevated "ethylene" production, a multifunctional plant hormone [Section 9.3.3 in U.S. EPA (2013)]. This ozone-induced elevated ethylene

production can lead to a dampening of the abscisic acid ("ABA") signal responsible for stomatal closure, resulting in damages to stomatal function. Less responsive stomata can increase water loss to the atmosphere ("H_2O"), reducing the plant's water use efficiency. Additionally, ROS may trigger leaf senescence and abscission (detachment from the plant) through oxidative stress to leaf biochemistry.

Plant carbon ("[CH_2O]") is affected by ozone in two major ways: through (1) decreases to gross photosynthesis via the mechanisms outlined above and (2) increases to carbon demands as more carbohydrates are used in "dark respiration" to support maintenance and repair processes and to produce antioxidants and secondary metabolites. Ozone-mediated changes in plant carbon budgets result in less carbon available for allocation to various pools: "reproductive organs," "leaves," "stems," "storage," and "roots," as well as maintenance, defense, and repair mechanisms. Changes in these organs can affect their function (e.g., diminished pollen production by flowers, diminished N uptake by roots), as well as affect dependent consumer organisms (e.g., altered detection of plant flowers and leaves by herbivores, altered abundance of belowground organisms). These changes can in turn alter ecosystem properties of storage (productivity, C sequestration) and cycling (biogeochemistry). Thus, changes in allocation can scale up to the population, community and ecosystem-level effects assessed in this document, including changes in soil biogeochemical cycling (Section 8.9), increased tree mortality (Section 8.4), shifts in community composition (Section 8.10), changes to species interactions (Section 8.6, Section 8.7, Section 8.10), declines in ecosystem productivity and carbon sequestration (Section 8.8), and alteration of ecosystem water cycling (Section 8.11).

8.2 Visible Foliar Injury and Biomonitoring

In the 2013 Ozone ISA the evidence was sufficient to conclude that there is a causal relationship between ambient ozone exposure and the occurrence of ozone-induced visible foliar injury on sensitive plant species across the U.S.(U.S. EPA, 2013). Visible foliar injury resulting from exposure to ozone has been well characterized and documented on many tree, shrub, herbaceous, and crop species through research beginning in 1958 (U.S. EPA, 2013, 2006, 1996, 1986, 1978; NAPCA, 1970; Richards et al., 1958). Ozone-induced visible foliar injury on certain plant species is considered diagnostic because such injuries have been verified experimentally in exposure-response studies (see Section 8.1.2.1) and are considered bioindicators for ozone exposure. Typical types of visible injury to broadleaf plants include stippling, flecking, surface bleaching, bifacial necrosis, pigmentation (e.g., bronzing), and chlorosis or premature senescence. Typical visible injury symptoms for conifers include chlorotic banding, tip burn, flecking, chlorotic mottling, and premature senescence of needles. At the time of the 2013 Ozone ISA, it was well understood that, although common patterns of injury develop within a species, these foliar lesions can vary considerably within and among taxonomic groups. A triad of conditions is necessary for visible foliar injury to occur. These conditions include the presence of ozone pollution, genetic susceptibility, and sufficient soil moisture to promote open stomata. In general, plants with higher stomatal conductance, allowing more ozone into the leaf, are more susceptible to injury. A lack of soil

moisture generally decreases stomatal conductance. Other factors, such as leaf age and light level, have also been shown to influence the amount of foliar injury.

As described in the PECOS tool (Table 8-2), the scope for new evidence reviewed in this section limits studies to those conducted in North America at concentrations occurring in the environment or experimental ozone concentrations within an order of magnitude of recent concentrations. Recent experimental evidence continues to show a consistent association between visible injury and ozone exposure (see Table 8-4). Studies reviewed in the current ISA provide further support for earlier observations that sensitivity to ozone varies within and between species. Since the 2013 Ozone ISA, several studies have further characterized modifying factors:

- Additional field studies have shown that dry periods in local areas tend to decrease the incidence and severity of ozone-induced visible foliar injury (Kohut et al., 2012; Smith, 2012).
- Data using additional species from greenhouse studies add to the evidence that sensitivity to ozone varies by time of day. Nighttime ozone exposure (<78 ppb) did not cause foliar injury in snap beans, (*P. vulgaris*), while daytime exposure (≥62 ppb) resulted in injury (Lloyd et al., 2018). Exposing Pima cotton (*Gossypium barbadense*) to pulses of ozone at different times throughout the day showed that sensitivity measured by foliar injury was lowest early in the photoperiod and reached a maximum in midafternoon (Grantz et al., 2013).
- Phenotypic variation in foliar sensitivity to ozone has been observed among genotypes for soybean. A comparison between two cultivars in a greenhouse study reported a mean foliar injury score of 16% for the ozone-tolerant Fiskeby III and a score of 81% for the ozone-sensitive Mandarin cultivar (Burton et al., 2016).
- In OTC exposure (mean 12-hour ozone concentration of 37 ppb for 118 days) foliar injury to loblolly pine seedlings (*Pinus taeda*) was not related to seedling inoculation with root-infecting fungi (Chieppa et al., 2015).

The use of bioindicator species to detect phytotoxic levels of ozone is a longstanding and effective methodology (Chappelka and Samuelson, 1998). To be considered a good bioindicator species, plants must (1) exhibit a distinct, verified response; (2) have few or no confounding disease or pest problems; and (3) exhibit genetic stability (U.S. EPA, 2013). Bioindicators are also currently being grown in ozone gardens in several places, including the St Louis Science Center, St. Louis, MO and the Appalachian Highlands Science Learning Center at Great Smoky Mountains National Park (Fishman et al., 2014). These gardens serve as a source of data on the effects of ambient ozone exposure on plants as well as an important educational outreach tool. The U.S. Department of Agriculture (USDA) Forest Service historically has assessed data on the incidence and severity of visible foliar injury on a variety of ozone-sensitive plant species throughout the U.S. (Smith, 2012). Biological indicators are especially useful in areas without ozone monitors; however, the approach requires expertise in recognizing signs and symptoms uniquely attributable to ozone exposure. Since the 2013 Ozone ISA, several additional studies have been conducted on bioindicator species:

- Cutleaf coneflower (*Rudbeckia laciniata* L. var. *digitata*) is an ozone bioindicator species native to Great Smoky Mountain National Park. It was recently shown that variety *ampla,* native to

Rocky Mountain National Park, displayed similar visible injury and may also serve as a bioindicator (Neufeld et al., 2018).

- Tree of heaven (*Ailanthus altissima*), an established invasive species found widely across the U.S., has been identified as an effective ozone bioindicator species by the National Park Service and Forest Service (Smith et al., 2008; Kohut, 2007). In greenhouse exposures, foliar injury occurred at 8-hour avg ozone exposure levels of 60 to 120 ppb, with greater injury corresponding to higher exposures (Seiler et al., 2014). In the field, an ambient ozone 3-month, 12-hour W126 value of 11.6 ppm-hour induced foliar injury (Seiler et al., 2014).

In addition to these studies, a recent global-scale synthesis of published ozone exposure studies documents foliar injury from ozone exposure in the field, across gradients, or in controlled ozone experiments in hundreds of species (Bergmann et al., 2017). In field and gradient studies involving ozone concentrations in ambient air, 245 plant species from 28 plant genera experienced ozone foliar injury (Bergmann et al., 2017). Many of the species that experience ozone foliar injury have populations native to the U.S. (see Table 8-3).

Table 8-3 Plant species that have populations in the U.S.[a] (USDA, 2015) that have been tested for ozone foliar injury as documented in the references listed with each in Bergmann et al. (2017).

Species	Ozone Causes Foliar Injury	References
Abies concolor	Y	Williams et al. (1977); Williams and Macgregor (1975)
Acer macrophyllum	Y	Temple et al. (2005)
Acer rubrum	Y	Davis and Skelly (1992); Simini et al. (1992); Findley et al. (1996)
Acer saccharum	Y	Gaucher et al. (2005); Pell et al. (1999); Rebbeck (1996a); Laurence et al. (1996); Kress and Skelly (1982); Noble et al. (1992); Tjoelker et al. (1993)
Achillea millefolium	Y	Bender et al. (2002); Bungener et al. (1999a); Bungener et al. (1999b)
Agrostis vinealis	Y	Hayes et al. (2006)
Alchemilla sp.	Y	Manning et al. (2002)
Alnus incana	Y	Mortensen and Skre (1990); Lorenz et al. (2005); Bussotti and Gerosa (2002); De Vries et al. (2003); Manning et al. (2002); Ozolincius and Serafinaviciute (2003)
Alnus viridis or *Alnus alnobetula*	Y	Vanderheyden et al. (2001); Skelly et al. (1999); Lorenz et al. (2005); De Vries et al. (2003)

Table 8 3 (Continued): Plant species that have populations in the U.S.[a] (USDA, 2015) that have been tested for ozone foliar injury as documented in the references listed with each in Bergmann et al. (2017).

Species	Ozone Causes Foliar Injury	References
Amorpha californica	Y	U.S. EPA (1980); Temple (1999)
Apocynum androsaemifolium	Y	Bergweiler and Manning (1999); Davis (2007a); Davis (2007b)
Apocynum cannabinum	Y	Kline et al. (2009)
Armeria maritima	N	Hayes et al. (2006)
Artemisia campestris	Y	Lorenz et al. (2005); De Vries et al. (2003)
Artemisia douglasiana	Y	Temple (1999); U.S. EPA (1980)
Artemisia dracunculus	Y	Temple (1999)
Aruncus dioicus	Y	Bussotti et al. (2003a)
Asclepias californica	Y	Temple (1999)
Asclepias exaltata	Y	Chappelka et al. (2007); Souza et al. (2006)
Asclepias fascicularis	Y	Temple (1999)
Asclepias incarnata	Y	Orendovici et al. (2003)
Asclepias syriaca	Y	Kline et al. (2009); Chappelka et al. (1997); Davis and Orendovici (2006); Davis (2007b); Davis (2011); Yuska et al. (2003); Lorenz et al. (2005)
Bignonia sp.	Y	Lorenz et al. (2005)
Bromus orcuttianus	Y	U.S. EPA (1980)
Calocedrus decurrens	Y	Williams et al. (1977)
Camissonia californica	Y	Thompson et al. (1984)
Camissonia claviformis	Y	Bytnerowicz et al. (1988); Thompson et al. (1984)
Camissonia hirtella	Y	Bytnerowicz et al. (1988)
Campanula rotundifolia	N	Ashmore et al. (1995); Hayes et al. (2006); Mortensen and Nilsen (1992); Ashmore et al. (1996)
Carex arenaria	Y	Jones et al. (2010)
Carex atrofusca	Y	Mortensen (1994b)
Carex echinata	Y	Hayes et al. (2006)

Table 8 3 (Continued): Plant species that have populations in the U.S.[a] (USDA, 2015) that have been tested for ozone foliar injury as documented in the references listed with each in Bergmann et al. (2017).

Species	Ozone Causes Foliar Injury	References
Carex nigra	Y	Franzaring et al. (2000)
Centaurea spp.	Y	Bussotti et al. (2006)
Cephalanthus occidentalis	Y	Kline et al. (2008)
Cercis canadensis	Y	Kline et al. (2008)
Chamerion angustifolium	Y	Skelly et al. (1999); Mortensen (1993)
Chenopodium album	Y	Bender et al. (2006); Bergmann et al. (1995); Bergmann et al. (1999); Pleijel and Danielsson (1997); Reiling and Davison (1992); Romaneckiene et al. (2008)
Circaea lutetiana	Y	Lorenz et al. (2005)
Cirsium acaule	N	Warwick and Taylor (1995)
Clarkia rhomboidea	Y	Wahid et al. (2011)
Collomia grandiflora	Y	Temple (1999); U.S. EPA (1980)
Comarum palustre	Y	Batty et al. (2001); Mortensen (1994b)
Conocarpus erectus	Y	Ceron-Breton et al. (2009)
Conyza canadensis	Y	Grantz et al. (2008)
Cordylanthus rigidus	Y	Temple (1999)
Cornus alba	Y	Skelly et al. (1999); Novak et al. (2003)
Cornus florida	Y	Davis (2011)
Cornus nuttallii	Y	Temple (1999)
Cornus spp.	Y	Bussotti and Gerosa (2002)
Cornus stolonifera	Y	Skelly et al. (1999)
Corylus cornuta	Y	Davis (2007a)
Crataegus spp.	Y	Bussotti and Gerosa (2002)
Cryptantha nevadensis	Y	Thompson et al. (1984)
Echinacea purpurea	Y	Szantoi et al. (2007)
Elymus glaucus	Y	U.S. EPA (1980)

Table 8 3 (Continued): Plant species that have populations in the U.S.[a] (USDA, 2015) that have been tested for ozone foliar injury as documented in the references listed with each in Bergmann et al. (2017).

Species	Ozone Causes Foliar Injury	References
Erigeron breweri	Y	U.S. EPA (1980)
Eriophorum angustifolium	Y	Hayes et al. (2006); Mortensen (1994b)
Eschscholzia parishii	Y	Thompson et al. (1984)
Eupatorium perfoliatum	Y	Orendovici et al. (2003)
Eupatorium sp.	Y	Fenn et al. (2002)
Festuca ovina	Y	Ashmore et al. (1995); Hayes et al. (2006); Pleijel and Danielsson (1997); Reiling and Davison (1992); Warwick and Taylor (1995); Ashmore et al. (1996)
Festuca rubra	Y	Ashmore et al. (1995); Bungener et al. (1999b); Bungener et al. (1999a); Hayes et al. (2006); Mortensen (1992); Ashmore et al. (1996);
Fraxinus americana	Y	Kress and Skelly (1982); Hildebrand et al. (1996)
Fraxinus pennsylvanica	Y	Kress and Skelly (1982); Lorenz et al. (2005)
Fraxinus spp.	Y	Davis (2007a)
Galium aparine	Y	U.S. EPA (1980)
Gayophytum diffusum	Y	Wahid et al. (2011)
Geum rivale	N	Batty et al. (2001)
Helianthus hirsutus	Y	Orendovici et al. (2003)
Humulus lupulus	Y	Manning and Godzik (2004); Manning et al. (2002); Blum et al. (1997)
Ipomoea nil	Y	Wan et al. (2014)
Juncus effusus	N	Hayes et al. (2006)
Lagunularia racemosa	Y	Ceron-Breton et al. (2009)
Lamium spp.	Y	Lorenz et al. (2005); Bussotti et al. (2006); De Vries et al. (2003)
Lepidium virginicum	Y	Wahid et al. (2011)
Liquidambar styraciflua	Y	Kress and Skelly (1982); Davis (2011)

Table 8 3 (Continued): Plant species that have populations in the U.S.[a] (USDA, 2015) that have been tested for ozone foliar injury as documented in the references listed with each in Bergmann et al. (2017).

Species	Ozone Causes Foliar Injury	References
Liriodendron tulipifera	Y	Rebbeck (1996a); Rebbeck (1996b); Cannon Jr et al. (1993); Simini et al. (1992); Kress and Skelly (1982); Davis and Skelly (1992); Chappelka et al. (1999b); Hildebrand et al. (1996)
Malacothrix glabrata	Y	Thompson et al. (1984)
Melica nitens	Y	Mortensen (1994b)
Mentha sp.	Y	Orendovici et al. (2003)
Mentzelia albicaulis	Y	Thompson et al. (1984)
Morus spp.	Y	Bussotti and Gerosa (2002)
Oenothera biennis	Y	Lorenz et al. (2005); De Vries et al. (2003)
Oenothera elata	Y	Wahid et al. (2011)
Oenothera rosea	Y	Skelly et al. (1999)
Oenothera sp.	Y	Skelly et al. (1999)
Oxalis acetosella	Y	Hayes et al. (2006)
Oxyria digyna	N	Hayes et al. (2006); Mortensen and Nilsen (1992); Mortensen (1993)
Parthenocissus quinquefolia	Y	Bussotti et al. (2003a); Gerosa and Ballarin-Denti (2003); Bussotti et al. (2003b); Davis and Orendovici (2006); Manning et al. (2002)
Pectocarya heterocarpa	Y	Thompson et al. (1984)
Pectocarya platycarpa	Y	Thompson et al. (1984)
Phleum alpinum/commutatum	Y	Pleijel and Danielsson (1997); Danielsson et al. (1999); Mortensen (1993)
Picea glauca	Y	Mortensen (1994a)
Picea sitchensis	Y	Lucas et al. (1988); Lucas et al. (1993); Mortensen (1994a)
Pinus contorta	Y	Mortensen (1994a); Williams et al. (1977)
Pinus ellioti	Y	Evans and Fitzgerald (1993); Dean and Johnson (1992); Byres et al. (1992)

Table 8 3 (Continued): Plant species that have populations in the U.S.[a] (USDA, 2015) that have been tested for ozone foliar injury as documented in the references listed with each in Bergmann et al. (2017).

Species	Ozone Causes Foliar Injury	References
Pinus jeffrey	Y	Temple et al. (2005); Miller et al. (1998); Williams and Macgregor (1975)
Pinus lambertiana	Y	Williams and Macgregor (1975); Williams et al. (1977)
Pinus leiophylla	Y	Fenn et al. (2002)
Pinus ponderosa	Y	Takemoto et al. (1997); Temple and Miller (1994); Temple et al. (1993); Beyers et al. (1992); Temple et al. (1992); Fenn et al. (2002); Jones and Paine (2006); Williams and Macgregor (1975); Williams et al. (1977)
Pinus pungens	Y	Neufeld et al. (2000)
Pinus rigida	N	Kress and Skelly (1982)
Pinus taeda	Y	Kress and Skelly (1982); Edwards et al. (1992); Qiu et al. (1992); Adams and O'Neill (1991); Adams et al. (1990); Shafer et al. (1993); Spence et al. (1990); Wiselogel et al. (1991); Chappelka et al. (1990)
Pinus virginiana	Y	Neufeld et al. (2000); Kress and Skelly (1982)
Plantago sp.	Y	Orendovici et al. (2003)
Platanus occidentalis	Y	Kress and Skelly (1982); Kline et al. (2008)
Platanus racemosa	Y	Temple et al. (2005)
Poa pratensis	Y	Bender et al. (2002); Bender et al. (2006); Bungener et al. (1999b); Bungener et al. (1999a); Mortensen (1992); Ashmore et al. (1996)
Polygonatum sp.	Y	Bussotti et al. (2006)
Populus spp.	Y	Davis (2007a); Bussotti and Gerosa (2002); De Vries et al. (2003)
Populus tremuloides	Y	Volin et al. (1998); Karnosky et al. (1999); Karnosky et al. (1996); Coleman et al. (1996); Yun and Laurence (1999)
Potentilla glandulosa	Y	Wahid et al. (2011); U.S. EPA (1980)
Prunus emerginata	Y	Temple (1999)
Prunus pensylvanica	Y	Davis (2007a)

Table 8 3 (Continued): Plant species that have populations in the U.S.[a] (USDA, 2015) that have been tested for ozone foliar injury as documented in the references listed with each in Bergmann et al. (2017).

Species	Ozone Causes Foliar Injury	References
Prunus serotina	Y	Skelly et al. (1999); Vanderheyden et al. (2001); Gunthardt-Goerg et al. (1999); Pell et al. (1999); Rebbeck (1996a); Rebbeck (1996b); Neufeld et al. (1995); Simini et al. (1992); Davis and Skelly (1992); Chappelka et al. (1997); Chappelka et al. (1999b); Chappelka et al. (1999a); Davis and Orendovici (2006); Davis (2007a); Davis (2007b); Davis (2011); Hildebrand et al. (1996); De Bauer et al. (2000); Bussotti and Gerosa (2002); Yuska et al. (2003)
Pseudotsuga menziesii	N	Runeckles and Wright (1996); Mortensen (1994a)
Quercus kelloggii	Y	Handley and Grulke (2008); U.S. EPA (1980); Temple et al. (2005)
Quercus phellos	N	Kress and Skelly (1982)
Quercus rubra	Y	Volin et al. (1998); Pell et al. (1999); Samuelson et al. (1996); Kelting et al. (1995); Edwards et al. (1994); Simini et al. (1992); Davis and Skelly (1992)
Ranunculus acris	Y	Hayes et al. (2006); Wyness et al. (2011); Mortensen (1993)
Rhamnus spp.	Y	Bussotti and Gerosa (2002)
Rhizophora mangle	Y	Ceron-Breton et al. (2009)
Rhus aromatica	Y	Kline et al. (2008)
Rhus copallina	Y	Davis and Orendovici (2006); Davis (2009)
Rhus typhina	Y	Wan et al. (2013); Wan et al. (2014)
Ribes spp.	N	Temple (1999)
Robinia pseudoacacia	Y	Skelly et al. (1999); Wang et al. (1986); Lorenz et al. (2005); Bussotti et al. (2003a); Bussotti and Gerosa (2002); Bussotti et al. (2006); Bussotti et al. (2003b); De Vries et al. (2003)
Rosa spp.	Y	Lorenz et al. (2005); Bussotti et al. (2003a); Gerosa and Ballarin-Denti (2003)
Rubus idaeus	Y	Hunova et al. (2011); Lorenz et al. (2005); Bussotti et al. (2003a); De Vries et al. (2003); Gerosa and Ballarin-Denti (2003); Ozolincius and Serafinaviciute (2003)
Rubus parviflorus	Y	Temple (1999)

Table 8 3 (Continued): Plant species that have populations in the U.S.[a] (USDA, 2015) that have been tested for ozone foliar injury as documented in the references listed with each in Bergmann et al. (2017).

Species	Ozone Causes Foliar Injury	References
Rubus spp.	Y	Lorenz et al. (2005); Bussotti et al. (2003a); Bussotti et al. (2006); Bussotti and Gerosa (2002); De Vries et al. (2003); Bussotti et al. (2003b)
Rudbeckia laciniata	Y	Szantoi et al. (2009); Chappelka et al. (2003); Davison et al. (2003)
Rumex acetosa	Y	Batty et al. (2001); Bender et al. (2002); Bender et al. (2006); Bergmann et al. (1999); Pleijel and Danielsson (1997); Manning and Godzik (2004); Reiling and Davison (1992); Ashmore et al. (1996); Mortensen (1993); Hayes et al. (2006)
Rumex sanguineus	Y	Bussotti et al. (2003a)
Rumex sp.	Y	Orendovici et al. (2003)
Salix amygdaloides	Y	Kline et al. (2008)
Salix eriocephala	Y	Kline et al. (2008)
Salix exigua	Y	Kline et al. (2008)
Salix lucida	Y	Kline et al. (2008)
Salix nigra	Y	Kline et al. (2008)
Salix sericea	Y	Kline et al. (2008)
Salix spp.	Y	Lorenz et al. (2005); Bussotti and Gerosa (2002); De Vries et al. (2003)
Sambucus canadensis	Y	Kline et al. (2008); Davis (2007b)
Sambucus nigra	Y	Cano et al. (2007); Kline et al. (2008); Lorenz et al. (2005); De Vries et al. (2003)
Sambucus racemosa	Y	Skelly et al. (1999); Cano et al. (2007); Vanderheyden et al. (2001); Lorenz et al. (2005); Bussotti et al. (2003a); De Vries et al. (2003); Godzik and Grodzinska (2002); Manning et al. (2002); Blum et al. (1997)
Sambucus spp.	Y	Bussotti and Gerosa (2002)
Sassafras albidum	Y	Chappelka et al. (1999b); Davis and Orendovici (2006); Davis (2011)
Scirpus cespitosus	Y	Hayes et al. (2006)
Scrophularia nodosa	Y	Bussotti et al. (2003a)

Table 8 3 (Continued): Plant species that have populations in the U.S.[a] (USDA, 2015) that have been tested for ozone foliar injury as documented in the references listed with each in Bergmann et al. (2017).

Species	Ozone Causes Foliar Injury	References
Sequoiadendron giganteum	Y	Williams et al. (1977)
Sicyos sp.	Y	Fenn et al. (2002)
Silene verecunda	Y	U.S. EPA (1980)
Silphium perfoliatum	Y	Orendovici et al. (2003)
Solanum nigrum	N	Bender et al. (2006); Bergmann et al. (1995); Bergmann et al. (1999); Bergmann et al. (1996a)
Solanum spp.	Y	Fenn et al. (2002)
Solidago albopilosa	N	Mavity and Berrang (1993)
Solidago canadensis	Y	Lorenz et al. (2005)
Solidago gigantea	Y	Lorenz et al. (2005)
Solidago spp.	Y	U.S. EPA (1980)
Solidago virgaurea	Y	Mortensen and Nilsen (1992); Mortensen (1993)
Spartina alterniflora	Y	Taylor (2002)
Stachys palustris	N	Batty et al. (2001)
Stachys spp.	Y	Bussotti et al. (2006)
Symphoricarpos albus	Y	Kline et al. (2008)
Symphoricarpos orbiculatus	Y	Kline et al. (2008)
Symphoricarpos spp.	Y	Kline et al. (2008); Lorenz et al. (2005)
Thalictrum minus	Y	Bussotti et al. (2003a); Gerosa and Ballarin-Denti (2003)
Tilia spp.	Y	Bussotti and Gerosa (2002)
Urtica dioica	N	Bender et al. (2006); Bergmann et al. (1999); Reiling and Davison (1992); Bergmann et al. (1996a); Bussotti et al. (2003a)
Vaccinium myrtillus	Y	Lorenz et al. (2005); Bussotti et al. (2006); De Vries et al. (2003); Godzik and Grodzinska (2002); Manning et al. (2002)
Vaccinium uliginosum	Y	De Vries et al. (2003); Gerosa and Ballarin-Denti (2003)

Table 8 3 (Continued): Plant species that have populations in the U.S.[a] (USDA, 2015) that have been tested for ozone foliar injury as documented in the references listed with each in Bergmann et al. (2017).

Species	Ozone Causes Foliar Injury	References
Verbesina occidentalis	Y	Chappelka et al. (2003)
Vernonia noveboracensis	Y	Orendovici et al. (2003)
Viburnum nudum	Y	Bergmann et al. (2017); Davis (2007a); Davis (2007b)
Viburnum spp.	Y	Bussotti and Gerosa (2002); Manning et al. (2002)
Vicia californica	Y	U.S. EPA (1980)
Vincetoxicum sp.	Y	Blum et al. (1997)
Vitis spp.	Y	Davis and Orendovici (2006); Davis (2009); Davis (2011)

In ozone-response categories, Y = ozone induces effect at tested exposures, N = ozone has no effect at tested exposures. Sixty-nine out of the 125 studies above have been cited in previous ISAs or AQCDs.

[a]Both native and introduced/naturalized plant species documented to occur in the U.S. are included.

As noted in the 2013 ISA (U.S. EPA, 2013), visible foliar injury usually occurs when sensitive plants are exposed to elevated ozone concentrations in a predisposing environment. A major modifying factor for ozone-induced visible foliar injury is the amount of soil moisture available to a plant during the year that the visible foliar injury is being assessed. This is because lack of soil moisture generally decreases stomatal conductance of plants and, therefore, limits the amount of ozone entering the leaf that can cause injury (Matyssek et al., 2006; Panek, 2004; Grulke et al., 2003; Panek and Goldstein, 2001; Temple et al., 1992; Temple et al., 1988). Consequently, many studies have shown that dry periods in local areas tend to decrease the incidence and severity of ozone-induced visible foliar injury; therefore, the incidence of visible foliar injury is not always higher in years and areas with higher ozone, especially with co-occurring drought (Smith, 2012; Smith et al., 2003). In a series of recent studies, researchers have found their spatial models of ozone injury in California improved significantly when GIS variables of plant water status were included (Kefauver et al., 2013; Kefauver et al., 2012b; Kefauver et al., 2012a). In a statistical modeling study, Wang et al. (2012) reported that incorporating ecological factors with ozone exposure and soil moisture improved model predictions of foliar injury in field plots (http://www.fia.fs.fed.us/, 1997−2007) from 24 states in Northeast and North Central U.S.

Although visible injury is a valuable indicator of the presence of phytotoxic concentrations of ozone in ambient air, it is not always a reliable indicator of other negative effects on vegetation [e.g., growth, reproduction; U.S. EPA (2013)]. The significance of ozone injury at the leaf and whole-plant levels depends on how much of the total leaf area of the plant has been affected, as well as the plant's age, size, developmental stage, and degree of functional redundancy among the existing leaf

area (U.S. EPA, 2013). Previous ozone AQCDs have noted the difficulty in relating visible foliar injury symptoms to other vegetation effects, such as individual plant growth, stand growth, or ecosystem characteristics (U.S. EPA, 2006, 1996). Thus, it is not presently possible to determine, with consistency across species and environments, what degree of injury at the leaf level has significance to the vigor of the whole plant. However, in some cases, visible foliar symptoms have been correlated with decreased vegetative growth (Somers et al., 1998; Karnosky et al., 1996; Peterson et al., 1987; Benoit et al., 1982) and impaired reproductive function (Chappelka, 2002; Black et al., 2000). Conversely, the lack of visible injury does not always indicate a lack of phytotoxic concentrations of ozone or a lack of ozone effects (Gregg et al., 2006, 2003).

8.2.1 Summary

Visible foliar injury from ozone exposure has been well characterized for over several decades, using both long-term field studies and laboratory approaches. Since the 2013 Ozone ISA, new research on bioindicator species and the further characterization of modifying factors have provided further support for these effects. Modifying factors for ozone-induced foliar injury include soil moisture, leaf age, and light level, genotype, and time of day of exposure. The use of biological indicators to detect phytotoxic levels of ozone is a longstanding and effective methodology, and recent evidence is supported by more information on sensitive species, such as the native cutleaf coneflower and the invasive tree of heaven as useful bioindicators. New information is consistent with the conclusions of the 2013 Ozone ISA that **the body of evidence is sufficient to infer a causal relationship between ozone exposure and visible foliar injury.**

Table 8-4 Ozone exposure and foliar injury.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Foliar Injury
Grantz et al. (2013)	Greenhouse; Kearney Research and Extension Center, Parlier, CA (36.598°N, 119.503°W)	*Gossypium barbadense*. (Pima cotton)	Each plant was exposed to a single 15-min pulse of O_3 (0.0, 0.5, 1.0, 1.5, 2.0 μmol/mol. Pulses were done at 2-h intervals throughout the daylight period. After a single pulse, plants were returned to greenhouse bench and left undisturbed for 6 days	Plant sensitivity (chlorophyll content, stomatal conductance, and noninjured leaf area) showed a clear diel trend with greatest sensitivity occurring in midafternoon. This trend was not closely related to gas exchange, whole-leaf ascorbate, or total antioxidant capacity.
Yendrek et al. (2015)	Greenhouse; Urbana, IL	*Pisum sativum* (garden pea), *Glycine max* (soybean), and *Phaseolus vulgaris* (common bean)	Six growth chambers—three fumigated with 151 ppb O_3 for 8 h for 45 days; three maintained at ambient with average of 12.5 ppb	The garden pea displayed no visual signs of O_3 damage, in contrast to soybean and common bean, both of which had signs of chlorosis. More extensive O_3 damage was observed in the common bean, including leaf bronzing and necrosis.
Burton et al. (2016)	Greenhouse; North Carolina State University in Raleigh, NC (36.3°N, 78.683°W)	*Glycine max* (soybean, two genotypes: tolerant Fiskeby III and sensitive Mandarin [Ottawa])	5 days of exposure in greenhouse chambers, 7 h/day, at 68−72 ppb O_3	Mean injury score in the mid canopy was 16% for Fiskeby III, and 81% for Mandarin (Ottawa). Injury scores were lower in younger leaves.
Lloyd et al. (2018)	Greenhouse; University Park campus of The Pennsylvania State University (40.806°N, 77.852°W)	*Phaseolus vulgaris* (snap bean, two genotypes; O_3 resistant [R123] and O_3 sensitive [S156])	O_3 treatments were a combination of O_3 concentration and treatment time as follows: (1) 45 ppb O_3, day-only; (2) 75 ppb O_3, day-only; (3) 45 ppb O_3 day + night; (4) 75 ppb O_3 day + night; (5) 30 ppb night-only treatment; and (6) 60 ppb night-only treatment	Nighttime O_3 exposure alone, at 62 ppb, did not cause foliar injury. When data were pooled across the day and day + night exposures times, mean daytime O_3 levels at 62 ppb caused foliar injury decreases in all three trials.

Table 8-4 (Continued): Ozone exposure and foliar injury.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Foliar Injury
Smith (2012)	Field; northeastern and north central U.S.	Most commonly sampled bioindicator species: *Rubus allegheniensis* (Allegheny blackberry), *Asclepias syriaca* (common milkweed), *Asclepias exaltata* (tall milkweed), *Prunus serotina* (black cherry), *Fraxinus americana* (white ash), and *Apocynum androsaemifolium* (spreading dogbane).	Ambient levels across the CONUS, values were grouped into three categories to represent low (SUM06 ≤ 10 ppm-h; N100 ≤ 5 h), moderate (SUM06 > 10 to 30 ppm-h; N100 5 to 30 h), and high (SUM06 > 30 ppm-h; N100 > 30 h) ozone exposure conditions	Foliar injury is significantly different between low- and high-ozone exposure sites. In all cases, injury was greater in high-ozone sites than low-ozone sites. The foliar injury response is also significantly different between low ozone exposure and high peak ozone exposure sites. When SUM06 and N100 are relatively low, the percentage of uninjured sites is much greater than the percentage of injured sites; and at all SUM06 and N100 exposures, when site moisture is limiting, the percentage of uninjured sites is much greater than the percentage of injured sites.
Kefauver et al. (2012b)	Gradient; Yosemite National Park (YOSE) and Sequoia and Kings Canyon National Park (SEKI), CA; Catalonia, Spain	California: *Pinus ponderosa* (ponderosa pine) and *Pinus jeffreyi* (Jeffrey pine) Spain: *Pinus uncinata* (mountain pine)	Ozone data were collected using passive monitors in both YOSE and SEKI. One U.S. EPA-certified active monitor was colocated at YOSE and SEKI. Average yearly O_3 mixing ratio in 2002 ranged from 35−65 ppb for all YOSE and SEKI sites. Yearly averages within sites were 49 ppb for YOSE and 46 ppb for SEKI	The ozone injury index (OII) was transferable to other conifer species and geographic regions (i.e., *P. uncinata* in Catalonia, Spain). Species-level image classifications produced 75% accuracy for YOSE yellow pines (i.e., Jeffrey and ponderosa pines combined) and 82% for SEKI yellow pines. Combining remote sensing indices with landscape GIS variables related to plant water status resulted in an improved regression for California sites.
Kohut et al. (2012)	Field; Rocky Mountain National Park, CO	*Rudbeckia laciniata* var. *ampla* (cutleaf coneflower), *Apocynum androsaemifolium* (spreading dogbane), *Populus tremuloides* (quaking aspen)	Monitoring of ambient levels 2006−2010. SUM06 = 26, 28, 24, 13, 27 ppm-h. W126 = 29.6, 33.2, 28.9, 19.9, 18.9 ppm-h. W126-3 mo = 19, 20, 18, 11, 19 ppm-h	Foliar injury in the form of ozone stipple was found on coneflower each year. The incidence of injured plants on sites with injury ranged from 5 to 100%. The severity of injury on affected foliage was generally <4% but occurred on some leaves at a level greater than 12% in 3 yr and in 1 yr on one plant at a level >75%. No foliar ozone injury was found on spreading dogbane or quaking aspen in any year of the survey.

Table 8-4 (Continued): Ozone exposure and foliar injury.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Foliar Injury
Kefauver et al. (2013)	Gradient; Yosemite National Park (YOSE) and Sequoia and Kings Canyon National Park (SEKI), CA; Catalonia, Spain	California: *Pinus ponderosa* (ponderosa pine) *and Pinus jeffreyi* (Jeffrey pine) Spain: *Pinus uncinata* (mountain pine)	Ozone data were collected using passive monitors in both YOSE and SEKI. One U.S. EPA-certified active monitor was colocated at YOSE and SEKI. Average yearly O_3 mixing ratio in 2002 ranged from 35−65 ppb for all YOSE and SEKI sites. Yearly averages within sites were 49 ppb for YOSE and 46 ppb for SEKI	The Ozone Injury Index (OII) was transferable to other conifer species and geographic regions (i.e., *P. uncinata* in Catalonia, Spain). Species-level image classifications produced 75% accuracy for YOSE yellow pines (i.e., Jeffrey and ponderosa pines combined) and 82% for SEKI yellow pines. The stepwise regression model of ozone injury at California sites using remote sensing vegetation indices combined with GIS landscape variables was significant.
Kefauver et al. (2012a)	Gradient; Yosemite National Park (YOSE) and Sequoia and Kings Canyon National Park (SEKI), CA; Catalonia, Spain	California: *Pinus ponderosa* (ponderosa pine) and *Pinus jeffreyi* (Jeffrey pine) Spain: *Pinus uncinata* (mountain pine)	Ozone data were collected using passive monitors in both YOSE and SEKI. One U.S. EPA-certified active monitor was colocated at YOSE and SEKI. Average yearly O_3 mixing ratio in 2002 ranged from 35−65 ppb for all YOSE and SEKI sites. Yearly averages within sites were 49 ppb for YOSE and 46 ppb for SEKI	Results show that the Ozone Injury Index (OII) was transferable to other conifer species and geographic regions (i.e., *P. uncinata* in Catalonia, Spain). OII by itself was poorly correlated to ambient ozone across all sites. Models were improved when GIS variables related to plant-water relations were included.
Chieppa et al. (2015)	OTC; research site located ~5 km north of Auburn University campus	*Pinus taeda* (loblolly pine) inoculated with either *Leptographium terebrantis* or *Grosmannia huntii* (fungal species associated with Southern Pine Decline)	Three ozone treatments in OTCs (12 h/day): charcoal filtered (~0.5% ambient air), nonfiltered air (ambient) and 2× ambient. The 1st 41 days were acclimatization then exposure, which continued 77 more days once seedlings were inoculated with fungus. Mean 12 h O_3 over the 118 days was 14 (CF), 23 (NF), and 37 (2×) ppb in the treatments. 12 h AOT40 values were 0.027 (CF), 1.631 (NF) and 31.2 (2×) ppm. Seasonal W126 values were 0.033 (CF), 0.423 (NF) and 21.913 (2×)	In elevated O_3, seedlings had 9.9× more needle injury and lower needle greenness (13.7%) than NF chambers. The two families selected for sensitivity to ophiostomatoid fungi had 3× more ozone injury compared with tolerant families; however, visible foliar injury was not affected by inoculation status of seedlings.

Table 8-4 (Continued): Ozone exposure and foliar injury.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Foliar Injury
Davis (2011)	Field; Mingo National Wildlife Refuge in southeastern Missouri	*Asclepias syriaca* (common milkweed), *Cercis canadensis* (redbud), *Cornus florida* (flowering dogwood), *Fraxinus* sp. (ash), *Liquidambar styraciflua* (sweetgum), *Prunus serotina* (black cherry), *Rhus aromatica* (fragrant sumac), *Rhus copallina* var. *latifolia* (winged sumac), *Sambucus canadensis* (black elderberry), *Sassafras albidum* (sassafras), and *Vitis* sp. (wild grape)	Cumulative ambient ozone levels (SUM60, ppb-h) monitored at the closest U.S. EPA monitor (Bonne Terre, MO) at time of survey were 1998 (44,886), 2000 (39,611), 2003 (38,465), 2004 (15,147)	Across all 4 survey yr and the seven species, 102 individuals out of 1,241 (8.22%) exhibited stipple. Percentage of bioindicator plants exhibiting stipple were wild grape (16.1%), common milkweed (16.0%), ash (7.5%), black cherry (6.7%), flowering dogwood (4.9%), sassafras (2.3%), and sweetgum (1.2%). By year, the incidence of symptomatic plants was 1998 (22.8%), 2003 (3.9%), 2000 (3.4%), and 2004 (2.5%). The cumulative SUM60 threshold value of ozone needed to cause foliar symptoms on ozone-sensitive plants within the refuge appears to be ~10,000 ppb-h.
Neufeld et al. (2018)	OTC; experiments conducted in Boone, NC. Rhizomes collected from Great Smoky Mountains National Park and Rocky Mountains National Park.	*Rudbeckia laciniata* var. *ampla* and var. *digitata* (cutleaf coneflower)	Three treatment groups: charcoal-filtered air (CF), nonfiltered air (NF), and nonfiltered air + 50 ppb O_3 (2012) or +30 ppb/+ 50 ppb (2013) (EO). In 2012, 24-h W126 was 0.1 ppm-h in the CF treatment, 2.0 ppm-h in the NF treatment, and 74.2 ppb in the EO treatment. 12-h AOT40 were 0.0, 2.0, and 24.1 ppm-h, respectively. In 2013, 24-h W126 were 0.1, 1.8, and 80.5 ppm-h, respectively. 12-h AOT40 were 1.0, 2.0, and 53.8 ppm-h, respectively. Plants were exposed for 47 days in 2012 and for 77 days in 2013.	In 2012 and 2013, injury levels in both varieties were higher in the EO treatment than in either the CF or NF treatments, which did not differ, but there were no statistically significant differences between the varieties. Stippling increased with time.

Table 8-4 (Continued): Ozone exposure and foliar injury.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Foliar Injury
Seiler et al. (2014)	Greenhouse, field; Penn State Russell E. Larson Agricultural Research Center, Rock Springs, PA	*Ailanthus altissima* (tree of heaven)	**2010 Greenhouse Study**: Charcoal-filtered control (<8 ppb daily hourly avg), exposures of 75 and 120 ppb 8 h/day, 5 days/week for 3 weeks 2011 **Greenhouse Study**: Charcoal-filtered control (<8 ppb daily hourly avg), exposures of 40, 60, 80,100, 120 ppb, 5 days/week for 4 weeks **2011 Field Study:** Seasonal SUM06 = 15.2 ppm-h., 3-mo 12-h W126 = 11.6 ppm-h. 3-yr mean of the 4th-highest daily max 8-h avg = 71.5 ppb for 2009−2011 and 1-yr 4th-highest daily max 8-h avg ozone concentration of 76 ppb. Calculated from U.S. EPA Aerometric Information Retrieval System located about 1.1 km northeast of field site	In greenhouse exposures, foliar injury occurred at 8-h avg O_3 exposure levels of 60 to 120 ppb, with greater injury corresponding to higher exposures. In the field, an ambient O_3 3-mo, 12-h W126 of 11.6 ppm-h induced foliar injury.

CF = charcoal-filtered air; EO = air + ozone treatment; N100 = the number of hours of ozone ≥ 100 ppb; NF = nonfiltered air; O_3 = ozone; OTC = open-top chamber; ppb = parts per billion; ppm = parts per million; SUM06 = seasonal sum of all hourly average concentrations ≥ 0.06 ppm; SUM60 = sum of hourly ozone concentrations equal to or greater than 60 ppb; µmol/mol = micromoles/mole; W126 = cumulative integrated exposure index with a sigmoidal weighting function; W126-3 mo = the running maximum 3-month, cumulative 12-hour W126 weighted value.

8.3 Plant Growth

In the 2013 Ozone ISA, the evidence was sufficient to conclude that there is a causal relationship between ambient ozone exposure and reduced growth of native woody and herbaceous vegetation (U.S. EPA, 2013). The 2013 Ozone ISA and previous ozone AQCDs concluded that there is strong and consistent evidence that exposure to ozone decreases photosynthesis and growth in numerous plant species (U.S. EPA, 2013, 2006, 1996, 1986). The evidence available at that time and discussed here found that ambient ozone concentrations cause decreased growth (measured as biomass accumulation) in annual, perennial, and woody plants, inclusive of crops, annuals, grasses, shrubs, and trees. A meta-analysis by Wittig et al. (2009) found that average ozone exposures of 40 ppb significantly decreased annual total biomass by 7% across 263 studies. Biomass declines were reported to be greater (11 to 17%) with elevated ozone exposures [average of 97 ppb; Wittig et al. (2009)]. Biomass declines were linked to reductions in photosynthesis [Section 9.3.5.1 in U.S. EPA (2013)], which are consistent with cumulative uptake of ozone into the leaf [Wittig et al. (2007); Figure 8-2]. Further, there is evidence ozone may change plant growth patterns by significantly reducing carbon allocated to roots in some species (Jones et al., 2010; Wittig et al., 2009; Grantz et al., 2006; Andersen, 2003; King et al., 2001).

As described in the PECOS tool (Table 8-2), the scope for new evidence reviewed in this section is limited to studies conducted in North America at ozone concentrations occurring in the environment or experimental ozone concentrations within an order of magnitude of recent concentrations. Plant growth, which is the increase in biomass over a period of time, may be determined using a number of metrics (e.g., the change in height, stem volume, leaf area, canopy density, or weight of plant material), and studies that examined these metrics were selected for review. The 2013 ISA broadly defined plant growth to include effects on plant reproduction. However, due to increased research and synthesis of ozone effects on plant reproduction, these endpoints are reviewed separately in Section 8.4.

8.3.1 Declines in Growth Rates

Since the 2013 Ozone ISA, there is more evidence from manipulative experiments that supports known detrimental effects of ozone on plant growth as well as models built with empirical data that scale up ozone exposure-response relationships of growth reductions to put these losses in context [e.g., Capps et al. (2016); Lapina et al. (2016); Table 8-6].

- Results from the aspen-only (*Populus tremuloides*) stands at the Aspen FACE ("free air" ozone and CO_2 exposure) experiment in Wisconsin show a decrease of 12−19% in the relative growth rate of three of five genotypes of aspen studied. Trees were exposed to ozone levels 1.5 times ambient over the period from 1998−2008 [Ambient W126 2.1−8.8 ppm-hour, Elevated 12.7−35.1 ppm-hour; Moran and Kubiske (2013)].

- When site-level results from the Aspen FACE experiment were scaled up using the forest landscape model (LANDIS II), ozone was found to significantly reduce landscape biomass (Gustafson et al., 2013).
- A meta-analysis of nine studies (inclusive of the Aspen FACE experiments) examining intra-specific variation in juvenile tree growth under elevated ozone found that elevated ozone generally reduced photosynthetic rate as well as height growth and stem volume (metrics used for biomass calculations and tracking growth rates) in multiple genotypes of silver birch (*Betula pendula*), quaking aspen (*Populus tremuloides*), and poplar hybrids (Resco de Dios et al., 2016).
- A study using the invasive Chinese tallow tree (*Triadica sebifera*) suggests ozone response may be genotype-specific; elevated ozone decreased both root and total biomass from U.S. seed sources, but had no effect on the biomass of Chinese seed sources (Wang et al., 2018).
- Using simulations of the GEOS-Chem model for 2010 data coupled with established U.S. EPA ozone exposure-response functions in seedlings, Lapina et al. (2016) estimated relative biomass loss at 2.5% for ponderosa pine (*Pinus ponderosa*) and 2.9% for aspen (*Populus tremuloides*) across the continental U.S.
- In another estimation of biomass loss of adult tree species across the U.S. for modeled spatially explicit ozone values, eastern cottonwood (*Populus deltoides*) and black cherry (*Prunus serotina*) show large annual losses in biomass under the authors' reference scenario (ambient ozone levels, W126 range 0−56 ppm-hour), 32, and 10%, respectively. Black cherry exhibits the greatest annual loss (2,210 tons of biomass/ha) of the 11 tree species studied, with twice the biomass loss potential of either eastern cottonwood or ponderosa pine. Biomass of quaking aspen (*Populus tremuloides*), tulip poplar (*Liriodendron tulipifera*), and various pine species also respond negatively to ozone, with losses ranging from 0.3−1.9% (Capps et al., 2016).

In addition to these studies, there is a recent global-scale synthesis of published ozone exposure studies that documents reductions in biomass due to ozone exposure in over a hundred species (Bergmann et al., 2017). Many of these species have populations native to the U.S., and a comprehensive list of U.S. species identified by Bergmann et al. (2017) as sensitive to ozone are presented below in Table 8-5.

Table 8-5 Plant species that have populations in the U.S. (USDA, 2015) that have been tested for ozone growth reduction as documented in the references listed with each species and synthesized in Bergmann et al. (2017).[a,b]

Species	Ozone Reduces Growth	References
Acer rubrum	Y	Davis and Skelly (1992); Simini et al. (1992); Findley et al. (1996)
Acer saccharum	Y	Gaucher et al. (2005); Pell et al. (1999); Rebbeck (1996a); Laurence et al. (1996); Kress and Skelly (1982); Noble et al. (1992); Tjoelker et al. (1993)
Achillea millefolium	N	Bender et al. (2002); Bungener et al. (1999a); Bungener et al. (1999b)
Agropyron smithii	Y	Volin et al. (1998)
Agrostis vinealis	N	Hayes et al. (2006)
Alnus incana	Y	Mortensen and Skre (1990); Lorenz et al. (2005); Bussotti and Gerosa (2002); De Vries et al. (2003); Manning et al. (2002); Ozolincius and Serafinaviciute (2003)
Andropogon gerardii	Y	Lewis et al. (2006)
Angelica archangelica	Y	Mortensen (1993)
Antennaria dioica	Y	Mortensen (1993)
Apocynum androsaemifolium	N	Bergweiler and Manning (1999); Davis (2007a); Davis (2007b)
Armeria maritima	Y	Hayes et al. (2006)
Campanula rotundifolia	Y	Ashmore et al. (1995); Hayes et al. (2006); Mortensen and Nilsen (1992); Ashmore et al. (1996)
Carex arenaria	Y	Jones et al. (2010)
Carex atrofusca	Y	Mortensen (1994b)
Carex echinata	N	Hayes et al. (2006)
Carex nigra	N	Franzaring et al. (2000)
Chamerion angustifolium	Y	Skelly et al. (1999); Mortensen (1993)
Chenopodium album	Y	Bender et al. (2006); Bergmann et al. (1995); Bergmann et al. (1999); Pleijel and Danielsson (1997); Reiling and Davison (1992); Romaneckiene et al. (2008)
Cirsium acaule	Y	Warwick and Taylor (1995)

Table 8-5 (Continued): Plant species that have populations in the U.S. (USDA, 2015) that have been tested for ozone growth reduction as documented in the references listed with each species and synthesized in Bergmann (2017).[a,b]

Species	Ozone Reduces Growth	References
Comarum palustre	Y	Batty et al. (2001); Mortensen (1994b)
Echinacea purpurea	Y	Szantoi et al. (2007)
Eriophorum angustifolium	N	Hayes et al. (2006); Mortensen (1994b)
Festuca ovina	Y	Ashmore et al. (1995); Hayes et al. (2006); Pleijel and Danielsson (1997); Reiling and Davison (1992); Warwick and Taylor (1995); Ashmore et al. (1996)
Festuca rubra	Y	Ashmore et al. (1995); Bungener et al. (1999b); Bungener et al. (1999a); Hayes et al. (2006); Mortensen (1992); Ashmore et al. (1996);
Fragaria vesca	Y	Mortensen (1993)
Fraxinus americana	Y	Kress and Skelly (1982); Hildebrand et al. (1996)
Fraxinus pennsylvanica	Y	Kress and Skelly (1982); Lorenz et al. (2005)
Geum rivale	Y	Batty et al. (2001)
Juncus effusus	Y	Hayes et al. (2006)
Liquidambar styraciflua	Y	Kress and Skelly (1982); Davis (2011)
Liriodendron tulipifera	Y	Rebbeck (1996a); Rebbeck (1996b); Cannon Jr et al. (1993); Simini et al. (1992); Kress and Skelly (1982); Davis and Skelly (1992); Chappelka et al. (1999b); Hildebrand et al. (1996)
Melica nitens	N	Mortensen (1994b)
Oxalis acetosella	N	Hayes et al. (2006)
Oxyria digyna	Y	Hayes et al. (2006); Mortensen and Nilsen (1992); Mortensen (1993)
Paspalum notatum	Y	Muntifering et al. (2000)
Phleum alpinum/commutatum	Y	Pleijel and Danielsson (1997); Danielsson et al. (1999); Mortensen (1993)
Picea glauca	N	Mortensen (1994a)
Picea rubens	Y	Finnveden (2000); Amundson et al. (1991); Laurence et al. (1997)
Picea sitchensis	Y	Lucas et al. (1988); Lucas et al. (1993); Mortensen (1994a)
Pinus contorta	N	Mortensen (1994a); Williams et al. (1977)

Table 8-5 (Continued): Plant species that have populations in the U.S. (USDA, 2015) that have been tested for ozone growth reduction as documented in the references listed with each species and synthesized in Bergmann (2017).[a,b]

Species	Ozone Reduces Growth	References
Pinus echinata	Y	Shelburne et al. (1993)
Pinus ellioti	Y	Evans and Fitzgerald (1993); Dean and Johnson (1992); Byres et al. (1992)
Pinus ponderosa	Y	Takemoto et al. (1997); Temple and Miller (1994); Temple et al. (1993); Beyers et al. (1992); Temple et al. (1992); Fenn et al. (2002); Jones and Paine (2006); Williams and Macgregor (1975); Williams et al. (1977)
Pinus pungens	N	Neufeld et al. (2000)
Pinus rigida	Y	Kress and Skelly (1982)
Pinus taeda	Y	Kress and Skelly (1982); Edwards et al. (1992); Qiu et al. (1992); Adams and O'Neill (1991); Adams et al. (1990); Shafer et al. (1993); Spence et al. (1990); Wiselogel et al. (1991); Chappelka et al. (1990)
Pinus virginiana	N	Neufeld et al. (2000); Kress and Skelly (1982)
Platanus occidentalis	Y	Kress and Skelly (1982); Kline et al. (2008)
Poa pratensis	Y	Bender et al. (2002); Bender et al. (2006); Bungener et al. (1999b); Bungener et al. (1999a); Mortensen (1992); Ashmore et al. (1996)
Polygonum viviparum	Y	Mortensen and Nilsen (1992)
Populus deltoides	Y	Wang et al. (1986)
Populus tremuloides	Y	Volin et al. (1998); Karnosky et al. (1999); Karnosky et al. (1996); Coleman et al. (1996); Yun and Laurence (1999)
Prunus serotina	Y	Skelly et al. (1999); Vanderheyden et al. (2001); Gunthardt-Goerg et al. (1999); Pell et al. (1999); Rebbeck (1996a); Rebbeck (1996b); Neufeld et al. (1995); Simini et al. (1992); Davis and Skelly (1992); Chappelka et al. (1997); Chappelka et al. (1999b); Chappelka et al. (1999a); Davis and Orendovici (2006); Davis (2007a); Davis (2007b); Davis (2011); Hildebrand et al. (1996); De Bauer et al. (2000); Bussotti and Gerosa (2002); Yuska et al. (2003)
Pseudotsuga menziesii	Y	Runeckles and Wright (1996); Mortensen (1994a)
Quercus phellos	Y	Kress and Skelly (1982)
Quercus rubra	Y	Volin et al. (1998); Pell et al. (1999); Samuelson et al. (1996); Kelting et al. (1995); Edwards et al. (1994); Simini et al. (1992); Davis and Skelly (1992)
Ranunculus acris	Y	Hayes et al. (2006); Wyness et al. (2011); Mortensen (1993)

Table 8-5 (Continued): Plant species that have populations in the U.S. (USDA, 2015) that have been tested for ozone growth reduction as documented in the references listed with each species and synthesized in Bergmann (2017).[a,b]

Species	Ozone Reduces Growth	References
Robinia pseudoacacia	Y	Skelly et al. (1999); Wang et al. (1986); Lorenz et al. (2005); Bussotti et al. (2003a); Bussotti and Gerosa (2002); Bussotti et al. (2006); Bussotti et al. (2003b); De Vries et al. (2003)
Rudbeckia laciniata	Y	Szantoi et al. (2009); Chappelka et al. (2003); Davison et al. (2003)
Rumex acetosa	Y	Batty et al. (2001); Bender et al. (2002); Bender et al. (2006); Bergmann et al. (1999); Pleijel and Danielsson (1997); Manning and Godzik (2004); Reiling and Davison (1992); Ashmore et al. (1996); Mortensen (1993); Hayes et al. (2006)
Schizachyrium scoparium	Y	Powell et al. (2003); Volin et al. (1998)
Scirpus cespitosus	Y	Hayes et al. (2006)
Silene acaulis	Y	Mortensen and Nilsen (1992)
Solanum nigrum	Y	Bender et al. (2006); Bergmann et al. (1995); Bergmann et al. (1999); Bergmann et al. (1996a)
Solidago albopilosa	Y	Mavity and Berrang (1993)
Solidago virgaurea	Y	Mortensen and Nilsen (1992); Mortensen (1993)
Spartina alterniflora	Y	Taylor (2002)
Stachys palustris	Y	Batty et al. (2001)
Urtica dioica	Y	Bender et al. (2006); Bergmann et al. (1999); Reiling and Davison (1992); Bergmann et al. (1996a); Bussotti et al. (2003a)

In ozone-response categories, Y = ozone induces effect at tested exposures, N = ozone has no effect at tested exposures
Sixty-five out of the 108 studies above have been cited in previous ISAs or AQCDs.

[a]Both native and introduced/naturalized plant species documented to occur in the U.S. are included.

[b]Data are found in the Supplemental Information in this publication.

8.3.2 Changes in Biomass Allocation

In addition to declines in plant growth rates, ozone alters patterns of carbon allocation, both belowground and aboveground (the portion of energy expended by the plant toward roots, stems, or leaves; see Figure 8-2). Changes in biomass allocation alter plant nutrient uptake, plant water use, and carbon fixation.

- Over the course of the Aspen FACE experiment (1998−2008), the effects of ozone on plant carbon allocation were dynamic through time and varied among the forest communities (Talhelm et al., 2014; Pregitzer and Talhelm, 2013; Rhea and King, 2012). Elevated ozone consistently suppressed leaf production in each of the three communities. There were effects on root biomass in 2006 consistent with Aspen FACE studies of previous years, with elevated ozone increasing small root (0−2 mm diameter) biomass in the aspen-only rings and decreasing small root biomass in the aspen-birch rings (Rhea and King, 2012). There were also effects of ozone on the distribution of roots across the soil profile, which are discussed in more detail in Section 8.9.2.
- Shifts in wood anatomy (change in growth, cell size, vessel density, and proportion) also occurred with elevated ozone at the Aspen FACE site (Kostiainen et al., 2014). Elevated ozone significantly decreased radial growth and diameters of wood fibers and vessels in quaking aspen. Most treatment responses were observed in the early phase of the experiment, indicating ontogenetic changes during wood maturation that are consistent with shifts in the trees' metabolic priority from growth to hydraulic transport in response to ozone.
- A study of the effects of short-term ozone exposure on loblolly pine seedlings found positive effects on aboveground growth, but the study authors attribute this finding to reduction in photosynthate transport to roots, which contributed to declines in seedling vigor (Chieppa et al., 2015). Even with the increased aboveground growth observed, ozone alterations to carbon transport and subsequent declines in seedling vigor and longevity may have negative impacts on forest establishment and regeneration.

8.3.3 Connections with Community Composition and Water Cycling

Studies published since the 2013 Ozone ISA have provided insight on ozone-mediated alterations to biomass allocations within an individual plant that are relevant to whole-plant growth and function. Additionally, the studies provide context for scaling up the long-known detrimental effects of ozone on photosynthesis and growth in numerous plant species to changes at the community and ecosystem level. While outside the scope of this assessment, decreases in photosynthesis due to ozone are well studied and quantified and are directly related to declines in plant biomass discussed here. Ozone-caused declines in canopy density and leaf area index, an important component of plant biomass, have similarly been well studied but are outside the scope of the current assessment (see Section 8.1.1.). These effects were, however, thoroughly reviewed in the 2013 Ozone ISA (U.S. EPA, 2013), and studies continue to be published in this area (U.S. EPA, 2008).

- Species-specific responses to ozone in terms of plant growth reductions and biomass allocation are a possible mechanism for community shifts. In a model simulation of long-term effects of ozone on a typical forest in the southeastern U.S. involving different tree species with varying ozone sensitivity, Wang et al. (2016) found that ozone significantly altered forest community composition and decreased plant biodiversity. Using published peer-reviewed data to place tree species into three sensitivity classes, Wang et al. (2016) applied either a 0, 10, or 20% growth reduction to species in the University of Virginia Forest Model Enhanced (UVAFME), a gap model which tracks the growth and survival of individual trees and species within a stand. Over the 500-year simulation, ozone-resistant species like white oak (*Quercus alba*) and American beech (*Fagus grandifolia*) dominate, and sensitive species like tulip poplar (*Liriodendron tulipifera*) and red maple (*Acer rubrum*) decline. Although the communities changed significantly, overall forest biomass and forest carbon storage did not decrease under high ozone conditions because tolerant species growth was enhanced after these species were freed from competition by the loss of ozone-sensitive species. The terrestrial community composition section (see Section 8.10) contains more information about scaling up biomass response of individual species and examining the ensuing compositional changes.
- Variable growth response between species may be a concern in agricultural systems as well. In a study of ozone's effects on the noxious weed Palmer's pigweed (*Amaranthus palmeri*), elevated ozone exposure and water stress had no effect on the daytime stomatal conductance, shoot growth, and root growth of this plant. The authors suggest that this weed species may have much higher tolerance to elevated ozone and moisture stress compared with crops, and therefore may become a more serious pest in the future because of this competitive advantage (Paudel et al., 2016).
- Changes in forest biomass may also affect ecosystem water use (see Section 8.11). Statistical models examining climate and ozone effects on late-season streamflow in several Appalachian forest watersheds were also found to accurately predict measurements of annual tree ring growth over 20 years in five native species in these forests—an important mechanistic step in understanding ecosystem-level effects of ozone exposure. The findings highlight the negative effects of ozone on tree growth and explicitly connect these declines to tree water use and seasonal watershed dynamics (Sun et al., 2012).

8.3.4 Summary

Previous studies showed strong and consistent evidence that ambient ozone concentrations cause decreased growth and biomass accumulation in annual, perennial, and woody plants, inclusive of crops, annuals, grasses, shrubs, and trees. Since the 2013 Ozone ISA, more evidence that supports this causal relationship has been published. In addition to reductions in plant growth rates, numerous studies from different ecosystems have found that ozone significantly changes patterns of carbon allocation belowground and aboveground which also supports previous knowledge. This evidence contributes to the understanding of ozone's effects on plant growth, biomass allocation, and development. Previous evidence and new evidence reviewed here **is sufficient to infer a causal relationship between ozone exposure and reduced plant growth.**

Table 8-6 Ozone exposure and plant growth and biomass.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Plant Growth and Biomass
Paudel et al. (2016)	Greenhouse; Parlier, CA	*Amaranthus palmeri* (Palmer amaranth)	Two runs of exposure 30 and 35 days. 12-h means of 4, 59, and 114 ppb	Elevated O_3 exposure and water stress had no effect on daytime stomatal conductance, shoot growth, and root growth. This weed species may have much more tolerance to elevated O_3 and moisture stress compared to crops that it competes with.
Sun et al. (2012)	Gradient; six watersheds in Appalachian mixed deciduous forests: Walker Branch and Littler River (eastern Tennessee), Cataloochee Creek (western North Carolina), James River and New River (Virginia), and Fernow Experimental Forest (West Virginia)	Mixed tree species in eastern forests	AOT60 at each watershed: 1.72 (WBWS), 2.6 (LR), 1.72 (CC), 0.82 (NR), 0.83 (JR), 0.74 (FEW); max hourly (in ppb): 68.2 (WBWS), 67.8 (LR), 68.2 (CC), 59.4 (NR), 58.7 (JR), 58.8 (FEW)	Empirical statistical models from data collected in six watersheds in Tennessee, North Carolina, Virginia, and West Virginia found that O_3 and climate are both significant predictors of late-season stream flow in forests, and models incorporating these environmental parameters also fit measurements of annual tree ring growth, which is an important mechanistic step in ozone effects on forested watersheds.
Rhea and King (2012)	FACE; Aspen FACE, near Rhinelander, WI (45.7°N, 89.5°W)	*Populus tremuloides* (quaking aspen), *Betula papyrifera* (paper birch)	Treatments up until the 2005 (when root samples were taken): ambient average W126 was 5.2 ppm-h and elevated O_3 was 27.3 ppm-h. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015).	Fine-root biomass in all-aspen (AA) and aspen-birch (AB) plots fumigated with ozone differed by community and soil depth. Biomass increased with depth in the AA (aspen clones) community by 10.2, 36.4, and 34.8% in the upper, middle, and lower soil layer relative to the control. In the AB community, root biomass decreased 16% in the shallow layer with a small increase at the middle soil layer, resulting in a total decrease of 11% across all layers. Total root length increased in the AA community to a greater extent than the AB community where smaller increases and some decreases were observed. A 33% decrease in root tissue density was observed across all soil layers in trees exposed to O_3. Specific root length increased with soil depth and O_3, with the greatest increases in the AA community.

Table 8-6 (Continued): Ozone exposure and plant growth and biomass.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Plant Growth and Biomass
Kostiainen et al. (2014)	FACE; Aspen FACE, near Rhinelander, WI (45.7°N, 89.5°W)	*Populus tremuloides* (quaking aspen), *Betula papyrifera* (paper birch)	Growing seasons 1998−2008. Ambient W126 2.1−8.8 ppm-h and elevated 12.7-35.1 ppm-h. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015).	Elevated CO_2 increased radial growth and cell diameters in aspen, while vessel density and proportion decreased. Elevated O_3 decreased growth and cell diameters, but increased vessel density and proportion. Neither CO_2 nor O_3 responses were consistent during the experiment. O_3 exposed trees had more and narrower vessels, which were packed more densely per unit wood area. In birch, the treatments had no major effects on wood anatomy or wood density.
Talhelm et al. (2014)	FACE; Aspen FACE, near Rhinelander, WI (45.7°N, 89.5°W).	*Populus tremuloides* (quaking aspen), *Betula papyrifera* (paper birch), *Acer saccharum* (sugar maple)	12 rings, factorial $CO_2 \times O_3$ with three chamber reps. Ambient O_3 W126 = 2.1−8.8 ppm-h, Elevated = 12.7−35.1 ppm-h. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015).	Over the 11 yr of the experiment, O_3 significantly reduced C content of stems and branches by 17%; and NPP by 10% however O_3 effects on NPP disappeared during final 7 yr of study; O_3 shifted fine roots toward soil surface.
Moran and Kubiske (2013)	FACE; Aspen FACE, near Rhinelander, WI (45.7°N, 89.5°W).	Clones of five genotypes of *Populus tremuloides* (quaking aspen), from the aspen-only sections of the experiment, 1997−2008	Full factorial: O_3 and CO_2, 1998−2008. Ozone: ambient W126 = 2.1−8.8 ppm-h, elevated W126 = 12.7−35.1 ppm-h. CO_2: ambient (360 ppm) or elevated (560 ppm). For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015).	Elevated O_3 decreases relative growth rate by 12−19% in three of the five genotypes.

Table 8-6 (Continued): Ozone exposure and plant growth and biomass.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Plant Growth and Biomass
Gustafson et al. (2013)	Model; Rhinelander, WI	*Betula papyrifera* (paper birch), *Acer saccharum* (sugar maple), and four clones of *Populus tremuloides* (quaking aspen)	Ambient O_3 W126 = 2.1−8.8 ppm-h and elevated = 12.7−35.1 ppm-h. Three chamber reps for each treatment, control, $+CO_2$, $+O_3$, and $+CO_2+O_3$. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015).	Site-level results from the Aspen FACE experiment were scaled up using the forest landscape model (LANDIS II). $+O_3$ reduced landscape biomass and the $+CO_2+O_3$ treatment was similar to the control; Total biomass was always lowest under the O_3 treatment.
Chieppa et al. (2015)	OTC; research site located ~5 km north of Auburn University campus	*Pinus taeda* (loblolly pine) inoculated with either *Leptographium terebrantis* or *Grosmannia huntii* (fungal species associated with Southern Pine Decline)	Three ozone treatments in OTCs (12 h/day): CF(~0.5% ambient air), NF, and 2× ambient. 1st 41 days were acclimatization then exposure continued 77 more days once seedlings were inoculated with fungus. Mean 12-h O_3 over the 118 days was 14 (CF), 23 (NF), and 37 (2×) ppb. 12-h AOT40 values were 0.027 (CF), 1.631 (NF), and 31.2 (2×) ppm-h. Seasonal W126 values were 0.03 (CF), 0.423 (NF) and 21.9 (2×) ppm-h.	Four loblolly pine families (two tolerant and two susceptible) were inoculated with root-infecting ophiostomatoid fungi following preacclimation to ozone (41 days). Seedling growth was not affected by inoculation but was affected by O_3. Seedling volume under 2× O_3 increased significantly compared with CF and NF seedlings and had greater aboveground and total dry matter yield than CF seedlings.

Table 8-6 (Continued): Ozone exposure and plant growth and biomass.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Plant Growth and Biomass
Neufeld et al. (2018)	OTC; experiments conducted in Boone, NC. Rhizomes collected from Great Smoky Mountains National Park and Rocky Mountains National Park	*Rudbeckia laciniata* var. *ampla* and var. *digitata* (cutleaf coneflower)	Three treatment groups: charcoal-filtered air (CF), nonfiltered air (NF), and nonfiltered air + 50 ppb O_3 (2012) or +30 ppb/+ 50 ppb (2013) (EO). In 2012, 24-h W126 was 0.1 ppm-h in the CF treatment, 2.0 ppm-h in the NF treatment, and 74.2 ppb in the EO treatment. 12-h AOT40 were 0.0, 2.0, and 24.1 ppm-h, respectively. In 2013, 24-h W126 were 0.1, 1.8, and 80.5 ppm-h, respectively. 12-h AOT40 were 1.0, 2.0, and 53.8 ppm-h, respectively. Plants were exposed for 47 days in 2012 and for 77 days in 2013.	In 2012 and 2013, injury levels in both varieties were higher in the EO treatment than in either the CF or NF treatments, which did not differ, but there were no statistically significant differences between the varieties. Stippling increased with time. Effects of O_3 on biomass accumulation were not significant.
Wang et al. (2018)	Greenhouse; Rice University, TX, with seeds collected from trees in China and North America	*Triadica sebifera* (tallow tree) seedlings, grown from seeds collected in native Chinese range and invasive American range	Factorial O_3 by CO_2 experiment for 78 days: ambient O_3 and ambient CO_2; elevated O_3 (100 ppb); elevated CO_2 (800 ppm); elevated O_3 + elevated CO_2	Elevated O_3 decreases U.S.-sourced *T. sebifera* root and total biomass, but does not affect the biomass of plants grown from seed sourced from China.

Table 8-6 (Continued): Ozone exposure and plant growth and biomass.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Plant Growth and Biomass
Lapina et al. (2016)	Model; continental U.S.	*Pinus ponderosa* (ponderosa pine) and *Populus tremuloides* (quaking aspen)	Exposure response functions for W126, AOT40, and mean metric (MX) for total ozone exposure were used to model relative loss estimates. The GEOS-Chem adjoint model was applied to estimate source-receptor relationships between tree biomass loss and emission sources.	This analysis of year 2010 data coupled with established U.S. EPA ozone exposure-response functions in seedlings estimates a nationwide biomass loss of 2.5% for ponderosa pine and 2.9% for aspen.
Capps et al. (2016)	Model; continental U.S. (CONUS)	*Populus deltoides* (eastern cottonwood), *Prunus serotina* (black cherry), *Populus tremuloide*s (quaking aspen), *Pinus ponderosa* (ponderosa pine), *Liriodendron tulipifera* (tulip poplar), *Pinus strobus* (eastern white pine), *Pinus virginiana* (Virginia pine), *Acer rubrum* (red maple), *Alnus rubra* (red alder)	Uses U.S. EPA-developed CMAQ model to model exposure values of W126 under three regulatory scenarios of maximum local decreases in W126: 1.3, 4, and 5.3%, as well as a reference (ambient, W126 range 0−56 ppm-h) over CONUS. See Figure 3 in paper.	Eastern cottonwood and black cherry show noticeable potential productivity losses of 32 and 10%, respectively, at ambient O_3 concentrations. Black cherry shows the greatest potential productivity losses of 2,210 tons of biomass per hectare with twice the biomass loss potential of either eastern cottonwood or ponderosa pine. The quaking aspen, tulip poplar, and various pine species also respond to ozone with potential productivity losses ranging from 0.3 to 1.9%.

AOT40 = seasonal sum of the difference between an hourly concentration at the threshold value of 40 ppb, minus the threshold value of 40 ppb; AOT60 = seasonal sum of the difference between an hourly concentration at the threshold value of 60 ppb, minus the threshold value of 60 ppb; C = carbon; CO_2 = carbon dioxide; FACE = free-air CO_2 enrichment; NPP = net primary production; ppb = parts per billion; ppm = parts per million; W126 = cumulative integrated exposure index with a sigmoidal weighting function.

8.4 Plant Reproduction, Phenology, and Mortality

In the 2013 Ozone ISA, there was no separate determination of causality for plant reproduction. Rather, evidence was sufficient to conclude that a causal relationship exists between ozone exposure and reduced plant growth, which at the time was broadly defined to encompass plant reproduction (U.S. EPA, 2013). In this ISA, due to increased research and synthesis of ozone effects on plant reproduction, evidence was evaluated for a separate causal statement for this endpoint. Studies in the 2013 Ozone ISA were in agreement with previous research reviewed in the 2006 Ozone AQCD and by Black et al. (2000), which included research going back to the 1970s, showing that ozone can affect plant reproductive function (U.S. EPA, 2013, 2006). For instance, paper birches (*Betula papyrifera*) at Aspen FACE that were exposed to years-long elevated ozone produced male flowers more frequently but produced seeds of lower weight that germinated less often than seeds from trees in ambient conditions (Darbah et al., 2008; Darbah et al., 2007). Additional research reviewed here strengthens the evidence that ozone affects plant reproduction, including newly summarized ozone response across species for a suite of reproductive metrics (Leisner and Ainsworth, 2012). New experiments have also isolated the effects of ozone on specific reproductive tissues and relative to reproductive developmental events. This evidence reinforces the previous understanding of the biological mechanisms for effects which are classified as either "direct" from exposure of reproductive tissues to ozone or "indirect" from reduction in photosynthesis that results in fewer total available resources to invest in flowers or seeds (Figure 8-2).

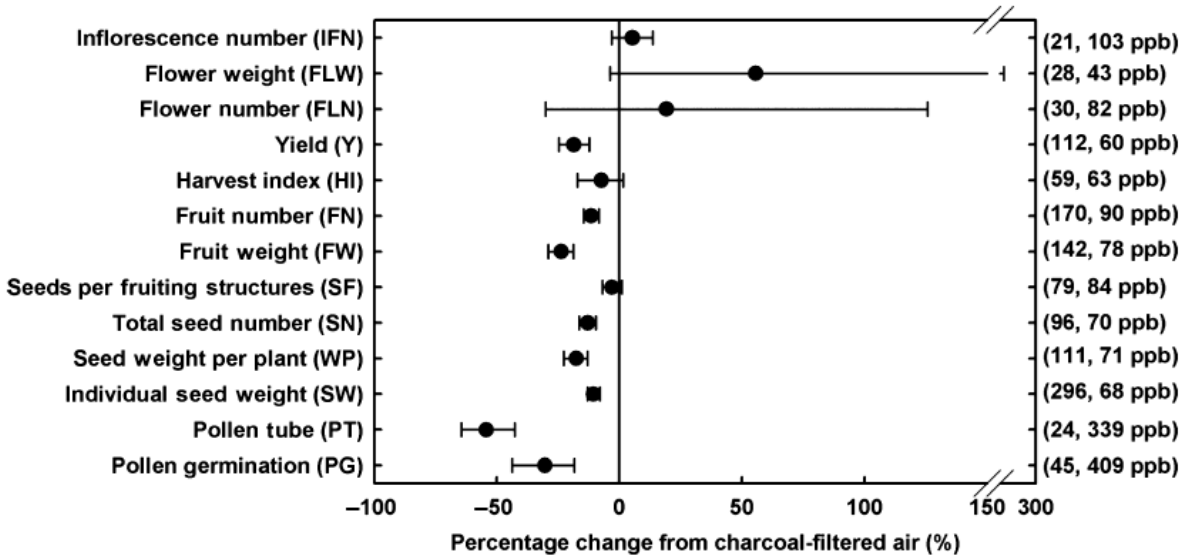
In the 2013 Ozone ISA, there was no determination of causality for plant phenology (i.e., timing of flowering or germination) or mortality (U.S. EPA, 2013). Black et al. (2000) reviewed the effects of ozone on the timing of flowering and germination, noting that responses vary considerably across species. Since then, new studies on the effects of ozone on phenology have been published, and those studies continue to show less consistent results than the studies on plant reproduction. With respect to plant mortality, the 2006 Ozone AQCD and 2013 Ozone ISA included studies identifying ozone as a contributor to tree mortality, however evidence was not sufficient to determine causality (U.S. EPA, 2013, 2006). Additional studies are reviewed here (Table 8-8), including one large multivariate analysis that showed ozone significantly increases mortality of trees in 7 out of 10 plant functional types that make up eastern and central U.S. forests (Dietze and Moorcroft, 2011).

As described in the PECOS tool (Table 8-2), the scope for this section includes studies on any continent in which alterations in plant reproduction (e.g., flower number, fruit number, fruit weight, seed number, rate of seed germination), phenology (e.g., timing of flowering or germination), and mortality (i.e., the fraction of individuals in a population that die over a given interval) were measured on the scale of individual plants in response to concentrations occurring in the environment or experimental ozone concentrations within an order of magnitude of recent concentrations (as described in Appendix 1).

8.4.1 Plant Reproduction

The recent literature shows that across most plant reproduction metrics (e.g., flower number, fruit number, fruit weight, seed number, rate of seed germination) and exposure concentrations that ozone has significant negative effects on plant reproduction. Although crop yield is sometimes considered a measure of reproduction, it is discussed separately in Section 8.5. Additional studies contribute to an increasingly refined understanding of how ozone affects plant reproduction in agricultural and horticultural species [e.g., snap bean (*P. vulgaris*), cowpea (*Vigna unguiculata*), pepper (*Capsicum annuum*), and petunia (*Petunia* hybrid); Yang et al. (2017); Tetteh et al. (2015); Taia et al. (2013); Burkey et al. (2012)], agricultural weeds (Li et al., 2013a), and pasture/grassland species [Gundel et al. (2015); Wang et al. (2015); Table 8-9].

- In a first of its kind study, Leisner and Ainsworth (2012) conducted a quantitative meta-analysis to understand the general magnitude and direction of the effects of ozone exposure on plant reproduction. Their conclusions were based on data from 128 studies and many plant species. They categorized ozone exposure concentration using daytime means (4-, 7-, 8-, or 12-hour daytime mean depending on data reported). Compared with charcoal-filtered air, most metrics of plant reproduction were reduced under elevated ozone (Figure 8-4). Furthermore, compared with ambient air (an average of 33 ppb across all studies), all metrics of plant reproduction were reduced under elevated ozone (Figure 8-6). For instance, in experiments that used charcoal-filtered air as the control, seed number decreased 16% (at an average exposure of 70 ppb), and fruit number decreased 9% (at an average exposure of 90 ppb). In experiments that used ambient air as the control, average fruit weight decreased 51% (at an average exposure of 98 ppb), which was the largest effect observed in this part of the meta-analysis, and seed number decreased approximately 10% (at an average exposure of 68 ppb). Some metrics significantly decreased even under the lowest exposure category (<40 ppb). A trend in larger negative responses under higher exposure levels existed for some metrics of plant reproduction, including fruit number and average fruit weight when ambient air was used as the control.

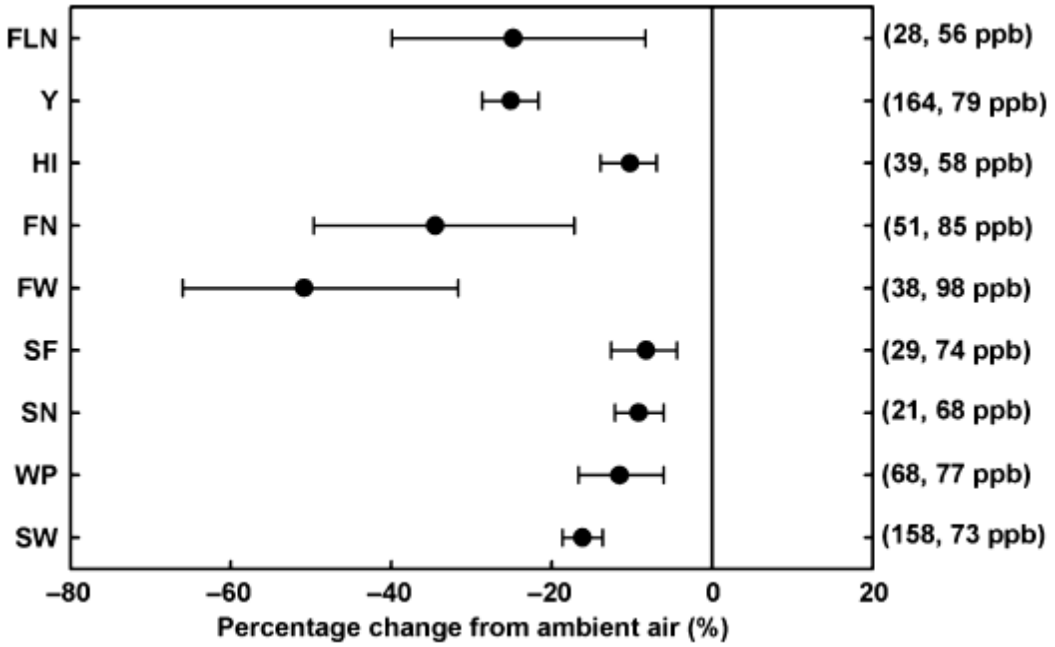


ppb = parts per billion.

Note: The parentheticals on the right of the panel show degrees of freedom for each data point in the panel and average exposure concentration represented by the effect.

Source: Permission pending Leisner and Ainsworth (2012).

Figure 8-3 Meta-analysis of the effects of ozone exposure (relative to charcoal-filtered air) on plant reproduction.



HI = harvest index; FLN = flower number; FN = fruit number; FW = fruit weight; ppb = parts per billion; SF = seeds per fruiting structure; SN = total seed number; SW = seed weight; WP = seed weight per plant; Y = yield.

Note: The parentheticals on the right of the panel show degrees of freedom for each data point in the panel and average exposure concentration represented by the effect.

Source: Permission pending, Leisner and Ainsworth (2012).

Figure 8-4 Meta-analysis of the effects of ozone exposure (relative to ambient air) on plant reproduction.

- Leisner and Ainsworth (2012) also analyzed the response in reproduction to ozone exposure of contrasting plant types (i.e., annual vs. perennial, monocot vs. dicot, C3 vs. C4, and indeterminate vs. determinate growth form) and found few significant differences in response magnitude or direction. One exception was that indeterminate plants had much greater reductions in fruit weight and fruit number than determinate plants (53.9% decrease vs. 4.4% increase, and 44.9% decrease vs. 7.1% decrease, respectively).
- Sanz et al. (2016) developed linear exposure (AOT40)-response and Phytotoxic Ozone Dose (POD1)-response curves for reproductive biomass in ozone-sensitive species of clover (*Trifolium* spp.) found in Europe. Reproduction was reduced significantly with increasing ozone exposure ($r^2 = 0.45$) and ozone dose ($r^2 = 0.69$).
- Gillespie et al. (2015) isolated the effects of ozone on particular reproductive tissues of tomato (*Lycopersicon esculentum*). Pollen grains exposed to ozone have significantly reduced germination and pollen tube growth in vitro. Reductions in pollen viability and pollen tube development in vivo tended to be greatest with exposure of pollen *and* the pollen recipient's stigma surface. Reduction in ovule fertilization, on the other hand, seemed to occur at approximately the same magnitude whether the pollen, stigma, or both were exposed to ozone. Finally, when developing fruits were exposed to ozone (but the rest of the plant was in charcoal-filtered air), fruit fresh weight and the number of seeds per fruit were reduced by 19 and

14%, respectively, relative to fruit developing in charcoal-filtered air, thus showing direct effects of ozone on developing fruit tissue.

- The timing of ozone exposure relative to reproductive development stages can affect reproductive outcomes in some cases. Flowers exposed to ozone early in their development tended to produce shorter fruits than flowers exposed later in their development. On the other hand, ozone exposure seemed to decrease the number of mature seeds per fruit by about the same amount regardless of flower developmental stage (Black et al., 2012).
- For noncultivated plants, authors have long hypothesized that air pollution could be a selective force driving the evolution of plant populations over generations, with potential consequences for community interactions (Bell et al., 1991). One recent study evaluated traits from three lines of the agricultural weed *Spergula arvensis* that were selected over generations under three different ozone concentrations (Landesmann et al., 2013). Selected lines appeared to vary in their seed germination rate under a range of laboratory conditions, but differences were not as clear for seed viability under more field-like conditions. Further evidence would be necessary to evaluate whether ozone-driven selection resulted in measurable changes in these lines that are relevant to field settings and interactions with other community members, including agricultural crops.

8.4.2 Plant Phenology

Several new studies of European grassland species have been published since the 2013 Ozone ISA that measure the effects of ozone on flowering phenology (e.g., time of first flowering, time of maximum flowering).

- No effect on timing of flowering was recorded for *Leontodon hispidus*, *Dactylis glomerate*, or *Anthoxanthum odoratum* over a range of ozone seasonal 24-hour means (21−103 ppb), either during greenhouse exposure or 2 months after exposure (Hayes et al., 2011). Similarly, no effects of two consecutive seasons of a range of ozone seasonal 24-hour means (19−73 ppb) were observed on timing of flowering in *Briza media, Sanguisorba minor,* or *Scabiosa columbaria* (Hayes et al., 2012b).
- There was no effect of ozone on flowering in *Briza maxima* during an open top chamber experiment, but 30 days after exposure the number of plants starting to flower was 66% lower in the 45−65 ppb mean ozone treatment group compared with the charcoal-filtered air treatment group (Sanz et al., 2011).
- Flowering sped up for *Lotus corniculatus* under increasing ozone, especially for well-watered plants (r^2 = 0.64 mean ozone vs. date to 20% maximum flower number). The date of maximum flowering was also earlier: an increase in the mean ozone concentration from 30 to 70 ppb corresponded with maximum flowering occurring 6 days earlier in both well-watered and drought conditions (Hayes et al., 2012b).

The effect of ozone on the timing of seed germination has also been recently studied. Germination is delayed in some species, sped up in others, or remains unaffected by ozone exposure (Abeli et al., 2017; Black et al., 2012). Leaf senescence can be considered a phenological event, although for the purposes of this review, it was considered a visible foliar injury (see Section 8.2).

8.4.3 Plant Mortality

Several new studies examined the impact of ozone exposure on plant mortality (i.e., the fraction of individuals in population that die over a given interval). All were focused on tree species, and the study results are consistent with previous evidence showing that ozone can affect tree mortality (Table 8-8).

- In the Aspen FACE experiment in Rhinelander, WI, the survival of sensitive aspen (*Populus tremuloides*) genotypes 271 and 259 declined significantly between 1997 and 2008 under elevated ozone concentrations relative to ambient conditions (Moran and Kubiske, 2013). In contrast, the survival of tolerant genotype 8L increased 14.8% under elevated ozone. Genetically based differences in ozone sensitivity could have implications for intra-specific diversity and evolution of wild populations (see Section 8.4.1).
- Dietze and Moorcroft (2011) conducted a large-scale analysis of factors contributing to annual mortality of trees in functional types (each characterized by different species) in the forests of the eastern and central U.S. The U.S. Forest Service's FIA database (http://www.fia.fs.fed.us/, version 2.1) was queried for data on tree mortality, and the analysis only included trees that were measured in consecutive censuses. Overall, ozone was ranked 9th on the list of 13 factors that forests were sensitive to, and ozone's effect was similar in magnitude to that of precipitation. Mortality in eight out of ten plant functional types was significantly correlated with ozone 8-hour max: seven experienced increasing mortality with increasing ozone exposure, while late successional conifers showed a slight decrease in mortality with increasing ozone exposure. Evergreen hardwoods were the functional type most sensitive to increasing ozone; they showed annual mortality ranging from 1% in areas of the country with relatively low ozone to 3% in areas of relatively high ozone. Assuming no replacement, a change in mortality rate from 1 to 3% would shift the time to 50% loss of a species from 69 to 24 years. Such changes in mortality are consistent with documented changes in community composition (Section 8.10).

8.4.4 Summary

Ozone exposure can affect plant reproduction. Over 100 studies of cultivated and noncultivated species have now been synthesized qualitatively and quantitatively. They show that diverse metrics of plant reproduction decline under ozone concentrations occurring either in the environment or under experimental conditions within an order of magnitude of recent concentrations. The biological mechanisms underlying ozone's effect on plant reproduction are twofold. They include both direct negative effects on reproductive tissues and indirect negative effects that result from decreased photosynthesis and other whole-plant physiological changes. Two metrics of plant reproduction, fruit number and fruit weight, show greater reductions under increased ozone when combined across species for ozone concentrations that span 40 to >100 ppb; other metrics do not show such reductions or do so across a narrower range of ozone concentrations. An exposure-response and a dose-response curve developed for legume species in Europe both show significant negative effects of ozone on plant reproductive biomass. Finally, experimental ozone exposure at multiple experimental settings (e.g., in vitro, whole plants in the laboratory, whole plants and/or reproductive structures in the greenhouse, and whole plant communities in field settings) convincingly show ozone independently

reduces plant reproduction. Therefore, previous evidence and new evidence reviewed here is **sufficient to infer a causal relationship between ozone exposure and reduced plant reproduction** (Table 8-7).

Studies of tree mortality indicate that ozone affects this endpoint. Multiple studies from different research groups show the co-occurrence of ozone exposure and increased mortality of trees. Evidence for plants other than trees is currently lacking. Studies linking ozone and tree mortality are consistent with known and well-established individual plant-level mechanisms that explain ozone phytotoxicity, including variation in sensitivity and tolerance based on age class, genotype, and species. Increased mortality is also consistent with effects at higher levels of biological organization, including changes in vegetation cover and decreased terrestrial biodiversity (Section 8.10). Relationships between ozone and mortality have been modeled for eastern and central U.S. forests; 7 out of 10 plant functional types show a significant positive correlation between 8-hour max ozone concentration and tree mortality. Experimentally, elevated ozone exposure has been shown to increase mortality in sensitive aspen genotypes. Therefore, previous evidence and new evidence reviewed here is **sufficient to infer a likely to be causal relationship between ozone exposure and tree mortality** (Table 8-8).

Table 8-7 Summary of evidence for causal relationship between ozone exposure and plant reproduction.

Rationale for Causality Determination	Key Evidence	Key References
Consistent evidence from multiple research groups under ozone concentrations occurring in the environment or experimental ozone concentrations within an order of magnitude of recent concentrations	Quantitative meta-analysis of >100 independent studies using different experimental approaches show statistically significant and sometime large (up to 50%) decreases in reproduction across crop and noncrop species with exposure to <40 ppb ozone. Independent studies synthesized using qualitative review have also shown consistent reduction in most measures of reproduction.	Leisner and Ainsworth (2012), U.S. EPA (2006), Sections AX9.5.4.1, AX9.5.4.4, AX9.5.5.1, AX9.6.2.4, AX9.6.2.5, AX9.6.4.2; U.S. EPA (2013), Section 9.4.3.3
Biologically plausible mechanisms are well established and show evidence for direct and indirect effects on reproductive tissues and function	Experimental exposure of whole plants and reproductive tissues in isolation demonstrate that the effect of ozone on plant reproduction can be indirect (via decreased available photosynthate) or direct (via changes in male or female function).	Gillespie et al. (2015), U.S. EPA (2006), Sections AX9.2, AX9.6.4.2; U.S. EPA (2013), Sections 9.3, 9.4.3.3
Exposure-response relationship is clear for some metrics of reproduction and not well resolved for others; exposure-response and dose-response curve exists for a set of legume species	Fruit number and fruit weight show a clear exposure-response relationship with exposure to ozone at a range of concentrations from 40 ppb to >100 ppb. Other measures show a exposure-response relationship over a narrower range of concentrations. Exposure-response and dose-response curves for *Trifolium* spp. show a significant negative relationship between ozone and reproductive biomass.	Leisner and Ainsworth (2012), Sanz et al. (2016)
Abundant experimental evidence isolates and demonstrates the effect of ozone on plant reproduction	Studies that compare plants grown in charcoal-filtered air or ambient air as a control with plants experimentally exposed to ozone demonstrate that exposure to ozone causes reduction in reproductive output.	Leisner and Ainsworth (2012), U.S. EPA (2006), Sections AX9.5.4.1, AX9.5.4.4, AX9.5.5.1, AX9.6.2.5, AX9.6.4.2; U.S. EPA (2013), Section 9.4.3.3

Table 8-8 Summary of evidence for likely to be causal relationship between ozone exposure and tree mortality.

Rationale for Causality Determination	Key Evidence	Key References
Consistent evidence from multiple research groups under ozone concentrations occurring in the environment or experimental ozone concentrations within an order of magnitude of recent concentrations	Independent studies show co-occurrence of increasing mortality and exposure to ozone in tree species from different forest types in the U.S. and in specific sensitive tree species in Mexico and Europe.	Dietze and Moorcroft (2011), Diaz-De-Quijano et al. (2016), U.S. EPA (2006), Sections AX9.6.2.1, AX9.6.2.2, AX9.6.2.3; U.S. EPA (2013), Section 9.4.7.1
Biologically plausible mechanisms are well established and support observed effects at higher levels of biological organization	Differences in mortality are consistent with known physiological mechanisms of ozone sensitivity and tolerance in age classes, genotypes, and species. Mortality due to ozone exposure is also consistent with observed changes in vegetation cover and terrestrial community composition.	Moran and Kubiske (2013), U.S. EPA (2006), Sections AX9.2 AX9.6.2.2, AX9.6.2.3, AX9.6.4.1; U.S. EPA (2013), Section 9.4.7.1
Independent effect of ozone modeled in one large-scale study but confounded in most observational studies	One empirical model of eastern and central U.S. forests shows a significant effect of ozone independent of other factors, most gradient studies cannot rule out other factors that contribute to mortality.	Dietze and Moorcroft (2011), U.S. EPA (2006), Sections AX9.6.2.1, AX9.6.2.2, AX9.6.2.3; U.S. EPA (2013), Section 9.4.7.1
Limited evidence from experimental studies that isolate the effect of ozone on tree mortality	The Aspen FACE study shows sensitive genotypes have increased mortality with ozone exposure compared with a tolerant genotype.	Moran and Kubiske (2013), U.S. EPA (2013), Section 9.4.7.1

This table is provided as an example of a causal table using the modified Hill criteria (U.S. EPA, 2015). Tables were only used if there was a change in causality from the 2013 Ozone ISA or if a new causality determination was warranted based on evaluation of the evidence.

Table 8-9 Ozone exposure and plant reproduction, phenology, and mortality.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Plant Reproduction and Mortality
Burkey et al. (2012)	FACE; Champaign, IL (40.033°N, 88.233°W)	*Phaseolus vulgaris* (snap bean) three genotypes (S156-O_3 sensitive; R123, R331-O_3 tolerant)	O_3 exposure from May−August 2006; exposure hours uncertain, perhaps 9:00 a.m. to 5:00 p.m. Ambient (control)—8-h mean = 43 ppb, 1-h max = 29−78 ppb, AOT40 = 5.3 ppm-h, SUM60 = 5.3 ppm-h +O_3—8-h mean = 59 ppb, 1-h max = 32−114 ppb, AOT40 = 16.3 ppm-h, SUM60 = 27 ppm-h +O_3+CO_2—8-h mean = 59 ppb, 1-h max = 33−112 ppb, AOT40 = 16.2 ppm-h, SUM60 = 26.7 ppm-h	Plant reproduction—sensitive genotype had 63% decline in pod weight per plant and similar result for seed weight per plant under elevated O_3; no significant difference for tolerant genotypes under elevated O_3. Sensitive genotype had 57% reduction in seed number per plant and 19% reduction in weight per seed under elevated O_3.
Taia et al. (2013)	OTC; Al Montazah botanical garden near Alexandria, Egypt	*Capsicum annuum* (pepper)	Three chambers exposed to ambient air or charcoal-filtered air 8 h/day 9:00 a.m.−5:00 p.m. for 75 days. Ambient: 8-h seasonal daily average = 78 (±8) ppb, AOT40 29,600 (±42); charcoal-filtered air: 15 (±3) ppb, AOT40 0	Plant reproduction—fruit length, fruit weight, and number of fruits per plant were all significantly lower in ambient chambers. Number of fruits reduced by 28%. Percentage pollen germination and pollen tube length were also lower in ambient chambers.

Table 8-9 (Continued): Ozone exposure and plant reproduction, phenology, and mortality.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Plant Reproduction and Mortality
Landesmann et al. (2013)	Other; University of Buenos Aires, Argentina (34.58°S, 58.58°W)	*Spergula arvensis* (annual agricultural weed corn spurry); populations grown under O_3 (0, 90, 120 ppb) for four generations in Corvallis, OR, before being sent to Argentina and grown for a generation in the field	(1) populations exposed to O_3 of 0, 90, or 120 ppb over four generations before the beginning of this study, and (2) maternal plants exposed to O_3 only for reproductive stage: ambient range 0−20 ppb, and elevated range 40−70 ppb O_3	Plant reproduction—generational O_3 exposure affects germination under hot and wet conditions, with the highest germination rate in the 90-ppb population and the lowest germination in the 120-ppb population (p = 0.022). Generational O_3 exposure affects germination under cold and dry conditions, with the highest germination rate in the 90-ppb population, and the lowest germination rate in the 0-ppb population (p = 0.16). For the 120-ppb population, germination rates were highest under cold and dry conditions. Exposure of mother plants to O_3 (40−70 ppb) as seeds were developing resulted in higher seed viability than in plants under ambient O_3 conditions.
Li et al. (2013a)	OTC; wheat fields in northern China	Agricultural weed *Descurainia sophia* (flixweed) grown alone or in competition with *Triticum aestivum* (winter wheat)	Three O_3 treatments: ambient (less than 40 ppb O_3), elevated (80 ± 5 ppb for 7 h/day for 30 days), highly elevated (120 ± 10 ppb for 7 h/day for 30 days)	Plant reproduction—elevated O_3 had no statistically significant effect on flixweed seed production, while highly elevated O_3 decreased flixweed seed production 24−29%.

Table 8-9 (Continued): Ozone exposure and plant reproduction, phenology, and mortality.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Plant Reproduction and Mortality
Gillespie et al. (2015)	Greenhouse; Close House, Northumberland, U.K.	*Lycopersicon esculentum* (tomato)	Pollen and ovule experiments: control = charcoal-filtered air (<5 nmol/mol O_3), treatment = CFA + 75 nmol/mol O_3 for 7 h/day, also combinations of these exposures (e.g., plant grown in CFA, then pollen exposed to 75 nmol/mol in separate chamber). Fruit experiment: control = charcoal-filtered air (<5 nmol/mol O_3), treatment = 100 nmol/mol O_3 for 8 h/day	Plant reproduction—pollen germination (on agar)—significant reduction in O_3/O_3 vs.O_3/CFA and CFA/O_3 vs. CFA/CFA which indicates direct effect of O_3 on pollen; pollen tube growth (on agar)—significantly reduced in O_3/O_3 vs. CFA/CFA and CFA/O_3 vs. CFA/CFA, which also suggests direct effect on pollen; pollen viability index (germinated vs. nongerminated pollen on stigma surface)—pollen from O_3 exposed plants used to pollinate an O_3 exposed stigma has 25% lower germination than other treatments; pollen tube development index (pollen tubes at base of the papillia vs. germinated pollen on stigman surface)—pollen from O_3 exposed plants used to pollinate an O_3 exposed stigma had lower pollen tube development than other treatments; ovule fertilization—percentage fertilized, viable ovules was reduced by 26% in O_3/O_3 crosses vs. CFA/CFA crosses; percentage nonviable, fertilized ovules, and nonfertilized ovules was lowest in CFA/CFA crosses; fruit fresh weight—19% lower in O_3; fruit dry weight—18% lower in O_3; number of seeds per fruit—14% lower in O_3 (all significant reductions).
Tetteh et al. (2015)	OTC; Fuchu, Tokyo, Japan	*Vigna unguiculata* (cowpea); two cultivars Blackeye and Asontem	88 days of exposure, 5 h O_3 addition per day (11:00 a.m.−4:00 p.m.); Filtered: 24-h avg = 13 ppb (daily min/max = 1−55), AOT0 = 9.2 ppm-h, AOT40 = 0.2; Ambient: 24-h avg = 26 ppm (daily min/max = 1−110), AOT0 = 18.6 ppm-h, AOT40 = 2.7; Ambient +O_3: 24-h avg = 39 ppb (daily min/max = 1−175), AOT0 = 27.3 ppm-h, AOT40 = 10.4	Plant reproduction—pod length per plant: significant effect of O_3 exposure (filtered = 14.05 cm, ambient = 13.8, amb + O_3 = 11.9). Number seeds per pod: significant effect of O_3 exposure (filtered = 10 seeds/pod, ambient = 9, amb + O_3 = 7.5). Number pods per plant: no effect of O_3 exposure.

Table 8-9 (Continued): Ozone exposure and plant reproduction, phenology, and mortality.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Plant Reproduction and Mortality
Black et al. (2012)	Lab; unable to tell where growth chambers were located (potentially U.K.)	*Brassica campestris* (field mustard)	Control = charcoal-filtered air, O_3 below detection limit. Treatment = 100.4 ± 1.16 ppb O_3 for 6 h (10:00 a.m.−4:00 p.m.) on four consecutive days between 17−20 days after sowing	Plant reproduction—number reproductive sites—ozone had no significant effect on the number of reproductive sites or in the relative proportion of sites that were aborted vs. produced a pod. Exception was reproductive sites exposed as buds, where ozone exposure increased development into pods from 10.7 to 15.7%; pod length decreased significantly for those from flowers that bloomed on Days 3, 4, or that remained as buds during ozone exposure (down by 16, 29, 25% respectively); pod weight (minus seeds) not significantly affected, except for an increase in those from flowers that bloomed Day 2 of exposure; pod number not affected by ozone exposure; number of seeds per pod, number of seeds per plant decreased by 33% in ozone-exposed plants; number of aborted seeds, prematurely germinated seeds significantly higher (129, 851%) in ozone-exposed plants; individual seed weight 11% higher in ozone-exposed plants; seed weight per plant 23% lower in ozone-exposed plants. Plant phenology: seeds exposed to ozone germinated more quickly but at the same final percentage as control seeds.
Dietze and Moorcroft (2011)	Gradient; eastern and central U.S., bounded to west by 98°W longitude	10 plant functional types each characterized by different species	Range of 0.050−0.114 ppm 8-h max	Plant mortality—8 of 10 plant functional types had a significant correlation with O_3; evergreen hardwoods plant functional type is most sensitive to O_3 increase; overall, eastern and central forests are 9th most sensitive (in terms of tree mortality) to O_3 (out of 13 variables).
Moran and Kubiske (2013)	FACE; Aspen FACE, near Rhinelander, WI (45.7°N, 89.5°W).	Clones of five genotypes of *Populus tremuloides* from the aspen-only sections of the experiment, 1997−2008	Full factorial: O_3 and CO_2, 1998−2008. Ozone: ambient (W126 2.1–8.8 ppm-h) or elevated (W126 12.7–35.1 ppm-h). CO_2: ambient (360 ppm) or elevated (560 ppm); for hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	Plant mortality—survival of two genotypes (271 and 259, which had respectively the highest and lowest survival under ambient conditions) declined significantly under elevated O_3. Survival of genotype 8L increased 14.8% under elevated O_3.

Table 8-9 (Continued): Ozone exposure and plant reproduction, phenology, and mortality.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Plant Reproduction and Mortality
Diaz-De-Quijano et al. (2016)	Gradient; central Catalan Pyrenees northeastern Spain	*Pinus uncinata* (mountain pine) stands	Along an altitudinal gradient with an annual average of 35 ppb at 1,040 m asl to 56 ppb at 2,300 m asl (2004 to 2007 but reached 38 and 74 ppb from April to September)	Plant mortality—along two transects (Guils, Meranges) in the Pyrenees, mortality of *P. unicata* was positively correlated with accumulated O_3 exposure over a period of 5 yr. Tree mortality increased with altitude (1 to 30% on Guils transect, 1 to 7.5% on Meranges transect). Explanatory variables for these observations included mean fortnightly O_3 levels, water availability, and stand characteristics. Higher percentage tree mortality was observed above a threshold of sum of fortnightly O_3 levels of 2,900 ppb. Authors note that O_3 exposure not established as the main cause of tree mortality due to other environmental and anthropogenic stressors.
Hayes et al. (2012b)	Mesocosm; U.K.	*Briza media, Festuca ovina* (data not collected), *Campanula rotundifolia, Sanguisorba minor, Scabiosa columbaria, Helianthemum nummularium, Lotus corniculatus*	Eight treatments varying in seasonal 24-h mean ozone, but with the same weekly profile (4 days of +10 to +25 ppb peaks followed by 3 days of +5 ppb peaks); exposure of same plants in two seasons (2009 and 2010) over 12 weeks each season, but flowering measurements taken weekly only in Season 2; 2010 seasonal 24-h mean ozone levels were: 19.0, 25.5, 34.8, 40.8, 51.2, 60.3, 66.2, 73.3 ppb	Plant phenology—no effect of O_3 on timing of flowering for *B. media, S. minor*, or *S. columbaria*. Flowering sped up for *L. corniculatus* under increasing O_3 levels, especially for the well-watered plants—r^2 = 0.64 mean ozone vs. date to 20% maximum flower number (drought somewhat dampened the effect). Date of maximum flowering was also earlier—an increase in the mean ozone concentration from 30 to 70 ppb corresponded with maximum flowering occurring 6 days earlier in both the well-watered and drought treatments. Plant reproduction: No effect of O_3 on maximum number of flowers *for L. corniculatus, B media,* or *S. minor*; *C. rotundifolia* showed a significantly lower number of maximum flowers under higher O_3 (but the range was small 2−10 flowers); *S. columbaria* also showed a main effect of O_3 with lower maximum bud number under high O_3, but the range was small (4−7 buds).

Table 8-9 (Continued): Ozone exposure and plant reproduction, phenology, and mortality.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Plant Reproduction and Mortality
Hayes et al. (2011)	Greenhouse; near Marchlyn Mawr, U.K.	Two communities: four plants of forb *Leontodon hispidus* and three plants of grass *Dactylis glomerata*; four plants of forb *Leontodon hispidus* and three plants of *Anthoxanthum odoratum*	Eight treatments: (1) Seasonal 24-h mean = 21.4 ppb (12-h mean = 21.1 ppb, daylight [7:00 a.m.−6:00 p.m.] AOT40 = 0.07 ppm-h, 24-h AOT40 = 0.07 ppm-h); (2) Seasonal mean = 39.9 ppb (12-h = 39.2 ppb, daylight AOT40 = 4.93 ppm-h, 24-h AOT40 = 10.91 ppm-h); (3) Seasonal mean = 50.2 ppb (12-h = 49.6 ppb, daylight AOT40 = 21.44 ppm-h, 24-h AOT40 = 41.29 ppm-h); (4) Seasonal mean = 59.4 ppb (12-h = 58.7 ppb, daylight AOT40 = 38.04 ppm-h, 24-h AOT40 = 72.19 ppm-h); (5) Seasonal mean = 74.9 ppb (12-h = 73.3 ppb, daylight AOT40 = 62.49 ppm-h, 24-h AOT40 = 119.82 ppm-h); (6) Seasonal mean = 83.3 ppb (12-h = 81.6 ppb, daylight AOT40 = 77.13 ppm-h, 24-h AOT40 = 147.42 ppm-h); (7) Seasonal mean = 101.3 ppb (12-h = 99.0 ppb, daylight AOT40 = 108.43 ppm-h, 24-h AOT40 = 206.70 ppm-h); (8) Seasonal mean = 102.5 ppb (12-h = 100.5, daylight AOT40 = 112.47 ppm-h, 24-h AOT40 = 214.34 ppm-h)	Plant phenology—there was no effect on timing of flowering or number of flowers during O_3 exposure or 2 mo after O_3 exposure. 2 mo after O_3 exposure ended, the proportion of living mature leaves on *L. hispidus* increased linearly with seasonal mean O_3 concentration. In the next growing season, there was no effect of the previous season's O_3 exposure on the number of flowers or seeds for *L. hispidus* or *D. glomerata*. The ratio of *L. hispidus* flowers to seed-heads in the second season decreased linearly with increasing first season mean O_3 concentration.

Table 8-9 (Continued): Ozone exposure and plant reproduction, phenology, and mortality.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Plant Reproduction and Mortality
Sanz et al. (2016)	OTC; experimental field located in a rural area in the northeastern Iberian Peninsula, Tarragona (40.41°N, 0.47°E)	Dehasa-type pasture species, Leguminoseae (three species)	Data analyzed from independent experiments, 45 day avg O_3 exposure length	Plant reproduction—an O_3 critical level for reproductive capacity AOT40 = 2.0 (1.5, 2.8) ppm-h and Phytotoxic Ozone Dose (POD)1 = 7.2 (1.1, 13.3) mmol/m^2 was developed from linear exposure response functions based on seed and flower production. Reproductive capacity had the lowest critical level of the endpoints evaluated.
Gundel et al. (2015)	OTC; Buenos Aires, Argentina	*Lolium multiflorum*	Low ozone = <10 ppb; High ozone = ~120 ppm for 2 h/day noon−2:00 p.m. for 5 consecutive days preanthesis	Plant reproduction—trend towards O_3 increasing seed viability under high temperature and humidity was not significant.
Sanz et al. (2011)	OTC; Mediterranean coast, Spain, (40.68°N, 0.78°E)	*Briza maxima*	O_3 as AOT40 index—ozone: charcoal-filtered (mean O_3 <10 ppb, AOT40 = 0); Ambient (mean O_3 <40 ppb, AOT40 = 1,367 ppb-h); Addition of 40 ppb O_3 from 7:00 a.m. to 5:00 p.m. for 5 days/week (mean O_3 = 40−65 ppb, AOT40 = 10,841 ppb-h) NH_4NO_3 addition to mimic 10, 30, or 60 kg N/ha	Plant phenology—while O_3 exposure ran, there was no significant effect of O_3 on phenology. 30 days after O_3 exposure ended, phenology differed by O_3 treatment: the number of plants starting to flower were 66% lower in added O_3 than in charcoal-filtered air, as the number of plants with mature seeds were 280−340% higher in the ambient and added O_3 treatments respectively, than in the filtered treatment. Nitrogen had no effects on phenology.

Table 8-9 (Continued): Ozone exposure and plant reproduction, phenology, and mortality.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Plant Reproduction and Mortality
Leisner and Ainsworth (2012)	Other; all locations included (but no summary of geographic distribution of studies included)	All species included (data set includes monocots and dicots, perennials and annuals, determinate and indeterminate growth habits, C3 and C4 plants)	Grouped and analyzed exposures in several ways: (1) Charcoal-filtered air vs. elevated O_3—elevated O_3 in multiple categories (4-, 7-, 8-, or 12-h daytime means depending on data reported): >100 ppb, 70−100 ppb, 40−70 ppb, <40 ppb; (2) Ambient air vs. elevated O_3—elevated O_3 in multiple categories (4-, 7-, 8-, or 12- h daytime means depending on data reported): >100 ppb, 70−100 ppb, 40−70 ppb, <40 ppb	Plant reproduction—(1) compared with charcoal-filtered air, most measurements of plant reproduction were reduced under elevated O_3 (but not flower measurements); for example, seed number, fruit number, and yield decreased by 16, 9, and 19%, respectively, all at slightly different average exposure levels. Some endpoints significantly decrease even under the lowest exposure category (<40 ppm). Some trends in larger negative responses under higher exposure levels, but overall there was no clear exposure-response across experiments and species. Yield was not significantly affected below 70 ppb, but decreased 45% at highest exposure level. (2) Compared with ambient air, all measurements of plant reproduction were reduced under elevated O_3—for example, yield, fruit weight, and seed number decreased by 25, 51% (the largest effect observed), and ~10%, respectively. Effects occurred even at the lowest exposure level (<40 ppb). There was a clear exposure-response with respect to yield and a clear trend for fruit number and fruit weight. Yield was down 52% at the highest exposure level. The response to O_3 by different types of plants was not significantly different in many cases. One exception was that indeterminate plants had much greater reductions in fruit weight and fruit number than did determinate plants.
Wang et al. (2015)	Global meta-analysis	98 herbaceous species tested for CO_2 effects on reproductive allocation in papers previously published in 1977−2011	Not specified	Without specific stressors, there is no effect of elevated CO_2 on plant allocation to reproductive biomass (n = 508). With ozone exposure, plant allocation to reproduction is 4% lower at elevated CO_2 (+CO_2+O_3) than at ambient CO_2 (+O_3).

Table 8-9 (Continued): Ozone exposure and plant reproduction, phenology, and mortality.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Plant Reproduction and Mortality
Ferreira et al. (2016)	Lab; Porto, Portugal	*Plantago lanceolata, Salix atrocinerea*	All exposures for 6 h; *Plantago*—control = 0 ppm; Treatment level + (approx. equal to European standard for health) = 0.065 ppm; Treatment level ++ = 0.124 ppm; *Salix*—control = 0 ppm; treatment level + (approx. equal to European standard for health) = 0.061 ppm; treatment level ++ = 0.118 ppm	Plant reproduction—*Plantago* and *Salix* pollen viability significantly declined with increasing O_3 exposure (e.g., ~56 and 23% lower, respectively, in ++O_3 treatment compared to control); Pollen germination was only significantly reduced at ++O_3 treatment; *Salix* pollen germination was significantly reduced at + and ++O_3 treatment, but O_3 treatments did not differ.
Abeli et al. (2017)	Lab; Alpine seeds collected on Mt. Cimone, Mt. Prado-Cusna and in the Dolomites in Italy; O_3 exposure inside incubators	*Achillea clavennae*, *Aster alpinus*, *Festuca rubra* subsp. *commutata*, *Festuca violacea* subsp. *puccinellii*, *Plantago alpina*, *Silene acaulis, Silene nutans*, *Silene suecica, Vaccinium myrtillus*	Control: Ambient air (0−1 ppb) “125_5” treatment: 125 ppb O_3 24 h/day for 5 days; “125_10” treatment: 125 ppb O_3 24 h/day for 10 days; “185_5” treatment: 185 ppb O_3 24 h/day for 5 days	Plant reproduction—significant differences in seed mortality for some species between all four germination conditions, but not in a consistent way. Combining all species, each treatment (compared with control) significantly delayed germination (125_5 = 0.71, 185_5 = 0.87, 125_10 = 1.17 day delay). Six of nine individual species had reduction in germination percentage for one or more of O_3 treatment at the end of O_3 exposure. Seven of nine species showed a significant effect of at least one O_3 treatment at 28 days after sowing, and effects ranged from increasing to decreasing germination percentage. Plant phenology—combining all species, 125_5 and 185_5 treatments did not affect mean germination time either at end of O_3 exposure or at end of the experiment. The 125_10 treatment significantly increased mean germination time by 1.25 days after O_3 exposure, but by the end of the experiment that difference did not exist. Individual species responded in different ways to treatments.

Table 8-9 (Continued): Ozone exposure and plant reproduction, phenology, and mortality.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Plant Reproduction and Mortality
Yang et al. (2017)	OTC; Zhangtou, Changping District, Beijing, China (40.20°N, 116.13°E)	*Tagetes erecta* (marigold) and four varieties of *Petunia* hybrid with pink, red, rose-red, or white flowers	Ozone exposure during growth period of marigold: Ambient average 37.1 ppb O_3 (AOT40 = 1.6 ppm-h); elevated average 99.2 ppb (AOT40 = 20.4 ppm-h); highly elevated average 145.2 ppb (AOT40 = 36.4 ppm-h); Ozone exposure during growth period of petunia: Ambient average 40.0 ppb O_3 (AOT40 = 4.0 ppm-h); elevated average 96.0 ppb (AOT40 = 25.0 ppm-h); highly elevated average 153.3 ppb (AOT40 = 47.6 ppm-h)	Plant reproduction—elevated O_3 (96.0 ppb) reduced flower diameter 7% and flower biomass 44% in white petunias, and reduced flower biomass 25% for pink petunias. Highly elevated O_3 (153.3 ppb) reduced flower diameter 7% in white petunias, 11% in rose petunias, 9% in red petunias, and 12% in pink petunias, and reduced floral biomass across all petunia varieties 20−40%. There were no effects of O_3 on marigold flower biomass or flower diameter.

AOT0 = seasonal sum of the difference between an hourly concentration at the threshold value of 0 ppb, minus the threshold value of 0 ppb; AOT40 = seasonal sum of the difference between an hourly concentration at the threshold value of 40 ppb, minus the threshold value of 40 ppb; asl = above sea level; C3 = plants that use only the Calvin cycle for fixing the carbon dioxide from the air; C4 = plants that use the Hatch-Slack cycle for fixing the carbon dioxide from the air; CO_2 = carbon dioxide; FACE = free-air CO_2 enrichment; kg N/ha = kilograms of nitrogen/hectare; n = sample size; NH_4NO_3 = ammonium/nitrate solution; nmol/m^2 = nanomole/meters squared; nmol/mol = nanomoles/mole; O_3 = ozone; OTC = open-top chamber; ppb = parts per billion; ppm = parts per million; SUM60 = sum of hourly ozone concentrations equal to or greater than 60 ppb; W126 = cumulative integrated exposure index with a sigmoidal weighting function.

8.5 Reduced Crop Yield and Quality

In the 2013 Ozone ISA, the evidence was sufficient to conclude there is a causal relationship between ozone exposure and reduced yield and quality of agricultural crops (U.S. EPA, 2013). The detrimental effect of ozone on crop production has been recognized since the 1960s, and a large body of research has subsequently characterized decreases in yield and quality of a variety of agricultural and forage crops. Ozone effects on cellular processes, plant metabolism, altered C allocation to vegetation and roots, and leaf-level physiology (Section 8.1.3 and Figure 8-2) underlie agricultural crop damage, which is measured as reduced crop yield and quality. The actual concentration and duration threshold for ozone damage varies from species to species, and sometimes even among genotypes of the same species Section 9.4.4.1 (U.S. EPA, 2013). Numerous experimental analyses have also demonstrated that the effects of ozone exposure vary depending on the growth stage of the plant. In studies reviewed in the 2013 Ozone ISA, increasing ozone concentration decreased nutritive quality of grasses, decreased macronutrient and micronutrient concentrations in fruits and vegetable crops, and decreased cotton fiber quality Section 9.4.4.2 (U.S. EPA, 2013).

As described in the PECOS tool (Table 8-2), the scope for new evidence reviewed in this section limits studies to those conducted in North America at ozone concentrations occurring in the environment or experimental ozone concentrations within an order of magnitude of recent concentrations (as described in Appendix 1). If data from other countries were included in meta-analyses with U.S. data (or incorporated into exposure-response functions for crops), these studies were also considered.

8.5.1 Field Studies and Meta-Analyses

Greenhouse, OTC, FACE, field, and gradient studies reviewed in the 2013 Ozone ISA clearly show negative impacts of ozone on crop yield at concentrations relevant to the then current conditions (U.S. EPA, 2013). In the 2013 Ozone ISA, the results of several meta analytic studies for soybean (Betzelberger et al., 2010; Morgan et al., 2006; Morgan et al., 2003), wheat (Feng et al., 2008), rice (Ainsworth, 2008), and potato, bean, and barley (Feng and Kobayashi, 2009) provided evidence that current levels of ozone decrease crop growth and yield. New field studies and meta-analyses of U.S. crop data and global analyses that include U.S. crop data further characterize effects on crop species, improve estimates of yield loss, and refine concentration response (Table 8-10).

- Ozone's effects on reproductive and developmental stages of the plant life cycle in a variety of crop and noncrop species were evaluated in a meta-analysis by Leisner and Ainsworth (2012). Grain or seed yield per unit area was decreased by 19% at an average ozone concentration of 60 ppb in ambient air compared with charcoal-filtered air (Figure 8-3). Compared with ambient air, yield decreased by 25%. Seed and fruit number were also frequently affected by elevated

ozone levels (Figure 8-4). Additional reproductive and developmental traits in the meta-analysis affected by ozone exposure discussed in Section 8.4 and Table 8-9 have relevance for crop yield.

- For soybean, additional studies at SoyFACE in Illinois report decreased seed/crop yield (Leisner et al., 2017; Ainsworth et al., 2014; Vanloocke et al., 2012) as well as timing of ozone damage and canopy senescence (Sun et al., 2014). Betzelberger et al. (2012) refined a exposure-response curve for soybean that previously relied on single concentrations over multiple years. A linear decrease in yield was observed across two growing seasons at SoyFACE at the rate of 37 to 39 kg per hectare per ppb cumulative ozone exposure over 40 ppb (AOT40); summed from the beginning of the growing season up to the date of measurement. All seven cultivars showed similar responses to ozone. Osborne et al. (2016) updated the exposure-response function for soybean by pooling relative yield data from 28 experimental studies after 1998 from the U.S., China, and India. This analysis identified a critical level of 32.3 ppb (7-hour seasonal mean) at which a statistically significant (5%) loss of yield can occur. Soybean cultivars varied in sensitivity to ozone with a yield loss at a 7-hour mean concentration of 55 ppb (used in the study to represent present day background levels) ranging from 13.3 to 37.9%.

- For wheat, meta-analyses using data from the U.S. and other countries provide further supporting evidence that current levels of ambient ozone decrease growth, quality, and yield (Pleijel et al., 2018; Broberg et al., 2015). A meta-analysis of ozone's effects on wheat grain quality based on 42 studies (OTC and FACE conducted in the U.S. Europe and Asia) indicated that ozone significantly and strongly reduced 1,000-grain weight. Volume weight and starch content were also significantly lower with higher ozone exposure (Broberg et al., 2015). Ozone OTC experiments with field-grown wheat from 33 studies in 9 countries, including the U.S., showed an average wheat yield loss per ppb ozone of 0.38% (Pleijel et al., 2018). Grain yield, grain mass, total aboveground biomass, starch concentration, starch yield, and protein yield were significantly decreased in nonfiltered air compared with charcoal-filtered air, with starch yield being the most strongly affected.

- New studies in nonsoybean legumes include evaluation of biomass and seed yield in ozone-exposed snap bean (*P. vulgaris*) under high and low vapor pressure deficit conditions (Fiscus et al., 2012). In elevated ozone treatments at high humidity (low vapor pressure deficit = VPD), snap bean yield was decreased by 55−72% with no significant yield loss at high VPD (Fiscus et al., 2012). Both mass per seed and number of seeds per plant were reduced. Lloyd et al. (2018) assessed the effect of nighttime ozone exposure on yield of snap bean. Nighttime ozone exposure alone, at 62 ppb, had no effect on yield. In combination with daytime ozone exposure, nighttime ozone concentrations up to 78 ppb did not affect yields or show a consistent effect on nocturnal stomatal conductance. When data were pooled across the day and day + night exposure times, mean daytime ozone levels ≥62 ppb caused significant yield losses. Burkey et al. (2012) considered the use of pod weight and seed weight per plant of a sensitive snap bean genotype as a bioindicator of ozone pollution. Under elevated ozone, the sensitive genotype showed a 63% decline in pod weight per plant and a similar decline for seed weight per plant. No significant differences were observed for tolerant genotypes under elevated ozone.

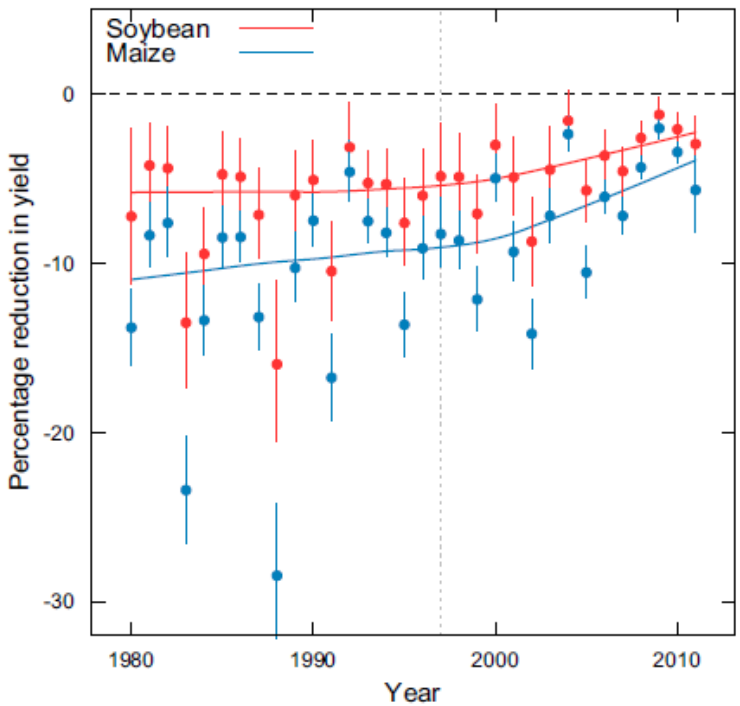
- A few recent studies conducted on U.S. southern piedmont grassland species have added to the evidence base for ozone effects on nutritive quality of forage (Gilliland et al., 2016; Gilliland et al., 2012).

- A study by Grantz and Vu (2009) reviewed in the 2013 Ozone ISA showed that a hybrid of sugarcane (*Saccharum* sp.) exhibited high sensitivity to ozone. However, in a follow-up comparative study of five hybrids of sugarcane, Grantz et al. (2012) found a wide range of sensitivities.

8.5.2 Yield Loss at Regional and National Scales

Global and U.S. modeling studies in the 2013 Ozone ISA found that ozone generally reduced crop yield and that different crops showed different sensitivity to ozone (Avnery et al., 2011; Van Dingenen et al., 2009; Tong et al., 2007). Newly available regional- and national-scale analyses of ozone's effects on major crops in the U.S., including soybean, wheat (*Triticum* sp.), and maize (*Zea mays*), have enabled further characterization and quantification of yield losses. These advances include estimates of yield loss based on field data, additional geographic refinement of crop ozone sensitivity, and for wheat and soybean, analyses of state-by-state sources and contribution of ozone precursors affecting crop loss.

- Regression analysis of historical ambient ozone concentrations (W126 calculated from hourly ozone data from U.S. EPA monitors), climate, and yield data for maize and soybean (rainfed only, irrigated fields excluded from analysis) in the U.S. from 1980 to 2011 was used to estimate yield losses (Mcgrath et al., 2015). Yield losses in the field averaged over the time period were approximately 10% for maize and 5% for soybean. The authors attribute a temporal improvement in crop loss to the more stringent ozone air quality standards implemented in 1997 (Figure 8-5). An unexpected observation from this analysis was that production losses for maize, a C4 plant thought to be less sensitive to ozone, were greater than for soybean, a C3 plant.



Note: Each point is a weighted mean of percentage reduction for all counties, where the value of a county was weighted by the harvested acreage of soybean or maize in that county. Percentage reduction was estimated by using a linear model incorporating climatic variables and ozone cumulative indices to predict yield using historical values of W126 or a value of 0 W126. The lines are a local regression analysis fit to the points. The black, dashed, horizontal line marks 0 change for reference. The gray, vertical, dotted line indicates when the U.S. EPA implemented more stringent standards for ozone emissions. Bars are 95% confidence intervals of yield reduction for that year.

Source: Permission pending, Mcgrath et al. (2015).

Figure 8-5 Estimated percentage reduction of soybean and maize yield in the U.S. from ozone for 1980−2011.

- For wheat and soybean, ozone exposure-response relationships of yield reductions were scaled up to the continental U.S. to put these losses in context. Relative yield losses were estimated to be 4.9% for wheat and 6.7% for soybean based on 2010 data using the GEOS-Chem model (Lapina et al., 2016). State-by-state percentage influence maps were generated for ozone damage. On a regional basis, the highest relative losses for wheat (12%) and soybean (25%) were in the eastern U.S. Kansas produces the most wheat but also experiences the greatest percentage of wheat loss due to ozone. The majority of NO_X emissions responsible for ozone-related wheat loss originate in Texas. For soybean, the highest loss occurs in Illinois which is most affected by NO_X emissions from Missouri. Twenty-seven percent of current soybean losses are attributed to combined NO_X emissions from Illinois, Missouri, and Indiana.
- Tai and Martin (2017) developed an empirical model (partial derivative linear regression [PDLR] model) from multidecadal data sets to estimate geographical variations across the U.S. in sensitivity to ozone of wheat, maize, and soybean. This approach takes into consideration strong ozone-temperature covariation and does not rely on pooled concentration-response functions. For all three crops, the revised sensitivities (calculated in latitude-longitude grid cells to account for

regional differences in temperature, water, and nutrient availability) are generally higher than previously indicated by concentration-response functions derived from experimental studies. Wheat yield sensitivities to ozone were statistically significant spatially along the northern U.S. border, maize sensitivity was spatially statistically significant at various locations across the U.S., and soybean sensitivity was spatially statistically significant in a band from the Great Plains to the south-central U.S. Crops in regions of elevated ozone and high water use, were more tolerant to ozone. The PDLR model coupled with ozone and temperature projections by the Community Earth System model from 2000−2050 have predicted average declines of U.S. wheat, maize, and soybean of 13, 43, and 28%, respectively.

- A modeling study considering the cobenefits associated with decreases of NO_X under the U.S. EPA Clean Power Plan (to regulate emissions of CO_2) estimated the effects on total production and biomass loss of four U.S. crops (potatoes, soybean, cotton, maize) under three policy scenarios and a reference (ambient air) scenario for the year 2020 (Capps et al., 2016). In this analysis, the CMAQ model was used to model exposure values of W126 and then apply these to crop distribution maps using published data to estimate biomass loss and potential productivity loss (PPL). At ambient ozone concentrations, modeled production loss is greatest for potatoes, soybean, and cotton, with these losses ranging from 1.5 to 1.9%. Scenario 1 (which is closest to current levels) results in an ozone impact reduction of <2% for each crop. Reductions in PPL of 8.4% (soybean) and 6.7% (cotton) in Scenario 2 (which is most similar to the final Clean Power Plan) and 6.6 and 3.8% in Scenario 3 (most stringent policy option) suggest that reduction in NO_X with CO_2 regulation will decrease agricultural yield losses associated with ozone.

8.5.3 Summary

The relationship between ozone exposure and reduced crop yield is well established in the scientific literature and continues to be an area of active research with hundreds of papers on this topic published since the 2013 Ozone ISA in the U.S. and other countries (U.S. EPA, 2009). There is a considerable amount of new research on major U.S. crops, especially soybean, wheat, and non-soybean legumes, including updated soybean exposure-response curves. Meta-analyses published since the 2013 Ozone ISA provide further supporting evidence that ozone decreases growth and yield of wheat and affects reproductive and developmental plant traits important to crop yield. Recent advances in characterizing ozone's effects on U.S. crop yield include further geographic and temporal refinement of ozone sensitivity and national-scale estimates of maize and soybean losses from ozone based on actual yield data. A few studies on grassland species add to the existing body of evidence in the 2013 Ozone ISA for ozone effects on nutritive quality. New information is consistent with the conclusions of the 2013 Ozone ISA that **the body of evidence is sufficient to infer a causal relationship between ozone exposure and reduced yield and quality of agricultural crops.**

Table 8-10 Ozone and crop yield and quality.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Crop Yield
Burkey et al. (2012)	FACE; SoyFACE, Champaign, IL (40.033°N, 88.233°W)	*Phaseolus vulgaris* (snap bean) Three genotypes S156—O_3 sensitive; R123, R331—O_3 tolerant	O_3 exposure from May−August 2006 Ambient (control)—8-h mean = 43 ppb 1-h max = 29−78 ppb AOT40 = 5.3 ppm-h SUM60 = 5.3 ppm-h; +O_3—8-h mean = 59 ppb 1-h max = 32−114 ppb AOT40 = 16.3 ppm-h SUM60 = 27 ppm-h; +O_3+CO_2—8-h mean = 59 ppb 1-h max = 33−112 ppb AOT40 = 16.2 ppm-h SUM60 = 26.7 ppm-h	Sensitive genotype declined 63% in pod weight per plant with similar result for seed weight per plant under elevated O_3 compared with control. No significant difference for tolerant genotypes under elevated O_3 compared with control.
Betzelberger et al. (2012)	FACE; SoyFACE, Champaign, IL (40.033°N, 88.233°W)	*Glycine max* (soybean) Seven cultivars	Eight 20-m-diameter SoyFACE plots with different O_3 concentrations were exposed ~8 h/day in two growing seasons (2009, 2010). Target concentrations were ambient, 40, 55, 70, 85, 110, 130, 160, 200 ppb in 2009, and ambient, 55, 70, 85, 110, 130, 150, 170, 190 ppb in 2010	An exposure-response for soybean was refined from previous estimates using target concentrations from ambient to 200 ppb/8 h. As ozone increased, a linear decrease in yield was observed at the rate of 37 to 39 kg/ha per ppb cumulative exposure >40 ppb. All seven cultivars showed similar responses to O_3 with the range of responses between 18 to 30 kg ha per ppb cumulative exposure >40 ppb. At the highest target concentration of 200 ppb (AOT40 of 67.4 ppm-h) yields declined 64%.
Grantz et al. (2012)	Other; California (36.6°N, 119.5°W)	*Saccharum* sp. (sugarcane) Four hybrids	Ozone exposure conditions in the continuously stirred tank reactors were the same each day, with nominal 12-h mean exposures of 4, 59, and 114 ppb and 8-h mean exposures of 3, 76, and 147 ppb. Leaf-level responses were measured following exposure to ozone for 7 weeks, then plants were excised and allowed to regrow before exposing shoots to ozone again for another 7 weeks.	The four hybrid clones exhibited a wide range of sensitivity to O_3 measured by net carbon assimilation. Hybrids containing a greater percentage of *Saccharum spontaneum* were less sensitive to ozone.

Table 8-10 (Continued): Ozone and crop yield and quality.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Crop Yield
Ainsworth et al. (2014)	FACE; SoyFACE, Champaign, IL (40.033°N, 88.233°W)	*Glycine max* (soybean) 11 genotypes	Eight ambient and eight +O_3 20-m-diameter plots exposed 8 h daily for the growing season. Ambient 8-h ozone concentration was 44 ppb, and for FACE plots ranged from 79 to 82 ppb.	Exposure to elevated O_3 resulted in approximately 30% avg decrease in seed yield.
Fiscus et al. (2012)	Other; USDA-ARS Plant Science Research Unit, 5 km south of Raleigh, NC	*Phaseolus vulgaris* (snap bean) Two genotypes S156—O_3 sensitive; R123—O_3 tolerant	Two ozone concentrations in charcoal-filtered air (12-h mean of 0 and 60 ppb) dispensed into outdoor plant environment chambers. Exposure started 18 days after planting at 1/3 of target concentrations and were gradually increased to reach full exposure levels at 21 days after planting. Experiment was 62 days in duration. For +O_3 concentration 12-h mean target resulted in daily AOT40 = 245, SUM06 = 534, W126 = 295 ppb-h. Two vapor pressure deficit levels tested (1.26 and 1.96 kPa).	In elevated O_3 treatments at high humidity (low vapor pressure deficit), yield was decreased by 55−72% with no significant yield loss under low humidity. Both mass per seed and number of seeds per plant were reduced. There was a difference in sensitivity in the two genotypes.
Broberg et al. (2015)	Other: OTC, FACE in Europe, Asia, and U.S.	*Triticum* sp. (wheat)	Elevated O_3 was at least 30 ppb, but no more than 100 ppb daily.	Meta-analysis of 42 studies showed O_3 significantly reduces 1,000-grain weight (strongly), volume weight, and starch concentration of wheat.
Osborne et al. (2016)	Other: OTC or FACE in U.S., Asia, and China 1982−2014	*Glycine max* (soybean) 48 cultivars	Ozone exposure data were all converted to seasonal 7-h mean from studies that reported concentration as 8-, 12-, or 24-h mean or 3-mo AOT40. Duration of O_3 exposure was at least 60% of growing season.	A exposure-response function was calculated by pooling relative yield data and plotting against the 7-h seasonal mean (M7) for 28 experimental studies. All data were scaled to theoretical yield at 0 ppb. 55 ppb was used to represent current background. Relative yield reduction at current background was 17.3%. A critical level at which statistically significant (5%) loss of yield can occur is 32.3 ppb M7. Cultivars varied in sensitivity to O_3 with a yield loss of 13.3 to 37.9% at 55 ppb M7. Sensitivity to O_3 increased by an average of 32.5% between 1960 and 2000.

Table 8-10 (Continued): Ozone and crop yield and quality.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Crop Yield
Mcgrath et al. (2015)	Other; county-level data from the U.S. department of Agriculture National Agricultural Statistics Service from 1980 to 2011	*Glycine max* (soybean), Zea *mays* (maize)	Regression analysis and statistical model of county-level data of maize and soybean production, and hourly O_3 data from the U.S. EPA AQS. Hourly O_3 at 2,700 sites from 1980 to 2011 obtained from U.S. EPA AQS, AOT40, SUM06, and W126 were calculated. Indices were summed over the growing season (J, J, A); only W126 data was reported because it was the most linear.	Average yield loss from 1980 to 2011 in rainfed fields was 9.8% for maize and 5.5% for soybean. Percentage losses of crop yield showed a temporal improvement in crop loss corresponding to implementation of O_3 standards in 1997. Current loss of production is estimated at 4−7%.
Vanloocke et al. (2012)	FACE; SoyFACE, Champaign, IL (40.04°N; 88.24°W)	*Glycine max* (soybean)	12-h mean O_3 in the experimental plots of 40, 46, 54, 58, 71, 88, 94, 116 ppb.	With increasing O_3, harvested seed yield decreased linearly; 64% reduction in yield at highest O_3 treatment compared with lowest.
Tai and Martin (2017)	Other; multidecadal U.S. crop yield and climate data to estimate geographical variation across the U.S.	*Glycine max* (soybean), *Triticum* (wheat), *Zea mays* (maize)	Three cumulative O_3 annual exposure metrics, AOT40, SUM-06, and W126, calculated from hourly ozone observations from the AQS and CASTNET networks averaged over 1993−2010.	An empirical (partial derivative linear regression) model incorporating the strong ozone-temperature covariation was used to calculate crop sensitivity to O_3. For all three crops, modeled sensitivities (calculated in latitude-longitude grid cells to account for regional differences in temperature, water, and nutrient availability) are generally higher than previously indicated by concentration-response functions derived from experimental studies.
Leisner and Ainsworth (2012)	Other; all locations included (but no summary of geographic distribution of studies included)	All species included (data set includes monocots and dicots, perennials and annuals, determinate and indeterminate growth habits, C3 and C4 plants)	Grouped/analyzed exposures in several ways: (1) Charcoal-filtered air vs. elevated O_3—elevated O_3 in multiple categories: >100 ppb, 70−100 ppb, 40−70 ppb, <40 ppb (2) Ambient air vs. elevated O_3—elevated O_3 in multiple categories: >100 ppb, 70−100 ppb, 40−70 ppb, <40 ppb	Grain or seed yield per unit area declined 19% at average O_3 concentration of 60 ppb compared with charcoal-filtered air. Compared with ambient air, yield decreased by 25% at an average O_3 concentration of 79 ppb.

Table 8-10 (Continued): Ozone and crop yield and quality.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Crop Yield
Leisner et al. (2017)	FACE; SoyFACE, Champaign, IL (40.04°N, 88.24°W)	*Glycine max* (soybean)	Elevated O_3 fumigation system increased O_3 to 100 ppb from 10:00 a.m. to 5:00 p.m. except when leaves were wet. Season-long 8-h avg ambient O_3 was 50.6 ppb, and the 8-h season-long elevated O_3 was 69.7 ± 1.3 ppb.	Number of seed pods per node was significantly reduced in O_3 treated soybeans. Average 3.4 pods per node (at 50.6 ppb ambient) decreasing to 2.8 pods per node in the elevated O_3 treatment (69.7 ppb).
Lloyd et al. (2018)	Greenhouse; Pennsylvania State University (40.80°N, 77.85°W)	*Phaseolus vulgaris* (snap bean) Two genotypes S156—O_3 sensitive; R123—O_3 tolerant	O_3 treatments were a combination of O_3 concentration and treatment time as follows: (1) 45 ppb O_3 day-only, (2) 75 ppb O_3 day-only, (3) 45 ppb O_3 day + night, (4) 75 ppb O_3 day + night, (5) 30 ppb night-only, (6) 60 ppb night-only.	Nighttime O_3 exposure alone, at 62 ppb, had no effect on the yield of either genotype. In combination with daytime O_3 exposure, nighttime concentrations up to 78 ppb did not impact yields. When data were pooled across the day and day + night exposures times, mean daytime O_3 levels (62 ppb) caused significant yield decreases. Under control conditions, R123 and S156 produced similar pod masses in two of the three trials. In all three trials, R123 produced significantly greater yields by mass than S156 with elevated O_3.
Sun et al. (2014)	FACE; SoyFACE, Champaign, IL (40.04°N, 88.24°W)	*Glycine max* (soybean) Two cultivars Dwight and IA3010	Nine plots fumigated with various O_3 concentrations from early vegetative stage to maturity. Daily 9-h avg concentrations over the 105 days were 37 (ambient), 40, 46, 54, 58, 71, 89, 95, and 116 ppb.	O_3 caused greater damage at later reproductive stages and in older leaves. Soybeans grown under O_3 levels of 116 ppb were senescent 1 week earlier than plants grown under ambient control (37 ppb). Average decrease of photosynthesis, total nonstructural carbohydrate levels, and many metabolites and amino acids (correlated to seed yield) was 7% for a 10-ppb increase in O_3. Loss of seed yield mainly due to loss of photosynthetic capacity and canopy senescence resulting in shorter growing season.
Gilliland et al. (2012)	OTC; Auburn University, Auburn, AL	*Lolium arundinacae* (tall fescue), *Paspalum dilatatum* (dallisgrass), *Cynodon dactylon* (common Bermuda grass), *Trifolium repens* (white clover)	Grasses in six OTC chambers (three chambers per treatment) exposed for 8 weeks. Mean monthly 12-h ambient NF was 21−32 ppb (average peak 49 ppb). Mean monthly 2× ambient was 37 to 56 ppb (average peak 102 ppb). Rabbits were fed (in the form of 50 g dried forage blocks) a mixture of the four plant species grown in OTCs at either ambient or 2× ambient O_3.	Neutral detergent fiber and acid detergent fiber digestibility was significantly lower for grasses grown under elevated O_3.

Table 8-10 (Continued): Ozone and crop yield and quality.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Crop Yield
Gilliland et al. (2016)	OTC; 5 km north of Auburn University Auburn, AL	*Lolium arundinacea* (tall fescue), *Paspalum dilatatum* (dallisgrass), *Cynodon dactylon* (common Bermuda grass), *Trifolium repens* (white clover)	12 OTC chambers (6 chambers ambient, 6 chambers 2× ambient, then treated to 3 levels of precipitation). Grasses were exposed for 4 mo with the mean 12-h O_3 concentration of 31 ppb (NF) and 56 ppb (2× ambient). Average peak O_3 = 39 (NF) and 77 ppb (2×). Peak average 1-h O_3 = 73 (NF) and 155 ppb (2×).	Three grass species pooled into one "grass" sample. Under elevated O_3, primary growth of grasses (dry matter yield) increased 19% compared with ambient, while clover decreased in nutritive quality (increase in acid detergent lignin). In regrowth, clover in 2× O_3 had a 60% decrease in DM yield, while grasses had lower concentrations of neutral detergent fiber, higher relative food value, and increased crude protein. Clover was sensitive to O_3 with decreased nutritive quality and higher response in regrowth harvests.
Lapina et al. (2016)	Other; continental U.S.	*Glycine max* (soybean), *Triticum* (wheat)	Exposure response functions for W126, AOT40, and mean metric for total O_3 exposure were used to model relative loss estimates. The GEOS-Chem adjoint model was applied to estimate source-receptor relationships between crop yield reduction and emission sources.	Analysis of year 2010 O_3 losses in wheat, soybean, and two tree species showed sources of O_3 in the U.S. and how individual states' emissions contribute to O_3 damage; the study suggests that most vegetation damage is attributable to local, not international sources. U.S. anthropogenic NO_X contributions were the highest total contributors (75−77%). State-by-state maps provide information on sources associated with vegetative damage. Relative yield loss: wheat 4.9% and soybean 6.7%.
Capps et al. (2016)	Other; U.S.	*Zea mays* (maize), *Gossypium* (cotton), *Solanum tuberosum* (potato), *Glycine max* (soybean)	Uses U.S. EPA-developed CMAQ model to model exposure values of W126 under three regulatory scenarios as well as a reference (ambient) over CONUS. Three CO_2-reduction scenarios were modeled. Scenario 1—closest to current levels, Scenario 2—most similar to the final Clean Power Plan, and Scenario 3—most stringent policy option.	At ambient O_3 concentrations, modeled production loss is greatest for potatoes, soybean, and cotton, with these losses ranging from 1.5 to 1.9%. Although potatoes show greatest impacts currently, the CO_2 mitigation strategies improve yields the least. These small changes are attributable to much of the potato potential productivity loss (PPL) arising from high W126 in southern California, which is not substantially altered in the policy scenarios. The PPLs for soybeans and cotton are reduced substantially in Scenario 2 and Scenario 3, resulting in 8.4% (6.6%) and 6.7% (3.8%) PPL reductions, respectively, for each crop. Scenario 1 reduces the O_3 impact by <2% for each crop.

Table 8-10 (Continued): Ozone and crop yield and quality.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Crop Yield
Pleijel et al. (2018)	Other; nine countries	*Triticum* sp. (wheat)	CF and NF in OTC treatments with daytime O_3 concentration converted to 7-h mean.	Average yield loss per ppb ozone of 0.38% across 33 studies. Grain yield, grain mass, total aboveground biomass, starch concentration, starch yield, and protein yield significantly declined in nonfiltered air compared with charcoal-filtered air in these studies, with starch yield (10.9%) being the most strongly affected.

AQS = (U.S. EPA) Air Quality System (database); AOT40 = seasonal sum of the difference between an hourly concentration at the threshold value of 40 ppb, minus the threshold value of 40 ppb; C3 = plants that use only the Calvin cycle for fixing the carbon dioxide from the air; C4 = plants that use the Hatch-Slack cycle for fixing the carbon dioxide from the air; CASTNET = Clean Air Status and Trends Network; CF = charcoal-filtered air; CO_2 = carbon dioxide; FACE = free-air CO_2 enrichment; kg/ha = kilograms/hectare; kPa = kilopascal; NF = nonfiltered air; NO_X = nitrogen oxides; O_3 = ozone; OTC = open-top chamber; ppb = parts per billion; ppm = parts per million; SUM06 = seasonal sum of all hourly average concentrations ≥ 0.06 ppm; SUM60 = sum of hourly ozone concentrations equal to or greater than 60 ppb; W126 = cumulative integrated exposure index with a sigmoidal weighting function.

8.6 Herbivores: Growth, Reproduction, and Survival

In the 2013 Ozone ISA there was no causality determination between ozone exposure and effects on herbivores. Reviewed studies included species-level responses (i.e., growth, reproduction, survival) and population- and community-level responses of herbivorous insects due to ozone-induced changes in plants. Ozone exposure can lead to changes in plant physiology (Figure 8-2), such as by modifying the chemistry and nutrient content of leaves (U.S. EPA, 2013; Menendez et al., 2009). These changes can have significant effects on herbivore physiology and behavior by affecting plant–herbivore interactions. In the 1996 Ozone AQCD, the 2006 Ozone AQCD, and the 2013 Ozone ISA, multiple studies showed statistically significant effects on insect growth, fecundity, and development. The effects, however, were highly context- and species-specific, there was no clear trend in directionality of response for most effects, and not all species tested showed a response (U.S. EPA, 2013, 2006, 1996). Studies on insect herbivores in previous ozone assessments included species from the orders Coleoptera (weevils, beetles), Hemiptera (aphids), and Lepidoptera (moths). There was no consensus in the 2013 Ozone ISA on how insects and other wildlife respond to elevated ozone.

Since that review, additional research has been published for more herbivorous insects, as well as for a few mammalian herbivores, at various levels of ozone exposure (see Table 8-13). As described in the PECOS tool, the scope for this section includes studies on any continent in which alterations in invertebrate and vertebrate responses were measured in individual species or at the population and community level to concentrations of ozone occurring in the environment or experimental ozone concentrations within an order of magnitude of recent concentrations (as described in Appendix 1).

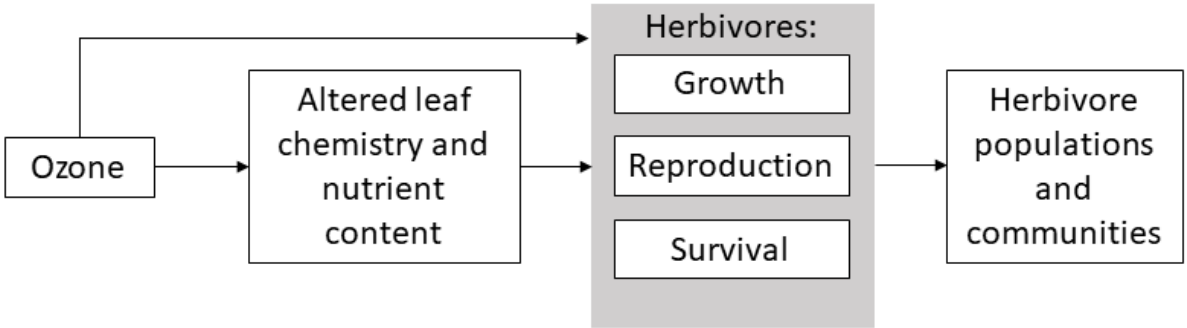


Figure 8-6 Conceptual model of ozone effects on herbivore growth, reproduction, and survival.

8.6.1 Individual-Level Responses

Consistently measured individual-level responses to ozone exposure include measures of growth, (including development time and adult and pupal mass) reproduction (e.g., fecundity, oviposition preference), and survival. A conceptual model of ozone effects on herbivores (Figure 8-6) illustrates cascading effects from individual-scale responses to populations and communities. Both the 1996 Ozone AQCD and 2006 Ozone AQCD stressed the variability in reported effects, including the lack of a consistent pattern in directionality and degree of response (U.S. EPA, 2006, 1996). In the 2013 Ozone ISA, a meta-analysis that included 16 studies published on insect herbivore species between 1996 and 2005 found that elevated ozone decreased development time and increased pupal mass in insect herbivores, with more pronounced effects occurring with longer durations of exposure (Valkama et al., 2007). In addition, for chewing insects, the meta-analysis found that relative growth rate increased under elevated ozone. There were no effects found on consumption, survival, or number of eggs laid (Valkama et al., 2007). In an assessment of five herbivore species (three moths and two weevils) only the growth of larvae of one moth species was affected (Peltonen et al., 2010).

Since the 2013 Ozone ISA, there is new evidence for endpoints related to growth, reproduction, and survival, as summarized below and in Table 8-14. As in the 2013 Ozone ISA, the insects encompass the orders Coleoptera, Hemiptera, and Lepidoptera. In addition to studies of insects, there are recent studies on a few mammalian herbivores. The new evidence describes ozone effects in a few additional species:

Growth:

- In the gypsy moth (*Lymantria dispar*) and tent caterpillar (*Malacosoma disstria*), exposure to 1.5× ambient ozone led to decreased growth (Couture et al., 2012). Female voles (*Microtus ochrogaster*) that consumed ozone-exposed plants showed reduced growth [1.5× ambient vs. control, Habeck and Lindroth (2013)].
- In the whitefly (*Bemisia tabaci*) and *L. dispar*, exposure to elevated ozone (72, 1.5× ambient, respectively) increased development time (Couture and Lindroth, 2012; Cui et al., 2012). However, at higher levels (238 vs. 50 ppb), development time decreased in *B. tabaci* (Hong et al., 2016). In the cabbage moth (*Pieris brassicae*), elevated ozone decreased development time [120 ppb vs. 15−20 ppb; Khaling et al. (2015)].
- In the leaf beetle (*Agelastica coerulea*) adults showed a preference to feed on leaves treated with elevated ozone [ambient vs. 60 ppb, Agathokleous et al. (2017)].
- Rabbits fed a mixture of common southern piedmont grassland species grown under concentrations of ozone 2 times (mean monthly 12 hour 37 to 56 ppb) ambient ozone had decreased digestible dry matter intake due to significantly lower neutral detergent fiber and acid detergent fiber digestibility compared to ambient [mean monthly 12 hour 21−32 ppb; Gilliland et al. (2012)].
- A loss in liveweight gain of 3.6 to 4.4% was predicted for lambs in the U.K. from 2007 to 2020 due to ozone effects on grasslands. With an ozone concentration increase from 20 to 30 ppb, liveweight gain was predicted to decrease by 12%. (Hayes et al., 2016).

Reproduction:

- In *B. tabaci*, exposure to elevated ozone (72 vs. 37 ppb) decreased fecundity (Cui et al., 2016b; Cui et al., 2012). However, at higher levels (238 vs. 50 ppb), fecundity increased (Hong et al., 2016). In *L. dispar*, exposure to 1.5× ambient levels decreased fecundity (Couture and Lindroth, 2012).
- Egg laying in the diamondback moth (*Plutella zylostella*) was significantly higher in the absence of ozone (when given a choice between artificial leaves fumigated with plant volatiles mixed with clean air or elevated ozone [80 ppb; Li and Blande (2015); see Section 8.7]. In the same lab study, plants exposed to herbivore-damaged neighbor plants had more eggs deposited on them at ambient ozone (10 ppb) than plants exposed to undamaged control plants. In presence of 80 ppb ozone, the preference for egg-laying on damaged plants was lost. Under field conditions, *P. zylostella* laid more eggs on plants exposed to control levels (10 ppb) compared with elevated ozone [30−80 ppb, Giron-Calva et al. (2016)].
- In *B. tabaci*, adults preferred control plants for oviposition [37 vs. 72 ppb, Cui et al. (2014)].

Survival:

- In *P. brassicae*, there was a nonsignificant trend whereby larval mortality tended to increase with increasing ozone levels [15−20 ppb, 70, 120 ppb; Khaling et al. (2015)]. Elevated ozone (50 and 150 ppb vs. 0.5 ppb) increased mortality in *Metopolophium dirhodum* aphids (Telesnicki et al., 2015).
- In *L. dispar*, survival of early instars decreased in response to feeding on leaves exposed to elevated ozone [1.5× ambient; Couture and Lindroth (2012)].
- At higher ozone levels, lifespan was prolonged in *B. tabaci* [238 vs. 50 ppb; Hong et al. (2016)].

8.6.2 Population- and Community-Level Responses

Changes in host plant quality resulting from elevated ozone can alter the population density and structure of associated insect herbivore communities, ultimately affecting ecosystem processes (Cornelissen, 2011). In the 2013 Ozone ISA, these population- and community-level responses included altered population growth rates in aphids (Menendez et al., 2010; Awmack et al., 2004), reduced total arthropod abundance at the Aspen FACE site (Hillstrom and Lindroth, 2008), and changes in genotypic frequencies of aphids over multiple generations (Mondor et al., 2005). Recent studies report metrics of altered population and community structure (e.g., population size, relative species abundance) adding to the evidence base for herbivore responses to ozone at higher levels of biological organization. New studies include:

- In a study from Aspen FACE, elevated ozone did not consistently influence arthropod community composition (Hillstrom et al., 2014).
- In a mesocosm study, past ozone exposure had no effect on the richness, diversity, or evenness of the arthropod community associated with the descendant plant community but did increase the relative abundance of carnivore arthropods while decreasing the relative abundance of herbivore arthropods (Martínez-Ghersa et al., 2017).

- Under low ozone conditions (1.5 ppb), the population size of *Rhopalosiphum padi* aphids was dependent on the symbiotic status of the host. However, under high ozone conditions (120 ppb), this difference disappeared (Ueno et al., 2016). In *M. dirhodum* aphids, ozone exposure did not affect population size, but did affect the proportion of dispersing aphids, with reduced dispersion in the ozone treatments [0.5 vs. 50, 150 ppb; (Telesnicki et al., 2015)].

8.6.3 Summary

Previous ozone assessments have summarized herbivorous insect-plant interactions and found information on a range of insect species in the orders Coleoptera, Hemiptera, and Lepidoptera (U.S. EPA, 2013, 2006, 1996). The majority of studies focused on growth and reproduction, while fewer studies considered herbivore survival and population and community-level responses to ozone. Although statistically significant effects were observed frequently, they did not provide any consistent pattern of response across growth (Table 8-11), reproduction (Table 8-12), and mortality endpoints. Recent studies reviewed here, including multiple experimental studies conducted by multiple research groups, expand the evidence base for the effects of elevated ozone on growth and reproduction in herbivores. Further, while effects were observed, there remains a more limited number of studies on the effects of ozone on survival and population/community-level responses. The effects of ozone exposure on plant biomass and biochemistry likely account, at least partially, for the observed changes across all endpoints that were assessed. It is also possible that variation in the herbivore responses to ozone stem from differences in study design, whereby ozone exposure was sometimes direct and other times indirect via effects on vegetation. Further uncertainties relate to differences in the plant consumption methods across species, for example chewing versus phloem-feeding in insects. Considering the large body of available evidence (Table 8-13) on growth and reproduction (i.e., 1996, 2006 AQCD, 2013 Ozone ISA, and more recent research efforts) and recognizing the above uncertainties, this ISA makes a new causality determination that **the body of evidence is sufficient to infer a likely to be causal relationship between ozone exposure and alteration of herbivore growth and reproduction.**

Table 8-11 Summary of studies reporting altered growth in herbivores.

Herbivore	Plant	Exposure ppb	Growth	Adult Mass	Pupal Mass	Development Time	Reference
Gypsy moth (*Lymantria dispar*); tent caterpillar (*Malacosoma disstria*)	Trembling aspen (*Populus tremuloides*); paper birch (*Betula papyrifera*)	50−100	↓				Couture et al. (2012)
Voles (*Microtus ochrogaster)*	*Solidago canadensis; Taraxacum officinale*	50−100	↓				Habeck and Lindroth (2013)
Pieris brassicae	*Brassica nigra*	120		↓		↓	Khaling et al. (2015)
Lymantria dispar	Trembling aspen (*Populus tremuloides*); paper birch (*Betula papyrifera*)	50−100	↓		↓	↑	Couture and Lindroth (2012)
Whitefly (*Bemisia tabaci*)	Tomato plant	72.2				↑	Cui et al. (2012)
Whitefly (*Bemisia tabaci*)	Tomato (*Lycoperiscon esculentum*)	238				↓	Hong et al. (2016)
Aphid (*Rhopalo-siphum padi*)	*Lolium multiflorum*	120		↓	↓		Ueno et al. (2016)
Aspen leaf beetle (*Chrysomela crotchi*)	Trembling aspen (*Populus tremuloides*)	50−100		↓		↑	Vigue and Lindroth (2010)[c]
Aphid (*Capegillettea betulaefoliae*)	Paper birch (*Betula papyrifera*)	50−60		No effect	No effect		Awmack et al. (2004)[c]
Epirrita autumnata	Silver birch (*Betula pendula*)	2× ambient	↓				Peltonen et al. (2010)[c]
Aphid	Broad bean	85	↓				Dohmen (1988)[a]
Aphid	Broad bean	100 (>24 h)	↓				Brown et al. (1992)[a]

Table 8-11 (Continued): Summary of studies reporting altered growth in herbivores.

Herbivore	Plant	Exposure ppb	Growth	Adult Mass	Pupal Mass	Development Time	Reference
Monarch butterfly	Milkweed	150−178	↑				Bolsinger et al. (1991); Bolsinger et al. (1992)[a]
Gypsy moth	Hybrid poplar (*P. trisitis* × *P. balsamifera*)		↓				Lindroth et al. (1993)[b]
Gypsy moth	Sugar maple (*Acer saccharum*)		No effect				Lindroth et al. (1993)[b]
Bug (*Lygus rugulipennis*)	Scots pine		↓				Manninen et al. (2000)[b]
Sawfly (*Gilpinia pallida*)	Scots pine		↑				Manninen et al. (2000)[b]
Colorado potato beetle (*Leptinotarsa decemlineata)*	Potato (*Solanum tuberosum)*		No effect				Costa et al. (2001)[b]
M. disstria	Aspen				↑		Percy et al. (2002)[b]
Tobacco hornworm (*Manduca sexta*)	Tobacco (*Nicotiana tabacum*)				↑		Jackson et al. (2000)[b]

[a]1996 Ozone AQCD.

[b]2006 AQCD.

[c]2013 ISA

Table 8-12 Summary of studies reporting altered reproduction in herbivores.

Herbivore	Plant	Exposure (ppb)	Fecundity	Oviposition Preference	Reference
Whitefly (*Bemisia tabaci*)	Tomato (*Lycopersicon esculentum*)	72	↓		Cui et al. (2016a)
Whitefly (*Bemisia tabaci*)	Tomato (*Lycopersicon esculentum*)	72	↓		Cui et al. (2012)
Whitefly (*Bemisia tabaci*)	Tomato (*Lycopersicon esculentum*)	238	↑		Hong et al. (2016)
Lymantria dispar	Trembling aspen (*Populus tremuloides*); Paper birch (*Betula papyrifera*)	50−100	↓		Couture and Lindroth (2012)
Diamondback moth (*Plutella zylostella*)	*Brassica oleracea*	80		↓	Li and Blande (2015)
Diamondback moth (*Plutella zylostella*)	*Brassica oleracea*	30−80		↓	Giron-Calva et al. (2016)
Colorado potato beetle (*Leptinotarsa decemlincata*)	Potato (*Solanum tuberosum*)		No effect		Costa et al. (2001)[b]
Beetle	Cottonwood	200	↓		Coleman and Jones (1988)[a]
Hornworm moth		Ambient+70%		↑	Jackson et al. (1999)[b]

[a]1996 Ozone AQCD.
[b]2006 AQCD.

Table 8-13 Summary of evidence for likely to be causal relationship between ozone exposure and alteration of herbivore growth and reproduction.

Rationale for Causality Determination	Key Evidence	Key References
Multiple experimental studies by multiple research groups show effects on growth in herbivorous insects, limited evidence in mammalian herbivores	Increased or decreased, herbivore growth, change in pupal or adult mass, altered development time	Hong et al. (2016), Ueno et al. (2016), Khaling et al. (2015), Habeck and Lindroth (2013), Couture et al. (2012), Couture and Lindroth (2012), Cui et al. (2012), Vigue and Lindroth (2010), Peltonen et al. (2010), Awmack et al. (2004) Section AX-9.3.3.1 U.S. EPA (2006)
Multiple experimental studies by multiple research groups show effects on reproduction in herbivorous insects	Increased or decreased fecundity, altered oviposition preference	Giron-Calva et al. (2016), Hong et al. (2016), Cui et al. (2016b), Li and Blande (2015), Couture and Lindroth (2012), Cui et al. (2012), Section AX-9.3.3.1 U.S. EPA (2006)

Table 8-14 Ozone exposure and effects on herbivores.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Insects and Other Wildlife
Cui et al. (2012)	OTC; Beijing, China (40.183°N,116.4°E)	Three genotypes (wild type, 35S, spr2) of tomato (*Lycopersicon esculentum*) and insect pest whitefly (*Bemisia tabaci*)	Two ozone treatments: current ambient O_3 (37.3 ppb) and twice the current ambient (72.2 ppb). All values are averages from 9:00 a.m. to 5:00 p.m.	In general, (varied by tomato genotype) whiteflies on ozone-treated plants had longer development time and reduced fecundity, which was correlated to phytochemical, enzymatic, and genetic alterations in the tomato plants. In response to O_3, there was an increased duration of larval stage with: 12.22% on wild type, 11.72% for 35S genotype, 4.64% for spr2 genotype; total length of larval and pupal stages: 9.65% for wild type, 9.71% for 35S genotype, and 5.41% for spr genotype; decreased fecundity: 19.33% reduction for whiteflies on wild-type tomato, 34.76% for 35S genotype, not significant for spr2; intrinsic rate of increase: 12.2% for wild type, 13.97% for 35S, not significant for spr2.
Li and Blande (2015)	Lab; Kuopio, Finland	Insect: *Plutella zylostella* Plant: *Brassica oleracea* var. *italica*	O_3: ambient (10 ppb), 80 ppb	Plants exposed to herbivore-damaged neighbor plants had more eggs deposited on them at ambient O_3 than plants exposed to undamaged control plants. At 10 ppb, there were significantly more eggs deposited on plants previously exposed to herbivore-damaged plants than those exposed to undamaged plants. In the presence of 80 ppb O_3, the preference for damaged plants was lost. When given a choice between artificial leaves fumigated with VPSCs mixed with clean air or elevated O_3, *P. xylostella* laid significantly more eggs in the absence of O_3.
Telesnicki et al. (2015)	OTC; (34.035°S, 58.029°W)	Insect: *Metopolophium dirhodum* Plant: *Triticum aestivum* cultivar Cronox	O_3: filtered air (0.5 ± 0.3 ppb), 50 ± 5 and 150 ± 10 ppb. Aphids received a single exposure to ozone during the 1st 6 h of daylight	The proportion of dead aphids was 0.054, 0.238, and 0.139 for control, 50 ppb, and 150 ppb O_3, respectively. The proportion of dispersed aphids was 0.654, 0.319, and 0.405 for control, 50 ppb, and 150 ppb O_3, respectively. The population of surviving aphids increased similarly for all treatments. The proportion of aphids dispersing from the diet cages was reduced by O_3 treatment.

Table 8-14 (Continued): Ozone exposure and effects on herbivores.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Insects and Other Wildlife
Cui et al. (2014)	OTC; Xiaotangshan County, Beijing, China (40.183°N, 116.4°E)	Insect: *Bemisia tabaci, Encarsia formosa* Plant: *Solanum esculentum* cultivar Castlemare, Jasmonic acid (JA) defence-enhanced genotype (JA-OE 35S)	O_3: ambient air (average = 37.3 ppm) and 72.2 ppm (~2× ambient), 8 h/day for 24 days	O_3 level, whitefly herbivory and tomato genotypes significantly affected the feeding and oviposition preferences of *B. tabaci.* Adult whiteflies preferred control plants over other treatments (i.e., O_3, herbivory and O_3 + herbivory treated) for feeding and oviposition. Compared with S35 plants, adult whiteflies preferred wild-type plants for feeding under control and herbivory treatments and for oviposition under control, O_3, herbivory, and O_3 + herbivory treatments. In a behavioral assay, parasitoids preferred O_3 + herbivory plants. Using an olfactometer, it was determined that the 35S plants were preferred by adult parasitoids under O_3, herbivory, and O_3 + herbivory treatments. Adult parasitoids showed no preference for either genotype under control conditions.
Hong et al. (2016)	Greenhouse; China	Insect: *Bemisia tabaci* Plant: tomato Fungi: *Beauveria bassiana*	Ambient (50 ± 10 ppb) and elevated (280 ± 20 ppb) 8 h/day for 40 days	Elevated O_3 shortened development time, prolonged adult lifespan, increased fecundity, increased the female ratio of offspring, and decreased the weight of newly enclosed adults. In the presence of elevated O_3 and fungal challenge, whitefly (adult and pupae) mortality increased, LC_{50} decreased, and the LT_{50} was shortened.
Cui et al. (2016b)	OTC; Observation Station for Global Change Biology, Beijing China (40.183°N, 116.4°E)	Insect: *Bemisia. tabaci* biotype B Plant: wild-type tomato (*L. esculentum* cultivar Castlemart), 35S: prosytemin transgenic tomato plants Virus: tomato yellow leaf curl virus	Ambient (37.3) and elevated (72.2 ppb). The OTCs were ventilated with air daily from 8:00 a.m. to 6:00 p.m. The experiment was terminated after 6 weeks	O_3 and tomato yellow leaf curl virus infection decreased *B. tabaci* fecundity and abundance, the greatest effect was observed for the combination of O_3 and infection.

Table 8-14 (Continued): Ozone exposure and effects on herbivores.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Insects and Other Wildlife
Couture and Lindroth (2012)	FACE; Aspen FACE, near Rhinelander, WI (45.7°N, 89.5°W)	Gypsy moth (*Lymantria dispar*) fed ozone exposed quaking aspen (*Populus tremuloides*) or paper birch (*Betula papyrifera*) leaves	Treatments for 1998-2008 were ambient O_3 W126 = 2.1 -8.8 ppm-h and elevated O_3 = 12.7-35.1 ppm-h. Ambient air CO_2 and elevated (560 ppm) CO_2. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015). For insect bioassays, insects were fed leaves from 11-yr-old ozone exposed trees for 7 days	Survivorship of early instars decreased by 16%, development time increased (5%, small but significant increase) across both tree species. Female pupal weight decreased 8%, while the effect on males was not significant. Development time was more influenced by tree species than by treatment. With O_3 exposure, insect egg production decreased by 28% in both tree species. The authors used statistical relationships to relate observed changes to alterations in foliar chemistry. With O_3-CO_2 interactions, effects on mortality and development time ameliorated.
Couture et al. (2012)	FACE; Aspen FACE, near Rhinelander, WI (45.7°N, 89.5°W)	Gypsy moth (*Lymantria dispar*) and forest tent caterpillar (*Malacosoma disstria*) fed ozone-exposed quaking aspen (*Populus tremuloides*) or paper birch (*Betula papyrifera*) leaves	Treatments for 1998−2007 were ambient O_3 W126 = 2.9−8.8 ppm-h and elevated O_3 = 13.1−35.1 ppm-h. Ambient air CO_2 and elevated (560 ppm) CO_2. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015). For insect bioassays, in 2007, insects were fed leaves from 11-yr-old ozone exposed trees for 7 days	Gypsy moth under elevated O_3: growth decreased with both aspen and birch. Consumption of both tree species increased by 10% and produced more frass (11% with aspen, 3% with birch). Finally, conversion of foliage to biomass decreased by 20 and 8% with aspen and birch, respectively. Tent caterpillar under elevated O_3: growth decreased with both aspen (32% response) and birch (7% response). Increased consumption of both aspen (37%) and birch (15%), produced more frass (23% increase with aspen), conversion of foliage to biomass decreased by 31 and 7% with aspen and birch, respectively. O_3-CO_2 interactions: Negative effects of O_3 on herbivores were offset to some degree by CO_2 but more so for gypsy moths than for tent caterpillars.

Table 8-14 (Continued): Ozone exposure and effects on herbivores.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Insects and Other Wildlife
Couture et al. (2015)	FACE research facility; Rhinelander, WI (45.7°N, 89.5°W)	Insect: specific insects not monitored, insect frass collected/analyzed Plants: two aspen genotypes (42 and 271, *Populus tremuloides*) and paper birch (*Betula papyrifera*)	Treatments for 2006−2008 were ambient O_3 W126 = 5.6, 4.9, 2.1 ppm-h and elevated O_3 = 14.6, 13.1, 12.7 ppm-h. Ambient air CO_2 and elevated (560 ppm) CO_2. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015). Leaves were collected from the lower and upper thirds of the canopies of 16 trees from each of the 12 rings, in June, July, and August of 2006, 2007, and 2008	Elevated O_3 elicited a "modest" decrease in canopy damage. Although organic deposition by insects was not affected by elevated O_3; N flux from the canopy to the soil decreased by 19% and the ratio of foliar C:N increased. Elevated CO_2 and O_3 (alone, not simultaneously) increased total abundance of herbivorous insects at the canopy level. Elevated O_3 decreased the negative effects of herbivory on ANPP.
Meehan et al. (2014)	FACE research facility; Rhinelander, WI (45.7°N, 89.5°W)	Aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*) and associated insect herbivore community	Treatments for 2006−2008 were ambient O_3 W126 = 5.6, 4.9, 2.1 ppm-h and elevated O_3 = 14.6, 13.1, 12.7 ppm-h. Ambient air CO_2 and elevated (560 ppm) CO_2. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	Dry matter C concentration was 6% higher under elevated O_3, N concentrations 6% lower under O_3, and C:N ratios were 10% higher under elevated O_3. Total C flux and tannins by herbivores were not affected by O_3. Elevated O_3 did not affect dry matter (13% reduction but NS), C, or tannin input, but had a small negative effect on N flux by herbivores.
Hillstrom et al. (2014)	FACE research facility; Rhinelander, WI	Insect: stratified sampling of canopy Plant: aspen genotypes (216, 217, 42E; *Populus tremuloides*) and paper birch (*Betula papyrifera*)	Treatments for 2005−2007 were ambient O_3 W126 = 7.3, 5.6, 4.9 ppm-h and elevated O_3 = 29.6, 14.6, 13.1 ppm-h. Ambient air CO_2 and elevated (560 ppm) CO_2: For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	The effects of elevated CO_2 and O_3 on arthropod abundance were species-specific and temporally variable. Unlike the results for aspen and birch trees exposed to elevated CO_2, the 10 most responsive arthropod species exhibited similar differences among species and years sampled in the elevated O_3 exposure group. Overall, the abundance of phloem-feeding increased and leaf chewing and galling guilds decreased under elevated CO_2. Elevated O_3 had the opposite effect. Effects on arthropod species richness were small and not believed to be biologically meaningful. While elevated CO_2 and elevated O_3 did not consistently influence arthropod community composition, tree genotype and the time of sample collection did.

Table 8-14 (Continued): Ozone exposure and effects on herbivores.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Insects and Other Wildlife
Giron-Calva et al. (2016)	Field; O_3 FACE facility; Laboratory; Kuopio, Finland (62.013°N, 27.035°E)	Insect: *Pieris brassicae, Plutella zylostella* Plant: *Brassica oleracea* var. *capitata, Brassica oleracea* var. *italica* cultivar Lucky	Laboratory: control (10 ppb) and elevated (30−80 ppb). Field: ambient and 1.5× ambient O_3; for hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	Under field conditions, female *P. brassicae* laid significantly more eggs on undamaged plants near other undamaged plants (cr-VOC) than on undamaged plants exposed to volatiles from nearby damaged plants (ir-VOC). In laboratory choice tests, there were no significant differences between oviposition on amb-cr-VOC plants and amb-ir-VOC plants. Similarly, there were no differences in oviposition between ozo-cr-VOC and ozo-ir-VOC plants. Unlike in the laboratory, more eggs were laid on amb-cr-VOC plants than on amb-ir-VOC plants. Elevated O_3 had no effect on oviposition preference. In laboratory tests conducted in large cages, *P. brassicae* laid "marginally" more eggs on amb-cr-VOC than on amb-ir-VOC plants. Significantly more eggs were laid on amb-cr-VOC plants than on ozo-cr-VOC plants and ozo-ir-VOC plants. *P. brassicae* laid significantly more eggs on amb-cr-VOC than on amb-ir-VOC plants. *P. brassicae* laid significantly more eggs on ozo-cr-VOC than on ozo-ir-VOC plants.
Agathokleous et al. (2017)	Sapporo Experimental Forest of Hokkaido University (43.1°N, 141.333°E)	Insect: Coleopteran leaf beetle (*Agelastica coerulea*) Plant: Japanese white birch (*Betula platyphylla* var. *japonica*)	Ambient (27.5 ± 11.6 ppb) and elevated (61.5 ± 13 ppb)	In the "no-choice assay," there were no statistical differences in the grazing behavior of adult beetles on leaves collected from ambient and elevated O_3. However, in the "choice assay," adults grazed 6 times more on leaves collected from elevated O_3 than on leaves collected from ambient O_3. 2nd instar grazed leaf area (no-choice vs. choice)—there were no statistical differences in the grazing behavior of larvae on leaves collected from ambient or elevated O_3 in either assay.

Table 8-14 (Continued): Ozone exposure and effects on herbivores.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Insects and Other Wildlife
Gilliland et al. (2012)	OTC/mammal feeding study Atmospheric deposition site of the school of forestry and wildlife Sciences, Auburn University, Auburn, AL	Tall fescue (*Lolium arundina*), dallisgrass and (*Paspalum dilatatum*), common Bermuda grass (*Cynodon dactylon*), and white clover (*Trifolium repens*) fed to New Zealand white rabbits (*Oryctolagus cuniculus*)	Six OTC chambers (three chambers per treatment). Grasses were exposed for 8 weeks. Mean monthly 12-h ambient was 21−32 ppb (average peak conc. 49 ppb). Mean monthly 2× ambient was 37 to 56 ppb (average peak concentration 102 ppb)	Neutral detergent fiber and acid detergent fiber digestibility was significantly lower for grasses grown under elevated ozone. Digestible dry matter intake (DM intake g/day × coefficient of apparent dry matter digestibility [percentage]) was 5.5 g/day greater in rabbits fed forage grown under NF (ambient) conditions compared with rabbits offered forage grown under 2× O_3. Decreased digestibility of O_3 forage was associated with increased concentrations of phenolics and lower neutral detergent fiber and acid detergent fiber digestibility.
Hayes et al. (2016)	Grassland ozone exposure experiments across four upland grassland types in locations throughout England	U.K. grassland species	Grasses collected from a range of O_3 exposure experiments in the U.K., with ozone concentrations ranging from 17 to 93 ppb	The authors predicted a loss in liveweight gain of 3.6 to 4.4% for lambs in the U.K. from 2007 to 2020. With O_3 conc. increase from 20 to 30 ppb, liveweight gain predicted to decrease by 12%.
Ueno et al. (2016)	OTC; Inland Pampa subregion, Buenos Aires, Argentina (34.583°S, 58.583°W)	Insect: *Rhopalosiphon padi* Plant: *Lolium multiflorum* Fungal endophyte: *Epichloe occultans*	120 ppb O_3	Aphid population size was significantly higher in the low-O_3 group in the presence of fungal endophyte, but not in the high-O_3 group. The proportion of nymphs to adults was significantly higher on fungal endophyte-free plants in the low-O_3 group where there were more adults on endophyte symbiotic plants compared with endophyte-free plants. Compared with low-O_3, the proportion of nymphs was increased in endophyte symbiotic plants, but the proportion of nymphs was lower in the absence of endophyte. The proportion of adult aphids was higher in endophyte-free plants under high O_3 than in symbiotic plants. The average body weight of insects at both instars (adult and nymph), between symbiotic and nonsymbiotic plants, was higher under low O_3.

Table 8-14 (Continued): Ozone exposure and effects on herbivores.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Insects and Other Wildlife
Habeck and Lindroth (2013)	Lab; plants: FACE site, Rhinelander, WI; wild-caught voles	Mammal: *Microtus ochrogaster* Plant: *Solidago canadensis* and *Taraxacum officinale*	Treatments for 1998−2007 were ambient O_3 W126 = 2.9−8.8 ppm-h and elevated O_3 = 13.1−35.1 ppm-h. Ambient air CO_2 and elevated (560 ppm) CO_2. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015).	Fumigation with elevated CO_2 or O_3 had no effect on the total amount of treatment diet consumed or the relative proportion of plant species consumed. Weanling male voles were unaffected by fumigation treatments, but female voles grew 36% less when fed plants harvested from the understory of O_3 rings. Total plant consumption by male voles was not related to any of the plant traits measured, but the growth rate of males was negatively associated with levels of ADF, ADL, and N. Total plant consumption by female voles was negatively associated with ADL and IVDMD, and positively associated with IVND and RP-N. The growth rate of female voles was positively associated with N, and negatively associated with CN, TNC, ADF, ADF:N, and ADL:N, although all of these associations were small.
Martínez-Ghersa et al. (2017)	Mesocosm; University of Buenos Aires, Argentina (34.58°S, 58.48°W)	Populations of agricultural weeds (mostly Eurasian annuals) from the seed bank in Corvallis, OR; planted and grown in Argentina and interacting with the Argentinian insect community	Plants are descended from populations exposed to 0 (charcoal-filtered), 90, or 120 ppb for 4 yr in OR. At the end of the fourth season, 5 cm top soil containing seed bank of the community resulting from 4-yr exposure to episodic ozone was removed from each chamber	There was a plant species richness and arthropod diversity linear relationship at 0 ppb historical O_3, but no relationship between plant species richness and arthropod diversity at 90 or 120 ppb historical O_3 exposure. Insects: Historical O_3 did not affect the richness, diversity, or evenness of the arthropod community associated with descendant plant community but did increase the relative abundance of carnivore arthropods, while decreasing the relative abundance of herbivore arthropods.

Table 8-14 (Continued): Ozone exposure and effects on herbivores.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Insects and Other Wildlife
Khaling et al. (2015)	Greenhouse; plant seeds: near Wageningen University, the Netherlands; Greenhouse: University of Eastern Finland, Kuipio, Finland. Insect eggs: Wageningen University, the Netherlands	Insect: *Pieris brassicae* Plant: *Brassica nigra*	Ambient, 70 and 120 ppb O_3. Ambient ozone concentrations fluctuated between 15 and 20 ppb; the other chambers had elevated concentrations from 4:00 a.m. to 8:00 p.m. and a basal concentration of 30 ppb from 8:00 p.m. to 4:00 a.m. Plants exposed for 5 days	Compared with ambient O_3, mean larval mass was significantly lower at 70 and 120 ppb O_3 after 3 and 6 days of treatment, but larval masses were not significantly different when comparing 70 and 120 ppb O_3. Compared with plants grown in ambient O_3, mean larval mass was significantly lower in larvae developing on plants pretreated with 70 and 120 ppb O_3 after 3 and 6 days of feeding, but larval masses were not significantly different when comparing the masses of larvae reared on plants pretreated with 70 or 120 ppb O_3. Compared with pupae feeding on plants exposed to 120 ppb O_3, pupae developing on plants pre-exposed to ambient O_3 had a significantly shorter larval period and achieved a significantly greater larval mass. There were no significant effects observed with 70 ppb O_3. Larval mortality tended to increase with increasing O_3 concentration, but the effect was not statistically significant for either concentration of O_3 tested. In dual choice assays, larvae consumed significantly more leaf material when feeding on plants pretreated with 120 ppb O_3 than plants pretreated with ambient O_3. There were no significant differences between ambient O_3 and 70 ppb O_3 or between 70 and 120 ppb O_3.

ADF = acid digestible fiber; ADL = acid digestible lignin; C = carbon; CN = carbon:nitrogen ratio; CO_2 = carbon dioxide; FACE = free-air CO_2 enrichment; IVDMD = in vitro dry matter digestibility; IVND = in vitro nitrogen digestibility; LC_{50} = median lethal concentration; LT_{50} = median lethal time; N = nitrogen; O_3 = ozone; OTC = open-top chamber; ppb = parts per billion; ppm = parts per million; RP-N = reducing power of protein-binding compounds on nitrogen digestibility; TNC = total nonstructural carbohydrates; VOC(s) = volatile organic compound(s); VPSC(s) = volatile plant signaling compound(s); W126 = cumulative integrated exposure index with a sigmoidal weighting function.

8.7 Plant-Insect Signaling

In the 2013 ISA there was no causality determination between ozone exposure and alteration of plant-insect signaling. Plants signal to other community members through the emission of volatile plant signaling compounds [VPSCs; Blande et al. (2014)]. Each signal emitted by plants has an atmospheric lifetime and a unique chemical signature comprised of different ratios of individual hydrocarbons that are susceptible to atmospheric oxidants like ozone (Yuan et al., 2009; Wright et al., 2005). Insects and other fauna discriminate between chemical signals of different plants. Scent-mediated ecological interactions include (1) host plant detection by herbivores, (2) attraction of pollinators and seed dispersers, and (3) plant attraction of natural enemies of insect herbivores (Figure 8-7). Evidence for ozone-mediated effects on plant-insect signaling are from studies that characterize scent plume emission/composition and studies that assess insect response to altered signals in ozone-enriched environments (Table 8-15). Ozone also interferes with VPSCs important in plant-plant interactions, such as emission of airborne signals to alert neighboring plants of insect attack and attraction of predators and parasitoids of herbivores (Giron-Calva et al., 2016; Li et al., 2016b; Li and Blande, 2015).

As described in the PECOS tool (Table 8-2), the scope for this section includes studies on any continent that assess altered plant insect signaling in response to concentrations of ozone occurring in the environment or experimental ozone concentrations within an order of magnitude of recent concentrations (as described in Appendix 1). Ozone effects on plant volatile chemical emissions were not specifically reviewed, rather identification of recent literature focused on plant-insect interactions, including plant signaling in response to herbivory. The effect of elevated ozone on plant insect signaling involves interactions with other biotic (species identity, lifestage) and abiotic (e.g., copollutants, elevated temperature, temporal) factors (Jamieson et al., 2017).

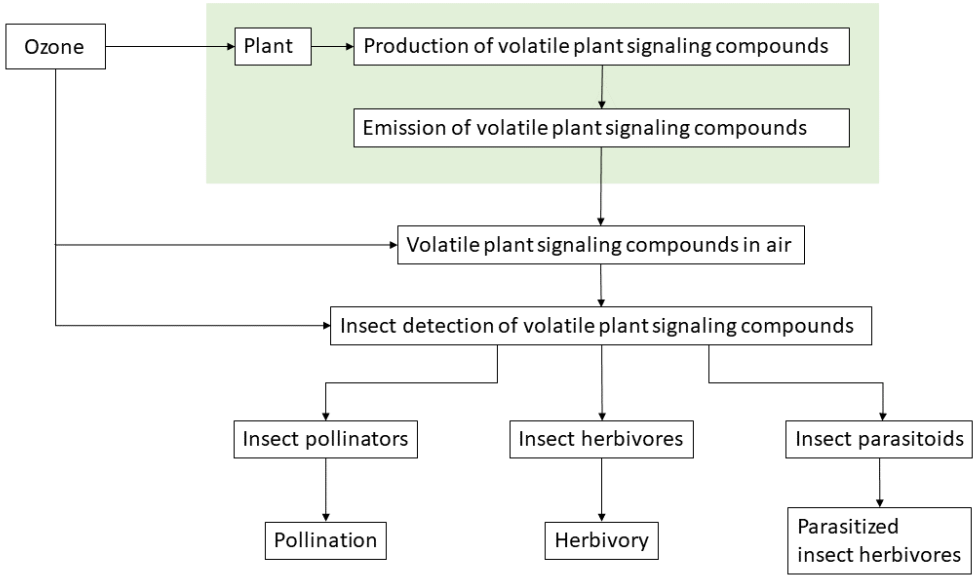


Figure 8-7 Conceptual model of ozone effects on volatile plant signaling compounds and plant-insect signaling.

8.7.1 Emission and Chemical Composition of Volatile Plant Signaling Compounds (VPSCs)

Studies in the 2013 Ozone ISA reported ozone alters the emission and chemical composition of VPSCs (Blande et al., 2010; Pinto et al., 2007; Vuorinen et al., 2004). Olfactory cues may travel shorter distances in ozone-enriched environments, reducing the effectiveness of chemical communication (Blande et al., 2010; Mcfrederick et al., 2008). Although not comprehensively reviewed for this ISA, elevated tropospheric ozone has been shown to alter plant production and emission of VPSCs and well as the atmospheric dispersion and lifespan of these compounds, thereby reducing the effectiveness of these signals (Juergens and Bischoff, 2017). Recent studies consistently show ozone effects on VPSCs, and that the emission and degradation of individual chemical signal components vary.

- *Ozone-induced VPSCs degradation:* Elevated ozone (≥50 ppb) degraded some plant VPSCs, changing the scent composition and reducing scent dispersion, potentially affecting (1) size of the scent plume, (2) ability of insects to detect the scent plume, and (3) time required to find the source of the scent plume (Mofikoya et al., 2017; Fuentes et al., 2016; Li et al., 2016b; Farré-Armengol et al., 2015; Li and Blande, 2015). In an enclosed ozone reaction system, Farré-Armengol et al. (2015) quantified degradation of several floral scent volatiles emitted by flowers (*Brassica nigra*) along a distance gradient. Degraded volatiles were first detected at 1.5 m in

80 ppb ozone, and the highest degradation levels (25−30%) were observed at 120 ppb ozone up to 4.5 m from the source (the farthest distance tested). Results of large eddy simulations show that ozone levels greater than 60 ppb degrade VPSCs, thus altering the chemical composition of the floral scent while increasing insect foraging times (Fuentes et al., 2016).

Plants emit VPSCs in response to herbivore feeding, and these signals are altered in combination with elevated ozone. Depending on the plant species studied, elevated ozone either increased or had no effect on VPSCs emissions in the presence of herbivory:

- *Herbivory and ozone-induced VPSCs emissions:* Tomato VPSC emissions increased 4.78-fold and 5.66-fold following exposure to elevated ozone (72.2 ppb) and whitefly herbivory stress, respectively. The combined effect of elevated ozone and whitefly herbivory further enhanced VPSC emissions (Cui et al., 2016b; Cui et al., 2014). Elevated ozone (up to 120 ppb) did not alter plant VPSC emissions by *Brassica nigra* (Khaling et al., 2016). However, simultaneous exposure to 120 ppb ozone and insect herbivore feeding stress for 24-hour increased emissions of several VPSCs beyond levels detected following insect feeding alone. The effect was not detectable 72 hours post exposure or in the presence of 70 ppb ozone (Khaling et al., 2016). Emissions of VPSCs varied by month in Scots Pine (Pinus sylvestris) subjected to herbivory stress and elevated ozone (Ghimire et al., 2017).

8.7.2 Pollinator Attraction and Plant Host Detection

Ozone effects on chemical signaling are evaluated in insect preference studies. Reduced detection of VPSCs may decrease the efficacy of insect pollination of native plants and crops, an important ecosystem service. This effect was demonstrated through a Lagrangian diffusion modeling study in the 2013 Ozone ISA in which the ability of pollinators to locate highly reactive VPSCs may have decreased from kilometers during preindustrial times to <200 meters at current ambient concentrations (Mcfrederick et al., 2008). One new empirical study tested detection response to elevated ozone in a pollinator species:

- *Pollinator attraction:* Under conditions of elevated ozone in experimental chambers, the degradation of VPSCs resulted in bumble bees (*Bombus terrestris*) orienting significantly less towards floral scent cues and exhibiting preference for artificial flowers closer to the ozone source [120 vs. 0 ppb; Farré-Armengol et al. (2015)].

As reported in the 2013 Ozone ISA, herbivorous insects use VPSCs to locate suitable host plants, and ozone can alter these interactions (Blande et al., 2010; Iriti and Faoro, 2009; Vuorinen et al., 2004; Jackson et al., 1999; Cannon, 1990). In an early study on VPSCs, ozone-induced emissions from red spruce pine needles were chosen less often than control needles by spruce budworm larvae (*Choristoneura fumiferana*), resulting in reduced plant host detection (Cannon, 1990). Subsequent studies showed that ozone can make a plant either more attractive or repellant to herbivores (Pinto et al., 2010; Jackson et al., 1999). Decreased detection of VPSCs by plant-eating insects may interrupt the ability of herbivores to locate plant hosts.

- *Plant host detection by insect herbivores:* In chamber studies, elevated ozone reduced the ability of insect herbivores to find their plant hosts (Li et al., 2016b; Fuentes et al., 2013). Striped

cucumber beetles (*Acalymma vittatum*) could not distinguish between clean air and air containing floral volatiles when the ozone concentration exceeded 80 ppb (Fuentes et al., 2013). Diamondback moth (*Plutella xylostella*) larvae oriented significantly more towards teflon filters exposed to nonozonated plant volatiles over filters exposed to plant volatiles mixed with elevated ozone (Li et al., 2016b). In addition, the larvae spent less time searching when placed on filters exposed to plant volatiles mixed with ozone [0 vs.100, but not 50 ppb, Li et al. (2016b)]. In OTCs, both ozone and herbivory by whiteflies (*Bemisia tabaci*) increased emissions of tomato plant (*Solanum esculentum*) VPSCs. Adult whiteflies preferred tomato plants exposed to ambient ozone levels over tomato plants exposed to elevated ozone for feeding [37.3 vs. 72.2 ppb, Cui et al. (2014)].

8.7.3 Plant Attraction of Natural Enemies of Herbivores

Plant defense responses include emission of VPSCs to attract predators and parasitoids that target the herbivores feeding on the plant. In studies reviewed in the 2013 Ozone ISA and new studies parasitoid-host attraction is either reduced, enhanced, or unaffected by elevated ozone (Cui et al., 2016b; Khaling et al., 2016; Cui et al., 2014; Pinto et al., 2008; Pinto et al., 2007; Gate et al., 1995). Altered plant signaling to natural enemies of herbivores disrupts predator-prey trophic interactions.

- *Effect of elevated ozone on parasitoid-host interactions:* The parasitoid *Cotesia glomerata* did not exhibit orientational bias for *Brassica nigra* plants exposed to elevated ozone [15−20 vs. 70 and 120 ppb, Khaling et al. (2016)]. The parasitoid *Encarsia formosa* preferred plants exposed to elevated ozone over control plants [37.3 vs. 72.2 ppb; Cui et al. (2014)]. Searching efficiency and the proportion of host larval fruit flies parasitized by *Asobara tabida* were reduced in the presence of 100 ppb ozone (Gate et al., 1995).
- *Combined effects of elevated ozone and insect herbivory on parasitoid-host interactions:* In field plots of potted cabbage plants, the behavior of the parasitoid *Cotesia plutella* was unaffected by elevated ozone [2× ambient; Pinto et al. (2008)]. In the absence of herbivory, the parasitoid *C. glomerata* did not exhibit preference for control or ozone exposed plants. (Khaling et al., 2016). When compared with plants only exposed to ozone, the parasitoid oriented toward plants exposed to 70 ppb ozone followed by herbivore feeding. The parasitoid oriented more towards plants exposed to a combination of 120 ppb ozone and herbivory more than herbivore-stressed plants that had not been exposed to ozone. However, in a wind tunnel assay, the strength of orientation toward insect-damaged plants was significantly reduced by 70 ppb ozone, but not by 120 ppb ozone (Khaling et al., 2016). The parasitoid *E. formosa* preferred insect-damaged plants exposed to elevated ozone over control plants [37.3 vs. 72.2 ppb, Cui et al. (2016b); Cui et al. (2014)].

8.7.4 Summary

In the 2013 Ozone ISA experimental and modeling studies reported altered insect-plant interactions mediated through chemical signaling. New empirical research from laboratory, greenhouse, OTC and FACE experiments expand the evidence for altered/degraded emissions of chemical signals from plants and reduced detection of VPSCs by insects, including pollinators, in the presence of ozone (Table 8-16). The interaction of ozone (>50 ppb) with VPSCs disrupts the production, emission,

dispersion, and lifespan of these compounds. Numerous preference studies in insects show altered plant host detection, reduced pollinator attraction, and shifts in plant host preference in the presence of elevated, yet environmentally relevant, ozone concentrations. Plant defense mechanisms (i.e., attraction of predators and parasitoids that target phytophagous insects) were either reduced, enhanced, or unaffected by elevated ozone. Considering the available evidence (i.e., the 2013 Ozone ISA and more recent research efforts) and recognizing uncertainties around how chemical signaling responses observed in the laboratory translate to natural environments (Table 8-13), this ISA makes a new causality determination that the body of evidence is **sufficient to infer a likely to be causal relationship between ozone exposure and alteration of plant insect signaling**.

Table 8-15 Summary of evidence for a likely to be causal relationship between ozone exposure and alteration of plant-insect signaling.

Rationale for Causality Determination	Key Evidence	Key References
Multiple experimental and modeling studies by multiple research groups show direct effects of ozone on VPSCs	Ozone disrupts the production, emission, dispersion, and lifespan of VPSCs	Vuorinen et al. (2004), Pinto et al. (2007), Mcfrederick et al. (2008) (model), Blande et al. (2010), Cui et al. (2014), Li and Blande (2015), Farré-Armengol et al. (2015), Cui et al. (2016b), Fuentes et al. (2016) (model), Khaling et al. (2016), Li et al. (2016b), Mofikoya et al. (2017), Ghimire et al. (2017)
Multiple experimental studies by multiple research groups show altered insect response to VPSCs in presence of ozone	Altered plant host detection and insect herbivory; reduced pollinator attraction and altered parasitoid attraction by plants	Cannon (1990), Gate et al. (1995), Fuentes et al. (2013), Cui et al. (2014), Farré-Armengol et al. (2015), Li et al. (2016b), Khaling et al. (2016), Cui et al. (2016b)

VPSC(s) = volatile plant signaling compound(s).

Table 8-16 Ozone exposure and plant insect signaling.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Plant Insect Signaling
Li and Blande (2015)	Lab; Kuopio, Finland	Insect: *Plutella zylostella* (diamondback moth) Plant: *Brassica oleracea*) (broccoli)	Ambient (~10 ppb, 24 h/day), 80 ppb 5 days (reduced to 30 ppb at night) for 5 days	Mixing VPSCs emitted by herbivore-damaged plants with O_3 resulted in complete degradation of some compounds and partial degradation of others. However, in a few instances, quantities of some VPSCs increased, suggesting that VPSCs degrade into other VOCs.
Khaling et al. (2016)	Greenhouse; plant seeds and insect eggs—near Wageningen University, the Netherlands Greenhouse; University of Eastern Finland, Kuipio, Finland	Insect: *Pieris brassicae* (large white moth), *Cotesia glomerata* Plant: *Brassica nigra* (black mustard)	Experiment #1: ambient (15−20 ppb), 70, 120 ppb from 4:00 a.m. to 8:00 p.m. and a basal concentration of 30 ppb for the remaining 8 h each day. Experiment #2: VOC emissions were sampled from ambient, 70, 120 ppb and herbivore feeding in combination with ambient, 70, 120 ppb for 24 and 72 h	Plant emissions were not significantly altered by O_3 exposure alone, but herbivore-feeding stress (24 and 72 h) induced emission of VPSCs. Simultaneous exposure to 120 ppb O_3 and insect herbivore feeding stress for 24 h increased emissions of several VPSCs beyond levels detected following insect feeding alone. The effect was not detectable 72 h post exposure or in the presence of 70 ppb O_3. The parasitoid did not show a preference for control or O_3 exposed plants. However, when compared to plants only exposed to O_3, the parasitoid oriented toward plants exposed to 70 ppb O_3 followed by herbivore feeding, but the same effect was not observed at 120 ppm O_3. In the absence of herbivory, the parasitoid did not show a preference between elevated O_3 and clean-air-exposed plants. Finally, the parasitoid oriented more towards plants exposed to 120 ppm O_3 and herbivory more than herbivore-stressed plants. The strength of parasitoid orientation toward herbivore-damaged plants over nondamaged plants was significantly affected by 70 ppb O_3, but not 120 ppb O_3.
Cui et al. (2014)	OTC; Xiaotangshan County, Beijing, China (40.183°N, 116.4°E)	Insect: *Bemisia tabaci* (whitefly), *Encarsia formosa* Plant: *Solanum esculentum* (wild-type tomato plants)	Ambient (37.3 ppb; average value from 9:00 a.m.−5:00 p.m. 2× ambient (72.2 ppb; average value from 9:00 a.m. to 5:00 p.m.) Exposure duration was 8 h/day for 24 days, excluding 2 days due to rain	Elevated O_3 levels increased VPSC emissions 4.85-fold in the wild-type tomato plants. Whitefly herbivory increased the total amount of plant VPSC emissions 5.12-fold. VPSC emissions were greatest for the O_3 + herbivory treatment. In a behavioral assay, adult parasitoids preferred insect-damaged plants exposed to elevated O_3 to elevated O_3 over control plants.

Table 8-16 (Continued): Ozone exposure and plant insect signaling.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Plant Insect Signaling
Li et al. (2016b)	Greenhouse; Kuopio, Finland	Insect: *Plutella xylostella* (diamondback moth) Plant: *Brassica oleracea* (cabbage) *Brassica oleracea* (broccoli)	Experiment #1: In a Y chamber bioassay, insect herbivore was given the choice between VPSCs from healthy plants vs. clean air (250 mL/min flow rate) Experiment #2: In a Y chamber bioassay, insect herbivore was given the choice between VPSCs + clean air vs. VPSCs+ O_3 (50, 100 ppb for 5 min, flow rate 300 mL/min) Experiment #3: Insect herbivore preference was evaluated through four choices between herbivore-induced cabbage VPSCs mixed with 50 ppb O_3 vs. clean air, herbivore induced cabbage VPSCs mixed with 100 ppb O_3 vs. clean air, constitutive cabbage VPSCs mixed with 100 ppb O_3 vs. clean air, and herbivore-induced broccoli VPSCs mixed with 100 ppb O_3 vs. clean air	Herbivore-induced VPSCs at both 50 and 100 ppb O_3 were significantly degraded, rendering the relative proportions of O_3-treated VPSC components different that the original VPSCs. Significantly more insect larvae oriented towards VPSCs from undamaged plants than charcoal-filtered air. Significantly more insect larvae oriented towards insect-infested plants than undamaged plants. Elevated O_3 degraded some VOCs emitted from herbivore-damaged plants; the effect appeared to be dependent on the concentration of O_3 present. Insect larvae preferred VPSCs exposed to filtered air over those mixed with 50 and 100 ppm O_3; preference was lost when the air supply was passed through an O_3 scrubber prior to mixing with VPSCs. Insect larvae preferred filters that had not been exposed to O_3 over filters exposed to VOCs that were mixed with O_3. Larvae spent significantly less time searching when placed on filters exposed to VOCs mixed with O_3 than on filters exposed to VOCs mixed with clean air. In both instances, effects were observed for constitutive and O_3 induced VOC blends and only in the 100 ppb O_3 treatment.
Mofikoya et al. (2017)	FACE; Kuopio, Finland (62.895°N, 27.625°E)	Insect: *Pieris xylostella* Plant: *Brassica oleracea* (white cabbage)	Ambient: (average 29 ppb), elevated: (average 42 ppb) from 8:00 a.m. to 10:00 p.m.	Compared to ambient O_3, plants at 0.5 m distance from the myrcene dispensers and exposed to elevated O_3 had lower myrcene emission rates. The effect was lost at the 1.5- and 3-m distances. Levels of myrcene were significantly lower at the 0.5-m distance from the dispenser in plots exposed to elevated O_3. Myrcene was not detectable at the 1.5- and 3-m distances in ambient or 42 ppb O_3 plots.

Table 8-16 (Continued): Ozone exposure and plant insect signaling.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Plant Insect Signaling
Cui et al. (2016b)	OTC; Observation Station for Global Change Biology, Beijing China (40.183°N, 116.4°E)	Insect: *Bemisia tabaci* (whitefly) Plant: *Solanum lycopersicum* (wild-type tomato)	Ambient (37.3 ppb; avg value from 9:00 a.m.−5:00 p.m.) for 3 weeks, in both 2010 and 2011 2× ambient (72.2 ppb; avg value from 9:00 a.m.−5:00 p.m.) Exposure duration was 8 h/day for 24 days, excluding 2 days due to rain	Elevated O_3 increased the total amount of plant VOC emissions 4.78-fold in the wild-type tomato plants. Whitefly herbivory increased the total amount of plant VOC emissions 5.66-fold in the wild-type tomato plants. Production of VPSCs was highest in the treatment of O_3 + herbivory. In a dual-choice Y-tube assay, adult parasitoids preferred O_3 + herbivory plants over all other treatments.
Ghimire et al. (2017)	Other; Kuopio, Finland (62.217°N, 27.583°E)	Insect: *Acantholyda posticalis* (great web-spinning pine sawfly) Plant: *Pinus sylvestris* (Scots pine)	O_3 exposure was 14 h/day, 7 days/week and calculated as daily average from 8:00 a.m.−10:00 p.m. 2011 and 2012: 1.48 ambient O_3 concentration 2013: 1.56× ambient O_3 concentration Daily average ozone concentrations (ppb) computed from daytime (8:00 a.m.−10:00 p.m.); hourly mean values for both ambient and elevated O_3 exposures graphed in Figure 1	Herbivore feeding alone significantly increased emissions of some VPSCs. BVOC emissions were exponentially related to the proportion of needles damaged by insect herbivores on the 7th feeding day in June. In July, herbivory increased emission rates of some VOCs from nondamaged branches, in combination with elevated O_3, emissions of MT-nx was further elevated. In August, herbivory stress did not alter emission of most VOC (post-feeding effect), except GLVs in the treatment with ambient O_3 and lower nitrogen. In September, post-feeding effects of herbivory significantly increased emission rates of MT-nx at elevated N level.

Table 8-16 (Continued): Ozone exposure and plant insect signaling.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Plant Insect Signaling
Fuentes et al. (2013)	Lab	Insect: *Acalymma vittatum* (striped cucumber beetle) Plant: *Cucurbita foetidissima*	Beetles exposed to two choices in a Y chamber, air flow 1 L/min. Duration of exposure no longer than 5 min. Three types of choice trials conducted: Filtered air vs. filtered + O_3 where filtered was 0 ppb and filtered + O_3 was 20, 40, 60, 80, 100, or 120 ppb. Filtered air vs. flower + O_3 where filtered air was 0 ppb and flower + O_3 was 0, 40, 80, or 120 ppb. Flower vs. flower + O_3 where flower was 0 ppb and flower + O_3 was 20, 40, 60, 80, 100, or 120 ppb	Under all choice conditions, beetles were equally likely to choose filtered air or filtered air + elevated O_3. At 0 ppb O_3, beetles chose flower over filtered air 83% of the time. At 40 ppb O_3, beetles chose flower + O_3 over filtered air 70% of the time. At 80 ppb O_3, beetles chose flower + O_3 63% of the time (not significantly different from no preference). At 120 ppb O_3, beetles were equally likely to pick filtered air as flower + O_3. At 20, 40, and 60 ppb O_3, beetles were statistically equally as likely to choose flower as flower + O_3. At 80, 100, and 120 ppb O_3, beetles chose flowers over flowers + O_3 between 75−80% of the time.
Farré-Armengol et al. (2015)	Lab	Insect: *Bombus terrestris* (buff-tail bumble bee) Plant: *Brassica nigra* (black mustard)	Flower VOC emission exposed to 0, 80, and 120 ppb. Bumble bees exposed to three choices in a cylindrical chamber, air flow 1 L/min. Duration of exposure 10 min. Three types of choice trials conducted: Floral scent from distance 0 at 0 ppb O_3 vs. clean air; floral scent from distance 3 at 120 ppb O_3 vs. clean air; floral scent from distance 0 at 120 ppb O_3 vs. floral scent from distance 3 at 120 ppb O_3	The concentration of floral scent volatiles decreased with increasing O_3 concentration and distance from the floral scent source. Degraded volatiles were first detected at 1.5 m in 80 ppb O_3 and the highest degradation levels (25−30%) were observed at 120 ppb O_3 up to 4.5 m from the source (the farthest distance tested). Because not all floral scents were degraded equally, O_3 altered the ratio of compounds in the scent blend. Bumble bees preferred floral scent at distance 0 m over scent-free, filtered air. Bumble bees showed no clear bias when presented with floral scent at a distance of 3 m with 120 ppb O_3 and filtered air. Bumble bees preferred floral scent at distance 0 and 120 ppb O_3 over floral scent at a distance of 3 m. More bumble bees landed more on artificial flowers associated with floral scent at distance 0 m with 0 ppb O_3 than artificial flowers associated with filtered air. More bumble bees landed on artificial flowers associated with floral scent from distance 0 at 120 ppb O_3 than artificial flowers associated with floral scent from distance 3 m at 120 ppb O_3.

Table 8-16 (Continued): Ozone exposure and plant insect signaling.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Plant Insect Signaling
Fuentes et al. (2016)	Other	Generic insects	Using large eddy simulations, modeled exposure to a range of O_3 concentrations (0 to 120 ppb vs. O_3 [ppb on a per volume basis])	Reactivity with >60 ppb O_3 reduces the lifetime of individual VPSCs by varying degrees. Degradation of VPSCs by elevated O_3 reduces the distance of floral scent dispersion. The composition of floral scent plumes changed following exposure to elevated O_3, changing the proportion of individual VOCs, and in some instances, reducing levels of some VOCs below the limit of analytical detection.

BVOC(s) = biogenic volatile organic compound(s); FACE = free-air CO_2 enrichment; GLV(s) = green leaf volatile(s); L/min = liters/minute; mL/min = milliliters/minute; MT-nx = total nonoxygenated monoterpenes; N = nitrogen; O_3 = ozone; ppb = parts per billion; ppm = parts per million; VOC(s) = volatile organic compound(s); VPSC(s) = volatile plant signaling compound(s).

8.8 Carbon Cycling in Terrestrial Ecosystems: Primary Productivity and Carbon Sequestration

In the 2013 Ozone ISA, the evidence was sufficient to conclude there is a causal relationship between ozone exposure and reduced plant productivity, and a likely to be causal relationship between ozone exposure and decreased terrestrial carbon sequestration (U.S. EPA, 2013). At the time of the 2013 Ozone ISA, there was evidence from long-term ecosystem-scale ozone fumigation experiments in forests, grasslands, and agricultural systems that ozone exposure could decrease plant productivity. Similarly, the 2013 Ozone ISA included findings from five models that incorporated the negative effects of ozone on leaf-level photosynthesis and plant growth into carbon-cycling models, which were then used to create regional-, national-, or global-scale estimates of the effect of ozone pollution on terrestrial plant productivity and carbon sequestration. Although the models and experiments varied widely in scale and scope, there were consistent observations of decreased plant productivity and a smaller flux of CO_2 into ecosystems.

For the current review, the scope is based on causality determinations in the 2013 Ozone ISA. As described in the PECOS (Table 8-2), the body of reviewed literature is different for the ecosystem productivity and for carbon sequestration. For ecosystem productivity, which was causal in the 2013 Ozone ISA, this synthesis will only include studies conducted in North America at ozone concentrations occurring in the environment or experimental ozone concentrations within an order of magnitude of recent concentrations (as described in Appendix 1), recognizing that there is a substantial body of research conducted in other countries that is out of scope of the current review. For studies of ozone effects on carbon sequestration, given the relatively small number of studies and the likely to be causal determination from the 2013 Ozone ISA, research from other countries examining ecosystems that were analogous to those in the U.S. were considered and included. Studies that were reviewed for this ISA include research that integrates ozone into carbon-cycling models at ecosystem to global scales and experiments using OTC and FACE systems to expose plants to ozone.

8.8.1 Terrestrial Primary Productivity

The terrestrial carbon cycle integrates processes at a variety of scales, ranging from organelles to individuals to biomes (Chapin et al., 2002). Gross primary productivity (GPP), which is the influx CO_2 from the atmosphere via photosynthesis at the ecosystem scale, is fundamental to global carbon cycling. However, because photosynthesis occurs simultaneously with autotrophic respiration, GPP is rarely measured directly, and estimates are most often derived from whole-ecosystem carbon flux measurements (eddy covariance) or models that scale measurements of leaf-level processes to ecosystems (Chapin et al., 2002). Researchers have used process models to quantify at the ecosystem scale the change in GPP

resulting from the widely documented decreases in photosynthesis at the leaf-scale caused by ozone exposure.

Since the 2013 Ozone ISA, two new studies have reported on the effects of ozone on gross primary productivity (Table 8-17):

- Working in three ecosystems with Mediterranean-type climates, Fares et al. (2013) conducted a statistical analysis of data from eddy covariance flux towers to quantify the effect of ozone on carbon assimilation (GPP). In California, ozone decreased carbon assimilation by 12% in a *Pinus ponderosa* forest in the Sierra Nevada and by 19% in an orange (*Citrus sinensis*) grove in the Central Valley; damage was greater in the orange grove because of higher average ozone concentrations and because irrigation supported higher stomatal conductance during periods of the day when ozone concentrations peaked. At the third site in Italy, ozone concentrations were lower and there was no detectable effect on GPP.
- Yue and Unger (2014) adopted the same ozone-damage thresholds and sensitivity coefficients that were used in the calibration of the MOSES model (described in the 2013 Ozone ISA) for use in the Yale Interactive Terrestrial Biosphere Model (YIBs), a biophysical vegetation model. Overall, inclusion of ozone damage improved the ability of the YIBs model to predict site-level GPP measured at eddy covariance flux towers at 40 sites in the U.S. and Canada. Decreases in GPP as a result of ozone ranged from 1 to 14% and were greatest at sites showing both high stomatal conductance and high growing season ozone concentrations. Modeled across the U.S., ozone decreased GPP by 2−5%, with stronger effects in the eastern U.S. (east of 95°W longitude, 4−8% decrease, with localized decreases of 11−17%) because of both higher stomatal conductance and higher ozone concentrations (Yue and Unger, 2014).

Carbon assimilated into plant tissue via photosynthesis is either respired or contributes to net primary productivity (NPP), which is often measured as the rate of plant biomass accumulation. Estimates of the effects of ozone on NPP have been derived from field experiments, such as Aspen FACE or SoyFACE, as well as from C-cycling models. While much of the research published since the 2013 Ozone ISA is confirmatory, other work has provided new mechanistic insight into the effects of ozone on NPP.

- Zak et al. (2011) and Talhelm et al. (2014) quantified NPP at the Aspen FACE experiment, which exposed tree communities composed of aspen *(Populus tremuloides*), aspen-birch (*Betula papyrifera*), or aspen-maple (*Acer saccharum*) planted 1 year before the experiment to elevated ozone (ambient W126 2.1−8.8 ppm-hour, elevated 12.7−35.1 ppm-hour) from 1998 to 2008. Although elevated ozone decreased cumulative NPP during the experiment by 10%, the effect of ozone on annual NPP gradually disappeared over the last 7 years of the experiment such that ozone had no significant effect on NPP during the last several years of the experiment (Talhelm et al., 2014; Zak et al., 2011). Zak et al. (2011) attributed the disappearance of the ozone effect on NPP to compensatory growth by ozone-tolerant individuals and species. Elevated ozone exposures were also much lower in the last 3 years of the experiment (W126 average of 13.5 ppm-hour) compared to the first 8 years of the experiment (W126 average of 27.4 ppm-hour) (Kubiske and Foss, 2015). Further, Talhelm et al. (2014) used an empirical model to attribute the disappearing ozone effect on NPP to canopy dynamics: ozone had a persistent negative effect on leaf biomass and canopy N (Talhelm et al., 2014) that created initial declines in NPP, but the marginal effect of the decrease in canopy N on NPP declined as canopy leaf area increased through time in the developing stand. Based on analysis of foliar insect herbivory patterns over

the last 3 years of the experiment, Couture et al. (2015) suggested reduced canopy damage (16% lower under elevated ozone) as another mechanism that contributed to the limited effect of ozone on NPP. Under ambient conditions, foliar herbivory decreased NPP by approximately 2%, but this effect was 23% smaller under elevated ozone.

- At the SoyFACE experiment in Illinois, Oikawa and Ainsworth (2016) studied soybean (*Glycine max*) plant growth, photosynthesis, canopy N (g per plant), and canopy development across treatments ranging from 37 to 116 ppb of ozone (9-hour means). Increasing exposure to ozone decreased canopy leaf area, canopy N, and aboveground plant mass. Each 10 ppb increase in ozone decreased whole-canopy net photosynthesis by 10%.
- In Finland, Kasurinen et al. (2012) conducted a free-air ozone fumigation (elevated: 30 ppb; ambient: 24 ppb averages for about 5 months) experiment using four genotypes of birch (*Betula pendula*) grown in pots for two growing seasons. There were no significant ozone effects ($p > 0.2$) on tree biomass in a midsummer harvest, but in sampling in autumn after the onset of leaf senescence, elevated ozone decreased overall leaf biomass and decreased stem biomass in two of the four birch genotypes included in the experiment.
- The FACE site in Kranzberger Forest, Germany examined ozone and CO_2 effects on *Fagus sylvatica* (European beech) and *Picea abies* (Norway spruce). In beech trees under elevated ozone treatments there was a significant decrease in the allocation of $^{13}CO_2$-labeled C to the stems (60%) and marginally significant increase in coarse root respiration. In spruce, flux of photosynthates to stem and coarse root respiration was slightly stimulated under elevated ozone.

Together, these new observations provide further evidence that ozone can decrease plant growth and NPP, but also help give a more nuanced understanding of how these effects vary among genotypes, species, communities, and environmental conditions. Models pair experimental observations of dose-response relationships for photosynthesis or growth with estimates of ozone exposure to estimate the effects on NPP at ecosystem or regional scales (see Table 9-2 in the 2013 Ozone ISA for a review). Because resources to conduct ecosystem-scale experiments are limited, these models provide important estimates of the consequences of ozone exposure for productivity and carbon cycling at larger temporal and spatial scales. New studies at larger scales provide the following insights:

- Similar to the Aspen FACE experiment, the effects of ozone on forest productivity were also dynamic in an analysis of long-term (500 years) forest productivity created by Wang et al. (2016) using the University of Virginia Forest Model Enhanced (UVAFME). In contrast to the physiological and ecosystem process models that have been widely used to model the effects of ozone on plant productivity, UVAFME is a gap model, which tracks the growth and survival of individual trees and species within a stand. Wang et al. (2016) applied UVAFME to a diverse southeastern U.S. broadleaf forest (32 species) using a scenario that assigned individual species sensitivities of 0, 10, and 20% growth reductions in response to ozone exposure. Ozone decreased forest productivity and biomass during the first 100 years, but then had a neutral or positive effect as more ozone-tolerant species grew more rapidly in response to a decrease in competition from more ozone-sensitive species.
- Gustafson et al. (2013) integrated the tree growth results from the Aspen FACE experiment into the LANDIS-II model, first in a scenario that modeled growth of the experimental stands for 180 years, then in a scenario that applied the results of Aspen FACE to a landscape simulation of forest cover and productivity. In both simulations, ozone favored both birch and maple relative to aspen, which was a function of both species' differences in ozone sensitivity, as well as greater longevity of these species.

- Ozone decreased stand-level NPP for three tree species (*Pinus sylvestris, Picea abies, Betula pendula*) growing in stands modeled with the 3-PG model representing six different geographic zones of Sweden, with effects ranging from 1.4 to 15.5% among species and climate scenarios (Subramanian et al., 2015).
- Based on empirical relationships between ozone exposure and tree growth derived primarily from the results of OTC experiments, de Vries et al. (2017) used a forest productivity model (EUgrow) that was coupled with the soil biogeochemical process model VSD to estimate that ozone decreased forest biomass across Europe by 4% over the simulation period of 1900 to 2005.
- Application of the landscape ecosystem process model, the Dynamic Land Ecosystem Model (DLEM), to the southeastern U.S. (Texas to Virginia, 1987−2007) by Tian et al. (2012) and to agricultural ecosystems across China (1980−2005) by Ren et al. (2012) produced estimates that ozone exposure decreased NPP by 3 and 10.5%, respectively. Consistent with DLEM simulations reviewed in the 2013 Ozone ISA, Tian et al. (2012) estimated larger effects on broadleaf trees (−3%) and crops (−7%) than on conifers (−0.5%).
- Using exposure-response relationships between W126 and plant growth published in the Welfare Risk and Exposure Assessment for Ozone (U.S. EPA, 2014) for 4 crops and 11 trees, Capps et al. (2016) examined the potential increases in crop and tree productivity that might result from regulations intended to limit greenhouse gas emissions from power plants in the U.S. Increases in productivity resulting from emissions controls were a function of the geographic distribution of both the plant species and the electric generating stations, as well as the physiological sensitivity of the plant species.

Results from the Aspen FACE experiment and the model simulation conducted by Wang et al. (2016) both suggest that the effects on ozone on NPP could be dynamic and temporary. However, the results of these experiments contrast with results from an 8-year FACE ozone experiment conducted in a 60-year-old beech (*Fagus sylvatica*)—spruce (*Picea abies*) forest in Germany (Matyssek et al., 2010). Although spruce growth increased under elevated ozone in this experiment, this increase amounted to only 5% of the lost stem volume of beech under elevated ozone. Thus, there are apparent limits in some systems to the extent that increased growth of ozone-insensitive species can compensate for decreased productivity of ozone-sensitive species. More broadly, the extent to which ozone affects terrestrial productivity will depend on more than just community composition, but other factors, which both directly influence NPP (i.e., availability of N and water) and modify the effect of ozone on plant growth (see Section 8.12: Modifying Factors).

8.8.2 Soil Carbon

Carbon in the soil can be bound in organisms (plant roots, microbial biomass, invertebrates) or bound in organic compounds within soil particles or aggregates. In some terrestrial ecosystems, including Aspen FACE's soils contain more carbon than is contained in the total plant biomass (Talhelm et al., 2014; Pregitzer and Euskirchen, 2004). Different forms of C within the soil have residence times ranging from decades to centuries (Schmidt et al., 2011), and soil C pools tend to respond more slowly than plant pools to environmental change (Tian et al., 2012). Ozone can alter terrestrial C storage through its effects

on plant biomass and NPP (Section 8.3 and Section 8.8.3), as well as through its effects on C in soils (Section 8.9.2). The experimental observations reviewed in Section 8.9.2 and in the 2013 Ozone ISA did not find a direct link between ozone, NPP, and soil C pools. Thus, although Talhelm et al. (2014) observed that ozone decreased soil C, the link between soil C and ozone may yet turn out to be as complex as that between soil C and elevated CO_2 (Terrer et al., 2018; van Groenigen et al., 2014).

8.8.3 Terrestrial Carbon Sequestration

Terrestrial carbon sequestration is the sum of C contained within biomass and soils within a defined ecosystem, typically quantified on a multiyear scale (Koerner, 2006; Chapin et al., 2002). As in the 2013 Ozone ISA, most assessments of the effects of ozone on terrestrial C sequestration are from model simulations. However, an assessment of the effect of ozone on ecosystem C content at the Aspen FACE experiment was published in 2014 (Table 8-17).

- At the conclusion of the Aspen FACE experiment after 11 years of fumigation, Talhelm et al. (2014) observed that elevated ozone decreased ecosystem C content (plant biomass, litter, and soil C to 1 m in depth) by 9%. Total tree biomass C was 15% lower under elevated ozone, with decreased woody biomass accounting for nearly all (98%) of the effect on tree biomass. With the exception of surface soil C, no other individual pool of C was significantly affected by ozone. The total pool of plant and litter C was closely related to cumulative NPP, but under elevated ozone, the pool of plant and litter carbon within the aspen-only forest community was significantly smaller than expected based on NPP, meaning that the biomass C produced under elevated ozone was more quickly returned to the atmosphere.

Several new model simulations provide further support for regional- and global-scale decreases in terrestrial C sequestration as a result of ozone pollution.

- Kvalevåg and Myhre (2013) used the Community Land Model (CLM), a terrestrial earth systems model, to understand the effect of tropospheric ozone pollution on global terrestrial C sequestration from 1900−2004. Here, a model scenario that included coupling between C and N cycling produced a lower estimate of the negative effect of ozone on global terrestrial C sequestration (8−26 Pg C/year) than a method comparable to a previous assessment [31−83 Pg C/year; Sitch et al. (2007)]. However, this decrease in terrestrial C sequestration was still estimated to have contributed up to 10% of the total increase in atmospheric CO_2 that occurred between 1900 and 2004 (Kvalevåg and Myhre, 2013).
- Tian et al. (2012) applied DLEM to the southeastern U.S. (Texas to Virginia) for the period of 1895−2007. As a single factor, ozone decreased overall C storage 2%, with larger effects on broadleaf forests (−5%) and croplands (−5%) than on conifer forests (−0.3%), paralleling changes in plant growth.
- de Vries et al. (2017) created a forest productivity model (EUgrow) that was coupled with the soil biogeochemical process model VSD to predict the effects of ozone pollution and other environmental factors on forest C pools in Europe. Ozone decreased forest carbon sequestration by approximately 6%.

- Ren et al. (2012) applied the agricultural module of DLEM to understand changes in soil C storage in Chinese agricultural land caused by ozone and other environmental factors from 1980 to 2005. In this study, ozone decreased the rate of soil C sequestration by 12.6%.

The results from the Aspen FACE experiment and the model simulations provide further evidence that ozone can decrease ecosystem C sequestration. Although the decreases in NPP were temporary in the Aspen FACE experiment and UVAFME simulation, the 10% decrease in cumulative NPP at Aspen FACE was associated with a 9% decrease in ecosystem C storage (Talhelm et al., 2014). The observed changes in NPP and ecosystem C storage at Aspen FACE are in part a demonstration of the influence of stand- or ecosystem-development processes on C cycling. As stands age and develop, they acquire structural features that increase ecosystem carbon storage, such as larger pools of coarse woody debris and larger soil organic horizons (Pregitzer and Euskirchen, 2004). At Aspen FACE, elevated ozone slowed stand development (Talhelm et al., 2014; Talhelm et al., 2012). At the landscape or biome scale, C storage is controlled by the demography of individuals and stands, with landscapes comprised of stands at varying points of development following natural and anthropogenic disturbances (Koerner, 2006). Thus, without a concomitant slowing of disturbances rates and landscape stand turnover, even temporary decreases in NPP caused by ozone may be meaningful for biome-scale carbon sequestration because stands at any given time since disturbance will contain less carbon.

8.8.4 Summary

Evidence on the effect of ozone exposure on ecosystem productivity comes from many different experiments with different study designs (open top chamber experiments, long-term ecosystem manipulation chamberless exposure experiments such as Aspen FACE, SoyFACE, FinnishFace) in a variety of ecosystems and models (including empirical models using eddy covariance measures, forest productivity models parameterized with empirical physiological and tree life history data, and various well-studied ecosystem models and scenario analysis). New information is consistent with the conclusions of the 2013 Ozone ISA that **the body of evidence is sufficient to infer a causal relationship between ozone exposure and reduced ecosystem productivity.**

The relationship between ozone exposure and terrestrial C sequestration is difficult to measure at the landscape scale. Most of the evidence regarding this relationship is from model simulations, although this endpoint was also examined in a long-term manipulative chamberless ecosystem experiment (Aspen FACE). Experiments at Aspen FACE found ozone exposure caused a 10% decrease in cumulative NPP and an associated 9% decrease in ecosystem C storage. Additional studies at this research site suggests that the effects of ozone on plant productivity will be paralleled by large and meaningful decreases in soil C, but the experimental observations reviewed did not find a direct link between ozone, NPP, and soil C pools. It is likely that stand age and development and disturbance regimes are complicating factors in the partitioning of ecosystem level effects of ozone exposure on carbon sequestration. Even with these limitations, the results from the Aspen FACE experiment and the model simulations provide further

evidence that is consistent with the conclusions of the 2013 Ozone ISA that **the body of evidence is sufficient to conclude that there is a likely to be causal relationship between ozone exposure and reduced carbon sequestration in ecosystems.**

Table 8-17 Ozone exposure effects on productivity and carbon sequestration.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Productivity
Oikawa and Ainsworth (2016)	FACE; SoyFACE, Champaign, IL (40.04°N, 88.24°W)	*Glycine max* (soybean)	Plots were fumigated from ~10:00 a.m. to 7:00 p.m. daily. Average [O_3] from 10:00 a.m. to 7:00 p.m. during the growth season was 36.9 ppb in the control (ambient [O_3]) plot, while it was 39.8, 46.3, 53.9, 58.4, 71.0, 88.3, 94.2, and 115.7 ppb in the eight elevated [O_3] plots. AOT40 (the hourly accumulated exposure over a threshold of 40 ppb) of these plots were 3.3, 3.8, 9.0, 16.8, 21.0, 31.4, 47.2, 52.9, and 67.4 ppm-h, respectively	This study scales decreased photosynthesis due to O_3 from the leaf to the canopy using a model dividing leaf canopy into horizontal layers and within each layer estimates light interception by the leaves. Leaf area and leaf nitrogen (N) per plant decreased with increasing (O_3; Figure 1a and b, respectively), as did leaf canopy-level photosynthesis (Figure 4a).
Talhelm et al. (2012)	FACE; Aspen FACE, Rhinelander, WI (45.675°N, 89.625°W)	*Betula papyrifera* (paper birch), *Acer saccharum* (sugar maple), *Populus tremuloides* (five genotypes of quaking aspen)	Fumigation 1998−2008 during daylight hours of the growing season. Ambient O_3 W126 2.1−8.8 ppm-h and elevated 12.7−35.1 ppm-h; elevated CO_2 515−540 ppm , ambient average 374. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	Exposure to O_3 (+O_3 and +O_3+CO_2 vs. ambient and +CO_2) decreased leaf mass by 13%, decreased leaf area by 18%, and decreased N mass by 16%.

Table 8-17 (Continued): Ozone exposure effects on productivity and carbon sequestration.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Productivity
Talhelm et al. (2014)	FACE; Rhinelander, WI	*Betula papyrifera* (paper birch), *Acer saccharum* (sugar maple), *Populus tremuloides* (five genotypes of quaking aspen)	Fumigation 1998−2008 during daylight hours of the growing season. Ambient O_3 W126 2.1−8.8 ppm-h and elevated 12.7−35.1 ppm-h; elevated CO_2 515−540 ppm, ambient average 374. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	O_3 significantly affected ecosystem C (−9%); C in stems and branches (−17%); C in 0−10 cm mineral soil (−11%); NPP (−10%), although O_3 effects on NPP disappeared during final 7 yr of study; NPP_{tree} (−11%); canopy N (−21%). O_3 shifted fine roots toward soil surface.
Fares et al. (2013)	Gradient; Italy and California	*Pinus ponderosa*, *Quercus* spp. and *P. pinea*, *Citrus sinensis*	Ambient data study over several years at three sites. Exposure duration varied based on site from 1 to 7 yr. Ambient concentrations were grouped as follows: low (<50 ppb), medium (>50 and <75 ppb), and high (>75 ppb)	As much as 12−19% of GPP reduction was explained by stomatal O_3 deposition in ponderosa pines and citrus trees. Stomatal O_3 deposition was not found to limit GPP at the oak site, likely due to higher stomatal resistance and low exposure to ozone. Reduction in GPP was more related to stomatal O_3 deposition than to O_3 concentration.
Gustafson et al. (2013)	FACE; Aspen FACE, Rhinelander, WI; model simulations into the future	*Betula papyrifera* (paper birch), *Acer saccharum* (sugar maple), *Populus tremuloides* (four genotypes of quaking aspen)	Fumigation 1998−2008 during daylight hours of the growing season. Ambient O_3 W126 2.1−8.8 ppm-h and elevated 12.7−35.1 ppm-h; elevated CO_2 515−540 ppm, ambient average 374. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	Measured total biomass was always highest under the elevated CO_2 treatment, lowest under the O_3 treatment, and the $+CO_2+O_3$ treatment was similar to the control. The O_3 treatment significantly affected abundance of all taxa except one aspen clone. By year 180, $+CO_2$ doubled aboveground productivity and $+O_3$ decreased productivity by half. $+O_3$ reduced forest biomass at the landscape scale.

Table 8-17 (Continued): Ozone exposure effects on productivity and carbon sequestration.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Productivity
Couture et al. (2015)	FACE; Aspen FACE, Rhinelander, WI (45.70°N, 89.50°W)	(1) Foliar herbivore insects: individual species not monitored, insect frass analyzed (2) Plants: *Populus tremuloides* (aspen, genotypes 42 and 271) and *Betula papyrifera* (paper birch)	Treatments for 2006−2008 were ambient O_3 W126 = 5.6, 4.9, 2.1 ppm-h and elevated O_3 = 14.6, 13.1, 12.7 ppm-h. Ambient air CO_2 and elevated (560 ppm) CO_2. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	Elevated O_3 elicited a modest decrease in canopy damage. Insect-mediated litter inputs (insect frass and greenfall) were not impacted by elevated O_3, but N flux from the canopy to the soil decreased by 19%, and the ratio of foliar C:N increased. Elevated O_3 decreased the negative effects of herbivory on ANPP.
Zak et al. (2011)	FACE; Aspen FACE, near Rhinelander, WI (45.70°N, 89.50°W)	*Betula papyrifera* (paper birch), *Acer saccharum* (sugar maple), *Populus tremuloides* (various genotypes of quaking aspen	Treatments for 2005−2008 were ambient O_3 W126 = 7.3, 5.6, 4.9, 2.1 ppm-h and elevated O_3 = 29.6, 14.6, 13.1, 12.7 ppm-h. Ambient air CO_2 and elevated (560 ppm) CO_2. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	No effect of O_3 on NPP was observed in the 10th through 12th yr of exposure. The authors speculate this was due to compensatory growth of O_3 tolerant genotypes and species. Elevated ozone had no effect on forest floor mass, N content, or ^{15}N content.
Wang et al. (2016)	Model of species-specific biomass in a small area with "typical temperate deciduous forest in the southeast U.S."	32 tree species. Dominant tree species are *Liriodendron tulipifera* (tulip poplar), *Acer rubrum* (red maple), *Acer saccharum* (sugar maple), *Quercus alba* (white oak), *Fagus grandifolia* (American beech), and *Quercus muehlenbergii* (chinkapin oak). Other species mostly pioneer species	Each of the 32 species was ranked based on O_3 sensitivity-resistant, intermediate, or sensitive. Species-specific biomass reductions due to O_3 exposure were 0, 10, and 20% for each of the three categories, respectively	As expected, O_3 resistant species (white oak, beech) dominate and sensitive species (tulip poplar, red maple) decline over the 500-yr simulation. Overall forest biomass and forest carbon storage do not decrease over time under high O_3 conditions because growth of tolerant species is enhanced due to decreased competition by the loss of O_3 sensitive species.

Table 8-17 (Continued): Ozone exposure effects on productivity and carbon sequestration.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Productivity
Tian et al. (2012)	Model; southeastern U.S. (30−37°N, 75−100°W; includes 13 states)	10 different plant functional types were mapped across the 13 state region	AOT40 simulated from 1895−2007 using the data set by Felzer et al. (2004)	Terrestrial ecosystems were a C source from 1895−1950, and a C sink from 1951−2007. Largest contributor to increased sink was CO_2, followed by N deposition. O_3 reduced C storage by 0.58 Pg C and NPP by 2.5% during the study period. O_3 × climate and O_3 × CO_2 interactions contributed to C gains in the study even though their interactions reduced NPP; the increase O_3 × CO_2 interaction was due to increased litter quantity and increasing soil C storage. O_3 greatest impact was in the NE portion of the study area due to increased emissions from affected broadleaf forest and cropland areas.
Yue and Unger (2014)	Model; U.S.	Eight primary functional vegetation types are identified, seven natural ecosystem types and cropland. Model uses either C3 or C4 photosynthesis	Hourly and daily max 8 h taken from 2005 data set, NASA Model-E2. Validated with CASTNET and AIRDATA; plant photosynthesis model used two levels—high O_3 sensitivity and low O_3 sensitivity	Total carbon uptake is estimated to be 4.43 Pg C during the growing season across the U.S. Simulated summertime GPP was 9.5 g C/m^2-day in the eastern U.S., and 3.9 g C/m^2-day in the western U.S. when the models included the high O_3 damage effect. Average GPP for the U.S. was 6.1 g C/m^2-day. O_3 reduces GPP 4−8% in east, with 11−17% decreases in “hot spots,” and very small reductions in the western U.S. due to stomatal limitations. Over all of U.S., total summer GPP reduced by 2−5% due to O_3. A 25% reduction in O_3 is estimated to reduce GPP by only 2−4% in the eastern U.S.

Table 8-17 (Continued): Ozone exposure effects on productivity and carbon sequestration.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Productivity
Capps et al. (2016)	Model; continental U.S. (CONUS)	*Zea mays* (maize), *Gossypium* sp. (cotton), *Solanum tuberosum* (potato), *Glycine max* (soybean), *Populus deltoides* (eastern cottonwood), *Prunus serotina* (black cherry), *Populus tremuloides* (quaking aspen), *Pinus ponderosa* (ponderosa pine), *Liriodendron tulipifera* (tulip poplar), *Pinus strobus* (eastern white pine), *Pinus virginiana* (Virginia pine), *Acer rubrum* (red maple), *Alnus rubra* (red alder)	Uses U.S. EPA-developed CMAQ model to model exposure values of W126 under three regulatory scenarios (summarized in Table 1) as well as a reference (ambient) over CONUS. Maximum W126 value for the reference case with no change in W126 = 56 ppm-h. Study proposes three scenarios in which maximum local decrease in W126 is 1.3, 4, and 5.3%	At ambient ozone concentrations, production loss is greatest for potatoes, soybean, and cotton (losses of 1.5 to 1.9 eastern cottonwood and black cherry demonstrate noticeable losses at ambient O_3 concentrations, 32, and 10%, respectively. Black cherry shows the greatest potential productivity losses of 2,210 t of biomass per hectare with twice the biomass loss potential of either eastern cottonwood or ponderosa pine. The quaking aspen, tulip poplar, and various pine species also respond to ozone with potential productivity losses ranging from 0.3 to 1.9%.
Ren et al. (2012)	Model; five different regions throughout China	Not specified	Two indices of O_3 were employed in each regional simulation, both expressed as AOT40 obtained from Felzer et al. (2005) The “control” was a constant level of O_3, the treatment simulation was based on historical O_3 levels in each region for 1980 through 2005, which showed dramatic increases in O_3 starting in 1995 (see Figure 2C)	O_3 decreased NPP and SOC by 10.7 and 12.6%, respectively, across all regions. O_3 had an increasingly negative impact over the study period. Land use change was the dominant factor controlling temporal and spatial variations in NPP and SOC. The combined contributions of climate variability, CO_2, N deposition, and O_3 accounted for less than 20% of changes in NPP and SOC. However, sensitivity analyses indicated that simulated effects of O_3 were doubled when combined with other climate factors.

Table 8-17 (Continued): Ozone exposure effects on productivity and carbon sequestration.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Productivity
Betzelberger et al. (2012)	FACE; SoyFACE, Champaign, IL (40.04°N, 88.24°W)	Seven cultivars of *Glycine max* (soybean)	Soybeans in eight 20-m-diameter SoyFACE plots with O_3 concentrations for 8 h/day in two growing seasons (2009, 2010): ambient, 40, 55, 70, 85, 110, 130, 160, 200 in 2009; and ambient, 55, 70, 85, 110, 130, 150, 170, 190 in 2010. 8-h, 24-h, and 1-h max mean as well as AOT40 and SUM06 for each plot in Table 2	An exposure-response for soybean was refined from previous estimates using seven cultivars and a range of target concentrations from ambient to 200 ppb/8 h. Harvest index (partitioning of carbon into seeds) was reduced by 12% over the range (for 2009 growing season only).
Pleijel et al. (2014)	Secondary analysis of previously published data; dose-response data from eight countries and three continents; analysis not fully described	*Triticum aestivum* (wheat)	Phytotoxic O_3 Dose (POD6) metric which is the stomatal O_3 uptake above a threshold of 6 nmol/m^2-s. Stomatal conductance was estimated from VPD, temperature, solar irradiance, and phenology. POD6 ranked on a relative scale, with zero POD6 being set to 1, meaning no effect; higher POD6 ranked <1. Full details not provided in this manuscript.	Although O_3 effects (vs. charcoal-filtered air) on aboveground biomass and yield were correlated, the effect on yield was larger than on aboveground biomass. Using the EMEP model for the year 2000 to model POD6 O_3 over Europe, O_3 caused a 9% reduction in biomass but a 14% reduction in yield. Analysis suggests that O_3 accounted for over 22.2 million tonnes of lost biomass in 2000, while a similar analysis using yield would result in a loss of 38.1 million tonnes, overestimating the loss by 15.9 million tonnes.

Table 8-17 (Continued): Ozone exposure effects on productivity and carbon sequestration.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Productivity
Cheng et al. (2011)	OTC; Lake Wheeler Experimental Station, NC (35.72°N, 78.67°W)	*Triticum aestivum* (wheat)—*Glycine max* (soybean) rotation: in November to June, O_3 tolerant soft red winter wheat (Coker 9486), and in June to November, soybean (multiple cultivars over 4-yr experiment)	Full factorial O_3 and CO_2 fumigation for 4 yr: (1) charcoal-filtered control (canopy height seasonal daily 12-h avg for June−November is 19.9 ppb O_3; canopy height seasonal daily 12 h avg for November−June is 20.7 ppb O_3) with ambient CO_2 (376 ppm June−November and 388 ppm November−June); (2) elevated O_3 (canopy height seasonal daily 12-h avg for June−November is 65.7 ppb O_3; canopy height seasonal daily 12-h avg for November−June is 49.8 ppb O_3); (3) elevated CO_2 (555 ppm June−November and 547 ppm November−June); (4) elevated O_3 and elevated CO_2, as described previously	Elevated O_3 reduces C and N input to soils from senesced soybean biomass by 12%. Elevated O_3 had no effect on soil C, soil N, or fungal and bacterial soil abundances or ratio assessed by PLFA.
Ritter et al. (2011)	FACE; Kranzberger Forest, Germany (48.417°N, 11.65°E)	*Fagus sylvatica* (European beech) and *Picea abies* (Norway spruce)	Ambient and 2× ambient O_3 (maximum O_3 concentrations restricted to <150 ppb). Trees exposed for 7 yr	In the 2× O_3 treatment in beech trees, there was a significant decrease in the allocation of $^{13}CO_2$-labeled C to the stems (60%) and marginally significant increase in coarse root respiration. In spruce, flux of photosynthates to stem and coarse root respiration was slightly stimulated under elevated O_3.

Table 8-17 (Continued): Ozone exposure effects on productivity and carbon sequestration.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Productivity
Kasurinen et al. (2012)	FACE; Ruohoniemi FACE, Kuopio, University of Eastern Finland, Finland (62.88°N, 27.62°E)	Clones of four genotypes from the wild population of *Betula pendula* (silver birch), as well as their associated mycorrhizal community and fruiting bodies of mycorrhizal fungi *Laccaria laccata*	Factorial O_3 by temperature treatment: mean ambient O_3 is 23.4 in 2007, 23.8 ppb in 2008 (AOT40 0.14 ppm-h in 2007,1.6 ppm-h in 2008); mean elevated O_3 is 28.1 ppb in 2007, 32.0 ppb in 2008 (AOT40 4.9 ppm-h in 2007, 9.0 ppm-h in 2008), fumigation is 800 to 2,200 daily in growing season; temperature treatment is ambient or elevated by infrared rings	O_3 marginally increases concentration of carbon fixed by trees in the soil (based on ^{13}C pulse-tracer, $p < 0.1$).
Subramanian et al. (2015)	Model; six zones in Sweden grouped according to similar O_3 levels within each zone	*Picea abies* (Norway spruce), *Pinus sylvestris* (Scots pine), and *Betula* sp. (birch)	AOT40 values were obtained from European Monitoring and Evaluation Program. Prehistoric treatment = AOT40 of 0; ambient treatment = AOT40 based on 4-yr avg per county, Counties were grouped into six regions with AOT40 values of 13.8, 13.0, 11.8, 10.5, 8.2, 3.7, and 7.1 ppm-h; increased treatment = AOT40 2× ambient	Ambient O_3 reduced modeled Norway spruce NPP 4.3−14.8% compared to prehistoric treatment. At increased O_3 (2× ambient), reductions were 8.5−30%. Ambient O_3 reduced modeled Scots pine NPP 4.5−15.5% compared with prehistoric treatment and increased O_3 reduced NPP 8.8−31.4%. Ambient O_3 reduced modeled birch NPP 1.4−4.3%, and increased O_3 reduced NPP 2.9−9.8%. When all species combined, modeled NPP decreased 3.7–12.5% in the ambient vs. prehistoric treatment. Total reductions in C sequestration of 3.7−14.9% in Swedish forests are estimated if current O_3 levels double.
de Vries et al. (2017)	Model; Europe	Main species include *Picea abies* (Norway spruce), *Pinus sylvestris* (Scots pine), other conifers (divided into northern and southern Europe), *Quercus* sp. (oak), *Fagus sylvestris* (beech), *Betula* sp. (birch), other broadleaves (northern and southern Europe)	O_3 uptake estimated using phytotoxic O_3 dose (POD), calculated using EMEP model. Two O_3 exposure relationships, linear with total biomass, and net annual increment (NAI). For 1900−2050, simulations, comparison used a scaling factor for O_3 relative to the reference O_3 exposure in 2005. Source of O_3 data unclear	Simulated European average total C sequestration in both forests and forest soils increased by 41% between 1950 and 2000 (mainly due to increased N and CO_2), with an additional 17% increased C sequestration expected between 2000 and 2050 (due to increased CO_2 and temperature). Effect of O_3 on tree C sequestration was −4% over 150 yr simulation and from 1900−2050 was −4.5 to −5% using linear O_3 biomass function and multiplicative model. Using net annual increment function (NAI) for O_3, the effect of O_3 was about −8.5% on C sequestration, regardless of model.

Table 8-17 (Continued): Ozone exposure effects on productivity and carbon sequestration.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Productivity
Hofmockel et al. (2011)	FACE; Aspen FACE, Rhinelander, WI (49.675°N, 89.625°E)	*Populus tremuloides* (quaking aspen), *Acer saccharum* (sugar maple), and *Betula papyrifera* (paper birch)	Samples taken 2003, 2004 and 2007. Treatments for 1998-2007 were ambient O_3 W126 = 2.9−8.8 ppm-h and elevated O_3 = 13.1−35.1 ppm-h. Ambient air CO_2 and elevated (560 ppm) CO_2.For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	Elevated O_3 reduced nitrogen in coarse particulate organic matter (cPOM) and fine particulate organic matter (fPOM) by 14%, which increased C:N ratios in all soil size fractions (coarse, fine, and mineral-associated organic matter) by 2−7%. Under elevated CO_2, elevated O_3 decreased storage of newly fixed C in whole soil by 22%, while increasing the storage of older C by 21% in cPOM and by 13% in fPOM.
Kvalevåg and Myhre (2013)	Model; global	Vegetation categorized into broadleaf, needle-leaf, shrub, C3 and C4 grasses	O_3 profiles were obtained from 1900−2004 from Oslo-CTM2 chem transport model. Also tested monthly average O_3 from MOZART chem transport model. Plants grouped into O_3 sensitivity groups (broadleaf trees, needle-leaf trees, shrubs, C3 and C4 grasses). Within sensitivity groups, a low and high simulation was run to estimate lower and upper limits of expected O_3 impacts on photosynthesis	Total ecosystem C was continuously decreased by O_3 reductions in photosynthesis between 1900 and 2004. In 2004, O_3 reduced total ecosystem C by 30 to 83 Pg C/yr in the C only case, and by 8 to 26 Pg C/yr in the C-N coupling example (which simulated N limitation). In 2004, the model estimates that 3−8 ppm of CO_2 is in the atmosphere due to O_3 effects on C sequestration.

^{13}C = carbon-13 isotope; ^{15}N = nitrogen-15, stable isotope of nitrogen; AIRDATA = (U.S. EPA) Air quality data collected at outdoor monitors across the U.S.; ANPP = annual net primary productivity; AOT40 = seasonal sum of the difference between an hourly concentration at the threshold value of 40 ppb, minus the threshold value of 40 ppb; C = carbon; C3 = plants that use only the Calvin cycle for fixing the carbon dioxide from the air; C4 = plants that use the Hatch-Slack cycle for fixing the carbon dioxide from the air; CASTNET = Clean Air Status and Trends Network; CO_2 = carbon dioxide; EMEP = European Monitoring and Evaluation Programme; FACE = free-air CO_2 enrichment; GPP = gross primary productivity; N = nitrogen; nmol/m^2 = nanomoles/meters squared; NPP = net primary productivity; O_3 = ozone; PLFA = phospholipid fatty acid; Pg C = petagrams (gigaton) carbon; POD6 = Phytotoxic Ozone Dose above a threshold of 6 nmol/m^2/s; ppm = parts per million; SOC = soil organic carbon; SUM06 = seasonal sum of all hourly average concentrations ≥ 0.06 ppm; VPD = vapor pressure deficit; W126 = cumulative integrated exposure index with a sigmoidal weighting function.

8.9 Soil Biogeochemistry

The 2013 Ozone ISA (U.S. EPA, 2013) concluded there is a causal relationship between O_3 exposure and the alteration of belowground biogeochemical cycles (U.S. EPA, 2013). This causality determination was based on the body of evidence known at that time. It has been documented since the 2006 Ozone AQCD (U.S. EPA, 2006) that while belowground roots and soil organisms are not exposed directly to O_3, belowground processes could be affected by O_3 through alterations in the quality and quantity of carbon (C) supply to the soils from photosynthates and litterfall (Andersen, 2003), although few studies had been conducted at that time. The 2013 Ozone ISA (U.S. EPA, 2013) presented evidence that O_3 alters multiple belowground endpoints including root growth, soil food web structure, soil decomposer activities, soil respiration, soil C turnover, soil water cycling, and soil nutrient cycling.

The scope for new evidence reviewed in this section limits studies to those conducted in North America (Table 8-18), while recognizing that a substantial body of research has been conducted in other countries, as described in the PECOS tool (Table 8-2). The endpoints reviewed in the 2013 Ozone ISA (U.S. EPA, 2013) are not systematically reviewed in the current ISA, however, some new studies are identified. The new evidence since the 2013 Ozone ISA (U.S. EPA, 2013) included in this assessment confirms O_3 affects soil decomposition (Section 8.10.1), soil carbon (Section 8.10.2), and soil nitrogen (Section 8.10.3) and is summarized in the following section (Figure 8-8).

Some mechanisms by which O_3 alters soil biogeochemistry are discussed in other sections of this ISA. For example, soil biogeochemistry can be altered by ozone-induced changes in plant productivity (Section 8.8.1) because changes in productivity often lead to decreases on C from leaves that form soil litter. Ozone can also alter root biomass and/or distribution across the soil profile (Section 8.3), which can alter the soil organisms that depend on roots as a primary source of carbon causing changes in soil organism (1) abundance, (2) activity, or (3) community composition. Ozone-induced changes to soil microbial communities and other root-associated communities of biota (Section 8.10.2) can alter carbon cycling and nitrogen cycling belowground, which may alter emission rates of C and N from the soil to the atmosphere, as well as C and N pools within the soil. Persistent or cumulative effects of O_3 on soil C and N pools can alter ecosystem C sequestration (Section 8.8.3) and soil fertility.

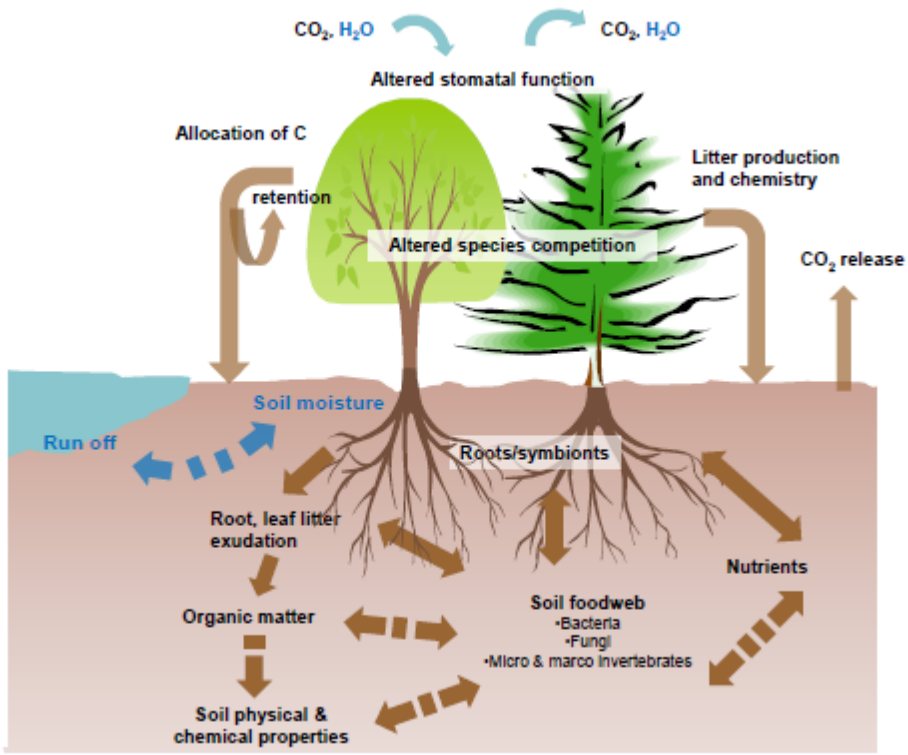


Figure 8-8 Conceptual diagram of ozone effects on belowground processes and biogeochemical cycles.

8.9.1 Decomposition

Soil decomposition is the breakdown and chemical transformation of senesced plant or animal matter by consumers (e.g., bacteria, fungus, archaea, or invertebrates). Within the soil profile, decomposition occurs most frequently in the leaf litter layer. Ozone-induced alteration of leaf chemistry can affect the rate at which a soil organism decomposes the leaf. Leaf litter chemistry was not within the scope of this review; however, it was reviewed in the 2006 AQCD (U.S. EPA, 2006) and 2013 Ozone ISA (U.S. EPA, 2013), which documented that although the responses are often species- and site-dependent, O_3 tends to alter litter chemistry. Most of the studies in the 2013 Ozone ISA evaluated forest trees and soils, with evidence often indicating mixed results. Some studies showed that ozone exposure decreases leaf litter nutrients (Liu et al., 2007; Kasurinen et al., 2006), increases leaf litter nutrients (Rodenkirchen et al., 2009; Parsons et al., 2008; Kozovits et al., 2005), or has no effect (Baldantoni et al., 2011; Rodenkirchen et al., 2009). Similarly, one study showed ozone-exposure increased leaf sugars, soluble phenolics, and fiber (Parsons et al., 2008), while another showed no effect (Kasurinen et al., 2006). The 2013 Ozone ISA (U.S. EPA, 2013) also documented that O_3 exposure via

litter nutrient alteration could be related to changes in decomposition rates, with some studies showing slight decreases and others showing no effect. Likewise, O_3 had mixed effects on the activity of cellulose-degrading enzyme that is associated with decomposer organisms, with some studies showing ozone-induced decreases and some studies showing no effect. Moreover, the 2013 Ozone ISA (U.S. EPA, 2013) documented mixed results on decomposition rates and associated metrics from O_3 exposure, with some studies showing slight reduction or increase and others showing no effect. Responses varied among species, sites, and exposure lengths.

New research from U.S. ecosystems also shows mixed results. The endpoints evaluated include the effects of O_3 on soil decomposition rates relative to labile litter (insect frass and agricultural plant residues) and recalcitrant litter (leaf litter and wood).

- *Labile litter:* Frass and crop residues are labile sources of nutrients for decomposer organisms in soils. Two studies report on O_3 effects on labile litter. A study at Aspen FACE found that elevated O_3 altered the N content, C:N, and condensed tannins of insect frass in trials of four insect species that fed on aspen leaves (Couture and Lindroth, 2014). In the OTC soy-wheat rotation at Lake Wheeler Experimental Station, NC, elevated O_3 reduced C and N inputs from soybean residues to soil by 12% (Cheng et al., 2011). These studies document that ozone-induces changes in labile litter N and C content.
- *Recalcitrant litter:* Elevated O_3 had no effect on woody litter chemistry or initial decomposition rates of *Populus tremuloides* logs or *Betula papyrifera* logs (Ebanyenle et al., 2016).

8.9.2 Soil Carbon

Soil carbon (C) is often a mix of inorganic and organic forms of C, the latter may be from living and/or dead plant, animal, fungal, archaeal, and bacterial organisms. The effects of O_3 on several aspects of soil C have been investigated. This section includes soil respiration, roots, C formation, methane emission, and perchlorate.

The 2006 Ozone AQCD (U.S. EPA, 2006) documented there was no consistent effect on soil respiration. Ozone could increase or decrease soil respiration, depending on the approach and timing of the measurements. The 2013 Ozone ISA (U.S. EPA, 2013) showed mixed results of O_3 exposure on roots (which contribute to soil respiration), and documented that long-term fumigation experiments, such as the Aspen FACE, suggested that ecosystem response to O_3 exposure can change over time. Observations made during the late exposure years can be inconsistent with those during the early years, highlighting the need for caution when assessing O_3 effects based on short-term studies. New studies since the 2013 Ozone ISA (U.S. EPA, 2013) show no effect of elevated O_3.

- *Soil respiration in crops:* New studies of CO_2 emissions from agricultural soils at SoyFACE in Illinois found no effect of elevated O_3 on CO_2 emissions, either at the site or measured in lab incubations of collected soils (Decock and Six, 2012; Decock et al., 2012).

In the 2013 ISA (U.S. EPA, 2013) it was known that O_3 could reduce the availability of photosynthates for export to roots, and thus, indirectly increase root mortality and turnover rates. The 2013 Ozone ISA (U.S. EPA, 2013) found mixed effects of O_3 on fine root biomass, with some studies finding increases (Grebenc and Kraigher, 2007; Pregitzer et al., 2006), and others finding no effect (King et al., 2001). New studies since the 2013 ISA (U.S. EPA, 2013) indicate there are ozone-induced effects on root distribution.

- *Root distribution in forests:* In Aspen FACE, 9 years of O_3 fumigation altered the distribution of tree roots across the top 1 m of the soil profile in the aspen-birch community (Rhea and King, 2012), while 11 years of O_3 fumigation shifted the distribution of tree fine roots within the mineral soil profile towards the soil surface across all tree communities (Talhelm et al., 2014). There are effects of elevated O_3 on tree root biomass in Aspen FACE, as well as in other tree and herb species.

The 2006 Ozone AQCD (U.S. EPA, 2006) documented that O_3 had the potential to alter soil C formation; however, very few experiments directly measured changes in soil organic matter content under O_3 fumigation. Studies documented in the 2013 Ozone ISA (U.S. EPA, 2013) found O_3 exposure resulted in mixed effects, either reducing or having no effect on soil C formation. Several studies have been published since the 2013 Ozone ISA (U.S. EPA, 2013) that indicate O_3 decreases soil carbon in shallow soils in some years and can differentially affect new and old carbon storage.

- *Soil carbon in forests:* In Aspen FACE, 11 years of O_3 fumigation decreased soil carbon in the top 10 cm of mineral soil by 11% (Talhelm et al., 2014). Soils sampled at Aspen FACE in the 5th, 6th, and 10th years of fumigation found no changes in total soil C pools in the top 20 cm of soil, but elevated O_3 decreased soil storage of new carbon while increasing storage of older carbon in particulate organic matter (Hofmockel et al., 2011), indicating a slowing of belowground C cycling which also affected N cycling (see Section 8.9.3).
- *Soil carbon in crops:* In an OTC soy-wheat rotation at Lake Wheeler Experimental Station, NC, elevated O_3 had no measurable effect on total soil organic C or extractable (K_2SO_4) soil C (Cheng et al., 2011). O_3 effects on soil C can alter long-term carbon storage in soils and plant biomass; for more on this topic, see Section 8.8.3 on terrestrial C sequestration.

No information was published in the 2006 O_3 AQCD (U.S. EPA, 2006) or the 2013 Ozone ISA (U.S. EPA, 2013) about root symbiont carbon endpoints (biomass or respiration). New information published since 2013 indicates O_3 had no effects.

- *Root symbionts in forests:* At Aspen FACE, elevated O_3 had no effect on hyphal biomass production, hyphal respiration, or sporocarp respiration by the mycorrhizal fungal symbionts associated with tree roots (Andrew et al., 2014). Effects of O_3 on the community composition of root-associated organisms are addressed in Section 8.10.2.

The 2013 Ozone ISA (U.S. EPA, 2013) found O_3 alters CH_4 emissions (Toet et al., 2011; Zheng et al., 2011; Morsky et al., 2008), and dissolved organic carbon (DOC) in U.K. peatland fen pore water (Jones et al., 2009). There are no new studies conducted in U.S. ecosystems that addressed the effects of O_3 on soil CH_4 emissions or DOC.

The 2013 Ozone ISA (U.S. EPA, 2013) did not address how O_3 reacts directly with soil particles and solutions. A recent study proposed that O_3 would react with soil to form perchlorate, which can be taken up by crop plants and could affect human consumers. In a greenhouse experiment, O_3 exposure of 204 ppb increased soil perchlorate concentration, while 102 ppb O_3 had no effect (Grantz et al., 2014). The study looked at leaf content and found no evidence of perchlorate in leaves.

8.9.3 Soil Nitrogen

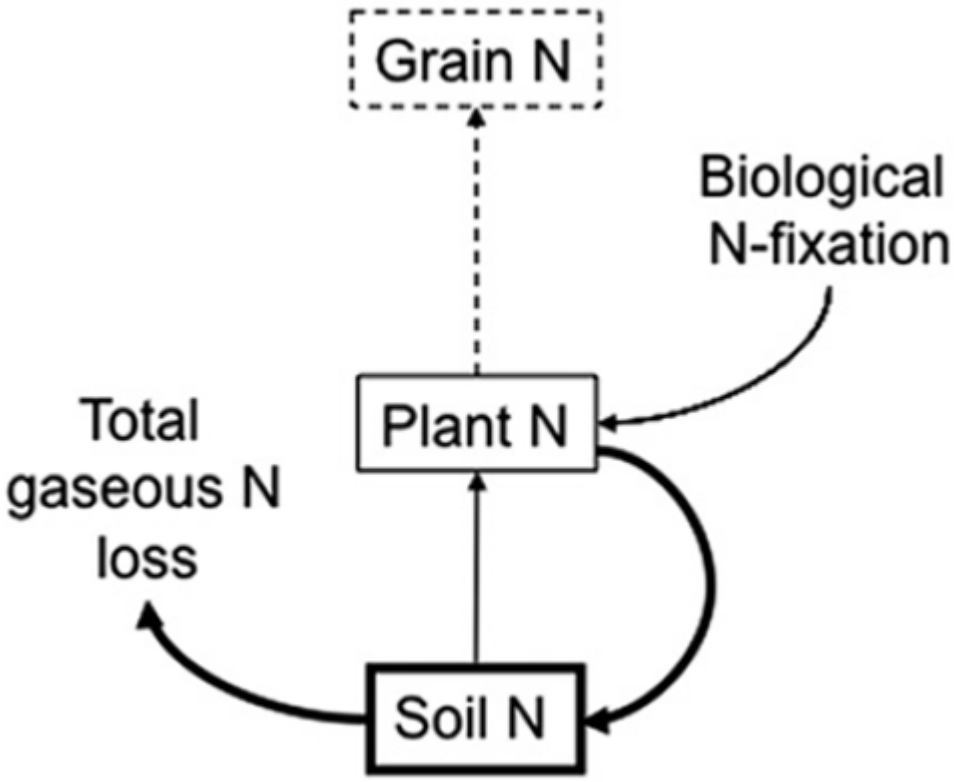
O_3 can alter the cycling of nitrogen in the soil via its direct effect on plants. Nitrogen is an important element to plant life as it is often the limiting nutrient for most temperate ecosystems. Nitrogen cycling may be measured by many different specific N pools or processes. The 2013 Ozone ISA (U.S. EPA, 2013) documented mixed results of O_3 effects on soil N pools and processes, with results indicating no effect in meadow N biomass or potential nitrification and denitrification (Kanerva et al., 2006). While ozone was shown to increase N release from litter in a forest (Stoelken et al., 2010), ozone decreased gross N mineralization (Holmes et al., 2006) at Aspen FACE and N release from soil litter (Liu et al., 2007). While in crops, O_3 decreased soil mineral N content (Pujol Pereira et al., 2011). In addition to empirical results, a model simulation for O_3 effects on N soil retention/stream flow showed that O_3 exposure decreased N retention, increasing stream export (Hong et al., 2006).

More recent studies from Aspen FACE and SoyFACE continue to find effects of O_3 on N cycling in soils by measuring endpoints of soil N, root N uptake, N transformations, and N emissions. These results support the findings in the 2013 Ozone ISA (U.S. EPA, 2013), showing that in forests, O_3 may decrease soil N content in some studies but have no effect in others and that O_3 did not affect forest root uptake of N. In crops, ozone did not affect soil N in field studies and showed mixed results, depending on the N chemical species, in lab studies.

- *Soil N in forests:* In Aspen FACE, elevated O_3 increased C:N ratios of soil organic matter by decreasing the N content of particulate organic matter (Hofmockel et al., 2011). At the stand level, elevated O_3 in Aspen FACE did not change the amount of N stored in the litter layer on the forest floor (Zak et al., 2011).
- *Soil N in crops:* In the OTC soy-wheat rotation at Lake Wheeler Experimental Station, NC, elevated O_3 had no measurable effect on soil N (Cheng et al., 2011). A set of studies conducted at SoyFACE found no effects of elevated O_3 on soil N or soil ^{15}N (Decock et al., 2012), although elevated O_3 significantly decreased surface soil ammonium in the field, and significantly increased soil nitrate in both field and lab incubation studies (He et al., 2014; Decock and Six, 2012).
- *Root N uptake in forests:* Elevated O_3 did not affect Aspen FACE stand uptake of a ^{15}N tracer and the incorporation of this ^{15}N into leaves (Zak et al., 2011), although there were differences between aspen genotypes in N uptake (see terrestrial community).
- *N transformations in crops:* Elevated O_3 in SoyFACE did not affect soil N transformation rates measured by ^{15}N tracers in incubations (Decock and Six, 2012), but decreased the abundance of

multiple microbial N cycling genes in surface soils (He et al., 2014). A model constructed using SoyFACE natural abundance ^{15}N values suggests that elevated O_3 accelerated N cycling by increasing both soybean belowground N allocation and N_2 emissions from soil [Decock et al. (2012); see Figure 8-9 below].

- *N emissions from meadow:* The 2013 ISA (U.S. EPA, 2013) found that elevated O_3 emissions decreased daily N_2O emissions in a Finnish meadow (Kanerva et al., 2007). At SoyFACE in Illinois, elevated O_3 did not alter N_2O emissions, but a model suggested that O_3 may affect N_2 emissions (Decock et al., 2012).



Note: Dashed lines indicate decreases, thin solid lines indicate no major change, and thick solid lines represent increases.
Source: Permission pending Decock et al. (2012).

Figure 8-9 Elevated ozone effect of accelerated senescence and reduced seed production soil N.

8.9.4 Summary

The 2013 Ozone ISA (U.S. EPA, 2013) presented evidence that O_3 was found to alter multiple belowground endpoints including root growth, soil food web structure, soil decomposer activities, soil respiration, soil C turnover, soil water cycling, and soil nutrient cycling. New evidence since the 2013 Ozone ISA (U.S. EPA, 2013) included in this assessment confirms O_3 effects on soil decomposition (Section 8.10.1), soil carbon (Section 8.10.2), and soil nitrogen (Section 8.10.3).

Decomposition of leaf litter in the soil may be altered by ozone-induced alteration of leaf chemistry. Leaf litter chemistry was not within the scope of this review; however, it was reviewed in the 2006 AQCD (U.S. EPA, 2006) and 2013 Ozone ISA (U.S. EPA, 2013). The 2013 Ozone ISA (U.S. EPA, 2013) documented mixed results on ozone-exposure effects on leaf litter decomposition with some studies showing slight reduction others showing no effect. Responses varied among species, sites, and exposure lengths. New studies in the current review do not change these observations.

There are new studies on the effects of ozone on several endpoints associated with soil C: soil respiration, root mortality, root symbionts and soil C formation. The 2006 Ozone AQCD (U.S. EPA, 2006) and the 2013 Ozone ISA (U.S. EPA, 2013) documented no consistent effect on soil respiration. New studies since the 2013 ISA show no effect of elevated O_3 on soil respiration. The 2013 ISA (U.S. EPA, 2013) documented that ozone could increase root mortality and turnover rates by reducing the availability of photosynthates for export to roots, while studies showed mixed effects of ozone on fine root biomass, with some studies finding increases and others finding no effect. New studies since the 2013 ISA indicate an ozone-induced effect on a new endpoint: root distribution. Studies documented in the 2013 Ozone ISA found ozone exposure resulted in mixed effects, either reducing or having no effect on soil C formation. Several new studies indicate O_3 decreases soil carbon in shallow forest soils. No information was published in the 2006 Ozone AQCD (U.S. EPA, 2006) or the 2013 Ozone ISA (U.S. EPA, 2013) about root symbiont carbon endpoints (biomass or respiration). New information published since 2013 indicates ozone had no effects on carbon in root symbionts. Overall, these new findings support the conclusions from the 2013 Ozone ISA (U.S. EPA, 2013) that there is no consistent effect of ozone on soil respiration and soil carbon formation. New evidence indicates ozone has effects on root distribution within the soil profile and no effect on carbon in root symbionts.

Ozone can alter the cycling of nitrogen in the soil via its direct effect on plants. Nitrogen is an important element to plant life as it is often the limiting nutrient for most temperate ecosystems. Nitrogen cycling may be measured by many different specific N pools or processes. The 2013 Ozone ISA (U.S. EPA, 2013) documented mixed results of ozone effects on soil N pools and processes, with results indicating no effect (NH_4^+ immobilization, gross nitrification, microbial biomass N and soil organic N), increasing rates (N release from litter), or decreasing rates/concentrations (gross N mineralization, soil mineral N content, and N release from soil litter). More recent studies from Aspen FACE and SoyFACE continue to find effects of ozone on N cycling in soils by measuring endpoints of soil N, root N uptake, N transformations, and N emissions. These results support the findings in the 2013 Ozone ISA (U.S. EPA,

2013) showing that in forests ozone may decrease soil N content in some studies, but have no effect in others. Also ozone did not affect forest root uptake of N. In crops, ozone did not affect soil N in field studies and showed mixed results, depending on the N chemical species, in laboratory studies.

Overall, the evidence does not change the conclusions from the 2013 Ozone ISA (U.S. EPA, 2013), and therefore, suggests that ozone can alter soil biogeochemical cycling of carbon and nitrogen, although the direction and magnitude of these changes often depends on the species, site, and time of exposure. Currently, there does not appear to be a consistent exposure-response relationship. **The body of evidence is sufficient to conclude that there is a causal relationship between ozone exposure and the alteration of belowground biogeochemical cycles**.

Table 8-18 Response of belowground processes and biogeochemical cycles to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Soil Biogeochemistry Endpoints
Decock et al. (2012)	FACE; SoyFACE, Illinois (40.056°N, 88.201°W)	(1) Soil collected from FACE for lab incubations in 2008, (2) field measurements of N_2O and CO_2 emissions in 2005 and 2006 soybean seasons, (3) soils collected from FACE for natural abundance ^{15}N study in July 2006	Ambient and elevated O_3 (target concentration of 1.23× ambient 2002−2006, and 2× ambient 2007−2008), in factorial combination with elevated CO_2. From 2001 to 2008, annual ambient average tropospheric O_3 concentrations ranged from 37.3 to 62.8 ppb	In lab soil incubations, six growing seasons of elevated O_3 did not affect soil N_2O emissions. Field measurements under 1.5× O_3 in 2005 and 2006 soybean seasons found no effect of elevated O_3 on CO_2 or N_2O emissions. Soils sampled in 2006 showed that four growing seasons of elevated O_3 had no effect on soil N or soil ^{15}N in soybean rhizosphere or bulk soil. Models of soil ^{15}N suggest that under elevated O_3, long-term soybean inputs of N to rhizosphere and bulk soil increase, while N losses from bulk soil increase (Figure 8-9 for conceptual diagram).
Decock and Six (2012)	Lab; SoyFACE, Illinois (40.056°N, 88.201°W)	Study in soybean (*Glycine max*) agroecosystem	Soils for the study collected from soybean plots at the SoyFACE agroecosystem with ambient and elevated O_3 (target concentration of 1.23× ambient)	Elevated O_3 significantly increased soil NO_3^-, and briefly increased soil NH_4^+ early in the incubation. Elevated O_3 did not affect mineral N transformation rates as determined by ^{15}N tracers, and did not affect potential CO_2 or N_2O emission rates.

Table 8-18 (Continued): Response of belowground processes and biogeochemical cycles to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Soil Biogeochemistry Endpoints
He et al. (2014)	FACE; SoyFACE, Illinois (40.056°N, 88.201°W)	Soil microbial community under soybean (*Glycine max*)	Ambient (~37.9 ppb), elevated O_3 (~61.3 ppb), soybeans also grown under elevated CO_2 (~550 ppm) and elevated CO_2 + elevated O_3	In elevated O_3 soybean crop surface soils, abundance of *niFH*, *narG*, *norB,* and *ureC* N-cycling genes were significantly decreased. There were no significant differences in the subsoil. For C cycling genes in elevated O_3 soil samples, most were unchanged while fungal arabinofuranosidase and endoglucanse increased significantly and xylanase, cellobiase and exochitanase decreased significantly. Soil N was quantified and NH was significantly lower in the surface soil and NO_3 significantly higher in subsoil of elevated O_3 plots compared to ambient.
Paudel et al. (2016)	Greenhouse; Parlier, CA	Palmer amaranth (*Amaranthus palmeri*	Two runs of exposure 30 and 35 days. 12-h means of 4, 59, and 114 ppb	Elevated O_3 exposure and water stress had no effect on root growth. This weed species may have much more tolerance to elevated O_3 and moisture stress compared to crops with which it competes.

Table 8-18 (Continued): Response of belowground processes and biogeochemical cycles to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Soil Biogeochemistry Endpoints
Cheng et al. (2011)	OTC; Lake Wheeler Experimental Station, NC (35.72°N, 78.67°W)	Wheat-soybean rotation: in November to June, O_3 tolerant soft red winter wheat (Coker 9486), and in June to November, soybean (multiple cultivars over 4-yr experiment)	Full factorial ozone and carbon dioxide fumigation for 4 yr: (1) charcoal-filtered control (canopy height seasonal daily 12-h avg for June−November is 19.9 nL/L O_3; canopy height seasonal daily 12-h avg for November−June is 20.7 nL/L O_3) with ambient CO_2 (376 μL/L June−November and 388 μL/L November−June); (2) elevated O_3 (canopy height seasonal daily 12-h avg for June−November is 65.7 nL/L O_3; canopy height seasonal daily 12-h avg for November−June is 49.8 nL/L O_3); (3) elevated CO_2 (555 μL/L June−November and 547 μL/L November−June); (4) elevated O_3 and elevated CO_2, as described previously	Elevated O_3 reduces C and N input to soils from senesced soybean biomass by 12%. Elevated O_3 had no effect on soil C, soil N, or fungal and bacterial soil abundances or ratio assessed by PLFA.

Table 8-18 (Continued): Response of belowground processes and biogeochemical cycles to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Soil Biogeochemistry Endpoints
Rhea and King (2012)	FACE; Aspen FACE, Rhinelander, WI (49.675°N, 89.625°E)	Quaking aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*)	Treatments up until the 2005 (when root samples were taken): ambient average W126 was 5.2 ppm-h and elevated O_3 was 27.3 ppm-h. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	This study assessed fine root responses to O_3 at deeper soil depths than typically studied. Fine root biomass in all-aspen (AA) and aspen-birch (AB) plots fumigated with ozone differed by community and soil depth. Biomass increased with depth in the AA (aspen clones) community by 10.2, 36.4, and 34.8 % in the upper, middle, and lower soil layer relative to the control. In the AB (aspen-birch) community root biomass decreased 16% in the shallow layer with a small increase at the middle soil layer resulting in a total decrease of 11% across all layers. Total root length increased in the AA community to a greater extent than the AB community where smaller increases and some decreases were observed. The authors suggested compensatory root growth occurred in the AB community where a decrease in length in the shallowest layer was mitigated by increased growth at the middle layer. A 33% decrease in root tissue density was observed across all soil layers in trees exposed to O_3. Specific root length increased with soil depth and O_3 with the greatest increases in the AA community.
Talhelm et al. (2014)	FACE; Aspen FACE, Rhinelander, WI (49.675°N, 89.625°E)	Aspen (*Populus* sp.), paper birch (*Betula papyrifera*), sugar maple (*Acer saccharum*)	Fumigation 1998−2008 during daylight hours of the growing season. Ambient O_3 W126 2.1−8.8 ppm-h and elevated 12.7−35.1 ppm-h; elevated CO_2 515−540 ppm, ambient average 374. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	Significant O_3 effects: Ecosystem C (−9%); mineral soil C 0−10 cm (−11%); canopy N (−21%); O_3 shifted fine roots toward soil surface.

Table 8-18 (Continued): Response of belowground processes and biogeochemical cycles to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Soil Biogeochemistry Endpoints
Couture and Lindroth (2014)	FACE; Aspen FACE, Rhinelander, WI (49.675°N, 89.625°E)	(1) Insect: gypsy moths (*Lymantria dispar*), forest tent caterpillars (*Malacosoma disstria*), white-marked tussock moths (*Orgyia leucostigma*), cecropia moths (*Hyalophora cecropia*) (2) Plants: single aspen genotype (42E, *Populus tremuloides*) and paper birch (*Betula papyrifera*)	Treatments for 1998−2007 were ambient O_3 W126 = 2.9−8.8 ppm-h and elevated O_3 = 13.1−35.1 ppm-h. Ambient air CO_2 and elevated (560 ppm) CO_2. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	Aspen grown under elevated O_3 (1) decreased frass N 26% from forest tent caterpillar, 16% from white marked tussock moths, and 12% from gypsy moths; (2) increased frass C:N 37% from forest tent caterpillar, 18% from white marked tussock moths, and 16% from gypsy moths; and (3) increased frass condensed tannins 37% from forest tent caterpillar, 17% from white marked tussock moths, and 17% from gypsy moths. Frass and aspen leaf litter chemistry were correlated, but elevated O_3 decreased the relative C:N of frass to leaf litter by 35% and increased the relative tannin concentration of frass to leaf litter by 20%.
Andrew et al. (2014)	FACE; Aspen FACE, Rhinelander, WI (49.675°N, 89.625°E)	Mycorrhizal fungi associated with aspen	Fumigation 1998−2008 during daylight hours of the growing season. Ambient O_3 W126 2.1−8.8 ppm-h and elevated 12.7−35.1 ppm-h; elevated CO_2 515−540 ppm, ambient average 374 ppm. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	No significant difference with treatment on net hyphal biomass production. No significant effects of treatment on hyphal respiration per unit or hyphal and sporocarp respiration on a mass-specific basis.
Zak et al. (2012)	FACE; Aspen FACE, Rhinelander, WI (49.675°N, 89.625°E)	Five genotypes of quaking aspen (*Populus tremuloides*) together; *P. tremuloides* genotype (216) with paper birch (*Betula papyrifera*); and *P. tremuloides* genotype (216) with sugar maple (*Acer saccharum*)	Fumigation 1998−2008 during daylight hours of the growing season. Ambient O_3 W126 2.1−8.8 ppm-h and elevated 12.7−35.1 ppm-h; elevated CO_2 515−540 ppm, ambient average 374 ppm. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	Elevated O_3 altered inter-specific competition for N in aspen, increasing genotype 8 plant N 93% and plant ^{15}N 171%, and decreasing genotype 271 plant N 40% and plant ^{15}N 27%, with no effect on plant competitiveness for N for the remaining three genotypes. Elevated O_3 did not alter species competition for soil N (measured as plant N and plant ^{15}N).

Table 8-18 (Continued): Response of belowground processes and biogeochemical cycles to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Soil Biogeochemistry Endpoints
Zak et al. (2011)	FACE; Aspen FACE, Rhinelander, WI (49.675°N, 89.625°E)	Paper birch (*Betula papyrifera*), sugar maple (*Acer saccharum*), various genotypes of quaking aspen (*Populus tremuloides*)	Treatments for 2005−2008 were ambient O_3 W126 = 7.3, 5.6, 4.9, 2.1 ppm-h and elevated O_3 = 29.6, 14.6, 13.1, 12.7 ppm-h. Elevated CO_2 treatment (560 ppm), ambient CO_2. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	Elevated ozone had no effect on forest floor mass, N content, or ^{15}N content in years 10−12 of the experiment.
Chieppa et al. (2015)	OTC; research field 5 km north of Auburn, AL	Loblolly pine (*Pinus taeda*) inoculated with root-infecting ophiostomatoid fungi (either *Leptographium terebrantis* or *Grosmannia huntii*, fungal species associated with Southern Pine Decline)	Three ozone treatments in OTCs (12 h/day): charcoal filtered (~0.5% ambient air), nonfiltered air (ambient), and 2× ambient. The 1st 41 days were acclimatization then exposure continued 77 more days once seedlings were inoculated with fungus. Mean 12-h O_3 over the 118 days was 14 (CF), 23 (NF), and 37 (2×) ppb in the treatments. 12-h AOT40 values were .027 (CF), 1.631 (NF), and 31.2 (2×) ppm. Seasonal W126 were 0.033 (CF), 0.423 (NF), and 21.913 (2×)	Seedlings under 2× O_3 had greater belowground dry matter yield than CF seedlings. Fungal lesion length was greater on 2× O_3 exposed seedlings but was not specific to either fungal species.
Ebanyenle et al. (2016)	FACE; Aspen FACE, Rhinelander, WI (49.675°N, 89.625°E)	Wood-decaying basidiomycete fungi (identified by sequencing of primer pair ITS1f/ITS4), growing on logs cut in 2009 from quaking aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*). Logs were grown and placed in each of the 4 FACE treatments in a full factorial design	Fumigation 1998−2008 during daylight hours of the growing season. Ambient O_3 W126 2.1−8.8 ppm-h and elevated 12.7−35.1 ppm-h; elevated CO_2 515−540 ppm, ambient average 374 ppm. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	O_3 had no effect on 1 yr of decomposition of logs, through effects on wood quality or effects on soil decomposition conditions. There was no statistically significant effect of O_3 on basidiomycete community composition, although there were fewer fungal species from logs in the O_3 plots than in the ambient O_3 plots.

Table 8-18 (Continued): Response of belowground processes and biogeochemical cycles to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Soil Biogeochemistry Endpoints
Hofmockel et al. (2011)	FACE; Aspen FACE, Rhinelander, WI (49.675°N, 89.625°E)	Quaking aspen (*Populus tremuloides*), sugar maple (*Acer saccharum*), and paper birch (*Betula papyrifera*)	Samples taken 2003, 2004 and 2007. Treatments for 1998−2007 were ambient O_3 W126 = 2.9−8.8 ppm-h and elevated O_3 = 13.1−35.1 ppm-h. Ambient air CO_2 and elevated (560 ppm) CO_2. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	Elevated O_3 reduced nitrogen in coarse particulate organic matter (cPOM) and fine particulate organic matter (fPOM) by 14%, which increased C:N ratios in all soil size fractions (from largest to smallest: coarse, fine, and mineral-associated organic matter) by 2−7%. Under elevated CO_2, elevated O_3 decreased storage of newly fixed C in whole soil by 22%, while increasing the storage of older C by 21% in cPOM and by 13% in fPOM.
Grantz et al. (2014)	Greenhouse; UC-Riverside, CA	Soybean, Pima cotton, bush bean, sorghum, maize	As 12-h means of 4 nL/L, 102 nL/L, 204 nL/L	The highest O_3 exposure (204 nL/L) increases perchlorate concentration in the potting soil 39% over soil perchlorate under the unexposed potting mix.
Tian et al. (2012)	Model; southeastern U.S., 75−100° west longitude, 30−37° north latitude. Includes 13 states	10 different plant functional types were mapped across the 13-state region	AOT40 simulated from 1895−2007 using data set by Felzer et al. (2004)	Southeast terrestrial ecosystems were a C source from 1895−1950, and a C sink from 1951−2007. Largest contributor to increased sink was CO_2, followed by N dep. O_3 reduced C storage by 0.58 Pg C during the period. The greatest impact by O_3 was in the northeast region of the study area due to increased emissions from impacted broadleaf forest and cropland areas.

^{15}N = nitrogen-15, stable isotope of nitrogen; AOT40 = seasonal sum of the difference between an hourly concentration at the threshold value of 40 ppb, minus the threshold value of 40 ppb; CF = charcoal-filtered air; CO_2 = carbon dioxide; FACE = free-air CO_2 enrichment; ITS1f/ITS4 = fungal specific primer pair; N_2O = nitrous oxide; NH_4^+ = ammonium; nL/L = nanoliters/liter; NO_3^- = nitrate; O_3 = ozone; OTC = open-top chamber; PLFA = phospholipid fatty acid; Pg C = petagrams (gigaton) carbon; ppb = parts per billion; ppm = parts per million; µl/L = microliters/liter; W126 = cumulative integrated exposure index with a sigmoidal weighting function.

8.10 Terrestrial Community Composition

In the 2013 Ozone ISA the evidence was sufficient to conclude there is a likely to be causal relationship between ozone exposure and alteration of terrestrial community composition of some ecosystems (U.S. EPA, 2013). Ozone altered aboveground plant communities such as conifer forests, broadleaf forests, and grasslands, and altered fungal and bacterial communities in the soil in both natural and agricultural systems (U.S. EPA, 2013). Ozone effects on individual plants can alter the larger plant community as well as the belowground community of microbes and invertebrates, which depend on plants as carbon sources. Ozone may alter community composition by uneven effects on co-occurring species, decreasing the abundance of sensitive species and giving tolerant species a competitive advantage (Figure 8-10). Field studies linked ozone exposure to conifer decline in the San Bernardino Mountains, in the Valley of Mexico, in alpine regions of southern France, and in the Carpathian Mountains. Evidence suggesting ozone-induced changes to competitive interactions among trees in broadleaf forests was drawn from the experiment at Aspen FACE and from European phytotron studies. Grassland studies suggested that ozone alters the ratios of grasses, forbs, and legumes in these communities to favor grasses, and that annual plant species are more sensitive to ozone than perennial species. In terms of belowground communities, sampling from FACE and mesocosm studies suggested that ozone altered fungal communities, including mycorrhizal species on which plants depend for water and nutrient acquisition, as well as bacterial communities.

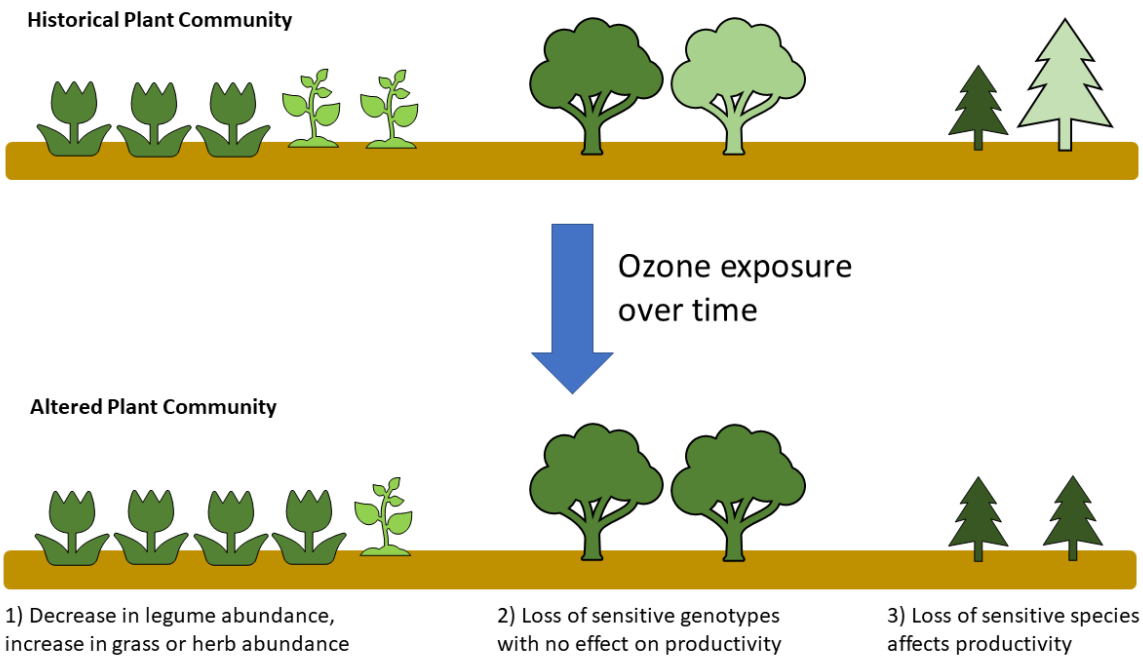


Figure 8-10 Mechanisms by which ozone alters plant communities.

As described in the PECOS tool (Table 8-2), studies on community composition will be considered for inclusion regardless of geography. Data of ozone alterations to suborganismal processes in eukaryotes (e.g., effects on gene expression, molecular or chemical composition) will not be reviewed from these studies. However, many microbial species in soil cannot be cultured in the laboratory, and sampling and analysis of biologically derived chemicals and genes within the soil represent standard methods for assessing microbial communities; these observations will be included. This section focuses on effects of ozone on groups of different species including, for the first time, effects on plants grouped by their shared phylogeny and on communities of animal consumers. The role of ozone in altering community composition continues to be an area of active research in the U.S. and in other regions of the world (Table 8-20).

8.10.1 Plant Community

8.10.1.1 Forest

In the 2013 Ozone ISA, evidence of ozone effects on forest composition was drawn from observational studies of conifer decline correlated with ozone exposure (Allen et al., 2007; de Lourdes de

Bauer and Hernandez-Tejeda, 2007; Wieser et al., 2006; Fenn et al., 2002; McBride and Laven, 1999; Miller, 1973) and from controlled exposure studies of broadleaf tree species in which ozone altered the growth or mortality of sensitive genotypes or species when sensitive and tolerant trees were grown together (Kubiske et al., 2007; Kozovits et al., 2005). New evidence suggests that ozone alters tree competitive interactions for nutrients, which partially determine forest community composition:

- Consistent with previous research on altered tree community composition at Aspen FACE, an empirical ^{15}N tracer study there showed that elevated ozone altered the relative competition for nutrients among aspen genotypes (Zak et al., 2012).

New studies extend the scope of evidence regarding forest community composition beyond the observational and controlled exposure studies summarized in the 2013 Ozone ISA to include synthesis and models (see also Section 8.4.3):

- Models using Aspen FACE data illustrate how ozone effects on tree biomass and productivity can scale to affect community composition at the genotype and species level. In models of aspen genotype survival and mortality, elevated ozone altered genotype abundance and exerted a selective pressure on aspens (Moran and Kubiske, 2013). In simulations using Aspen FACE data of northern forests at the landscape level over centuries, elevated ozone altered species abundance and the speed of replacement and succession (Gustafson et al., 2013).
- A model of forest community development through time that included ozone effects on biomass without including ozone effects on competitive interactions showed that ozone effects on biomass alone can alter community succession within a century (Wang et al., 2016). This study used published peer-reviewed data to place tree species into three sensitivity classes, applied either a 0, 10 or 20% growth reduction to species in the University of Virginia Forest Model Enhanced (UVAFME), a gap model which tracks the growth and survival of individual trees and species within a stand. This provides additional biological plausibility to the finding of the 2013 Ozone ISA that differences between species in ozone sensitivity leads to decline of ozone-sensitive trees in terrestrial communities.

8.10.1.2 Grassland and Agricultural Land

In the 2013 Ozone ISA, there was evidence of ozone effects on grassland community composition in controlled experimental exposure studies, in models, and in reviews. Experimental exposure or model studies found ozone shifted grass:legume dominance (Hayes et al., 2009; Volk et al., 2006) or grass:forb dominance (Hayes et al., 2010) in grassland communities. High environmental heterogeneity made it difficult to ascribe causality solely to ozone for changes in plant community composition in several European experiments (Stampfli and Fuhrer, 2010; Bassin et al., 2007; Volk et al., 2006), while high annual variation in a U.S. study of agricultural weeds had a stronger impact on plant community composition than did detected effects on ozone-sensitive species (Pfleeger et al., 2010).

Key new studies include experimental ozone exposures that allow evaluation of ozone effects on grassland community composition in analyses that explicitly include environmental or annual heterogeneity. In a seeded pasture of three legume, two grass, and one forb species in Spain, ozone was a

more powerful explanatory factor than N for plant community biomass variation and explained 8−11% of community biomass variation (Calvete-Sogo et al., 2016). In a restored English grassland of 47 species, ozone accounted for 10 and 40% of variation in herb and legume species composition in the 1st and 2nd years, respectively, of fumigation (Wedlich et al., 2012).

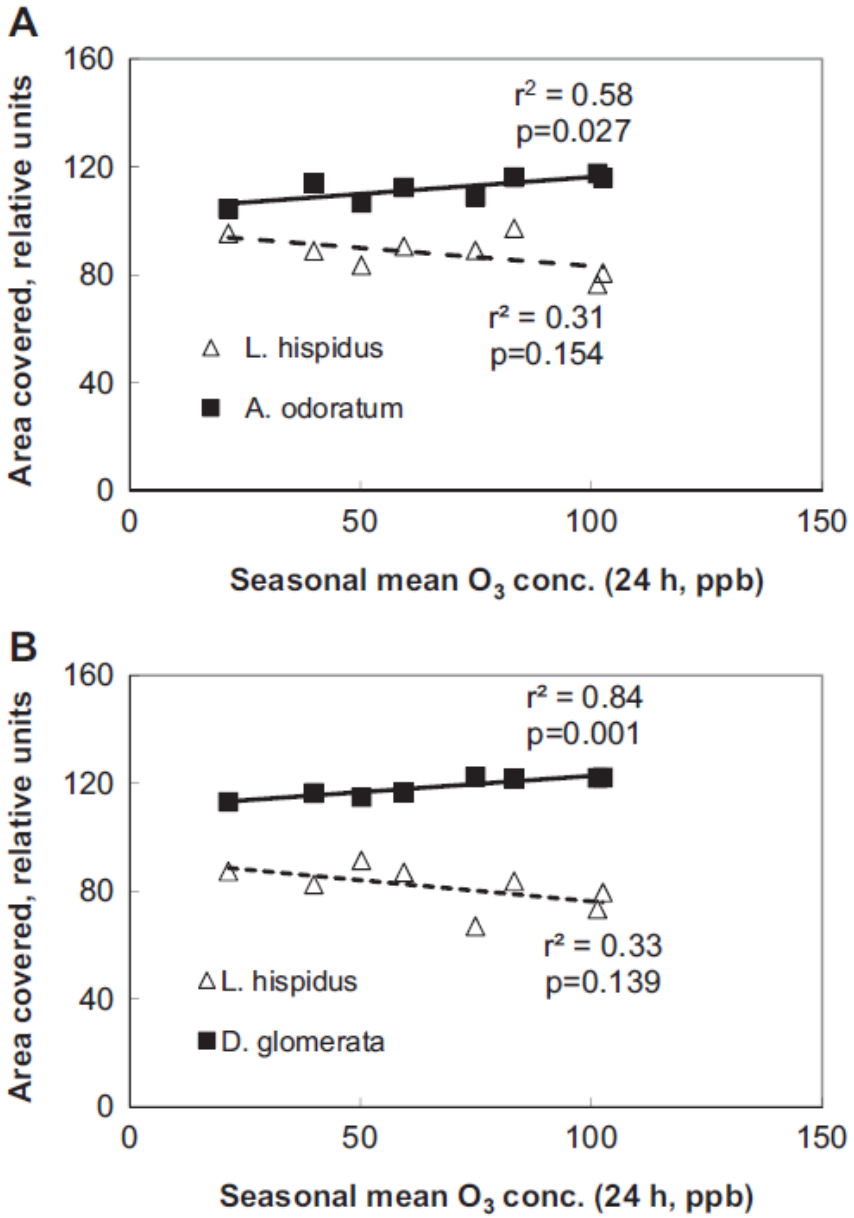
The 2013 Ozone ISA included a review that identified grasslands as the most sensitive European plant communities to ozone effects (Mills et al., 2007) and another that identified annual plants or plants with high leaf N concentration as particularly sensitive to ozone concentration in European species (Hayes et al., 2007).

New studies include a synthesis and a large-scale gradient exposure observational study that confirm these findings while setting critical levels to protect European grasslands. Across an ambient gradient of 64 grasslands in the U.K., ozone is the strongest predictor of plant species cover in single factor models, with a change in species composition at an AOT40 of 3.1 ppm hour; and there are species associated with low ozone sites and species associated with high ozone sites [see Table 8-18 for full description; Payne et al. (2011)].

Another set of new studies synthesized ozone response information from an array grassland and herbaceaus species from experiments around the world (Bergmann et al., 2017; van Goethem et al., 2013). Many of the species in these synthesis studies occur in the U.S. While these syntheses did not specifically measure community composition, they do demonstrate that different species differ in ozone sensitivity and is a mechanism for affecting community composition in grassland and agricultural lands. In an analysis of ozone exposure-biomass loss studies of 25 annual grassland species and 62 perennial grassland species that occur in northwestern Europe, annuals were significantly more sensitive to ozone than were perennial species, with a projected 10% reduction in biomass across the community of grassland annual plants at an AOT40 of 0.8 ppm hour and a 10% reduction in perennial grassland community biomass at an AOT40 of 1.1 ppm hour (van Goethem et al., 2013). There are 17 species from this analysis that are native to the U.S. according to the USDA PLANTS database (USDA, 2015), 12 of which experience biomass reduction in response to ozone (see also Section 8.13.2). In a global synthesis of ozone effects on plants (Bergmann et al., 2017), 47.5% of the 223 herbaceous plant species experimentally exposed to ozone experienced effects in growth, productivity, C allocation, or reproduction. This synthesis of all tested plant species to ozone exposure suggests that in considering how ozone may shift composition from a mix of tolerant and sensitive species to a community of only tolerant species, the sensitive species affected include roughly half of tested herbaceous species. The two syntheses described have the strength of combining the results of many researchers, but there are some limitations to this approach that include variation in experimental design, intra-species variation in response, and potential differences in species response when grown together in competition.

New studies confirm the 2006 Ozone AQCD and the 2013 Ozone ISA finding that ozone can shift community composition towards ozone-tolerant grass species:

- In a seeded pasture of leguminous clover and three grass species in Alabama, experimentally elevated ozone (56 ppb ozone) increased the biomass of grass species but had no effect on clover biomass (Gilliland et al., 2016), effectively increasing the relative biomass of grass to legumes in the community.
- In a greenhouse study of grass and forbs in competition, grass cover increased linearly with elevated ozone in a 12-hour mean range of 21 to 103 ppb [Hayes et al. (2011); Figure 8-11].
- In an experimental fumigation in a Swiss high-elevation pasture reported in Bassin et al. (2007), there was no effect of 7 years of elevated ozone on relative abundance of plants grouped as forbs, grasses, or sedges, but elevated ozone did increase the abundance of a dominant grass species in the community (Bassin et al., 2013).



Source: Permission pending Hayes et al. (2011).

Figure 8-11 Relationship between ozone concentrations and cover of grass *A. odoratum* grown in competition with forb *L. hispidus* (top graph), and cover of grass *D. glomerata* grown in competition with forb *L. hispidus*.

A new study that directly tests the ozone response of an agricultural weed along with its crop competitor suggests that Pfleeger et al. (2010) and newer studies should be used to infer the responses of weeds within the context of crop production, where elevated ozone may favor weeds over crops:

- In a Chinese competition experiment between wheat and the cosmopolitan agricultural aggressive weed species flixweed (*Descurainia sophia*, introduced in the U.S., state-listed as a noxious weed, see USDA-NRCS), wheat biomass declined at 30-day exposures of 80 and 120 ppb ozone, but flixweed biomass was not affected by either level of elevated ozone (Li et al., 2013a).
- The community of agricultural weeds described by Pfleeger et al. (2010) was transported to Argentina and planted under ambient ozone exposures to test whether historical experimentally elevated ozone exposure had altered community composition. Historical exposure of ozone over four generations had no effects on richness, diversity, or evenness of cosmopolitan agricultural weeds, although 90 or 120 ppb ozone did increase the seedling density of the two dominant weeds (Martínez-Ghersa et al., 2017).

8.10.2 Microbes

The 2013 Ozone ISA documented effects of ozone on soil microbial communities, with changes in proportions of bacteria or fungi as a result of experimental ozone exposure in grassland mesocosms (Kanerva et al., 2008; Dohrmann and Tebbe, 2005), peatland mesocosms (Morsky et al., 2008), and forest mesocosms (Pritsch et al., 2009; Kasurinen et al., 2005); as well as changes in soil microbial communities in an agricultural ecosystem (Chen et al., 2010) and changes specifically in fungal communities in forest ecosystems at Aspen FACE (Edwards and Zak, 2011; Chung et al., 2006). Soil communities can be assessed based on the sequencing of genetic material within a sample; these analyses can target broad phylogenetic groups (e.g., fungi, bacteria, archaea) or a subset of the community with a common metabolic ability (e.g., nitrification, methane generation). Phospholipid fatty acid (PLFA) analysis allows coarse characterization of soil communities based on the abundance of fatty acids in a soil sample; different fatty acids are associated with the cell membranes of different groups of fungi or bacteria. Ordination analysis of multiple microbial taxa allows assessment of broader microbial community responses to ozone. Many new studies consider taxon-specific effects of ozone on bacteria (Section 8.10.2.1), fungi (Section 8.10.2.2), and archaea (Section 8.10.2.3), and several new studies consider metrics of soil microbial community change across taxa, as well as:

- At SoyFACE, elevated ozone altered the soil microbial community (bacteria, fungi, archaea, and unidentified prokaryotes) based on sequencing of functional genes related to carbon, nitrogen, phosphorus, and sulfur cycling (He et al., 2014). Effects were detected for individual functional genes involved in C fixation, in the breakdown of C substrates including forms of hemicellulose and chitin, in N fixation, in denitrification, and in ammonification. These changes in microbial community were consistent with effects of elevated ozone on N cycling at SoyFACE (see Section 8.9).
- In a greenhouse exposure of snap beans to ozone, elevated ozone altered microbial community structure based on PLFA data (Wang et al., 2014). In a wheat-rice rotation at Shuangpiao Farm,

China, elevated ozone reduced functional diversity of rhizosphere microbial communities determined by incubation on different carbon substrates (Chen et al., 2015).

- Elevated ozone had no effect on bacterial:fungal abundance quantified by PLFA in a wheat-soy rotation in Lake Wheeler, NC (Cheng et al., 2011). Elevated ozone increased the archaea:bacteria and decreased the fungi:bacteria ratios in soil associated with wheat (Li et al., 2013b).

8.10.2.1 Bacteria

The 2013 Ozone ISA found decreases in bacterial abundance (measured as PLFA biomass) in response to elevated ozone in meadow (Kanerva et al., 2008) and forest (Pritsch et al., 2009) mesocosms, as well as increases in Gram-positive bacteria in peatland mesocosms (Morsky et al., 2008). Many new studies assess the effect of elevated ozone on soil bacteria:

- *Bacterial abundance in agriculture:* In a rice-wheat rotation at Lake Wheeler Farm, NC, experimentally elevated ozone had no effect on bacterial abundance when assessed by PLFA analysis (Cheng et al., 2011). In contrast, in a rice-wheat rotation at Shuangpiao Farm, China, elevated ozone increased bacterial abundance in both rhizosphere and bulk soil when assessed by PLFA (Chen et al., 2015).
- *Bacterial communities in natural and agricultural FACE studies:* At Aspen FACE, elevated ozone increased soil bacterial richness assessed by 16S rRNA sequencing, but did not affect the functional composition of the bacterial communities (Dunbar et al., 2014). At Ruohoniemi FACE in Finland, elevated ozone increased bacterial abundance on senesced silver birch leaves, but affected bacterial abundance during leaf decomposition only at particular stages of decomposition on particular birch genotypes (Kasurinen et al., 2017). A greenhouse study using rice plants assessed the effects of 30 days of elevated ozone on bacterial communities on leaf surfaces and root surfaces (Ueda et al., 2016). There were no effects on broad community metrics (diversity, richness, or evenness of bacteria assessed by 16S sequencing), but elevated ozone increased the genetic variance of leaf-surface bacteria and decreased the relative abundance on root surfaces of the numerically dominant operational taxonomic units [OTUs; Ueda et al. (2016)]. In the ozone FACE rice-wheat rotation at Jiangdu City, China, elevated ozone decreased the abundance of dominant bacterial groups determined by 16S sequencing and altered the phylogenetic diversity of the bacterial communities (Feng et al., 2015). In maize cultivated at SoyFACE in Illinois, elevated ozone altered bacterial community composition in maize endosphere (i.e., plant microbiome) and soil for one of two tested maize genotypes (Wang et al., 2017).
- *Evaluation of specific bacterial taxa:* Experimentally elevated ozone affected the diversity and evenness of methanotrophic bacteria assessed by qPCR and T-RFLPs of the gene *pmoA* in soil communities associated with wheat at the Changping Seed Management Station in China, and effects varied by ozone exposure and by soil depth (Huang and Zhong, 2015). In the ozone FACE rice-wheat rotation at Jiangdu City, China, elevated ozone did not affect the abundance of methanogenic bacteria assessed by 16S rRNA primers specific to methanogens [but see section on archaea; Zhang et al. (2016)], but did decrease the abundance of anoxygenic phototrophic purple bacteria in soil, which are important for carbon cycling in flooded soils (Feng et al., 2011). In German mesocosms of European beech, elevated ozone altered root-associated actinobacterial community composition seasonally without affecting functional diversity (Haesler et al., 2014). Similarly, at Shuangpiao Farm, China, elevated ozone decreased actinomycete abundance in soil based on PLFA abundances (Chen et al., 2015). In an Argentinian OTC pasture experiment,

elevated ozone reduced the number of *Rhizobium* nodules on clover roots (Menendez et al., 2017). These results can inform the assessment of ozone effects on belowground processes and biogeochemistry in Section 8.9.

8.10.2.2 Fungi

The 2013 Ozone ISA found effects of ozone exposure on soil fungi. Studies found that ozone exposure decreased fungal biomass in meadow mesocosms (Kanerva et al., 2008), marginally increased fungal abundance (quantified by PLFA profiling) in peatland mesoscosms (Morsky et al., 2008), and altered fungal community composition in some studies of forest soils (Edwards and Zak, 2011; Chung et al., 2006; Kasurinen et al., 2005), although some forest studies found no effects of ozone on fungi (Pritsch et al., 2009). Previous reviews have also found that ozone interactions with fungi that cause disease (U.S. EPA, 2006). A number of new studies have evaluated the effects of ozone on fungi; as in the 2013 Ozone ISA, evidence of effects is mixed:

- Studies show effects of elevated ozone on mycorrhizal fungi in some but not all ecosystems. In 2013, a study from Aspen FACE found effects of ozone exposure on community composition of mycorrhizal fungi (Edwards and Zak, 2011), and a German lysimeter study observed visible differences in root mycorrhizal communities using microscopy (Kasurinen et al., 2005). In more recent Aspen FACE studies, there was no effect of elevated ozone on respiration by mycorrhizal hyphae in the soil or by mycorrhizal mushrooms (Andrew et al., 2014). Elevated ozone had no effect on ectomycorrhizal community composition in aspen root tips assessed by sequencing using ITS IF and ITS4 primers, but increased the abundance of four ectomycorrhizal taxa (Andrew and Lilleskov, 2014). Experimentally elevated ozone at Ruohoniemi FACE in Finland increased mycorrhizal colonization in silver birch roots (Kasurinen et al., 2012), but increased root fungal colonization in Scots pine roots (quantified as ergosterol concentration) without increasing mycorrhizal colonization (Rasheed et al., 2017). Elevated ozone had no effect on arbuscular mycorrhizal communities sequenced in soy roots at SoyFACE (Cotton et al., 2015), but did reduce mycorrhizal colonization in a greenhouse experiment with snap beans in which mycorrhizal inoculation was a controlled treatment (Wang et al., 2014).
- There are effects of ozone on some but not all fungi involved in decomposition. In an Aspen FACE study of mushrooms produced by saprophytic basidiomycete fungal communities in logs, elevated ozone had no effect on basidiomycete community composition (Ebanyenle et al., 2016). Experimentally elevated ozone at Ruohoniemi FACE in Finland altered fungi in roots and litter,, increasing fungal abundance on senesced leaf litter and altering fungal abundance in decomposing leaf litter with leaf genotype-specific effects, as quantified by qPCR (Kasurinen et al., 2017).
- There are effects of ozone on some mushroom-forming fungal taxa (basidiomycetes and ascomycetes). There were qualitative decreases under elevated ozone in species richness of basidiomycete mushrooms on logs at Aspen FACE (Ebanyenle et al., 2016), while qPCR of Aspen FACE soil samples showed that elevated ozone increased the relative abundance of basidiomycete to ascomycete fungal biomass in the soil without altering broader fungal community composition (Dunbar et al., 2014). At Ruohoniemi FACE in Finland, elevated ozone increased mushroom production in a year when mushroom production was high enough to quantify in all rings (Kasurinen et al., 2012).

- Consistent with the 2013 Ozone ISA, new evidence of ozone effects across all fungal species is mixed. Elevated ozone had no effect on fungal abundance broadly quantified by PLFA in a wheat-soy rotation in Lake Wheeler, NC (Cheng et al., 2011). Elevated ozone reduced fungal PLFA abundance in rhizosphere and bulk soil in a wheat-rice rotation at Shuangpiao Farm, China (Chen et al., 2015).
- Some new studies have reported that elevated ozone may interact with fungi that cause disease in plants. Elevated ozone may interact with plant pathogens to affect plant survival. In an exposure experiment in Alabama in which loblolly pines were inoculated with two fungal pathogens associated with Southern Pine Decline, elevated ozone increased the length of fungal lesions on plant roots (Chieppa et al., 2015). In an OTC experiment in India involving wheat and the fungal disease *Bipolaris sorokiniana*, elevated O_3 increased the frequency of leaf lesions, the production of disease spores, and decreased by half the latency stage of the disease (Mina et al., 2016).

8.10.2.3 Archaea

The 2013 Ozone ISA did not address the effects of ozone on archaeal community composition. The effects of elevated ozone on archaea have been assessed by three studies conducted at the ozone FACE soy-wheat rotation in Jiangdu City, China. Elevated ozone increased the archaea:bacteria ratio in soil associated with wheat (Li et al., 2013b). In rice-associated soil, elevated ozone decreased the richness, diversity, and abundance of the methanogenic archaeal community and decreased abundance of the dominant archaeal methanogen *Methanosaeta* (Zhang et al., 2016; Feng et al., 2013).

8.10.3 Consumer Communities

This section addresses the effects of ozone on communities of animal consumers via effects on vegetation and plant roots. The 2013 Ozone ISA did not address this topic within the context of altered terrestrial community composition. The effects of ozone on aboveground herbivores or the ecosystem service of pollination are addressed in more detail in Section 8.6 and Section 8.7.

- Ozone affects aboveground communities of invertebrates, which include both herbivores and insectivores. In Aspen FACE, elevated ozone altered the abundance of some arthropod species with trends towards effects on particular feeding guilds and decreased cumulative arthropod species richness in aspen canopy (Hillstrom et al., 2014). In a community of cosmopolitan agricultural weeds grown under four generations of elevated ozone, there was a strong linear relationship between plant species richness and aboveground arthropod diversity in a community that had grown for four generations at 0 ppb historical ozone, but no relationship between plant and arthopod diversity in communities grown for four generations at 90 or 120 ppb historical ozone (Martínez-Ghersa et al., 2017).
- Ozone affects belowground communities of invertebrates, including herbivores, detritovores, and higher level consumers. In soils under elevated ozone (110 ppb), the diversity index of nematodes decreased and the dominance index increased (Bao et al., 2014), while in a different FACE experiment, elevated ozone (60 ppb) changed the proportion of fungivorous nematodes in soil (Li et al., 2016a). In contrast, a separate study found no change in the nematode populations in soils

exposed to elevated levels [50, 60 ppb; Payne et al. (2017)]. However, in these soils, there was an increase in abundance and loss of diversity of testate amoeba (Payne et al., 2017).

8.10.4 Summary

The 2013 Ozone ISA found the evidence sufficient to conclude that there is a likely to be causal relationship between ozone exposure and the alteration of community composition of some ecosystems. Evidence of this relationship was presented for forest communities of trees; grassland communities of grasses, herbs, and legumes; and soil microbial communities of bacteria and fungi. Recently published papers extend the evidence for each of these topics (Table 8-19 and Table 8-20).

Table 8-19 Summary of evidence for a causal relationship between ozone exposure and terrestrial community composition, based on Table 2 from the Preamble.

Aspect of Ecological Weight of Evidence	Key Evidence	Key References
Relevant pollutant exposures	Defined in PECOS tool	Table 8-2
	Studies at relevant O_3 exposures	2006 Ozone AQCD (U.S. EPA, 2006); 2013 Ozone ISA (U.S. EPA, 2013)
Studies in which chance, confounding, and other biases are ruled out with reasonable confidence	Models of forest tree community composition in the U.S.	Gustafson et al. (2013); Wang et al. (2016)
	Grassland studies	Calvete-Sogo et al. (2016); Wedlich et al. (2012); Payne et al. (2011)
Controlled exposure studies (lab or small- to medium-scale field study)	Forest: Aspen FACE	2013 Ozone ISA (U.S. EPA, 2013); Zak et al. (2012).
	Grassland plants	2006 AQCD (U.S. EPA, 2006); 2013 Ozone ISA (U.S. EPA, 2013); Gilliland et al. (2016); Calvete-Sogo et al. (2016); Wedlich et al. (2012)
Studies with large scale of inference	Models of regional forest composition in the U.S.	Gustafson et al. (2013); Wang et al. (2016)
	Global synthesis of woody and herbaceous plant responses to controlled exposure of O_3, grouped by plant family (relevant to natural plant communities)	Bergmann et al. (2017)
	Grassland plant studies at national or European scale	Payne et al. (2011); van Goethem et al. (2013); U.S. EPA (2013)

Table 8-19 (Continued): Summary of evidence for a causal relationship between ozone exposure and terrestrial community composition, based on Table 2 from the Preamble.

Aspect of Ecological Weight of Evidence	Key Evidence	Key References
	Previous U.S. EPA syntheses	2006 Ozone AQCD (U.S. EPA, 2006); 2013 Ozone ISA (U.S. EPA, 2013)
Multiple studies by multiple research groups	Forest (studies in the U.S., Europe)	Section 8.10.1.1
	Grassland (studies in the U.S., Argentina, China, U.K., Spain, Switzerland, Europe)	Section 8.10.1.2
Many lines of evidence	Forest (experimental exposure, observations at ambient exposures, synthesis, multiple models)	2006 AQCD (U.S. EPA, 2006); 2013 Ozone ISA (U.S. EPA, 2013); Section 8.10.1.1
	Grassland and agricultural land (experimental exposure, observations at ambient exposures, synthesis, multiple models)	2006 Ozone AQCD (U.S. EPA, 2006); 2013 Ozone ISA (U.S. EPA, 2013); Section 8.10.1.2
	Grassland: exposure-response relationships	van Goethem et al. (2013); Hayes et al. (2011); Payne et al. (2011)
	Soil microbial communities	Section 8.10.3
	Aboveground and belowground invertebrate communities	Section 8.10.4

FACE = free-air CO_2 enrichment; O_3 = ozone.

In forests, previous evidence included correlational studies across ambient gradients of ozone exposure that found effects of ozone on conifer species, and studies with controlled experimental exposure of trees that found effects of ozone on deciduous trees. Key new studies (Wang et al., 2016; Gustafson et al., 2013) show that observational and experimental observations of ozone effects on tree species extend to affect regional forest composition in the eastern U.S. Additionally, a global-scale synthesis of research on ozone effects on plants confirms that some plant families are more susceptible to ozone damage than others (Bergmann et al., 2017), which is consistent with studies reviewed in previous ISA and AQCDs (U.S. EPA, 2013, 2006, 1996, 1986). This lends biological plausibility to a mechanism by which elevated ozone alters terrestrial community composition by inhibiting or removing ozone-sensitive plant species or genotypes, and thereby altering competitive interaction to favor the growth or abundance of ozone-tolerant species or genotypes.

In grasslands, previous evidence included multiple studies from multiple research groups to show that elevated ozone shifts the balance among grasses, forbs, and legumes in European grassland communities. There are new studies with findings consistent with earlier research, including a study in the U.S. that found elevated ozone affected the ratio of grass-to-legume biomass (Gilliland et al., 2016). There are also new studies from European grasslands that found exposure-response relationships between

ozone and community composition (Hayes et al., 2011; Payne et al., 2011), including a study that calculated AOT40 values for 10% reduction in biomass for 87 grassland species (van Goethem et al., 2013), some of which also grow in the U.S.

In soil microbial communities, previous evidence included studies that found effects on the ratio of bacteria to fungi in soil communities, as well as effects on community composition of mycorrhizal fungi. New studies confirm that elevated ozone alters soil microbial taxa, although as with previous evidence, the strength and direction of effects are not consistent across ecosystems. This may be due to the proposed mechanism of ozone effects on soil microbial communities, namely, that ozone indirectly affects soil communities via effects on plant chemistry and plant carbon allocation, which alter the substrates on which soil microbial communities subsist (Figure 8-12). This mechanism also explains an aspect of altered community composition not directly addressed in the 2013 Ozone ISA: the alteration of invertebrate community composition from effects that elevated ozone has on plants, as documented in several recent studies.

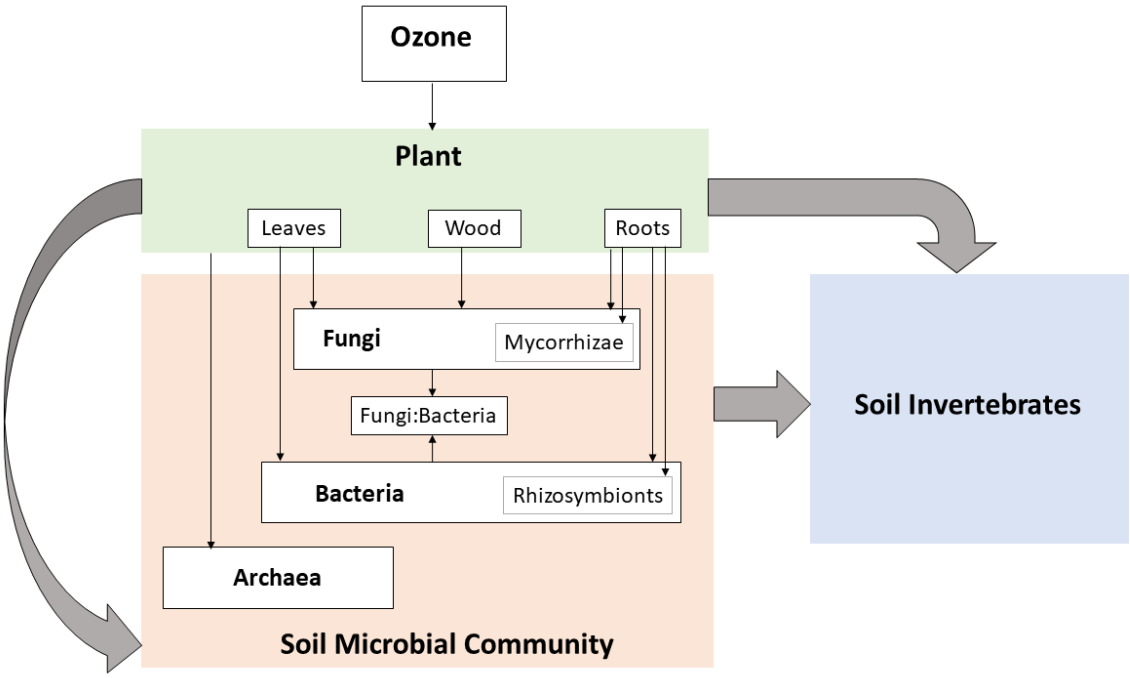


Figure 8-12 Biological plausibility of ozone effects on soil microbial communities and soil invertebrate communities.

The 2013 Ozone ISA presented multiple lines of evidence that elevated ozone alters terrestrial community composition, and recent evidence strengthens our understanding of the effects of ozone on

plant communities, while confirming that the effects of ozone on soil microbial communities are diverse. **The body of evidence is sufficient to conclude that there is a causal relationship between ozone exposure and the alteration of community composition of some ecosystems.**

Table 8-20 Terrestrial community composition response to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects
Agathokleous et al. (2015)	Meta-analysis	473 wild plant species tested for O_3 effects in 195 previously published papers	Multiple	Within the published literature testing O_3 effects on plants, 80% of wild plant species experience negative effects of O_3 exposure, representing 210 genera and 85 families.
Li et al. (2013b)	O_3 FACE site; Jiangdu City, Jiangsu, China (32.58°N, 119.70°E)	Two cultivars of *Triticum aestivum* (wheat), grown November−June in annual wheat-rice rotation: O_3 sensitive Yannong 19 and Yangmai 16	Ambient: 40 ppb, elevated: 60 ppb 9:00 a.m.−6:00 p.m. from March 3 to May 31, 2010	Elevated O_3 had no effect on number of detected genes. Elevated O_3 reduced Simpson's evenness of soil microbial functional genes, but only altered the abundance of gene *fhs*, which decreased under elevated O_3. Elevated O_3 changed the soil community associated with Yannong 19 cultivar, decreasing the fungi:bacteria ratio and increasing the archaea:bacteria ratio.
Feng et al. (2013)	O_3 FACE site; Jiangdu City, Jiangsu, China (32.585°N, 119.70°E)	Surface soil samples pulled from *Oryza sativa* (rice) in vegetative (July) and flowering (September) stages in 2010, cultivated in annual rice-wheat rotation	FACE: Three ambient O_3 rings, three elevated rings with mean 60 ppb O_3 during rice season, fumigated 9 a.m. to sunset (target was 1.5× ambient O_3, not to exceed 250 ppb O_3)	Elevated O_3 decreased the diversity 18% and the richness 39% of the methanogenic archaeal soil community under vegetative rice. Elevated O_3 decreased total abundance of the dominant archaeal methanogen *Methanosaeta* 35% in soils under vegetative rice and 44% in soils under flowering rice, and decreased its relative abundance within the methanogenic archaea as well. Elevated O_3 had a stronger influence on methanogenic archaeal community composition than did rice lifestage.

Table 8-20 (Continued): Terrestrial community composition response to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects
He et al. (2014)	FACE; SoyFACE, Illinois (40.056°N, 88.201°W)	Soil microbial community under *Glycine max* (soybean)	Ambient (~37.9 ppb), elevated O_3 (~61.3 ppb) soybeans also grown under elevated CO_2 (~550 ppm) and elevated CO_2 + elevated O_3	In elevated O_3 soybean crop surface soils, abundance of *niFH*, *narG*, *norB,* and *ureC* N-cycling genes were significantly decreased. There were no significant differences in the subsoil. For C-cycling genes in elevated O_3 soil samples, most were unchanged while fungal arabinofuranosidase and endoglucanse increased significantly and xylanase, cellobiase and exochitanase decreased significantly. Soil N was quantified; NH_4-N was significantly lower in the surface soil and NO_3-N significantly higher in subsoil of elevated O_3 plots compared to ambient.
Li et al. (2013a)	OTC; wheat fields, north China	Agricultural weed *Descurainia sophia* (flixweed), grown alone or in competition with *Triticum aestivum* cultivar Liangxing 99 (winter wheat), planted October., fumigated April, harvested May	Three O_3 treatments: ambient (<40 ppb O_3), elevated (80 ± 5 ppb for 7 h/day for 30 days), highly elevated (120 ± 10 ppb for 7 h/day for 30 days)	Wheat biomass and yield decline in response to competition under elevated and high ozone, whereas competition does not affect flixweed biomass or yield at any ozone exposure.

Table 8-20 (Continued): Terrestrial community composition response to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects
Chen et al. (2015)	OTC; winter wheat-rice rotation at Shuangpiao Farm, Jianxing City, Zhejiang Province, China (31.88°N, 121.30°E)	Soil microbial communities under wheat grown November 2006−May 2007, assessed as functional diversity by incubations with 31 different C substrates for community-level physiological profiles (CLPPs), and assessed as microbial community structure by phospholipid fatty acid analysis (PLFAs)	Three O_3 treatments March−May 2007: control, AOT40 = 0 (charcoal-filtered air); elevated O_3, AOT40 = 1,585 ppb-h (2 h daily of 50 ppb, 4 h daily of 100 ppb, and 2 h daily of 150 ppb O_3); highly elevated O_3, AOT40 = 9,172 ppb-h (2 h daily of 100 ppb, 4 h daily of 150 ppb, and 2 h daily of 200 ppb O_3)	Principal component analysis (PCA) of CLPPs shows that elevated and highly elevated O_3 affect microbial functional diversity in rhizosphere (root-associated) soil. PCA of PLFAs shows that elevated and highly elevated O_3 affect microbial structure and abundance in rhizosphere and nonrhizosphere soil. Highly elevated O_3 reduced Shannon-Weaver diversity 10% in rhizosphere and 4% in nonrhizosphere soils relative to control soil functional diversity, and reduced rhizosphere soil functional richness 24% relative to control. In nonrhizosphere soils, both elevated and highly elevated O_3 increased bacterial abundance (elevated O_3: 4% increase over control relative abundance, highly elevated O_3: 5% increase over control relative abundance) and decreased fungal abundance (22 and 28%). In rhizosphere soils, highly elevated O_3 increased bacterial abundance 1%, decreased actinomycete abundance 23%, and decreased fungal abundance 11%.
Huang and Zhong (2015)	OTC; Seed Management Station of Changping, Beijing, China (40.20°N, 116.12°E)	Methanotrophic bacteria in soil under winter wheat; soil methanotrophs assessed by qPCR of *pmoA* gene and 16S rRNA primers specific to type 1 and type 2 methanotrophs, and by T-RFLP of *pmoA*	Fumigation for 9 h/day, April−June 2010, with four different treatments: control (charcoal-filtered), low O_3 (nominally 40 ppb O_3), moderate O_3 (nominally 80 ppb), and high O_3 (nominally 120 ppb)	O_3 exposure affects soil methanotroph communities at different soil depths. In 0−10 cm soil, low O_3 increases Shannon diversity of methanotrophs 30% and increases evenness 32% relative to diversity in control soils, while high O_3 decreases diversity 13%. In 10−20 cm depth soil, low O_3 decreases diversity 18% and evenness 18%, moderate O_3 decreases diversity 13%, and high O_3 increases diversity 31% and increases evenness 22%. In 20−40 cm depth soil, moderate O_3 increases diversity 19%, and high O_3 decreases diversity 23% and decreases evenness 23%.

Table 8-20 (Continued): Terrestrial community composition response to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects
Feng et al. (2015)	O_3 FACE site; Jiangdu City, Jiangsu, China (32.585°N, 119.70°E)	Soil bacteria community assessed by bacterial 16S rRNA in surface soil samples pulled from *Oryza sativa* (rice) in vegetative (July) and flowering (September) stages in 2012, cultivated in annual rice-wheat rotation	Ambient O_3, seasonal 7-h (9:00 a.m.−4:00 p.m.) mean for 2012 was 33.7 ppb (AOT40 = 5.2 ppm-h); elevated O_3, seasonal 7-h mean for 2012 was 42.6 ppb (AOT40 = 13.4 ppm-h)	Elevated O_3 altered soil bacterial community composition associated with two different rice cultivars, decreasing the relative abundance of dominant bacteria Acidobacteria, Bacteroidetes, and Chloroflexi, and increasing the relative abundance of Proteobacteria.
Cotton et al. (2015)	FACE; SoyFACE, Illinois (40.056°N, 88.201°W)	Sequencing of arbuscular mycorrhizal fungi in *Glycine max* cultivar Pioneer 93B15 (soybean) roots, 54−62 days after planting	Factorial CO_2 and O_3 treatment: (1) ambient CO_2 and ambient O_3; (2) ambient CO_2 and elevated O_3 (1.2× ambient in 2004 and 2006, 1.6× ambient in 2008); (3) elevated CO_2 (550 ppm) and ambient O_3; (4) elevated CO_2 and elevated O_3	No effects of elevated O_3 on arbuscular mycorrhizal fungi. richness, evenness, or community composition.
Zhang et al. (2016)	O_3 FACE site; Jiangdu City, Jiangsu, China (32.585°N, 119.70°E)	Methanogenic archaea and methanotrophic bacteria, sequenced by 16S rRNA primers specific in surface soil samples pulled from *Oryza sativa* (rice) in vegetative (July) and flowering (September) stages in 2012, cultivated in annual rice-wheat rotation	Ambient O_3, seasonal 7-h (9:00 a.m.−2:00 p.m.) mean for 2012 was 33.7 ppb (AOT40 = 5.2 ppm-h); Elevated O_3, seasonal 7-h mean for 2012 was 42.6 ppb (AOT40 = 13.4 ppm-h)	O_3 decreased methanogenic archaeal abundance 20−21% under both rice cultivars in their vegetative growth phase. O_3 did not affect methanotrophic bacterial abundance. O_3 decreased methanogenic archaeal diversity: 22−41% for phylogenetic diversity under both rice cultivars in their vegetative phase, and 25−59% by the Chao index for both rice cultivars in their vegetative phase. Elevated O_3 affected soil methanogenic archaeal diversity more strongly under vegetative rice than under flowering rice.

Table 8-20 (Continued): Terrestrial community composition response to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects
Feng et al. (2011)	O_3 FACE site; Jiangdu City, Jiangsu, China (32.585°N, 119.70°E)	Soils sampled from rice in 2009: anoxygenic phototrophic purple bacteria assessed by pufM and bacterial 16S rRNA genes and by counts of representative purple nonsulfur bacteria *Rhodopseudomonas palustris*	Ambient O_3 mean for 2009 ~40 ppb; elevated O_3 ~60 ppb (nominally, 50% higher than ambient) from 9:00 a.m. to sunset, daily, unless leaves were wet or ambient O_3 was lower than 20 ppb. When the target O_3 was higher than 250 ppb, the set point was fixed at 250 ppb to prevent the plants from being exposed to extraordinarily high O_3	Abundance of anoxygenic phototrophic purple bacteria and *R. palustris* were significantly lower in elevated O_3 when quantified from rice in vegetative growth and seed set stages (no effect of O_3 when rice was flowering). There was less *R. palustris* diversity (number of genotypes) in elevated O_3 soils than in ambient O_3 soils.
Bao et al. (2014)	OTC; Shenyang Experimental Station of Ecology, Chinese Academy of Sciences, (41.517°N, 123.367°E)	*Glycine max* cultivar Tiefeng 29 (soybean), nematodes	Ambient (~45 ppb) and elevated (110 ± 10 ppb). Exposed to elevated ozone or/and UV-B radiations for 8 h (9:00 a.m.−5:00 p.m.) per day in the middle of the photoperiod from June 20 to September 7	Soybean growth stage-dependent effects on the abundance of bacterivores and fungivores were reported for elevated O_3. The ratios of bacterivores and fungivores:plant parasites and omnivores-carnivores:plant parasites were significantly affected by elevated O_3. The observed effect was soybean growth stage-dependent for omnivore-carnivore: plant parasites, but not for bacterivores and fungivores:plant parasites. Indices of nematode diversity, enrichment and community structure decreased under elevated O_3. The nematode dominance index increased under elevated O_3.
Li et al. (2016a)	FACE; Jiangsu Province, China (32.583°N, 119.70°E)	Arthropod: nematode population; FACE soil: collected from rice-wheat rotation system (*Oryza sativa*, *Triticum aestivum*); Greenhouse plants: Yangfumai 2 (Y2), Yannong 19 (Y19), Yangmai 15 (Y15), Yangmai 16 (Y16), rice cultivar (II-you)	FACE site: ambient (40 ppb) and elevated (60 ppb)	Other than the ratio between fungal and bacterial PLFAs, no other cultivar or O_3 exposure effects were detected. Although the total number of nematodes and the number of bacterivorous and plant parasitic nematodes were not affected by previous O_3 exposure or wheat cultivar, the number of fungivorous nematodes increased (except for Y2 cultivar) in soils previously exposed to O_3. The number of omnivorous-predatory nematodes differed between cultivars.

Table 8-20 (Continued): Terrestrial community composition response to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects
Martínez-Ghersa et al. (2017)	Mesocosm; University of Buenos Aires, Argentina (34.58°S, 58.58°W)	Populations of agricultural weeds (mostly Eurasian annuals) from the seed bank in Corvallis, OR; planted and grown in Argentina and interacting with the Argentinian insect community	Plants are descended from populations from U.S. EPA mesocosm experiments in Oregon. O_3 exposures for the 4 yr of experiment were: charcoal-filtered air with low O_3 and two elevated treatments (targets of 90 and 120 ppb). The ozone profile was developed based on the regional air quality data from the Midwest (U.S.) and consisted of an episodic pattern of varying daily peak concentration. Each chamber received the same episodic ozone exposure profile each year. Hourly requested peaks ranged from 1 to 155 ppb for the 90 ppb treatments and 1 to 219 ppb for the 120 ppb ozone treatments. The high peaks lasted for 1 h	There was no effect of historical O_3 exposure on descendant plant community richness, diversity, or evenness. Historical O_3 exposure increased the seedling density of the two dominant plants in descendant communities: 90 and 120 ppb increased *Spergula arvensis* density 30−50%, and *Calandrinia ciliata* density increased 109 and 217%, respectively. The relative abundance of the other plant species declined in response to O_3. There was linear relationship between plant species richness and arthropod diversity at 0 ppb historical O_3 (Spearman's $r = 0.71$), but no relationship at 90 or 120 ppb historical O_3 exposure. Historical O_3 does not affect the richness, diversity, or evenness of the arthropod community associated with descendant plant community, but does increase the relative abundance of carnivore arthropods while decreasing the relative abundance of herbivore arthropods ($p < 0.05$).
Menendez et al. (2017)	OTC; University of Buenos Aires, Argentina (34.59°S, 58.58°W)	6-week-old *Trifolium repens* (white clover) and *Rhizobium* spp. (N fixing bacteria in root nodules on clover)	Charcoal-filtered ambient O_3 in 2010−2011 (concentrations not reported), elevated O_3 for 4 h/day over 5 days at maximum 90−120 ppb O_3	O_3 exposure reduced the number of *Rhizobium* nodules on clover roots by 35%.
Wang et al. (2017)	FACE; SoyFACE, Illinois (40.056°N, 88.201°W)	Soil, rhizosphere, and root endosphere-associated microbial communities in maize crop grown under elevated O_3	O_3 plots were enriched with O_3 to a target concentration of 100 ppb by fumigation (summer 2014)	No change in α-biodiversity was observed in endosphere, rhizosphere or soil with elevated O_3. There were significant differences in β-biodiversity of microbial communities in the endosphere and soil. Microbial community composition shifted by maize genotype, specifically in the endosphere samples of inbred B73 and the soil where both hybrids were grown under elevated O_3.

Table 8-20 (Continued): Terrestrial community composition response to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects
Mina et al. (2016)	OTC; Indian Agriculture Research Institute, (28.583°N, 77.20°E)	Plant: wheat (*Triticum aestivum* cultivar PBW343); Pathogen: *Bipolaris sorokiniana*	Ambient (12−72 ppb) and elevated (ambient O_3 + 25−30 ppb). Wheat plants were pretreated with different O_3 levels for 8 h/day from seedling to 65-day-old stage	The maximum number of lesions/leaf and spores/25mm^2 were detected in elevated O_3. Elevated O_3 also shortened the *Bipolaris* latency period from 20 to 10 days or less. Antioxidants interfered with the growth of *Bipolaris* on wheat plants. Compared to charcoal-filtered air, elevated O_3 reduced PR protein content and chitinase activity alone and in combination with *Bipolaris*. The effect of elevated O_3 alone and O_3 + *Bipolaris* on PR protein content and chitinase activity were reversed by the addition of antioxidants.
Cheng et al. (2011)	OTC; Lake Wheeler Experimental Station, NC (35.72°N, 78.67°W)	Wheat-soybean rotation: in November to June, O_3 tolerant *Triticum aestivum* cultivar Coker 9486 (soft red winter wheat), and in June to November, soybean (multiple cultivars over 4 yr experiment)	Full factorial O_3 and CO_2 fumigation for 4 yr: (1) charcoal-filtered control (canopy height seasonal daily 12-h avg for June−November is 19.9 ppb O_3; November−June is 20.7 ppb O_3) with ambient CO_2 (376 ppm June−November and 388 ppm November−June); (2) elevated O_3 (canopy height seasonal daily 12-h avg for June−November 65.7 ppb O_3; November−June 49.8 ppb O_3); (3) elevated CO_2 (555 ppm June−November and 547 ppm November−June); (4) elevated O_3 and elevated CO_2, as above	Elevated O_3 had no effect on soil C, soil N, or fungal and bacterial soil abundances or ratio assessed by PLFA.

Table 8-20 (Continued): Terrestrial community composition response to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects
Kasurinen et al. (2012)	FACE; Ruohoniemi FACE, Kuopio, University of Eastern Finland, Finland (62.88°N, 27.62°E)	Clones of four genotypes from the wild population of *Betula pendula* (silver birch) as well as their associated mycorrhizal community and fruiting bodies of *Laccaria laccata* (mycorrhizal fungi)	Factorial O_3 by temperature treatment: mean ambient O_3 is 23.4 ppb in 2007, 23.8 ppb in 2008 (AOT40 = 0.14 ppm-h in 2007,1.6 ppm-h in 2008); mean elevated O_3 is 28.1 ppb in 2007, 32.0 ppb in 2008 (AOT40 = 4.9 ppm-h in 2007, 9.0 ppm-h in 2008), fumigation 800 to 2,200 daily, from spring leaf out to autumn; temperature treatment is ambient or elevated by infrared rings above the canopy	Elevated O_3 increases mycorrhizal infection in roots by 9% at ambient temperatures (T) and 5% at elevated T. Elevated O_3 increases mushroom count 660% in ambient T and 230% in elevated T above respective ambient O_3 treatments.
Andrew et al. (2014)	FACE; Aspen FACE, Rhinelander, WI (49.675°N, 89.625°E)	Mycorrhizal fungi	Fumigation 1998−2008 during daylight hours of the growing season. Ambient O_3 W126 2.1−8.8 ppm-h and elevated 12.7−35.1 ppm-h; elevated CO_2 515−540 ppm, ambient average 374 ppm. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	No significant difference with treatment on net hyphal biomass production. No significant effects of treatment on hyphal respiration per unit or hyphal and sporocarp respiration on a mass-specific basis.
Andrew and Lilleskov (2014)	FACE; Aspen FACE, Rhinelander, WI (49.675°N, 89.625°E)	Plot areas planted with *Populus tremuloides*. ectomycorrhizal fungal root tip communities	Fumigation 1998−2006 during daylight hours of the growing season. Ambient O_3 W126 2.9−8.8 ppm-h and elevated 14.6−35.1 ppm-h; elevated CO_2 (560 ppm), ambient CO_2.For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	Soil properties were a stronger determinant of EMF root tip community structure than O_3 treatment. The relative abundances of four taxa (*Tomentella* sp. "A," *Tomentella* sp. "C," *Sebacina* (*Serendipita*) sp., and *Hebeloma crustuliniforme* species group were increased under elevated O_3.

Table 8-20 (Continued): Terrestrial community composition response to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects
Moran and Kubiske (2013)	FACE; Aspen FACE, Rhinelander, WI	Clones of five genotypes of *Populus tremuloides* (aspen), from the aspen-only sections of the experiment, 1997−2008	Full factorial: O_3 and CO_2, 1998−2008. Ozone: ambient (W126 2.1−8.8 ppm-h) or elevated (W126 12.7−35.1 ppm-h). CO_2: ambient (360 ppm) or elevated (560 ppm); for hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	In model runs of genotype abundance after 12 yr of O_3 exposure, O_3 increased the relative abundance of genotypes 216 and 8L by 6 and 26% respectively, and decreased the relative abundance of genotypes 42E and 259 by 9 and 44% respectively.
Gustafson et al. (2013)	LANDIS Model using Aspen FACE data; Rhinelander, WI	*Acer saccharum* (sugar maple), *Betula papyrifera* (paper birch), four clones of *Populus tremuloides* (aspen)	Target over the course of the Rhinelander experiment was 30−50 ppb for control, and 60−80 ppb for elevated	Overall, total biomass was lowest under the O_3 treatment. The O_3 treatment significantly affected abundance of all taxa except one clone. Simulations suggest that O_3 will affect forest composition at the landscape scale. Simulations suggest that O_3 will cause an increase in birch at the expense of aspen. By year 180, elevated O_3 decreased productivity by half. Elevated O_3 reduced landscape biomass.
Zak et al. (2012)	FACE; Aspen FACE, Rhinelander, WI (49.675°N, 89.625°E)	Five genotypes of *Populus tremuloides* (quaking aspen) together; *P. tremuloides* genotype (216) with *Betula papyrifera* (paper birch); and *P. tremuloides* genotype (216) with *Acer saccharum* (sugar maple)	Fumigation 1998−2008 during daylight hours of the growing season. Ambient O_3 W126 2.1−8.8 ppm-h and elevated 12.7−35.1 ppm-h; elevated CO_2 515−540 ppm, ambient average 374 ppm. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	Elevated O_3 altered inter-specific competition for N in aspen, increasing genotype 8 plant N by 93% and plant ^{15}N by 171%, and decreasing genotype 271 plant N by 40% and plant ^{15}N by 27%, with no effect on plant competitiveness for N for the remaining three genotypes. Elevated O_3 did not alter species competition for soil N (measured as plant N and plant ^{15}N).

Table 8-20 (Continued): Terrestrial community composition response to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects
Wang et al. (2016)	Gap Model (UVAFME) representing "a typical temperate deciduous forest in the southeast U.S."	32 total species were used in the model; 10 dominant tree species: *Acer rubrum* (red maple), *Acer saccharum* (sugar maple), *Carya cordiformis* (bitternut hickory), *Fagus grandifolia* (American beech), *Liriodendron tulipifera* (tulip poplar), *Quercus alba* (white oak), *Quercus montana* (chestnut oak), *Quercus rubra* (red oak), *Quercus velutina* (black oak), *Prunus serotine* (black cherry)	Each of the 32 species was ranked based on O_3 sensitivity (resistant, intermediate, or sensitive). Growth reduction parameters were 0, 10, and 20% for each of the three categories, respectively	O_3 resistant species dominate and sensitive species decline over the 500-yr simulation. Overall forest biomass and forest carbon storage do not decrease over time under high O_3 conditions because tolerant species growth is enhanced as they are released from competition by the loss of O_3 sensitive species. O_3 reduced biodiversity over time.
Chieppa et al. (2015)	OTC; research field 5 km north of Auburn, AL	*Pinus taeda* (loblolly pine) inoculated with either *Leptographium terebrantis* or *Grosmannia huntii* (root infecting ophiostomatoid fungal species associated with Southern Pine Decline)	O_3 12 h/day. The first 41 days were acclimatization; O_3 exposure continued 77 more days once seedlings were inoculated with fungus. Mean 12 h O_3 over the 118 days was 14 (charcoal-filtered), 23 (ambient), and 37 (2× ambient) ppb. 12-h AOT40 values were 0.027 (CF), 1.631 (ambient) and 31.2 (2×) ppm-h. Seasonal W126 were 0.033 (CF), 0.423 (ambient) and 21.913 (2×)	Seedlings under 2× O_3 had greater belowground dry matter yield than CF seedlings. Fungal lesion length was greater on 2× O_3 exposed seedlings but was not specific to either fungal species.

Table 8-20 (Continued): Terrestrial community composition response to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects
Hillstrom et al. (2014)	FACE research facility; Rhinelander, WI	(1) Tree canopy arthropods sampled 3× each summer 2005−2007 (2) Plants: *Populus tremuloides* genotypes (216, 217, 42E) and *Betula papyrifera* (paper birch)	(1) O_3: ~1.5× ambient. (2) CO_2: ~560 ppm; for hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	Elevated O_3 did not affect total arthropod abundance on aspen or birch, but did significantly alter the abundance of certain species, tending to decrease the abundance of phloem-feeders and increase the abundance of leaf chewing and galling feeding guilds. Elevated O_3 did not affect arthropod species richness in any single summer for either aspen or birch, but elevated O_3 significantly decreased arthropod richness 15% cumulatively sampled across all 3 yr in aspen canopies (p = 0.03). Elevated O_3 did not affect arthropod community composition in aspen canopies in any single year or across all years. Elevated O_3 altered community arthropod community composition in birch canopies only in 2007, but had no effect across all years. genotype was an important determinant of community composition in aspen canopies.
Dunbar et al. (2014)	FACE; Aspen FACE, Rhinelander, WI (49.675°N, 89.625°E)	Fungal and bacterial communities in top 5 cm of mineral soil in *Populus tremuloides* FACE soils, July 2007; quantified by qPCR, clone library surveys, and gene pyrotag surveys of bacterial 16S rRNA, fungal 18S rRNA, and fungal LSU rRNA	Treatments for 1998−2007 were ambient O_3 W126 = 2.9−8.8 ppm-h and elevated O_3 = 13.1−35.1 ppm-h. Ambient air CO_2 and elevated (560 ppm) CO_2. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	Bacterial communities had higher richness under elevated O_3 than under ambient O_3. Elevated O_3 increased the ratio of Basidiomycota:Ascomycota abundance (fungal mushroom-producing taxa) based on fungal rRNA clone libraries. O_3 did not affect the functional composition of the bacterial or fungal soil communities.
Haesler et al. (2014)	Mesocosm; soil from a mixed beech/spruce stand, Eurasburger forest, Augsburg, Germany (48.30°N, 11.08°E)	Soil actinobacterial communities associated with *Fagus sylvatica* (European beech) characterized by t-RFLP and clone libraries of actinobacteria-specific 16s rRNA primers and type 2 polyketide synthases (PKS)	Ambient O_3 (range 20−80 ppb) and elevated O_3 (twice ambient concentrations not exceeding 150 ppb)	Elevated O_3 altered actinobacterial community composition as measured by T-RFLP in summer but not in spring or fall. Elevated O_3 did not affect actinobacterial evenness or functional diversity (PKS).

Table 8-20 (Continued): Terrestrial community composition response to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects
Kasurinen et al. (2017)	FACE; Ruohoniemi FACE, Kuopio, University of Eastern Finland, Finland (62.88°N, 27.62°E)	Bacterial and fungal communities subsisting *Betula pendula* (silver birch) leaf litter from clones of two distinct birch genotypes (gt14 and gt15). Microbial abundance assessed by qPCR (bacterial primers pE and pF', fungal primers ITS3 and ITS4) at leaf drop, 217 days of decomposition, and 257 days decomposition	Factorial O_3 by temperature treatment: mean summer ambient O_3 is 24.2 ppb in 2009, 30.7 ppb in 2010 (AOT40 = 0.14 ppm-h in 2009, 1.1 ppm-h in 2010); mean elevated O_3 is 33.6 ppb in 2009, 43.7 ppb in 2010 (AOT40 = 4.7 ppm-h in 2009, 10.7 ppm-h in 2010); temperature treatment is ambient or elevated by infrared rings above the canopy	Elevated O_3 increases bacterial abundance 196% (p = 0.002) and fungal abundance 61% (p = 0.095) in freshly fallen litter. Effects of elevated O_3 on fungal abundance via changes to soil microbial conditions varied with birch genotype: in birch gt14 leaves, elevated O_3 reduced fungal abundance 24%, and in birch gt15 leaves, elevated O_3 increased fungal abundance 44%. Ozone Interactions: effects of elevated O_3 on microbial abundance during decomposition via changes in litter quality only occurred in interactions with birch genotype, warming, and decomposition stage (p < 0.1). Effects of elevated O_3 on bacterial abundance via changes to soil microbial conditions only occurred in interactions with birch genotype and decomposition stage (p < 0.1).
Rasheed et al. (2017)	FACE; Ruohoniemi FACE, Kuopio, University of Eastern Finland, Finland (62.88°N, 27.62°E)	*Pinus sylvestris* (Scots pine) seedlings and their root-colonizing fungi (quantified as root ergosterol) and rhizosphere soil microbial community (quantified by PLFA)	O_3 in full factorial design with air warming treatment (+1°C), N fertilization (+120 kg N/ha-yr), and insect herbivore treatment (+4 larval sawfly, *Acantholyda posticalis*). O_3 exposure 2011−2013: ambient O_3 monthly averages during the growing seasons 17.7−38.3 ppb O_3 (AOT40 = 0.25 ppm-h in 2011, 0.52 ppm-h in 2012, and 1.27 ppm-h in 2013); elevated O_3 monthly averages during the growing seasons 26.7−55.0 ppb O_3 (AOT40 = 6.29 ppm-h in 2011, 9.58 ppm-h in 2012, and 29.28 ppm-h in 2013)	In 2013, elevated O_3 increased the extent of fungal colonization in pine roots (measured as ergosterol) with no main effect on mycorrhizal colonization or soil fungi:bacteria.

Table 8-20 (Continued): Terrestrial community composition response to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects
Ueda et al. (2016)	Greenhouse; soil from Meckenheim, Germany (50.6°N, 7.0°E)	Bacterial communities assessed by 16S rRNA sequencing, bacteria on rice leaf surfaces (phyllosphere) and rice root surfaces (rhizoplane)	Ozone fumigation for 30 days, 900−1,600, beginning with 10-week-old rice plants: control (5 ± 4 ppb O_3), elevated (85 ± 34 ppb O_3)	Elevated O_3 did not affect diversity (inverse Simpson index), richness, evenness, or functional diversity of phyllosphere bacteria or rhizoplane bacteria. The genetic variance of phyllosphere bacteria was higher in elevated O_3 than control O_3 (HOMOVA, p = 0.021); although, O_3 did not affect relative abundance of the most abundant phyllosphere bacterial OTUs. Elevated O_3 decreased the relative abundance of two of the most abundant rhizoplane bacterial OTUs, Rhodospirillaceae (nonsulfur photosynthetic bacteria) and Clostridiales (obligate anaerobe).
Ebanyenle et al. (2016)	FACE; Aspen FACE, Rhinelander, WI (49.675°N, 89.625°E)	Wood-decaying basidiomycete fungi (identified by sequencing of primer pair ITS1f/ITS4), growing on logs cut in 2009 from *Populus tremuloides* (quaking aspen) and *Betula papyrifera* (paper birch). Logs were grown and placed in each of the four FACE treatments in a full factorial design	Fumigation 1998−2008 during daylight hours of the growing season. Ambient O_3 W126 2.1−8.8 ppm-h and elevated 12.7−35.1 ppm-h; elevated CO_2 515−540 ppm, ambient average 374 ppm. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	There was no statistically significant effect of O_3 on basidiomycete community composition, although there were fewer fungal species from logs in the O_3 plots than in the ambient O_3 plots.

Table 8-20 (Continued): Terrestrial community composition response to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects
Hayes et al. (2011)	Greenhouses near Marchlyn Mawr, U.K.	Two communities: four plants of forb *Leontodon hispidus* and three plants of grass *Dactylis glomerata*; four plants of forb *Leontodon hispidus* and three plants of grass *Anthoxanthum odoratum*	Eight treatments: (1) seasonal 24-h mean 21.4 ppb (12-h mean 21.1 ppb, daylight (7:00 a.m.−6:00 p.m.) AOT40 = 0.07 ppm-h, 24-h AOT40 = 0.07 ppm-h); (2) seasonal mean 39.9 ppb (12 h = 39.2 ppb, daylight AOT40 = 4.93 ppm-h, 24-h AOT40 = 10.91 ppm-h); (3) seasonal mean 50.2 ppb (12 h = 49.6 ppb, daylight AOT40 = 21.44 ppm-h, 24-h AOT40 = 41.29 ppm-h); (4) seasonal mean 59.4 ppb (12 h = 58.7 ppb, daylight AOT40 = 38.04 ppm-h, 24-h AOT40 = 72.19 ppm-h); (5) seasonal mean 74.9 ppb (12 h = 73.3 ppb, daylight AOT40 = 62.49 ppm-h, 24-h AOT40 = 119.82 ppm-h); (6) seasonal mean 83.3 ppb (12 h = 81.6 ppb, daylight AOT40 = 77.13 ppm-h, 24-h AOT40 = 147.42 ppm-h); (7) seasonal mean 101.3 ppb (12 h = 99.0 ppb, daylight AOT40 = 108.43 ppm-h, 24-h AOT40 = 206.70 ppm-h); (8) seasonal mean 102.5 ppb (12 h = 100.5, daylight AOT40 = 112.47 ppm-h, 24-h AOT40 = 214.34 ppm-h)	Grass cover increases linearly with increasing seasonal O_3 mean.

Table 8-20 (Continued): Terrestrial community composition response to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects
Payne et al. (2011)	Gradient; 64 acidic grassland sites stratified by N deposition and climate, U.K.	Entire plant community, acidic grasslands	Site-specific O_3 exposures from the U.K. Air Pollution Information System	In terms of single factor models, O_3 is the strongest predictor of species cover. Within the multiple-factor model, only current total inorganic N deposition and mean annual total evapotranspiration are stronger predictors of species cover than O_3. Cluster analysis identifies a change in species composition at an AOT40 of 3,150 ppb-h. *Nardus stricta* (grass), *Deschampsia flexuosa* (grass), and *Juncus effusus*(sedge) are indicator species for low-O_3 sites; *Pseudoscleropodium purum* (moss), *Festuca rubra* (grass), and *Dicranum scoparium* (moss) are indicator species for high-O_3 sites. O_3 affects species composition but not species richness across the gradient.
Bassin et al. (2013)	FACE; Alp Flix, Sur, Switzerland (9.65°N, 46.53°E)	Pasture turf: 107 vascular plant species: 84 forbs, 11 grasses, 6 legumes, 6 sedges. Initial and control community dominated by *Nardus stricta, Carex sempervirens,* and *Festuca* spp., which together on average comprise 35% cover in plots	Ambient (mean during growing season 45−47 ppb O_3); elevated (120% ambient O_3), high elevated (160% ambient O_3) fumigated 24 h April to October, 2004−2010. Crossed with an N addition experiment: ambient N deposition 4 kg N/h/yr, +5 kg, +10 kg, +25 kg, +50 kg N/ha/yr	Plant diversity of mesocosms was high and varied among mesocosms before the experiment started, and analyses do not account for initial conditions. Elevated and highly elevated O_3 had no effect on biomass of functional groups (i.e., relative abundance of forbs, sedges, or grasses; legumes not included) across all years. Elevated O_3 (120% ambient) increased *N. stricta* abundance 22%, and highly elevated O_3 (160% ambient) increased *N. stricta* abundance 40%, in the last 3 yr of the study (2008−2010).
Wedlich et al. (2012)	High Keenley Fell; northern England, U.K. (approximately 54.9°N, 2.3°W)	Restored and managed mesotrophic grassland with 47 plant species (grasses, herbs, legumes), dominated by *Festuca rubra, Holcus lanatus*, and *Anthoxanthum odoratum*	Ambient: annual maximum monthly mean was 45 ppb; moderately elevated: annual maximum monthly mean was 50 ppb (June−August ambient +4 ppb in 2008 and ambient +3 ppb in 2009); elevated: annual maximum monthly mean was 65 ppb (June−August ambient +14 ppb in 2008 and ambient +8 ppb in 2009)	In 2008, O_3 explained 9.5% of the variation in herb and legume species biomass composition (p = 0.01), and in 2009, O_3 explained 40.3% of the variation in herb and legume species biomass composition (p = 0.002).

Table 8-20 (Continued): Terrestrial community composition response to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects
Calvete-Sogo et al. (2016)	OTC; La Higueruela agricultural research farm, Toledo, Spain (40.05°N, 4.43°W)	Pasture comprised of six annual plants: three legumes (*Trifolium striatum, Trifolium cherleri*, and *Ornithopus compressus*); two grasses (*Briza maxima and Cynosurus echinatus*); and the herb *Silene gallica*	Fumigation for 39 days starting in early April: charcoal-filtered air with maximum of daily mean 29 ppb O_3 (AOT40 = 3 ppb-h); ambient with maximum of daily mean O_3 42 ppb (AOT40 = 760 ppb-h); moderate O_3 with maximum of daily mean 61 ppb (AOT40 = 5,771 ppb-h); and elevated O_3 with maximum of daily mean 73 ppb (AOT40 = 10.316 ppb-h)	O_3 was a more powerful explanatory factor than N addition in redundancy analysis of aboveground biomass (O_3 explained 8.4% of total variability, p = 0.027), total living biomass (O_3 explained 11.1% of variability, p = 0.007), and senesced biomass (O_3 explained 10.6% of variability, p = 0.012) of the pasture plant community. O_3 interactions: O_3 and N interactions did not have significant effects on whole-community metrics.
Gilliland et al. (2016)	OTC; research site located ~5 km north of Auburn University campus	*Trifolium repens* (white clover) and three grass species pooled into "grasses" (*Lolium arundinacea, Paspalum dilatatum, Cynodon dactylon*)	Exposure for 4 mo with the mean 12-h O_3 concentration of 31 ppb (NF) and 56 ppb (2× ambient), average peak O_3 = 39 ppb (NF) and 77 (2×), peak average 1-h O_3 = 73 (NF) and 155 (2×), 12-h AOT40 1.8 ppm-h (NF) and 29.8 ppm-h (2×), seasonal 12-h W126 1.6 ppm-h (NF) and 42.5 ppm-h (2× ambient)	Elevated O_3 increased primary growth of grasses (dry matter yield) 19%. In mowed pasture, elevated O_3 decreased clover yield 60% and increased grass yield 40%. There was no effect of ozone on cover of clover or grass.
van Goethem et al. (2013)	Meta-analysis; northwestern Europe (mapping of sensitivity is for a square area of 50 to 61°N, and 11°E to 11°W)	25 annual grassland species, 62 perennial grassland species, 9 tree species	OTC, FACE, or solardomes. All experimental treatments were at >40 ppb for at least 21 days, with mean hourly O_3 never exceeding 100 ppb. Control treatments were charcoal-filtered air or ambient air	Annual grassland species were significantly more sensitive to O_3 >40 ppb than were perennial grassland species. Mean 10% reduction in biomass occurred at 0.84 ppm-h for annual species and 1.14 ppm-h for perennial grassland species. Exposure-response relationships for 96 European plants (biomass reduction vs. AOT40) are listed.

Table 8-20 (Continued): Terrestrial community composition response to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects
Wang et al. (2014)	Plant growth chambers with soil from a wheat-snap bean agricultural rotation; Haidian District, Beijing City, China	Soil microbial communities assessed by PLFA on soil in mesoscosms of *Phaseolus vulgaris* (snap bean, one sensitive and one tolerant genotype), either inoculated or not with *Glomus aggregatum* (an arbuscular mycorrhizal fungi)	60-day O_3 exposure: ambient, 20 ± 5 ppb (AOT40 = 0); elevated, 70 ± 10 ppb (AOT40 = 19.6)	Elevated O_3 decreased root mycorrhizal colonization 44% in sensitive genotype and 24% in tolerant genotype. Elevated O_3 changed soil microbial community structure of both bean genotypes based on PCA of PLFA data.
Bergmann et al. (2017)	Meta-analysis; global; peer-reviewed papers, book chapters, reports, and conference proceedings published 1980 to unspecified mid 2010s	Seed-bearing plants: Grouped into herbaceous plants (298 species in 47 plant families: wild native or pasture species), woody plants (165 species, 39 families, 69 genera), crops (agricultural or horticultural). Also assessed ferns, mosses, lichens, vertebrates	Multiple study designs, grouped into experimental O_3 exposures (growth chambers where O_3 did not exceed 100 ppb, greenhouse, solardome, OTC, FACE) or ambient gradient O_3 exposures for vascular plants	Among herbaceous plant families with at least 10 species tested, O_3 sensitivity to foliar injury is Onagraceae > Fabaceae > Cyperaceae > Lamiaceae > Asteraceae > Poaceae. Among 135 woody plant species tested, ozone causes foliar injury in 86% of broadleaf and 72% of conifer species. In field and gradient O_3 observations, 245 plant species and 28 genus-level plant groups experience O_3 foliar injury. 47.5% of the 223 herbaceous plant species experimentally exposed to O_3 experience effects in growth, productivity, C allocation, or reproduction. These effects are more common across annuals/biennials than perennials. 70% of Fabaceae species tested are sensitive to these effects of O_3. Among woody plant species, 53% of the conifer and 67% of the broadleaf species experimentally exposed to O_3 experience effects in growth, productivity, C allocation, or reproduction. The woody plant families Myrtaceae, Oleaceae, Salicaceae, and Betulaceae are particularly sensitive to foliar injury and growth effects of O_3. O_3 effects have been tested on 2 fern species, 10 moss species, and 31 lichen species; there are O_3 effects at physiological scales.

Table 8-20 (Continued): Terrestrial community composition response to ozone exposure.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects
Payne et al. (2017)	Mesocosm; peat sampled from wet, heath peatland, U.K.	Microscopic algae (desmids, diatoms), protozoa (ciliates, flagellates, testate amoebae), and microscopic animal consumers (nematodes, rotifers) sampled from *Sphagnum papillosum* stems	Experimental O_3 for 3.5 yr: ambient air (25 ppb O_3), low O_3 (ambient + 10 ppb for 24 h/day), moderate O_3 (ambient + 25 ppb O_3 24 h/day), elevated O_3 (ambient + 35 ppb 8 h/day in summer, +10 ppb rest of year)	Testate amoeba community structure was significantly affected by ozone. Moderate and elevated O_3 decreased testate amoeba species richness 31%. Low and elevated O_3 increased grouped flagellate and ciliate abundance. Exposure indices: authors indicated that O_3 effects on microscopic food web in peat generally start at moderate O_3 exposures.

^{15}N = nitrogen-15, stable isotope of nitrogen; 16s rRNA = bacteria-specific primer; 18s rRNA = fungi-specific primer; AOT40 = seasonal sum of the difference between an hourly concentration at the threshold value of 40 ppb, minus the threshold value of 40 ppb; C = carbon; CO_2 = carbon dioxide; EMF=ectomycorrhizal fungal; FACE = free-air CO_2 enrichment; kg/ha = kilograms/hectare; Lsu rRNA = fungi-specific primer; N = nitrogen; N/h/yr = kg nitrogen/hectare/year; NF = nonfiltered air; O_3 = ozone; OTC = open-top chamber; OTU(s) = operational taxonomic unit(s); ppb = parts per billion; ppm = parts per million; PR protein = pathogenesis related protein; *puf*M = primer for nonsulfur bacteria; qPCR = quantitative polymerase chain reaction; T-RFLP = terminal restriction fragment length polymorphism; W126 = cumulative integrated exposure index with a sigmoidal weighting function.

8.11 Water-Cycling

In the 2013 Ozone ISA, the evidence was sufficient to conclude there is a likely to be causal relationship between ozone exposure and the alteration of ecosystem water cycling (U.S. EPA, 2013). Plants are responsible for a part of ecosystem water cycling through root uptake of soil moisture and groundwater, as well as transpiration through leaf stomata to the atmosphere; changes to this part of the water cycle may, in turn, affect the amount of water moving through the soil, running off overland or through groundwater, and flowing through streams.

Ozone can affect water use in plants and ecosystems through several mechanisms, including damage to stomatal functioning and loss of leaf area (Figure 8-2), which may affect plant and stand transpiration. Although the 2013 Ozone ISA found no clear universal consensus on leaf-level stomatal conductance response to ozone exposure, many studies reported incomplete stomatal closure and loss of stomatal control in several plant species, which result in increased plant water loss [Section 9.4.5; U.S. EPA (2013)]. Additionally, ozone has been found to alter plant water use through decreasing leaf area index, accelerating leaf senescence and causing changes in branch architecture, which can significantly impact stand-level water cycling. Some key studies attempted to scale up these effects of ozone on leaf physiological measurements to ecosystems, connecting increased plant water loss to changes in soil moisture, runoff, and streamflow, both through empirical study and modeling (Paoletti and Grulke, 2010; Felzer et al., 2009; Mclaughlin et al., 2007a; Mclaughlin et al., 2007b; Hanson et al., 2005).

As described in the PECOS tool (Table 8-2), the scope for evidence reviewed and assessed includes studies on any continent in which alterations in water acquisition and hydraulic transport (based on plant structural changes), stomatal response, and plant water use were measured on the scale of individual plants in response to ambient exposures and experimentally elevated ozone exposures within an order of magnitude of recent concentrations. Many metrics are used to evaluate effects of ozone on ecosystem water cycling including stomatal conductance, sap flow, vessel size and density, soil moisture, and stream flow. The evidence presented here also includes studies from any continent where models (both empirical statistical models and mechanistic models) were developed and assessed to examine ozone effects on plant water use and ecosystem water cycling.

8.11.1 Structural Changes in Plants

In addition to well-documented ozone-mediated declines in leaf area and longevity, new evidence identifies a relationship between ozone and changes in wood anatomy associated with water transport. Additional studies find ozone alters plant biomass allocation by decreasing root growth and density, which may result in lower drought tolerance and changes to soil moisture and runoff (Table 8-19). Both alterations are important mechanisms for ozone effects on ecosystem water cycling.

- Recent results from the long-term Aspen FACE experiment show ozone causes significant changes in wood anatomy (along with changes in leaf area index and longevity reviewed in the 2013 Ozone ISA) and vessel architecture. Ozone-exposed trees had more and narrower vessels which were packed more densely per unit wood area, indicating that trees prioritized hydraulic safety over water transport efficiency. These developmental shifts in wood anatomy are one mechanism for changes in tree water use efficiency, and thus, ecosystem water cycling (Kostiainen et al., 2014).
- Because plants rely on their root systems for water uptake, shifts in carbon allocation away from roots can significantly alter water cycling. In a study by Hayes et al. (2012a), *Dactylis glomerata*, previously thought to be a species insensitive to ozone, showed increasing sensitivity with increasing ozone concentration as seen by large reductions in root biomass, with a 50% reduction between highest and lowest ozone treatments. Ozone was found to shift biomass allocation away from roots in several other studies (Grantz et al., 2016; Fiscus et al., 2012; Rhea and King, 2012; Calatayud et al., 2011), and changes in root biomass in response to ozone exposure seems to be species specific.

8.11.2 Impaired Stomatal Function

Ozone-mediated impairment of stomatal function has been documented for decades (Keller and Häsler, 1984), although impairment seems to be species specific, and the extent of its prevalence is not clear. Studies continue to show reduced sensitivity of stomatal closing in response to various factors (light, vapor pressure deficit, temperature, soil moisture) when exposed to ozone ("sluggish stomata") in a number of species (Table 8-21).

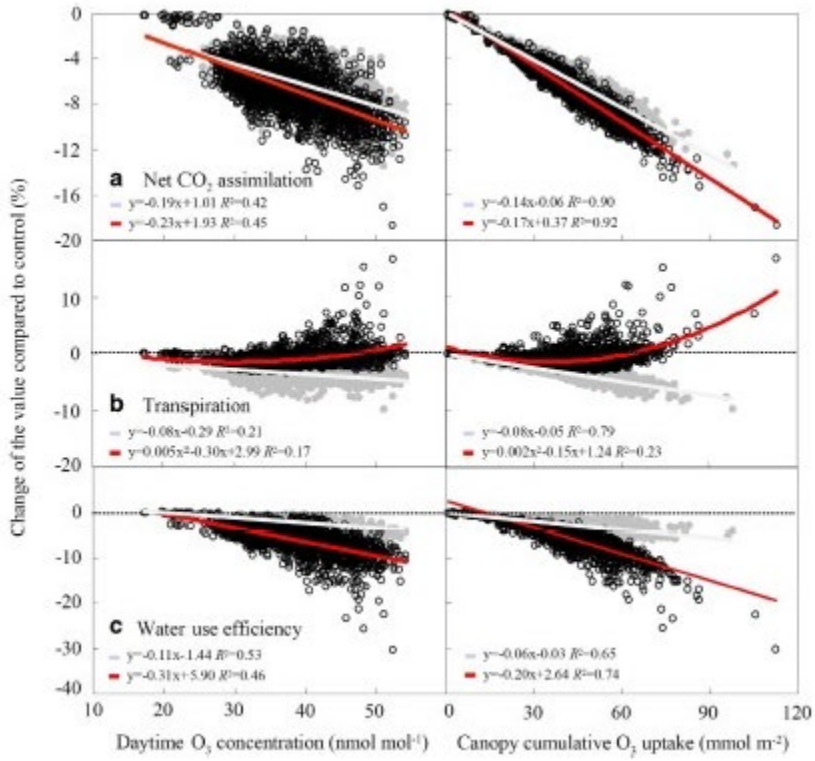
- A meta-analysis synthesized studies of ozone effects on stomatal response in 68 species (including trees, crops and grassland); 10% showed a sluggish stomatal response to elevated ozone, 24% showed an increased stomatal opening under elevated ozone, and 44% displayed stomatal closure in response to ozone. Trees were the most adversely affected, with 73% showing an altered stomatal response. Four tree species exhibited sluggish stomata and 13 showed stomatal opening in response to ozone (Mills et al., 2016; Mills et al., 2013). This study provides a comprehensive look at prevalence of ozone impairment to stomatal functioning across multiple plant species and growth forms.
- Under increased ozone, a sluggish response of stomata was observed in a growth chamber experiment using poplar trees (three genotypes of a *Populus deltoides* × *Populus nigra* hybrid) in reaction to changes in light intensity, CO_2 concentration, and vapor pressure deficit. The speed of the responses varied by genotype and appeared to explain some of the genotype-related sensitivity seen in poplar trees (Dumont et al., 2013).
- An ozone-FACE experiment in Japan shows leaves of Siebold's beech (*Fagus crenata*) grown in elevated ozone took a significantly longer time to close stomata (+27 and +73%, in August and September, respectively) and slower rate of decrease in stomatal conductance (−26 and −64%) than leaves of trees grown in ambient conditions in response to decreasing light (Hoshika et al., 2012b). Models of transpiration created for this data set were found to better fit the data when stomatal conductance was adjusted for ozone exposure (Hoshika et al., 2012a). Reduced stomatal sensitivity was also reported for *Betula platyphylla* var. *japonica* at this FACE site (Hoshika et al., 2018), as well as *Ailanthus altissima*, *Fraxinus chinensis*, and *Platanus orientalis* in OTC experiments in China (Hoshika et al., 2014).

- A study of two grassland species shows that ozone causes a lack of stomatal sensitivity to changes in environmental conditions. The widely distributed grassland species *Dactylis glomerate* consistently exhibited reduced sensitivity of the stomatal closing response to all the environmental parameters studied (light, vapor pressure deficit, temperature, soil moisture) in elevated ozone treatments. In a co-occurring species, *Ranunculus acris,* stomatal conductance was a found to be less responsive to light, vapor pressure deficit, and temperature under high ozone (Wagg et al., 2013). In another study of *Dactylis glomerate*, elevated ozone caused stomatal insensitivity to drought conditions between 3 and 9 weeks. Lack of stomatal control was shown on leaves that developed after a midseason harvest, implying that the results seen are not due to long-term exposure damage to leaf architecture, but develop on the plant under adverse conditions (Hayes et al., 2012a).
- Sluggish stomatal response have also been reported in ozone sensitive *Phaseolus vulgaris* [snapbean; Hoshika et al. (2016)], but not in *Glycine max* (Bernacchi et al., 2011).
- Finally, numerous studies outside of the scope of this review have considered ozone effects on leaf-level physiological processes, particularly photosynthetic and gas exchange measurements, and the biochemical mechanisms of ozone response (U.S. EPA, 2009).

8.11.3 Models of Plant Water Use

A few studies have attempted to assess ozone effects on plant water demands across large regions using various modeling methods; significant differences exist between estimations generated by models that assume stomatal closure with ozone exposure and those which take into account stomatal impairment from ozone.

- A conceptual model of leaf atmospheric boundaries developed using data collected in a pasture from C3 grasses to assess changes in evapotranspiration estimated that ozone reduced maximum evapotranspiration by 7.5% (Super et al., 2015). Grasses are relatively ozone tolerant, and stomatal closure may help limit ozone effects (Section 8.10).
- Using the Community Land Model, Lombardozzi et al. (2015) estimated that present-day ozone exposure reduces transpiration globally by 2−2.4%. Larger reductions in GPP compared to transpiration decreased water-use efficiency 5−10% in the eastern U.S., and increased surface runoff more than 15% in eastern North America. However, uncertainties arise when trying to estimate transpiration over large geographical regions consisting of different species with a simple transpiration function. Additionally, this study did not consider stomatal sluggishness which could modify the transpiration results.
- Models that do not incorporate sluggish stomatal response may significantly underestimate plant water loss. When accounting for it, transpiration decreases until 30 ppb ozone and then increases with increasing ozone exposure. A significant part (10%) of the water use efficiency (WUE) at North American sites may be explained by ozone exposure with ozone-induced stomatal sluggishness. The contribution of ozone to declines in WUE is estimated at 4.5 to 8.8 % in different regions of the Northern Hemisphere [Hoshika et al. (2015); Figure 8-13].



Note: Effects of ozone-induced stomatal sluggishness were included (black open circles and red lines) or excluded (gray circles and gray lines). The percentage of change of each parameter was calculated relative to "control run" (no ozone effect).
Source: Permission pending Hoshika et al. (2015).

Figure 8-13 Percentage change of modeled net carbon dioxide (CO_2) assimilation, transpiration, and water use efficiency in temperate deciduous forests in the Northern Hemisphere in relation to daytime mean ozone concentration or cumulative canopy ozone uptake (years 2006−2009). (a) Net CO_2 assimilation, (b) transpiration, and (c) water use efficiency were simulated by the offline coupling simulation of SOLVEG-MRI-CCM2.

8.11.4 Ecosystem Water Dynamics

New work examines the influence of environmental measures, inclusive of ozone exposure and climate, on late-season stream flow in forests in the eastern U.S. and shows that ozone effects scale up from leaf level through to ecosystem level. The 2013 Ozone ISA reviewed the work of Mclaughlin et al. (2007a); Mclaughlin et al. (2007b), which used field measurements to link ozone to changes in tree sap flow and scale up to the ecosystem level. Building on this, Sun et al. (2012) built empirical statistical models from data collected in six watersheds in Tennessee, North Carolina, Virginia, and West Virginia

and found that ozone and climate are both significant predictors of late season stream flow in forests; these predictor variables were also significant when applied to measurements of tree radial growth. Findings from this study support the assertion that ambient ozone concentrations in Appalachian forests decrease efficiency of tree water use through lowered stomatal control, which in turn, reduces streamflow in forested watersheds. When statistical models were partitioned to examine the contribution of ozone and climate variables to predictions of streamflow, Sun et al. (2012) also found statistically significant negative interaction effects between climate and ambient ozone levels that resulted in a net decrease in late season streamflow.

8.11.5 Drought and Ozone

Several studies have tested the interactive effects of ozone and drought on plant stomatal function, water use, growth, and performance; these are discussed in the section on modifying factors (Section 8.12).

8.11.6 Summary

During the review for the 2013 Ozone ISA, the widely held assumption that ozone exposure consistently reduced stomatal conductance in plants was being challenged. Several studies found increased conductance, suggesting stomatal dysfunction in response to ozone exposure; other studies found ozone caused a loss of stomatal control, incomplete stomatal closure at night, and a decoupling of photosynthesis and stomatal conductance. The relationship of stomatal response to ozone exposure continues to be an active area of research. There is mounting biologically relevant, statistically significant, coherent, and cohesive evidence from multiple studies of various types about the mechanisms of ozone effects on plant water use and ecosystem water cycling (reduced leaf area, reduced leaf longevity, changes in root and branch biomass and architecture, changes in vessel anatomy, stomatal dysfunction, reduced sap flow). Additionally, there are a few strong studies which scale up these changes to effects at the ecosystem level and show significant effects. The most compelling evidence is from six watersheds in eastern forests and from Aspen FACE. This new information supports and strengthens the conclusions of the 2013 Ozone ISA. **The body of evidence is sufficient to conclude that there is a likely to be causal relationship between ozone exposure and the alteration of ecosystem water cycling.**

Table 8-21 Ozone exposure and water cycling.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Water Cycling
Hoshika et al. (2011)	Lab study; location not stated	*Phaseolus vulgaris*, S156 (snap bean)	Short-term 1 h, low (48 ppb), middle (87 ppb), high (150 ppb), and control (0 ppb)	Steady-state stomatal conductance decreased by 27% in low O_3 and 75% in high O_3 under well-watered conditions. There was no effect of O_3 treatment in water-stressed conditions. High O_3 exposure caused higher nocturnal stomatal conductance than control plants under well-watered conditions.
Fiscus et al. (2012)	USDA-ARS Plant Science Research Unit field site 5 km south of Raleigh, NC	Two genotypes of *Phaseolus vulgaris* (snap bean)	Two O_3 concentrations (charcoal-filtered air) dispensed into outdoor chambers (12-h mean of 0 and 60 ppb). Exposures started 18 days after planting at 1/3 target concentrations and increased to full exposure at 21 days after planting. Experiment ran 62 days. For elevated O_3 daily AOT40 = 245, SUM06 = 534, W126 = 295 ppb-h. There were also two vapor pressure deficit (VPD) levels tested (1.26 and 1.96 kPa)	In low VPD treatment with elevated O_3, daily water use significantly increased 23 to 38%.
Grantz et al. (2016)	Greenhouse; Parlier, CA (36.60°N, 119.50°W)	*Gossypium barbadense* (Pima cotton)—O_3 sensitive cultivar	Approximate 12-h mean O_3 concentrations were 4, 59, and 114 ppb, with peak concentrations at solar noon	Midday stomatal responses at are not representative of the morning or evening. Lowest responsivity was observed during periods of rapid stomatal movement in the morning and evening. Maximum responsivity corresponded to previously determined maximum plant sensitivity to short-term pulse exposures in the cotton plants, not with maximum gas exchange active regulation. A clear diel pattern emerged with stomatal responsivity increasing in the early morning through midafternoon then decreasing in the early evening.

Table 8-21 (Continued): Ozone exposure and water cycling.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Water Cycling
Paudel et al. (2016)	Greenhouse; Parlier, CA (36.60°N, 119.50°W)	*Amaranthus palmeri* (Palmer amaranth)	Two runs of exposure 30 and 35 days. 12-h means of 4, 59, and 114 ppb	Elevated O_3 exposure and water stress had no effect on the daytime stomatal conductance, shoot growth, and root growth. This agricultural weed species may be more tolerant to elevated O_3 and moisture stress than crop species.
Vanloocke et al. (2012)	FACE; SoyFACE, Champaign, IL	*Glycine max* (soybean)	12-h means of 40, 46, 54, 58, 71, 88, 94, 116 ppb	With increasing ozone treatment, yield (−64%), canopy evapotranspiration (−26%), and water use efficiency (−50%) decreased. The sensible heat flux, water use efficiency, and canopy temperature increased linearly. These results indicate that O_3 could alter meteorological conditions through warmer surface temperatures and perturb the hydrologic cycle via decreased water vapor release to the atmosphere.
Hoshika et al. (2016)	Greenhouse; location not stated	*Phaseolus vulgaris* (snap bean) ozone sensitive genotype S156	Elevated O_3 exposure level 149 ± 3 ppb, control 3 ± 1 ppb	Elevated O_3 induced stomatal sluggishness only under high light intensity (1,500 μmol/m-s); stomata needed 53% more time to half Gs under high light × elevated O_3.
Bernacchi et al. (2011)	FACE; SoyFACE, Champaign, IL (40.056°N, 88.201°W)	*Glycine max* (soybean)	2002−2006; 8-h max (ppb): ambient = 35−55, elevated = 46−68; AOT40 (ppm-h): ambient = 3−35, elevated = 25−65; SUM06 (ppm-h): ambient = 4−21, elevated = 15−39	Elevated O_3 reduced evapotranspiration for four of five growing seasons. O_3 decreased seasonal water use by 12% in 2002, 14% in 2003, 13% in 2005, and 11% in 2006. In 2004, there was no significant effect of O_3. Under elevated O_3, canopy temperatures were consistently warmer. The results suggest that future increased O_3 exposure could lead to alterations in the local and regional hydrologic cycles in areas of high intensity soybean cultivation.

Table 8-21 (Continued): Ozone exposure and water cycling.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Water Cycling
Sun et al. (2012)	Gradient; six watersheds: Walker Branch and Littler River (eastern Tennessee), Cataloochee Creek (western North Carolina), James River and New River (Virginia), and Fernow (West Virginia)	Appalachian mixed deciduous forests	AOT60 at each watershed: 1.72 (WBWS), 2.6 (LR), 1.72 (CC), 0.82 (NR), 0.83 (JR), 0.74 (FEW); maximum hourly (in ppb): 68.2 (WBWS), 67.8 (LR), 68.2 (CC), 59.4 (NR), 58.7 (JR), 58.8 (FEW)	O_3 and climate are both significant predictors of late season stream flow, regardless of the seasonal timescale used for these parameters. Models incorporating O_3 and climate capture the variation and magnitude of stream flow, and also fit annual tree ring growth (an important mechanistic step in O_3 effects on forested watersheds). Models generated from data from southern watersheds in Tennessee (where O_3 levels are higher) have better predictive power throughout the study area than those in the north. Ambient O_3 concentrations in Appalachian forests decrease efficiency of tree water use through lowered stomatal control and that reduces streamflow in forested watersheds.
Kostiainen et al. (2014)	FACE; Aspen FACE, Rhinelander, WI	*Populus tremuloides* (quaking aspen) clones, *Betula papyrifera* (paper birch)	Fumigation 1998−2008 during daylight hours of the growing season. Ambient O_3 W126 2.1−8.8 ppm-h and elevated 12.7−35.1 ppm-h; elevated CO_2 515−540 ppm , ambient average 374. For hourly ozone concentrations during experimental ozone treatment, see Kubiske and Foss (2015)	Elevated CO_2 increased radial growth and cell diameters in aspen, while vessel density and proportion decreased. Elevated O_3 decreased growth and cell diameters, but increased vessel density and proportion. Neither CO_2 nor O_3 responses were consistent across years. O_3 exposed trees had more and narrower vessels, which were packed more densely per unit wood area.
Kefauver et al. (2012a)	Gradient; Yosemite National Park (YOSE) and Sequoia and Kings Canyon National Park (SEKI), CA; Catalonia, Spain	California: *Pinus ponderosa* (ponderosa pine) and *Pinus jeffreyi* (Jeffrey pine) Spain: *Pinus uncinata* (mountain pine)	Passive monitors in YOSE and SEKI colocated with one U.S. EPA-certified active monitor per park. Average yearly O_3 mixing ratio in 2002 ranged from 35 to 65 ppb for all YOSE and SEKI sites. Yearly averages within sites were 49 ppb for YOSE and 46 ppb for SEKI	Ozone Injury Index by itself was poorly correlated to ambient O_3 across all sites. Models improved when GIS variables related to plant water status were included (YOSE, $R^2 = 0.36$, $p < 0.001$; SEKI, $R^2 = 0.33$, $p = 0.007$).

Table 8-21 (Continued): Ozone exposure and water cycling.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Water Cycling
Hoshika et al. (2014)	OTC; China	*Ailanthus altissima* (tree of heaven), *Fraxinus chinensis* (Chinese ash), and *Platanus orientalis* (Oriental planetree)	42, 69, 100 ppb avg from 9:00 a.m. to 6:00 p.m. for 3 mo	O_3 did not affect stomatal density. Elevated O_3 exposure slowed stomatal dynamics in these tree species. Time for 50% decrease of stomatal conductance increased with increasing stomatal O_3 flux.
Holmes (2014)	Gradient; U.S. and Europe	Many trees species	O_3 concentrations not given. Trends over 1995−2010	As a result of O_3 AOT40 decreasing by approximately half (~20 to 10 in the Midwest) over the period 1995−2010, forest WUE likely increased by ~0.33% per year in the midwestern U.S. and slightly less in the northeastern U.S.
Dumont et al. (2013)	Lab; France	Three Euramerican *Populus deltoides* × *Populus nigra* (poplar) genotypes (Carpaccio, Cima, and Robusta)	Elevated O_3 at 120 ppb for 13 h/day and charcoal-filtered air. Treatments run for 18 days	O_3 significantly decreased stomatal conductance and photosynthesis for the three genotypes. Under increased O_3, a sluggish response of stomata was observed in reaction to blue light intensity, CO_2 concentration and VPD, and lower amplitude of the response to variations in light intensity. Speed of responses varied by genotype and appeared to explain some of the genotype-related sensitivity.
Hoshika et al. (2012b)	FACE; Sapporo Experimental Forest, Hakkaido University, northern Japan (13.067°N, 141.333°E)	*Fagus crenata* (Siebold's beech)	Daytime O_3: ambient = 26 ppb, elevated = 54 ppb; AOT40: ambient = 0.3, elevated = 11.9 ppm-h	Leaves under elevated O_3 had lower stomatal conductance (25 and 31% in September and October, respectively) than control leaves and had a steeper decline in photosynthesis after September. Leaves in elevated O_3 had significantly longer time to close stomata (+27 and +73%, in August and September, respectively) and slower rate of decrease of stomatal conductance (−26 and −64%) than leaves of trees grown in ambient conditions in response to decreasing light.

Table 8-21 (Continued): Ozone exposure and water cycling.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Water Cycling
Hoshika et al. (2012a)	FACE; Sapporo Experimental Forest, Hakkaido University, northern Japan (13.067°N, 141.333°E)	*Fagus crenata* (Siebold's beech)	The target O_3 concentration was 60 ppb during daylight hours. Mean daytime O_3 concentrations were 25.7 ± 11.4 ppb (ambient) and 56.7 ± 10.5 ppb (elevated). Fumigation was August 6−November 11, 2011	Gs under elevated O_3 was lower than under ambient conditions after 2 weeks of exposure. The ratio of daily maximum Gs (elevated O_3:ambient O_3) decreased linearly with both cumulative O_3 uptake and AOT40, although the determination of coefficient was substantially higher with cumulative exposure calculation (r^2 = 0.67 vs.0.44). Jarvis algorithm better fits data when Gs is adjusted for O_3 exposure.
Hoshika et al. (2015)	Not site-specific (although model parameters were taken from FACE); Sapporo Experimental Forest, Hakkaido University, northern Japan (13.067°N, 141.333°E)	Northern Hemisphere temperate forests	Modeled response covers a range of O_3 exposures—daytime concentrations of 15 to 55 ppb; canopy cumulative O_3 uptake from 0 to 115 ppb	The O_3 induced decline of net CO_2 assimilation at the average daytime O_3 concentrations of 37.2 ± 6.2 nmol/mol was 6.6 ± 2.1% and 6.0 ± 1.8% (incorporating sluggish stomatal response and without). O_3 further reduced CO_2 assimilation at higher concentrations. Without the inclusion of stomatal sluggishness parameters, transpiration showed a linear decline as O_3 increased. When sluggishness was included, transpiration decreased until 30 nmol/mol of O_3 concentration or 37 mmol/m^2 of canopy cumulative O_3 uptake, and then increased with increasing O_3 exposure or uptake. O_3 decreased WUE as compared to control, with higher exposure causing greater declines in WUE the contribution of O_3 to the decline in WUE ranged from 4.5 ± 1.9 to 8.8 ± 3.0% in different regions of the Northern Hemisphere. When taking sluggishness into account, authors estimated that a 8−10 ppb decrease in O_3 concentrations would yield an increase of 2−3% in WUE of temperate forests, while only a ~1% increase of WUE at the same change in O_3 was found without sluggish stomatal response.

Table 8-21 (Continued): Ozone exposure and water cycling.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Water Cycling
Gao et al. (2017)	OTC; Seed Station Field of Changping, northwest Beijing, China	O_3 sensitive clone (546) of *Populus deltoides* (eastern cottonwood)	Three O_3 treatments: charcoal-filtered (CF), ambient (ambient), and elevated (E−O_3) for 96 days (June−September). Mean O_3 was 33.5 ± 2.4 ppb in the CF treatment, 51.1 ± 4.1 ppb in the ambient treatment, and 78.2 ± 5.5 ppb in the E−O_3 treatment. Accumulated exposures in the CF, ambient, and E−O_3 treatments expressed as AOT40 were 4.3, 16.0, and 38.7 ppm-h, respectively. Two irrigation treatments: drought treatment had 51.9% less water, while well-watered plants were watered to capacity. Average soil water content of well watered and drought treatments was 24.8 ± 0.38% (95% CI) and 12.8 ± 0.47%, respectively	Elevated O_3 significantly reduced total biomass, stem diameter, stem biomass, and leaf biomass. Interactions between O_3 and water stress were significant for leaf, stem, and total biomass of the plants, with lower relative biomass reductions in drought-stressed plants. Leaf senescence, was also reduced in reduced watered plants in comparison to well-watered plants. For O_3 dose-response, modeled as biomass changes, model performance was significantly better when using POD (flux) compared with AOT40 (R^2 = 0.829, p = 0.012 vs. R^2 = 0.560, p = 0.087). Using the flux model, the O_3 critical level (CL) for preventing a 4% biomass loss in this poplar clone under different water regimes was between 5.27 mmol/m^2 PLA and 4.09 mmol/m^2 PLA.
Hoshika et al. (2018)	FACE; Sapporo Experimental Forest, Hokkaido University, northern Japan	*Betula platyphylla* var. *japonica* (Japanese white birch) and *Quercus mongolica* var. *crispula* (Mongolian deciduous oak)	There were two plots, one with elevated O_3 (target of 60 ppb) and one with ambient O_3. Fumigation occurred August to November 2011, and May to November 2012. Daytime hourly mean O_3 concentrations in ambient and elevated O_3 were 25.7 ± 11.4 ppb and 56.7 ± 10.5 ppb during the experimental period in 2011, and 27.5 ± 11.6 ppb and 61.5 ± 13.0 ppb in 2012	Elevated O_3 significantly decreased white birch stomatal conductance 28% in early summer, and 10% in late summer. Elevated O_3 reduced stomatal sensitivity of white birch to VPD and increased stomatal conductance under low light conditions. In contrast, no significant effects of O_3 were observed in deciduous oak.

Table 8-21 (Continued): Ozone exposure and water cycling.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Water Cycling
Xu et al. (2017)	OTC; Shenyang Arboretum, China	Greenhouse grown 1 yr old *Lonicera maackii* (bush honeysuckle)	30 days of O_3 treatments in ppb (range, mean, AOT40): control (1.2−58.3, 41.5, 165.3), drought (0.9−62.1, 39.8, 170.9), elevated O_3 (75.4−125.0, 85.3, 12,073.5), drought × O_3 (68.9−119.0, 84.9, 11,685.2); soil water content: control (65.9%), drought (35.6%), elevated O_3 (62.5%), drought × O_3 (38.4%)	All treatments significantly decreased stomatal size as compared with control, and O_3 significantly decreased WUE in single and combined treatments (about 30%).
Bohler et al. (2013)	Greenhouse study; 14-h light period, location not given	10 cm tall clones of *Populus tremula* × *P. alba* (*Populus* × *canescens* [poplar]—clone INRA 717-1-B4).	Factorial design of O_3 by drought: O_3 treatments: charcoal-filtered air, charcoal-filtered air + 120 ppb of O_3 for 13 h/day. Drought treatment: maintained soil water content at 35%	Differences in Gs were observed on Day 10, when O_3 × drought treatment had a lower Gs than control (roughly 0.18 mmol/m^2-s vs. 0.3 mmol/m^2-s), with no significant effect of drought alone or O_3 alone.
Wagg et al. (2013)	Greenhouse; England	*Ranunculus acris* (meadow buttercup), *Dactylis glomerata* (orchard grass)	Low O_3: 16−34 ppb seasonal mean, high O_3: 73−90 ppb seasonal mean	For *D. glomerata*, the maximum stomatal conductance increased 50% in high O_3 compared to low O_3 *D. glomerata* grown in high O_3 exhibited reduced sensitivity of stomatal closing response to the environmental parameters of light, vapor pressure deficit, temperature, soil moisture. At high O_3, *R. acris* stomatal conductance was less responsive to light, vapor pressure deficit, and temperature.

Table 8-21 (Continued): Ozone exposure and water cycling.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Water Cycling
Hayes et al. (2012a)	Greenhouse; Bangor, North Wales, U.K. (elevation 610 m, grid ref SH613619)	*Dactylis glomerata* (orchardgrass)	Treatments in ppb and ppb-h (season mean, mean daily maximum, season AOT40): ambient-20: (16.2, 20.8, 0); ambient: (33.9, 41.56, 2.00); ambient+12 (44.1, 53.85, 13.95); ambient+24 (50.7, 62.4, 24.80); ambient+36 (62.0, 80.6, 44.33); ambient+48 (72.6, 89.1, 58.04); ambient+60 (88.9, 108.4, 84.65); ambient+72 (89.5, 110.7, 85.12)	At 3 weeks, there was drought-induced lowering of stomatal conductance. After 9 weeks of elevated O_3 exposure, stomatal conductance was only significantly different between watering treatments at low/moderate levels of increased O_3. At 19 weeks, stomatal conductance (i.e., drought response) in newly developed leaves of reduced water plants was only significantly lower at ambient O_3. Modifying models of cumulative O_3 flux to incorporate this loss of stomatal control results in significantly higher values of cumulative O_3 uptake in leaves. *Dactylis glomerata* shows increasing sensitivity with increasing O_3, due to 50% reduction in root biomass between highest and lowest O_3 treatments. Shoot biomass increased slightly with increasing O_3.
Super et al. (2015)	Model using data collected in Cabauw pasture in the Netherlands (51.91°N, 4.93°E)	Unspecified C3 grasses	Measured O_3 exposures ranged diurnally from 0 to 28 ppb	In a conceptual model of leaf atmospheric boundaries to assess changes in evapotranspiration, O_3 reduced maximum evapotranspiration 7.5%.
Lombardozzi et al. (2015)	Mode based on literature reviews; global	Vegetation	Global concentrations 2002−2009, generated by CAM model. Global growing season mean hourly O_3 concentrations from 2002 to 2009 ranged approximately from 0 to 55 ppb	The model estimated that ambient O_3 reduced GPP by 8−12% and transpiration by 2−2.4% globally. GPP and transpiration decreased as much as 20 and 15%, respectively, in the eastern U.S., Europe, and southeast Asia. Model did not include stomatal sluggishness responses.

Table 8-21 (Continued): Ozone exposure and water cycling.

Study	Study Type and Location	Study Species	Ozone Exposure	Effects on Water Cycling
Mills et al. (2016)	Reanalysis of papers reviewed from numerous study locations	68 species (including trees, crops, and seminatural grassland species)	Multiple studies with multiple exposure values	Of the 68 species, 22% showed no change in stomatal conductance, 10% showed a slowed (sluggish) stomatal response to elevated O_3, 23.5% showed an increased stomatal opening under elevated O_3, and 44% displayed stomatal closure in response to O_3. Tree species were the most adversely affected with 73% of species showing an altered stomatal response, with 13 species showing stomatal opening and 15 showing stomatal closure in response to O_3. Crops tended to respond to O_3 stress with stomatal closure (occurring in 75% of the species), while increased or sluggish stomatal response was only reported in 19% of the crops. For the eight grassland species included, responses were more or less evenly spread across the four categories of stomatal response. There is a tendency for stomatal opening to occur at lower concentrations.
Calatayud et al. (2011)	OTC; Spain	*Lamottea dianae,* a perennial forb endemic to the Mediterranean	24-h avg (ppb): CF = 11, NF+30 = 40, ambient = 32; 12-h avg (ppb): CF = 10, NF+30 = 66, ambient = 46; 8-h avg (ppb): CF = 13, NF+30 = 74, ambient = 49; AOT40 (ppm-h): CF = 0, NF+30 = 36, ambient = 11	Visible symptoms began in 3 days at an AOT40 of 2 ppm-h in the NF+30 treatment. Mature leaves in NF+30 had a 26% reduction in photosynthesis and a 25% decrease in WUE at saturating light conditions compared to CF. NF+30 significantly reduced belowground biomass compared to CF.

C3 = plants that use only the Calvin cycle for fixing the carbon dioxide from the air; C4 = plants that use the Hatch-Slack cycle for fixing the carbon dioxide from the air; CAM = plants that use the crassulacean acid metabolism for fixing the carbon dioxide from the air; CF = charcoal-filtered air; CO_2 = carbon dioxide; FACE = free-air CO_2 enrichment; GPP = gross primary production; Gs = stomatal conductance; kPa = kilopascal; NF+30 = nonfiltered air plus 30 ppb ozone; nmol/m^2 = nanomole/meters squared; O_3 = ozone; OTC = open-top chamber; POD = phytotoxic ozone dose; ppb = parts per billion; ppm = parts per million; SUM06 = seasonal sum of all hourly average concentrations ≥ 0.06 ppm; μmol/m^2/s = micromoles/meters squared/second; VPD = vapor pressure deficit; W126 = cumulative integrated exposure index with a sigmoidal weighting function; WUE = water use efficiency.

8.12 Modifying Factors

It is important to acknowledge that ozone is just one of the environmental and anthropogenic factors simultaneously influencing ecosystem function and that the human influence on ecosystems is ubiquitous (Lewis and Maslin, 2015). To varying degrees, these other factors may exacerbate or negate the effects of ozone. Research into how interactions with biotic and abiotic factors, both natural and anthropogenic, is diverse and includes topics such as UV-B radiation (Bao et al., 2014), pathogens (Mina et al., 2016; Chieppa et al., 2015), shifts in community genetic and species composition (Menendez et al., 2017; Moran and Kubiske, 2013), and land use/land cover change (Tian et al., 2012). The degree to which biotic and abiotic factors may modify the effects of ozone on ecosystems was comprehensively reviewed in the 2006 Ozone AQCD and updated in the 2013 Ozone ISA. Consequently, this section focuses on three factors that have received considerable research attention since the 2013 Ozone ISA: nitrogen enrichment, increases in atmospheric CO_2, and climate change. These topics were not systematically reviewed for this ISA; however, some key citations are highlighted.

8.12.1 Nitrogen

Because oxidized nitrogen is a precursor to ozone formation, many ecosystems that are exposed to chronic ozone pollution also experience elevated rates of N deposition (Fowler et al., 1998), and the combined effects of these two anthropogenic pollutants on plants and ecosystems have been a topic of research for decades (Takemoto et al., 2001; Grulke et al., 1998; Darrall, 1989). The 2013 Ozone ISA reviewed several mechanisms wherein elevated N deposition might exacerbate or negate the effects of ozone. First, because leaf-level photosynthesis is positively correlated with both foliar N concentrations and stomatal conductance (Wright et al., 2004) and N deposition has been linked to increased photosynthetic capacity in some ecosystems (Fleischer et al., 2013), greater N deposition may lead to higher ozone flux into the leaf and further ozone damage. Conversely, because N limitation often limits plant productivity (Yue et al., 2016), N deposition can stimulate plant growth and NPP (Horn et al., 2018; Thomas et al., 2010), overshadowing the effects of ozone on these processes. Additionally, the 2013 Ozone ISA also cited the possibility that increased photosynthesis as a result of N deposition could help plants produce antioxidants that neutralized ozone damage.

Nitrogen deposition can also decrease plant biodiversity, eliminating rare species and favoring species with rapid growth rates (Suding et al., 2005). In a national analysis of the growth and mortality of 71 tree species, 53 of the species exhibited positive, negative, or unimodal (threshold) changes in growth or mortality with increasing N deposition (Horn et al., 2018). Among forests in the northeastern U.S., the growth of some ozone-sensitive trees such as black cherry (*Prunus serotina*) and tulip poplar (*Liriodendron tulipifera*) increased with greater N deposition, while the only three species showing

decreased growth rates with greater N deposition were evergreen conifers, which tend to be less sensitive to ozone (Thomas et al., 2010). Each of these effects of N deposition on plant communities—decreased biodiversity, greater abundance of rapidly growing plants, and the promotion of ozone-sensitive broadleaf species at the expense of conifers—could make ecosystems more sensitive to the negative effects of ozone. However, in a survey of grassland community composition in the U.K., Payne et al. (2011) found that while N deposition and ozone each had effects on community composition, there were no interactions between these effects. Similarly, Bassin et al. (2013) observed no significant interactive effects between ozone and N for plant community composition or biomass in a 7-year factorial N addition and ozone experiment in alpine grassland mesocosms in Switzerland. In a short-term (39 days) OTC experiment in a Mediterranean annual grassland in Spain, Calvete-Sogo et al. (2016) observed several significant ozone × N for the growth of individual species. In another ozone and N deposition experiment in a Mediterranean ecosystem in Spain with the annual grass *Briza maxima*, the high N deposition treatment negated the increase in leaf senescence caused by ozone in other treatments, but the increase in grass lignin concentration caused by ozone persisted (Sanz et al., 2011). In applying the DLEM to 20th century C cycling in the southeastern U.S., Tian et al. (2012) found significant effects of ozone and N deposition on NPP and C sequestration, but no interactive effects between the two.

Although gas-phase forms of N such as NO_X and PAN can cause direct foliar injury and phytotoxicity (Greaver et al., 2012; Riddell et al., 2012) that would be potentially additive to similar damage caused by ozone, the most recent Integrated Science Assessment for Oxides of Nitrogen, Oxides of Sulfur, and Particulate Matter-Ecological Criteria [Second External Review Draft U.S. EPA (2018)] concluded that concentrations of these gas-phase N forms in the U.S. rarely reach levels high enough to be damaging.

8.12.2 Carbon Dioxide

The effects of elevated atmospheric CO_2 on plants and terrestrial ecosystems have been well studied over the past several decades (Norby and Zak, 2011; Curtis and Wang, 1998). Elevated CO_2 broadly stimulates photosynthesis and often increases plant growth and NPP (Norby and Zak, 2011). Because these effects contrast with those of ozone, it has long been hypothesized that elevated CO_2 may counteract the effects of ozone on plants (Dickson et al., 2000). However, like ozone and N deposition, the effects of CO_2 extend beyond changes in photosynthesis and growth to include changes in other properties and processes such as tissue chemistry (Norby and Zak, 2011), ecosystem water use (Norby and Zak, 2011), and trophic interactions (Andrew et al., 2014; Couture et al., 2012). Further, like N deposition, the effects of elevated CO_2 are often species specific, and the shifts in plant community composition observed under elevated CO_2 can have consequences for biogeochemical cycling and other processes (Bradley and Pregitzer, 2007). The diverse and complex effects of elevated CO_2 make it difficult to fully predict how ozone might interact with elevated CO_2.

Research on the combined effects of CO_2 and ozone were reviewed in detail in the 2006 Ozone AQCD and the 2013 Ozone ISA concluded that the bulk of the research to date showed that increased CO_2 could protect plants from ozone damage. In the time since the 2013 Ozone ISA, numerous new papers have been published from the Aspen FACE and SoyFACE experiments, both of which include elevated CO_2 and ozone treatments. At SoyFACE, there was a significant interaction between CO_2 and ozone wherein elevated CO_2 did not affect snap bean (*Phaseolus vulgaris*) pod yield, but did ameliorate the negative effect of ozone (Burkey et al., 2012). In addition, some elements of soil microbial community composition also responded uniquely to the combined CO_2 and ozone treatment (He et al., 2014). However, the effects of CO_2 and ozone at SoyFACE were generally additive and often offsetting, including in aspects of the agroecosystem that are less directly tied to the leaf-level physiological effects, such as mycorrhizal community composition (Cotton et al., 2015) and rates of soil N cycling (Decock et al., 2012).

As at SoyFACE, CO_2 and ozone had largely offsetting effects on most ecosystem properties and processes at Aspen FACE. In analyses conducted at the end of the Aspen FACE experiment, Talhelm et al. (2014) and Zak et al. (2011) found few statistically significant interactions between ozone and CO_2 in measures of growth, productivity, ecosystem C pools, or ecosystem N pools. Instead, the combined treatment (CO_2 + ozone) created changes that were similar to the additive effects of the CO_2 and ozone treatments individually (Talhelm et al., 2014; Zak et al., 2011). Likewise, Hofmockel et al. (2011) observed no significant $CO_2 \times$ ozone interactions among particulate and mineral-associated fractions of soil organic matter. The relative competitive ability of tree species or aspen genotypes to acquire soil N was altered by CO_2 and ozone as individual treatments, but there were no significant interactions between the gases (Zak et al., 2012). Similarly, although CO_2 and ozone each individually affected the growth and survival of the five different aspen genotypes in ways that altered community genetic composition, community composition in the CO_2 + ozone treatment was similar to ambient conditions (Moran and Kubiske, 2013). There were some significant $CO_2 \times$ ozone interactions at higher trophic levels, such as for ectomycorrhizal root tip community composition (Andrew and Lilleskov, 2014), arthropod species abundance (Hillstrom et al., 2014), and gypsy moth larvae growth (Couture et al., 2012), but these effects tended to be small, vary by year, or to ameliorate the effect of ozone.

Overall, this body of research suggests that increases in atmospheric CO_2 to levels predicted by midcentury can help ameliorate many of the effects of ozone on terrestrial ecosystems. However, because responses to CO_2 and ozone are each species specific, the combined exposure to CO_2 and ozone can cause some shifts in community composition and concomitant shifts in ecosystem function. Moreover, results from combined CO_2 and ozone exposure experiments do not suggest that elevated CO_2 will prevent ozone damage, but instead create ecosystem outcomes that look similar to the current period (early 21st century) rather than the potential increases in NPP, greater C sequestration, and other changes that could be observed under low ozone conditions in a higher-CO_2 environment.

8.12.3 Weather and Climate

Variation in climate and weather can potentially alter both conditions that lead to the formation, transport, and persistence of ozone in the troposphere (Jacob and Winner, 2009) as well as the vulnerability of plants and ecosystems (Anav et al., 2018; Anav et al., 2017); this section of text focuses on how changes in climate and weather have already and may in the future modify the effects of ozone on ecosystems. The degree to which climate and weather alter the effects of ozone is context specific because damage to terrestrial ecosystems caused by ozone is largely a function of stomatal uptake (Anav et al., 2017). Changes in climate that cause shifts in plant cover from ozone-insensitive species such as needle-leaf evergreens and C4 grasses to ozone-sensitive species, such as some broadleaf deciduous trees and C3 grasses, may increase the portion of the landscape at risk for ozone damage. Conversely, changes in climate that restrict stomatal conductance during periods of the day and growing season that experience high ozone concentrations would limit damage to vegetation. As an example, central and southern California has some of the highest ozone concentrations in the U.S. (Mahmud et al., 2008). Seasonal peaks in ozone in California occur during the summer (Mahmud et al., 2008; Geyh et al., 2000) and daily peaks occur during late afternoon (Fares et al., 2013). However, ozone damage to natural vegetation in California is constrained by the predominance of conifers and other species with low stomatal conductance, as well as the presence of a Mediterranean climate that concentrates precipitation and the growth of vegetation to winter and spring months (Fares et al., 2013). Climate change is expected to increase the number of high ozone summer days in California (Mahmud et al., 2008), but also accelerate the timing of seasonal snowmelt, shift the growing season to earlier in the year, and increase summer plant moisture deficits (Westerling et al., 2011). Thus, climate change in California could simultaneously increase exposure while limiting plant vulnerability by creating conditions that would decrease stomatal conductance during high ozone periods. Conversely, when applying the DLEM model to 20th century C cycling in the southeastern U.S., Tian et al. (2012) found a significant interaction between ozone and climate that decreased NPP. These examples highlight both the range of potential ozone-climate interactions, as well as the degree to which these interactions are context specific.

There have been relatively few field experiments that manipulated both ozone and temperature. A warming (~+0.8°C) and ozone (1.2× ambient) experiment was conducted in Finland, first using potted birch (*Betula pendula*) trees and then with potted Scots pine (*Pinus sylvestris*). For birch, warming increased tree growth and ozone tended to decrease tree growth (particularly a decrease in foliar biomass), but the only significant interactive effect between warming and ozone was that warming ameliorated the acceleration of leaf senescence caused by ozone (Kasurinen et al., 2012). As part of the birch portion of the experiment, Kasurinen et al. (2017) observed that the two treatments each altered the leaf litter chemistry and the soil abundance of bacteria and fungi, but there were no meaningful interactions between the treatments, and treatment effects on litter decomposition were weak. Growth responses for the pine were similar: warming increased growth rates, whereas ozone caused negative effects that were weak aside from decreases in older needle biomass (Rasheed et al., 2017). Only in the 1st year of the experiment was there a significant warming × ozone effect on growth, wherein the negative effect of

ozone on needle biomass was larger in the warming treatment. The pine portion of the experiment also included fertilization and herbivory treatments, and belowground processes such as allocation to root biomass, mycorrhizal colonization, and the rate of root ramification were subject to complex three- and four-way interactions between experimental factors (Rasheed et al., 2017). There were no interactive effects on pine sawfly (*Acantholyda posticalis*) foliar herbivory, but the combination of ozone and warming increased herbivory-induced emissions of sesquiterpenes and oxidated monoterpenes (Ghimire et al., 2017).

There have been more experiments involving ozone and drought stress. Because drought stress decreases stomatal conductance, it can limit ozone effects on plant growth and leaf gas exchange (Gao et al., 2017; Xu et al., 2017; Bohler et al., 2013; Hoshika et al., 2011). However, outcome of these experiments are not always straightforward; in a drought and ozone experiment on Shantung maple (*Acer truncatum*) seedlings, drought stress reduced or alleviated ozone effects on leaf chlorophyll, tree height growth, and stem diameter growth, but tended to exacerbate ozone effects on photosynthesis and stomatal conductance (Li et al., 2015). In the San Joaquin Valley of California, neither drought nor ozone affected daytime stomatal conductance in the invasive weed *Amaranthus palmeri* (Paudel et al., 2016).

Ozone may also exacerbate the effects of climate change on vegetation. Although ozone often decreases stomatal conductance, there is also evidence from multiple experiments that ozone may lead to decreased stomatal responsiveness to changing environmental conditions such as water stress (Wagg et al., 2013; Uddling et al., 2009). At Aspen FACE, this loss of stomatal control was apparently linked to increases in canopy conductance, particularly later in the growing season (Sun et al., 2012; Uddling et al., 2009). The accuracy of model predictions of streamflow in six Appalachian watersheds improved when both ozone and climate were included with in the model, with higher ozone linked to increases in water use, decreases in soil moisture, and lower streamflow (Sun et al., 2012). In addition to hydrologic effects, the decrease in stomatal responsiveness to drought stress caused by ozone may increase stomatal fluxes of ozone and ozone damage under low moisture conditions (Hayes et al., 2012a).

Overall, the body of research examining ozone interactions with climate has grown considerably since the 2013 Ozone ISA. However, the context-specific nature of the outcomes and key mechanisms makes it difficult to make broad generalizations about how climate and ozone interact to influence ecosystems.

8.12.4 Summary

Other factors may exacerbate or negate the effects of ozone on plants, these include nitrogen deposition, CO_2, and climate variables. Nitrogen deposition often co-occurs with increased ozone exposure. At the individual plant level, nitrogen deposition may either increase ozone flux and damage through increased stomatal conductance and higher amounts of photosynthetic machinery, or decrease ozone damage through increased antioxidant production. Effects of increased nitrogen on plant growth

may overshadow the detrimental effects of ozone on the same. At community and ecosystem scales, the species-specific responses to both increased nitrogen and tropospheric ozone result in significant impacts on species composition, although there is very little evidence of interactive effects between the two. For CO_2, research found the response to elevated CO_2 was also species specific. The diverse and complex effects of elevated CO_2 on plant physiology make it difficult to fully predict how ozone might interact with elevated CO_2 in plants. At the individual plant level, increased CO_2 exposure may either protect plants from ozone exposure or overshadow the negative effects. In general, at larger scales, research finds combined exposure to CO_2 and ozone can cause some shifts in community composition and concomitant shifts in ecosystem function. With respect to climate, modeling studies found a significant interaction between ozone and climate that decreased NPP. Relatively few field experiments have manipulated both ozone and temperature, but they find stronger effects of warming on plant growth and function than the effects of ozone, with little evidence of interactive effects. Drought was once thought to have a protective effect from ozone exposure, limiting ozone flux into the leaf as stomata close to prevent water loss. However, more research into ozone-mediated impairment of stomatal function suggest that for some species this assumption is false, and ozone exposures may be much higher than once thought.

8.13 Exposure Indices/Exposure Response

Exposure indices and exposure-response information for vegetation and related ecosystem effects of ozone are critical for understanding the effects of current and future ozone exposures and evaluating potential air quality standards. For over 60 years, controlled ozone exposure experiments have yielded a wealth of information on exposure indices appropriate for vegetation and exposure response relationships. This topic has been thoroughly described and supported by hundreds of studies in the 2013 Ozone ISA (U.S. EPA, 2013) and previous AQCDs (U.S. EPA, 2006, 1996). In this section, new relevant information was considered with what was previously known pertaining to species in the U.S. There is some brief discussion of advances in European dose and exposure models for context of potential advances in this area.

The main conclusions from the 1996 and 2006 Ozone AQCDs and 2013 Ozone ISA regarding indices based on ambient exposure are still valid. These key conclusions can be restated as follows:

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

No recent information available since the 2013 Ozone ISA alters these basic conclusions. The 2013 Ozone ISA and previous AQCDs focused on the research used to develop various exposure indices (e.g., SUM06, AOTx, W126, see Section 8.1.2.2) to help quantify effects on growth and yield in crops, perennials, and trees (primarily seedlings). The performance of indices was compared through regression analyses of earlier studies designed to support the estimation of predictive ozone exposure-response models for growth and/or yield of crops and tree (seedling) species.

8.13.1 Exposure Indices

Exposure indices are metrics that quantify exposure as it relates to measured plant damage (e.g., reduced growth). In the over 60 years of research, many forms of exposure metrics have been used, including 7-, 12-, and 24 hour avg. The current secondary standard form of the 4th highest 8 hour max avg over 3 years is rarely reported in the vegetation research. The most useful metrics in vegetation research have been differentially weighted hourly concentrations that are cumulative during the growth of plants. The 2013 Ozone ISA primarily discussed SUM06, AOTx, and W126 exposure metrics (see Section 8.1.2.2 for definitions). These remain the common concentration-based indices discussed in the literature since the 2013 Ozone ISA. These three types of metrics performed well in a recent study of observations of maize and soybean yield and W126 was the preferred metric because it was potentially the most sensitive index (Mcgrath et al., 2015). Other studies also report various types of mean concentration exposures, which are generally less robust than the metrics discussed above. The indices described in Section 8.1.2.2 have a variety of relevant time windows that may be applied based on time of day and season. In general, ozone concentrations have applied time windows during the daytime (e.g., 8:00 a.m.−8:00 p.m.) when stomata are open and during the active growing season (e.g., 3 months during the warm season; see Section 9.5.3 of 2013 Ozone ISA). In recent study, Mills et al. (2018) described the distributions and trends of W126, AOT40 and 12 hour avg metrics at vegetated sites across the globe and found the highest values were in the mid latitudes of the northern hemisphere where the density of ozone monitors are the greatest.

Another approach for improving risk assessment of vegetation response to ambient ozone is based on determining the ozone concentration from the atmosphere that enters the leaf (i.e., flux or deposition). Much work has been published in recent years, particularly in Europe, in using mathematically tractable flux models for ozone assessments at the regional, national, and European scale (Feng et al., 2017; Mills et al., 2011; Matyssek et al., 2008; Paoletti and Manning, 2007; Emberson et al., 2000b; Emberson et al., 2000a). While some efforts have been made in the U.S. to calculate ozone flux into leaves and canopies (Turnipseed et al., 2009; Uddling et al., 2009; Bergweiler et al., 2008; Hogg et al., 2007; Grulke et al., 2004; Grantz et al., 1997; Grantz et al., 1995), little information has been published relating these fluxes to effects on vegetation. Recently, Grantz et al. (2013) reported short-term ozone flux and related it to leaf injury in cotton in California. The authors reported that cotton leaves were most sensitive in the midafternoon, possibly due to changes in detoxification. They suggested with more

research a sensitivity parameter may function well with the W126 metric. However, there remains much unknown about ozone stomatal uptake in vegetation at larger scales and how much uptake results in an injury or damage, which depends to some degree on the amount of internal detoxification occurring with each particular species. Those species having high amounts of detoxification potential may, in fact, show little relationship between ozone stomatal uptake and plant response (Musselman and Massman, 1999). The lack of data in the U.S. and the lack of understanding of detoxification processes have made this technique less viable for vulnerability and risk assessments in the U.S.

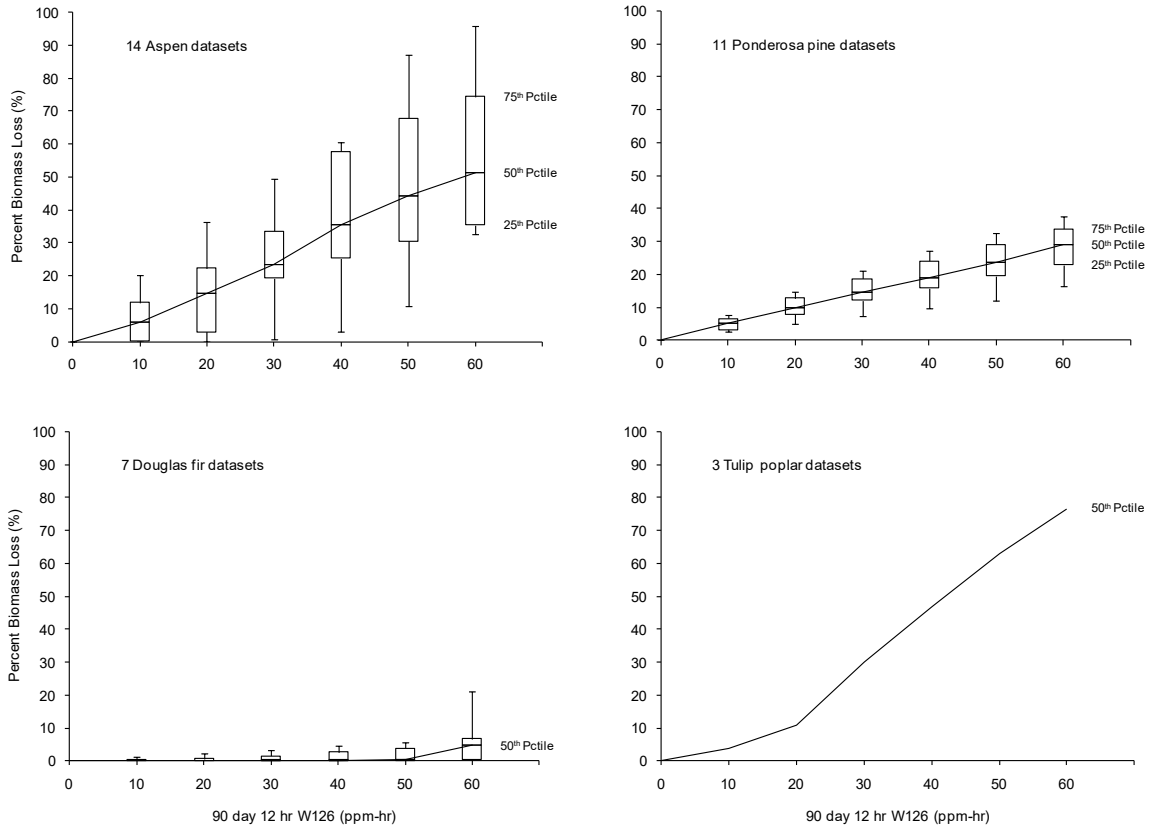
8.13.2 Exposure Response

The characterization of the effects of ozone on plants is contingent not only on the choice of the index used (i.e., W126, AOT40) to summarize ozone exposure (see above), but also on quantifying the response of the plant variables of interest at specific values of the selected index. The many factors that determine the response include species, genotype and other genetic characteristics, biochemical and physiological status, previous and current exposure to other stressors, and characteristics of the exposure itself. This section reviews results that have related specific quantitative observations of ozone exposure with quantitative observations of plant responses, and the predictions of responses that have been derived from those observations through empirical models.

Extensive exposure-response information on a wide variety of plant species has been produced by two long-term projects that were designed with the explicit aim of obtaining quantitative characterizations of the response of such an assortment of crop plants and tree seedlings to ozone under North American conditions: the NCLAN project for crops, and the U.S. EPA National Health and Environmental Effects Research Laboratory, Western Ecology Division (NHEERL-WED) tree seedling project. The NCLAN project was initiated by the U.S. EPA in 1980 primarily to improve estimates of yield loss under field conditions and to estimate the magnitude of crop losses caused by ozone throughout the U.S. (Heck et al., 1991; Heck et al., 1982). The cultural conditions used in the NCLAN studies approximated typical agronomic practices, and the primary objectives were (1) to define relationships between yields of major agricultural crops and ozone exposure as required to provide data necessary for economic assessments and development of ozone NAAQS, (2) to assess the national economic consequences resulting from ozone exposure of major agricultural crops, and (3) to advance understanding of cause-and-effect relationships that determine crop responses to pollutant exposures.

NCLAN experiments yielded 54 exposure-response curves for 12 crop species, some of which were represented by multiple cultivars at several of six locations throughout the U.S. The NHEERL-WED project was initiated by U.S. EPA in 1988 with the same objectives for tree species, and yielded 49 exposure-responses curves for multiple genotypes of 10 tree species grown for up to 3 years in Oregon, Michigan, and the Great Smoky Mountains National Park. Both projects used OTCs to expose plants to three to five levels of ozone. Eight of the 54 crop data sets were from plants grown under a

combination of ozone exposure and experimental drought conditions. These two programs are explained in detail in Section 9.5 of the 2013 Ozone ISA. Figure 8-14 shows an example of some of the exposure-response information from the NHEERL-WED on tree seedlings.

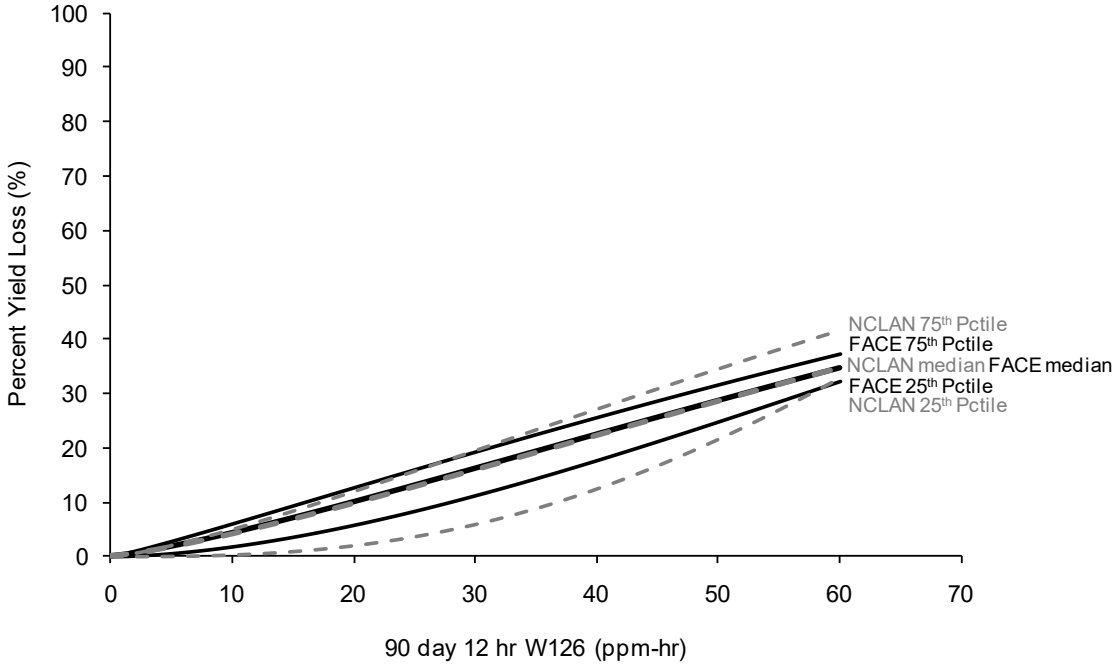


Note: Curves were standardized to 90-day W126. The number of studies available for each species is indicated on each plot.
Source of Weibull parameters: permission pending Lee and Hogsett (1996).

Figure 8-14 Quantiles of predicted relative biomass loss for four tree species in NHEERL-WED experiments. Quantiles of the predicted relative aboveground biomass loss at seven exposure values of 12-hour W126 for Weibull curves estimated using nonlinear regression on data for four tree species grown under well-watered conditions for 1 or 2 years.

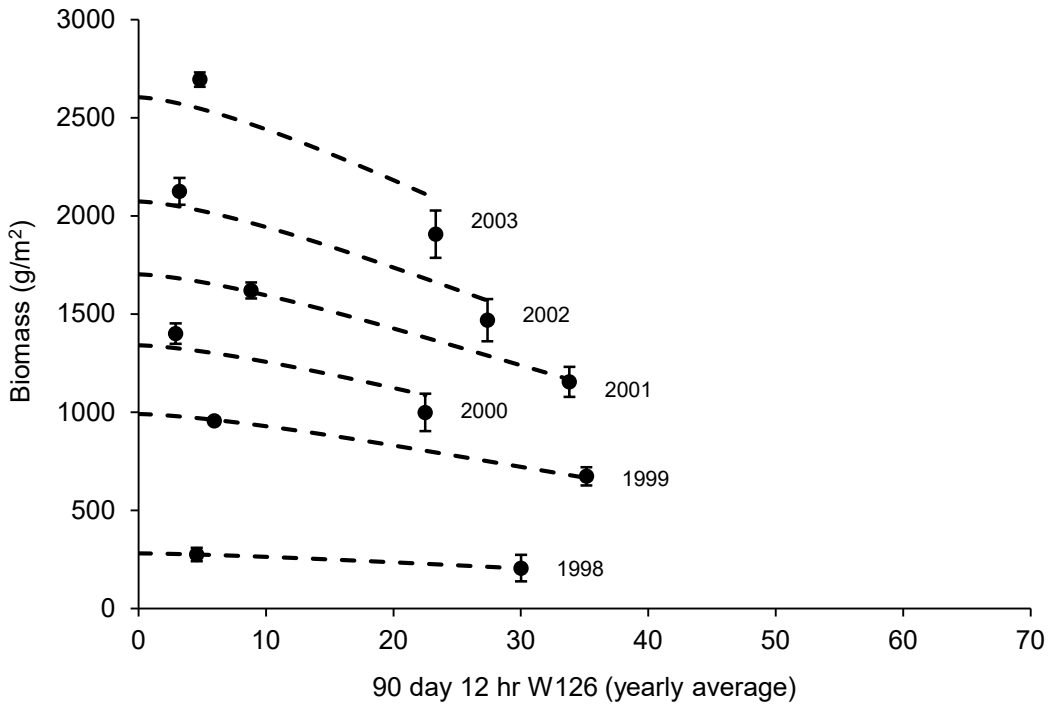
In the 2013 Ozone ISA, yield and growth results for aspen trees and soybean that had provided extensive exposure-response information in those projects have become available from studies that used FACE technology, which is intended to provide conditions much closer to natural environments (Pregitzer et al., 2008; Morgan et al., 2006; Morgan et al., 2004; Dickson et al., 2000). The NCLAN and

NHEERL-WED data with exposure measured as W126, was used to derive single-species median models for soybean and aspen from studies involving different genotypes, years, and locations. The resulting models were used to predict the change in yield of soybean and biomass of aspen between the two levels of exposure reported in the later FACE experiments. Results from these new experiments were exceptionally close to predictions from the models (Figure 8-15, Figure 8-16). The accuracy of model predictions for two widely different plant species provided support for the validity of the corresponding multiple-species models for crops and trees in the NCLAN and NHEERL-WED projects. However, variability among species in those projects indicates that the range of sensitivity is likely quite wide. This was confirmed by a study with cottonwood in a naturally occurring gradient of exposure (Gregg et al., 2006), which established the occurrence of species with responses substantially more severe than predicted by the median model for multiple species.



Source: Permission pending Betzelberger et al. (2010); Morgan et al. (2006); Lee and Hogsett (1996).

Figure 8-15 Comparison of composite functions for the quartiles of 7 curves for 7 genotypes of soybean grown in the SoyFACE experiment, and for the quartiles of 11 curves for 5 genotypes of soybean grown in the NCLAN project.



Note: Black dots are aspen biomass/m^2 for 3 FACE rings filled with an assemblage of 5 clonal genotypes of aspen at Aspen FACE; bars are SE for 3 rings; dashed line is median composite model for 4 clonal genotypes and wild-type seedlings in 11 NHEERL-WED 1-year OTC studies. Aspen FACE ozone data updated from Kubiske and Foss (2015). Single year 12 hour W126 is shown rather than the cumulative yearly average (average of each current and previous year) shown in Figure 9-20 of the 2013 Ozone ISA.
Source: Permission pending King et al. (2005), and Lee and Hogsett (1996).

Figure 8-16 Comparison between aboveground biomass observed in Aspen FACE experiment in 6 years and biomass predicted by the median composite function based on NHEERL-WED.

Since the 2013 Ozone ISA, there have been a few new experimental studies that add more exposure-response relationship information to the large historical database available on U.S. plants. In a new experimental study, Betzelberger et al. (2012) studied seven soybean cultivars at the SoyFACE experiment in Illinois. They found that the cultivars showed similar responses in a range of ozone exposures expressed as AOT40. These results support conclusions of previous studies (Betzelberger et al., 2010) and the 2013 Ozone ISA that sensitivity of current soybean genotypes is not different than early genotypes; therefore, soybean response functions developed in the NCLAN program remain valid. A study by Neufeld et al. (2018) provided information on foliar injury response on two varieties of cutleaf coneflower (*Rudbeckia laciniata*). For example, one variety had statistically detectable foliar injury when the 24-hour W126 index reached 23 ppm-hour (12-hour AOT40 = 12 ppm-hour). Gao et al. (2017) studied an ozone-sensitive hybrid cottonwood and found a strong relationship between biomass loss and

ozone exposure measured as AOT40 and phytotoxic ozone dose. This study was performed in China, but this species does occur in the U.S.

Despite the limited number of recent U.S. exposure-response studies, U.S. and international syntheses have highlighted response function information for grassland and other plant species that occur in the U.S. In a study by van Goethem et al. (2013), AOT40 response relationships were calculated for 87 grassland species that occur in Europe. Seventeen of these species are native to the U.S. and 65 additional species have been introduced to the U.S. and may have significant ecological, horticultural, or agricultural value (USDA, 2015). This study has the most significant amount of new exposure-response information for plants in the U.S. (see Table 8-22). A soybean synthesis study used some U.S. studies along with studies from other countries to create composite exposure-response functions based on a 7-hour mean metric. This study had limitations because the 7-hour means for many studies had to be converted from other published metrics and some soybean cultivars included in the study may not be used in the U.S. However, the same general patterns were seen with sensitivity of soybean yield to ozone as reported in the 2013 ISA (Osborne et al., 2016). Tai and Martin (2017) developed an empirical model (partial derivative linear regression [PDLR] model) from multidecadal data sets to estimate geographical variations across the U.S. in sensitivity to ozone of wheat, maize, and soybean. This approach takes into consideration strong ozone-temperature covariation and does not rely on pooled concentration-response functions. Several European studies have added to the exposure-response information to the literature, but these studies mainly focused on European plant species (Abeli et al., 2017; Payne et al., 2017; Sanz et al., 2016; Hayes et al., 2011).

Table 8-22 Grassland species that occur in the U.S. with biomass loss exposure-response functions as a function of AOT40 calculated from previously published open-top chamber (OTC) experiments by van Goethem et al. (2013).[a,b]

Species	Duration	*a*	*b*	Exposure (ppm-h) for 10% Biomass Reduction	R^2	Status in U.S.	Reference
Trifolium striatum	Annual	−0.046	0.9	1.94	0.95	Introduced	Gimeno et al. (2004)
Medicago minima	Annual	−0.049	0.97	1.98	0.98	Introduced	Gimeno et al. (2004)
Trifolium angustifolium	Annual	−0.046	0.96	2.06	0.85	Introduced	Gimeno et al. (2004)
Matricaria chamomilla	Annual	−0.051	1.06	2.08	0.16	Introduced	Bergmann et al. (1995), Bergmann et al. (1996b)
Rumex acetosa	Perennial	−0.048	1.02	2.14	0.22	Introduced	Hayes et al. (2006), Pleijel and Danielsson (1997), Power and Ashmore (2002), Ashmore et al. (1996)
Malva sylvestris	Perennial	−0.047	1.06	2.28	0.63	Introduced	Bergmann et al. (1995), Bergmann et al. (1996b)
Papaver dubium	Annual	−0.041	0.98	2.4	0.93	Introduced	Bergmann et al. (1996b)
Vaccinium vitis-idaea	Perennial	-0.042	1.03	2.46	0.98	Native	Mortensen and Nilsen (1992)
Phleum alpinum	Perennial	−0.04	1.14	2.84	0.8	Native	Danielsson et al. (1999), Pleijel and Danielsson (1997)
Leontodon hispidus	Perennial	−0.031	0.97	3.18	0.49	Introduced	Pleijel and Danielsson (1997), Ashmore et al. (1996)

Table 8 22 (Continued): Grassland species that occur in the U.S. with biomass loss exposure-response functions as a function of AOT40 calculated from previously published open top chamber (OTC) experiments by van Goethem et al. (2013).[a,b]

Species	Duration	*a*	*b*	Exposure (ppm-h) for 10% Biomass Reduction	R^2	Status in U.S.	Reference
Cirsium arvense	Perennial	−0.03	0.99	3.24	0.04	Introduced	Bergmann et al. (1995), Hayes et al. (2006), Power and Ashmore (2002), Bergmann et al. (1996b)
Phleum pratense	Perennial	−0.027	1.09	3.96	0.46	Introduced	Danielsson et al. (1999), Kohut et al. (1988), Mortensen and Nilsen (1992)
Dianthus deltoides	Perennial	−0.024	0.97	3.98	1	Introduced	Pleijel and Danielsson (1997)
Trifolium subterraneum	Perennial	−0.026	1.08	4.14	0.46	Introduced	Gimeno et al. (2004)
Lolium rigidum	Annual	−0.021	0.89	4.26	0.5	Introduced	Gimeno et al. (2004)
Trifolium glomeratum	Annual	−0.019	0.95	4.92	0.66	Introduced	Gimeno et al. (2004)
Campanula rotundifolia	Perennial	−0.021	1.02	4.98	0.07	Native	Hayes et al. (2006), Ashmore et al. (1996)
Matricaria matricarioides	Annual	−0.021	1.1	5.3	0.25	Introduced	Bergmann et al. (1995), Bergmann et al. (1996b)
Avena sterilis	Annual	−0.019	1.01	5.4	0.09	Introduced	Gimeno et al. (2004)
Senecio vulgaris	Annual	−0.017	1.14	6.72	0.39	Introduced	Bergmann et al. (1995), Pleijel and Danielsson (1997)
Aegilops geniculata	Annual	−0.013	0.96	7.6	0.51	Introduced	Gimeno et al. (2004)

Table 8 22 (Continued): Grassland species that occur in the U.S. with biomass loss exposure-response functions as a function of AOT40 calculated from previously published open top chamber (OTC) experiments by van Goethem et al. (2013).[a,b]

Species	Duration	*a*	*b*	Exposure (ppm-h) for 10% Biomass Reduction	R^2	Status in U.S.	Reference
Nardus stricta	Perennial	−0.012	0.99	8.3	0.73	Introduced	Hayes et al. (2006), Ashmore et al. (1996)
Trifolium repens	Perennial	−0.011	0.94	8.76	0.89	Introduced	Bungener et al. (1999b), Ashmore et al. (1996)
Hieracium pilosella	Perennial	−0.011	0.97	8.78	0.06	Introduced	Pleijel and Danielsson (1997), Ashmore et al. (1996)
Silene acaulis	Perennial	−0.009	0.94	10.2	0.52	Native	Mortensen and Nilsen (1992)
Bromus sterilis	Annual	−0.009	0.98	10.92	0.19	Introduced	Gimeno et al. (2004)
Chrysanthemum leucanthemum	Perennial	−0.009	1.01	11.26	0	Introduced	Bungener et al. (1999b)
Lychnis flos-cuculi	Perennial	−0.013	1.5	11.38	0.52	Introduced	Bungener et al. (1999b), Power and Ashmore (2002), Tonneijck et al. (2004), Franzaring et al. (2000)
Holcus lanatus	Perennial	−0.009	1	11.52	0.6	Introduced	Hayes et al. (2006), Tonneijck et al. (2004), Ashmore et al. (1996)
Chrysanthemum segetum	Annual	−0.008	0.96	11.96	0	Introduced	Pleijel and Danielsson (1997)
Festuca rubra	Perennial	−0.008	1	12.5	0.22	Native	Bungener et al. (1999b), Hayes et al. (2006), Ashmore et al. (1996)

Table 8 22 (Continued): Grassland species that occur in the U.S. with biomass loss exposure-response functions as a function of AOT40 calculated from previously published open top chamber (OTC) experiments by van Goethem et al. (2013).[a,b]

Species	Duration	*a*	*b*	Exposure (ppm-h) for 10% Biomass Reduction	R^2	Status in U.S.	Reference
Chenopodium album	Annual	−0.007	0.94	13.28	0	Native	Bergmann et al. (1995), Pleijel and Danielsson (1997), Bergmann et al. (1996b)
Briza maxima	Annual	−0.006	0.87	13.82	0.08	Introduced	Gimeno et al. (2004)
Tragopogon orientalis	Perennial	−0.007	1.01	14.26	0.85	Introduced	Bungener et al. (1999b)
Hypochaeris radicata	Perennial	−0.006	0.95	15.02	0.54	Introduced	Pleijel and Danielsson (1997), Ashmore et al. (1996)
Centaurea jacea	Perennial	−0.006	1.01	15.76	0.74	Introduced	Bungener et al. (1999b)
Trifolium pratense	Perennial	−0.007	1.09	15.82	0.8	Introduced	Bungener et al. (1999b), Kohut et al. (1988), Ashmore et al. (1996)
Bromus arvensis	Annual	−0.006	1.03	16.3	0.01	Introduced	Pleijel and Danielsson (1997)
Taraxacum officinale	Perennial	−0.006	1.08	17.96	0.37	Native	Bungener et al. (1999b)
Poa annua	Annual	−0.005	0.98	18.14	0.88	Introduced	Pleijel and Danielsson (1997)
Poa pratensis	Perennial	−0.005	0.96	18.48	0.06	Native*	Bungener et al. (1999b), Ashmore et al. (1996)
Papaver rhoeas	Annual	−0.005	0.91	19.06	0.78	Introduced	Pleijel and Danielsson (1997)
Eupatorium cannabinum	Perennial	−0.005	1.07	19.78	0.72	Introduced	Franzaring et al. (2000)

Table 8 22 (Continued): Grassland species that occur in the U.S. with biomass loss exposure-response functions as a function of AOT40 calculated from previously published open top chamber (OTC) experiments by van Goethem et al. (2013).[a,b]

Species	Duration	*a*	*b*	Exposure (ppm-h) for 10% Biomass Reduction	R^2	Status in U.S.	Reference
Briza media	Perennial	−0.005	0.99	21.5	0.75	Introduced	Pleijel and Danielsson (1997), Ashmore et al. (1996)
Anthoxanthum odoratum	Perennial	−0.004	0.94	21.9	0.38	Introduced	Hayes et al. (2006), Pleijel and Danielsson (1997), Hayes et al. (2010), Ashmore et al. (1996)
Bromus hordeaceus	Annual	−0.004	0.98	23.34	0.7	Introduced	Gimeno et al. (2004)
Saxifraga cernua	Perennial	−0.004	1.04	24.26	0.17	Native	Mortensen and Nilsen (1992)
Polygonum viviparum	Perennial	−0.003	1	29.32	0.99	Native	Mortensen and Nilsen (1992)
Achillea millefolium	Perennial	−0.003	1.04	31.4	0.99	Native	Bungener et al. (1999b)
Achillea ptarmica	Perennial	−0.003	1.02	35.08	0.53	Introduced	Franzaring et al. (2000)
Lotus corniculatus	Perennial	−0.003	0.96	36.82	0.04	Introduced	Bungener et al. (1999b), Ashmore et al. (1996)
Knautia arvensis	Perennial	−0.003	1.02	40.88	0.58	Introduced	Bungener et al. (1999b)
Deschampsia flexuosa	Perennial	−0.002	1.08	59.96	0.64	Native	Ashmore et al. (1996)
Crepis biennis	Annual	−0.002	1.08	67.36	0.04	Introduced	Bungener et al. (1999b)
Salvia pratensis	Perennial	−0.001	1.08	83.38	0.75	Introduced	Bungener et al. (1999b)

Table 8 22 (Continued): Grassland species that occur in the U.S. with biomass loss exposure-response functions as a function of AOT40 calculated from previously published open top chamber (OTC) experiments by van Goethem et al. (2013).[a,b]

Species	Duration	*a*	*b*	Exposure (ppm-h) for 10% Biomass Reduction	R^2	Status in U.S.	Reference
Dactylis glomerata	Perennial	−0.001	0.9	150.42	0.34	Introduced	Bungener et al. (1999b), Pleijel and Danielsson (1997), Ashmore et al. (1996)
Plantago lanceolata	Perennial	−0.001	0.96	192.58	0.96	Introduced	Bungener et al. (1999b), Hayes et al. (2006), Pleijel and Danielsson (1997), Tonneijck et al. (2004), Franzaring et al. (2000), Ashmore et al. (1996)
Phalaris arundinacea	Perennial	0.027	0.89	-	0.76	Native*	Pleijel and Danielsson (1997)
Festuca pratensis	Perennial	0.018	0.92	-	0.13	Introduced	Pleijel and Danielsson (1997)
Anthyllis vulneraria	Perennial	0.017	0.98	-	0.72	Introduced	Pleijel and Danielsson (1997), Ashmore et al. (1996)
Silene dioica	Perennial	0.015	0.91	-	0.92	Introduced	Bungener et al. (1999b)
Silene vulgaris	Perennial	0.014	0.97	-	0.83	Introduced	Pleijel and Danielsson (1997)
Galium saxatile	Perennial	0.011	0.91	-	0.05	Introduced	Hayes et al. (2006), Hayes et al. (2010), Ashmore et al. (1996)
Molinia caerulea	Perennial	0.01	0.88	-	0.39	Introduced	Tonneijck et al. (2004), Franzaring et al. (2000)

Table 8 22 (Continued): Grassland species that occur in the U.S. with biomass loss exposure-response functions as a function of AOT40 calculated from previously published open top chamber (OTC) experiments by van Goethem et al. (2013).[a,b]

Species	Duration	*a*	*b*	Exposure (ppm-h) for 10% Biomass Reduction	R^2	Status in U.S.	Reference
Salix herbacea	Perennial	0.008	1.07	-	0.98	Native	Mortensen and Nilsen (1992)
Deschampsia caespitosa	Perennial	0.008	1.01	-	0.83	Native	Ashmore et al. (1996)
Carex bigelowii	Perennial	0.007	1	-	0.1	Native	Hayes et al. (2010)
Agrostemma githago	Annual	0.006	1.05	-	0.83	Introduced	Pleijel and Danielsson (1997)
Saxifraga cespitosa	Perennial	0.006	0.92	-	0.15	Native	Mortensen and Nilsen (1992)
Calluna vulgaris	Perennial	0.006	0.92	-	0.04	Introduced	Foot et al. (1996)
Festuca ovina	Perennial	0.005	1.04	-	0.03	Introduced	Hayes et al. (2006), Pleijel and Danielsson (1997), Hayes et al. (2010), Ashmore et al. (1996)
Lolium perenne	Perennial	0.003	0.98	-	0.1	Introduced	Bungener et al. (1999b), Ashmore et al. (1996)
Agrostis capillaris	Perennial	0.003	1	-	0.84	Introduced	Hayes et al. (2006), Hayes et al. (2010), Ashmore et al. (1996)
Centaurea cyanus	Annual	0.002	1.03	-	0.02	Introduced	Pleijel and Danielsson (1997)
Aegilops triuncialis	Annual	0.002	1.04	-	0.81	Introduced	Gimeno et al. (2004)
Carum carvi	Perennial	0.002	0.96	-	0.03	Introduced	Bungener et al. (1999b)

Table 8 22 (Continued): Grassland species that occur in the U.S. with biomass loss exposure-response functions as a function of AOT40 calculated from previously published open top chamber (OTC) experiments by van Goethem et al. (2013).[a,b]

Species	Duration	*a*	*b*	Exposure (ppm-h) for 10% Biomass Reduction	R^2	Status in U.S.	Reference
Onobrychis viciifolia	Perennial	0.002	1.1	-	0.43	Introduced	Bungener et al. (1999b)
Arrhenatherum elatius	Perennial	0.001	0.91	-	0.51	Introduced	Bungener et al. (1999b), Ashmore et al. (1996)
Trisetum flavescens	Perennial	0.001	1.1	-	0.61	Introduced	Bungener et al. (1999b)
Bromus erectus	Perennial	0.001	1.05	-	0.99	Introduced	Bungener et al. (1999b), Ashmore et al. (1996)
Alopecurus pratensis	Perennial	0.001	0.96	-	0.24	Introduced	Pleijel and Danielsson (1997), Ashmore et al. (1996)

[a]Both native and introduced/naturalized plant species documented to occur in the U.S. are included.

[b]Data are found in the Supplemental Information in this publication.

Note: "Duration describes the life cycle of the plant (annual or perennial). Columns "a" and "b" represent variables in the exposure-response relationship with ozone, $y = ax + b$ derived by linear regression for exposure (in AOT40) and proportion of biomass compared to charcoal-filtered air treatment. Column "Exposure…" represents the AOT40 ozone exposure that reduces species biomass by 10%. Species that exhibited a biomass reduction in response to ozone have a negative value for a, and species appear in the table in descending order of sensitivity to ozone (i.e., most sensitive species at the top, most tolerant species at the bottom of table). Column "R^2" is the coefficient of determination from linear regression for the exposure-response relationship. The column "Status in U.S." is based on the USDA (2015) determination of whether species are native to the U.S. (Native), are introduced to the U.S. (Introduced), or have populations with native progenitors as well as populations with introduced progenitors (Native*).

ER functions for this table are from the OZOVEG database (Hayes et al., 2007). Six out of the sixteen studies above have been cited in previous ISAs or AQCDs."

8.13.3 Summary

Exposure indices are metrics that quantify exposure as it relates to plant response (e.g., reduced growth). These indices are summary measures of ozone concentrations over time intended to provide a consistent metric for reviewing and comparing exposure-response effects obtained from various studies. Given the current state of knowledge and the best available data, exposure indices that cumulate and differentially weight the higher hourly average concentrations and also include the midlevel values (e.g., the W126 or AOT40 metrics) continue to offer the most defensible approach for use in developing response functions and comparing studies, as well as for defining future indices for vegetation protection.

Since the 2013 Ozone ISA, there have been a limited number of new experimental studies that add more exposure-response relationship information (see Table 8-23). However, U.S. and international syntheses have highlighted response function information for grassland and other plant species that occur in the U.S. (see Table 8-22), thus adding many new species with exposure-response information. Previous reviews of the NAAQS have included exposure-response functions for the yield of many crop species, and for the biomass accumulation of tree species. They were based on large-scale experiments designed to obtain clear exposure-response data and are updated by using the W126 metric to quantify exposure. In more recent years, extensive exposure-response data obtained in more naturalistic settings have become available for yield of soybean and growth of aspen. In the 2013 Ozone ISA, the exposure-response median functions were validated based on previous data by comparing their predictions with the newer observations (see Section 9.6 of the 2013 Ozone ISA). These functions continue to provide very accurate predictions of effects in naturalistic settings. Although these median functions provide reliable models for groups of species or group of genotypes within a species, the original data along with recent results consistently show that some species, and some genotypes within species are much more severely affected by exposure to ozone.

Finally, with what was known at the time of the 2013 Ozone ISA added to the new information reported in this ISA, the knowledge base is stronger on exposure indices and exposure-response for vegetation. The cumulative weighted indices (W126 and AOT40) and exposure-response relationships presented in this section continue to be used in analyses in the scientific literature and are the best available approach for studying the effects of ozone exposure on vegetation in the U.S.

Table 8-23 Exposure indices and exposure response.

Study	Study Type and Study Location	Study Species	Ozone Exposure	Relevant Results
Betzelberger et al. (2012)	FACE; SoyFACE, Champaign, IL	Seven cultivars of soybean (*Glycine max*)	Soybeans in eight 20-m diameter soyFACE plots with different O_3 concentrations were exposed for ~8 h each day in two different growing seasons (2009, 2010). Target concentrations were ambient, 40, 55, 70, 85, 110, 130, 160, 200 in 2009, and ambient, 55, 70, 85, 110, 130, 150, 170, 190 in 2010. 8-, 24-, and 1-h max mean as well as AOT40 and SUM06 calculated for each plot shown in Table 2 of this study.	All seven cultivars showed similar responses to O_3 with the range of responses between 18 to 30 kg ha per nL/L cumulative exposure over 40 nL/L. At the highest target concentration of 200 nL/L (AOT40 of 67.4 ppm-h) yields were reduced 64%. This paper improves the estimate of soybean response from an earlier paper where one concentration was used over multiple years to develop an exposure-response curve. For the first time, a significant effect on duration of canopy and size of canopy was observed with O_3 exposure. Interception efficiency was estimated to be reduced by 20% at the highest target concentration.
Grantz et al. (2013)	Greenhouse; Kearney Research and Extension Center, Parlier, CA	*Gossypium barbadense* (Pima cotton)	Each plant was exposed to a single 15-m pulse of O_3 (0.0, 0.5, 1.0, 1.5, 2.0 µmol/mol). Pulses were done at 2-h intervals throughout the daylight period. After a single pulse, plants were returned to greenhouse bench and left undisturbed for 6 days	All three leaf injury measures declined with increasing dose as indications of O_3 induced injury. For chlorophyll content, early in the photoperiod (700 h), the slope was shallow and nonsignificant, but in midafternoon (1,500 h), the sensitivity increased substantially and the slope (a) became significant ($a = -1.84$ m^2/mmol, $r^2 = 0.3$). Similar results for D-R of conductance (at 1,500 h $a = -1.84$ m^2/mmol, $r^2 = 0.83$). The slope for D-R of noninjured leaf area was shallow but significant at 700 h ($a = -0.54$, $r^2 = 0.15$). The slope and its significance increased to maxima at 1,700 h ($a = -2.57$, $r = 0.52$).

Table 8-23 (Continued): Exposure indices and exposure response.

Study	Study Type and Study Location	Study Species	Ozone Exposure	Relevant Results
Osborne et al. (2016)	Other; 28 experimental studies (OTC or FACE) between 1982−2014 from the U.S., Asia, and China	48 soybean cultivars	Ozone exposure data all converted to seasonal 7-h mean from studies that reported concentration as 8-h mean, 12-h mean, 24-h mean, or 3-mo AOT40. Duration of O_3 exposure was at least 60% of growing season	This study updates the exposure-response function for O_3 in soybean using data after 1998 from the U.S., China, and India and examines temporal and geographical trends in sensitivity. The exposure-response function was calculated by pooling relative yield data and plotting against the seasonal mean at 7 h (M7). All data was scaled to theoretical yield at 0 ppb, 55 ppb was used to represent present-day background levels. Relative yield reduction at present concentration was 17.3%. significant (5%) loss of yield can occur is 32.3 ppb M7. Previous exposure-response function for soybean based on U.S. data may have underestimated yield losses in Asia where some cultivars appear to be more sensitive. Cultivars varied in sensitivity to ozone with a yield loss at a 7-h mean concentration of 55 ppb ranging from 13.3 to 37.9%. Sensitivity to O_3 increased by an average of 32.5% between 1960 and 2000. Sensitivity was higher in India and China compared with the U.S. Also, sensitivity has appeared increase over time in soybeans.

Table 8-23 (Continued): Exposure indices and exposure response.

Study	Study Type and Study Location	Study Species	Ozone Exposure	Relevant Results
Tai and Martin (2017)	Other; modeling study using multidecadal U.S. crop yield and climate data	Soybean (*Glycine max*), wheat (*Triticum*), maize (*Zea mays*)	Three cumulative ozone annual exposure metrics AOT40, SUM06, and W126 calculated from hourly ozone observations from the AQS and CASTNET networks averaged over 1993−2010	Instead of relying on pooled concentration response functions which do not account for cultivar sensitivity to ozone and temperature differences, authors developed an empirical model (partial derivative linear regression [PDLR] model) from multidecadal data sets to estimate geographical variations across the U.S. in sensitivity of wheat, maize, and soybean to ozone. This approach takes into consideration the strong ozone-temperature covariation. For all three crops, the revised sensitivities (calculated in latitude-longitude grid cells to account for regional differences in temperature, water, and nutrient availability) are, in general, higher than previously indicated by concentration-response functions derived from experimental studies. Wheat yield sensitivities to ozone were statistically significant spatially along the northern U.S. border, maize sensitivity was spatially statistically significant at various locations across the U.S., and soybean sensitivity was spatially statistically significant in a band from the Great Plains to the south-central U.S. Crops in regions of elevated ozone and high-water use, were more tolerant to ozone. The PDLR model coupled with ozone and temperature projections from 2000 to 2050 by the Community Earth System model predict average declines of U.S. wheat, maize, and soybean of 13, 43, and 28% respectively.
Feng et al. (2017)	Other; global data set of O_3 experiments in temperate, Mediterranean, and subtropical climates	57 tree species for foliar injury, 9 European tree species for biomass	Elevated O_3 experiments; O_3 exposures are expressed as AOT40 values and Phytotoxic O_3 dose	Phytotoxic O_3 dose (POD) based on leaf mass is a stronger predictor of biomass reduction (r^2 = 0.56) than is POD based on leaf area (r^2 = 0.42).

Table 8-23 (Continued): Exposure indices and exposure response.

Study	Study Type and Study Location	Study Species	Ozone Exposure	Relevant Results
Gao et al. (2017)	OTC; Seed Station Field of Changping, northwest Beijing, China	O_3 sensitive clone (546) of eastern cottonwood (*Populus deltoides*)	Three ozone treatments: charcoal filtered, ambient, and elevated O_3; Plants fumigated 96 days (June−September). Mean O_3 was 33.5 ± 2.4 ppb in the CF treatment, 51.1 ± 4.1 ppb in the NF treatment, and 78.2 ± 5.5 ppb in the E-O_3 treatment. AOT40 were 4.3, 16.0, and 38.7 ppm-h, respectively. Two irrigation treatments were also applied	For O_3 exposure-response, which was modeled in response to biomass changes, model performance was significantly better when using POD (flux) compared with AOT40 (R^2 = 0.829, p = 0.012 vs. R^2 = 0.560, p = 0.087). Using this accumulated flux model, The O_3 critical level (CL) for preventing a 4% biomass loss in this poplar clone under different water regimes was between 5.27 mmol/m^2 PLA and 4.09 mmol/m^2 PLA, depending on which threshold (maximum biomass at zero O_3 exposure) was used.
Neufeld et al. (2018)	OTC; experiments conducted in Boone, NC. Rhizomes collected from Great Smoky Mountains National Park and Rocky Mountains National Park	*Rudbeckia laciniata* var. *ampla* and var. *digitata* (cutleaf coneflower)	Three treatment groups: charcoal-filtered air (CF), nonfiltered air (NF), and nonfiltered air + 50 ppb O_3 (2012) or +30 ppb/+ 50 ppb (2013) (EO). In 2012, 24-h W126 was 0.1 ppm-h in the CF treatment, 2.0 ppm-h in the NF treatment, and 74.2 ppb in the EO treatment. 12-h AOT40 were 0.0, 2.0, and 24.1 ppm-h, respectively. In 2013, 24-h W126 were 0.1, 1.8, and 80.5 ppm-h, respectively. 12-hour AOT40 were 1.0, 2.0, and 53.8 ppm-h, respectively. Plants were exposed for 47 days in 2012 and for 77 days in 2013.	In 2012 and 2013, injury levels in both varieties were higher in the EO treatment than in either the CF or NF treatments, which did not differ, but there were no statistically significant differences between the varieties. Stippling increased with time. Effects of O_3 on biomass accumulation were nonsignificant.

Table 8-23 (Continued): Exposure indices and exposure response.

Study	Study Type and Study Location	Study Species	Ozone Exposure	Relevant Results
Hayes et al. (2011)	Greenhouse; near Marchlyn Mawr, U.K.	Two communities: four plants of forb *Leontodon hispidus* and three plants of grass *Dactylis glomerata*; four plants of forb *Leontodon hispidus* and three plants of *Anthoxanthum odoratum*	Eight treatments: (1) seasonal 24-h mean 21.4 ppb (12-h mean 21.1 ppb, daylight (7:00 a.m.−6:00 p.m.) AOT40 = 0.07 ppm-h, 24-h AOT40 = 0.07 ppm-h); (2) seasonal mean 39.9 ppb (12 h = 39.2 ppb, daylight AOT40 = 4.93 ppm-h, 24-h AOT40 = 10.91 ppm-h); (3) seasonal mean 50.2 ppb (12 h = 49.6 ppb, daylight AOT40 = 21.44 ppm-h, 24-h AOT40 = 41.29 ppm-h); (4) seasonal mean 59.4 ppb (12 h = 58.7 ppb, daylight AOT40 = 38.04 ppm-h, 24-h AOT40 = 72.19 ppm-h); (5) seasonal mean 74.9 ppb (12 h = 73.3 ppb, daylight AOT40 = 62.49 ppm-h, 24-h AOT40 = 119.82 ppm-h); (6) seasonal mean 83.3 ppb (12 h = 81.6 ppb, daylight AOT40 = 77.13 ppm-h, 24-h AOT40 = 147.42 ppm-h); (7) seasonal mean 101.3 ppb (12 h = 99.0 ppb, daylight AOT40 = 108.43 ppm-h, 24-h AOT40 = 206.70 ppm-h); (8) seasonal mean 102.5 ppb (12 h = 100.5, daylight AOT40 = 112.47 ppm-h, 24-h AOT40 = 214.34 ppm-h)	Exposure indices: there was a linear relationship between 24-h mean ozone and 12-h mean ozone treatments (r^2 = 0.9999). There were linear relationships between seasonal O_3 concentration and root biomass, leaf retention, reproductive phenology in the following season, and grass cover.

Table 8-23 (Continued): Exposure indices and exposure response.

Study	Study Type and Study Location	Study Species	Ozone Exposure	Relevant Results
Sanz et al. (2016)	OTC; OTC experimental field located in a rural area in the northeast of the Iberian Peninsula, Tarragona, Spain	Pasture species, Leguminosae (three species)	Data analyzed from independent experiments, 45 day avg O_3 exposure length	An O_3 critical level for reproductive capacity AOT40 = 2.0 (1.5, 2.8) ppm-h and Phytotoxic Ozone Dose (POD) 1 = 7.2 (1.1, 13.3) mmol/m^2 was developed from linear exposure-response functions based on seed and flower production (see Figure 1 for AOT40 and Figure 2 for POD1). Reproductive capacity had the lowest critical level of the endpoints evaluated.
van Goethem et al. (2013)	Other; northwestern Europe (mapping of sensitivity is for a square area of 50 to 61°N, and 11°E to 11°W)	25 annual grassland species, 62 perennial grassland species, 9 tree species	OTC, FACE, or solardomes. All experimental treatments were at >40 ppb for at least 21 days, with mean hourly O_3 never exceeding 100 ppb. Control treatments were charcoal-filtered air or ambient air	Annual grassland species were significantly more sensitive to O_3 >40 ppb than were perennial grassland species. Mean 10% reduction in biomass occurred at 0.84 ppm-h for annual species and 1.14 ppm-h for perennial grassland species. Exposure-response relationships for 96 European plants (biomass reduction vs. AOT40) reported.
Abeli et al. (2017)	Lab; alpine seeds collected on Mt. Cimone, Mt. Prado-Cusna, and in the Dolomites in Italy; O_3 exposure inside incubators	*Achillea clavennae* *Aster alpinus*, *Festuca rubra* subsp. *commutata* (Gaudin) Markgr.-Dann, *Festuca violacea* subsp. *puccinellii* (Parl.) Foggi, GrazRossi & Signorini, *Plantago alpina* L., *Silene acaulis* (L.) Jacq., *Silene nutans* L., *Silene suecica* (Lodd) Greuter & Burdet, *Vaccinium myrtillus* L.	Control: ambient air (0−1 ppb), 125_5 treatment: 125 ppb O_3 24 h/day for 5 days, 125_10 treatment: 125 ppb O_3 24 h/day for 10 days, 185_5 treatment: 185 ppb O_3 24 h/day for 5 days	Combining all species, each treatment (compared to control) significantly delayed germination (125_5 = 0.71, 185_5 = 0.87, 125_10 = 1.17 day delay). Individual species varied in their responses. Six of nine species had reduction in germination percentage for one or more of the O_3 treatments at end of the O_3 exposure. Seven of nine species showed significant effect of at least one O_3 treatment at 28 days after sowing, and effect ranged from increasing to decreasing germination percentage. Combining all species, 125_5 and 185_5 treatments did not affect mean germination time either at the end of the O_3 exposure or at the end of the experiment. The 125_10 treatment significantly increased mean germination time by 1.25 days after O_3 exposure, but by the end of the experiment, that difference did not exist.

Table 8-23 (Continued): Exposure indices and exposure response.

Study	Study Type and Study Location	Study Species	Ozone Exposure	Relevant Results
Payne et al. (2017)	Mesocosm; peat sampled from wet, heathy peatland, U.K	Microscopic algae (desmids, diatoms), protozoa (ciliates, flagellates, testate amoebae), and microscopic animal consumers (nematodes, rotifers) sampled from *Sphagnum papillosum* stems	Experimental O_3 for 3.5 yr: ambient (average 25 ppb O_3), low O_3 (ambient + 10 ppb for 24 h/day), moderate O_3 (ambient + 25 ppb O_3 24 h/day), elevated O_3 (ambient + 35 ppb daytime 8 h/day in summer, +10 ppb rest of year)	Authors indicated that O_3 effects on microscopic food web in peat generally start at moderate O_3 exposures.

AOT40 = seasonal sum of the difference between an hourly concentration at the threshold value of 40 ppb, minus the threshold value of 40 ppb; CF = charcoal-filtered air; D = dose; EO = elevated-ozone treatment; E-O_3 = elevated-ozone treatment; kg/ha = kilograms/hectare; NF = nonfiltered air; nL/L = nanoliters/liter; OTC = open-top chamber; PLA = projected leaf area; ppm = parts per million; S = sensitivity; SUM06 = seasonal sum of all hourly average concentrations ≥ 0.06 ppm; µmol/mol = micromoles/mole; W126 = cumulative integrated exposure index with a sigmoidal weighting function.

8.14 References

Abeli, T; Guasconi, DB; Mondoni, A; Dondi, D; Bentivoglio, A; Buttafava, A; Cristofanelli, P; Bonasoni, P; Rossi, G; Orsenigo, S. (2017). Acute and chronic ozone exposure temporarily affects seed germination in alpine plants. Plant Biosyst 151: 304-315. http://dx.doi.org/10.1080/11263504.2016.1174169.

Adams, MB; Kelly, JM; Taylor, GE, Jr; Edwards, NT. (1990). Growth of five families of Pinus taeda L during three years of ozone exposure. New Phytol 116: 689-694. http://dx.doi.org/10.1111/j.1469-8137.1990.tb00555.x.

Adams, MB; O'Neill, EG. (1991). Effects of ozone and acidic deposition on carbon allocation and mycorrhizal colonization of Pinus taeda L seedlings. Forest Sci 37: 5-16.

Agathokleous, E; Saitanis, CJ; Koike, T. (2015). Tropospheric O-3, the nightmare of wild plants: a review study. Journal of Agricultural Meteorology 71: 142-152. http://dx.doi.org/10.2480/agrmet.D-14-00008.

Agathokleous, E; Sakikawa, T; Abu ElEla, SA; Mochizuki, T; Nakamura, M; Watanabe, M; Kawamura, K; Koike, T. (2017). Ozone alters the feeding behavior of the leaf beetle Agelastica coerulea (Coleoptera: Chrysomelidae) into leaves of Japanese white birch (Betula platyphylla var. japonica). Environ Sci Pollut Res Int 24: 17577-17583. http://dx.doi.org/10.1007/s11356-017-9369-7.

Ainsworth, EA. (2008). Rice production in a changing climate: a meta-analysis of responses to elevated carbon dioxide and elevated ozone concentration. Global Change Biol 14: 1642-1650. http://dx.doi.org/10.1111/j.1365-2486.2008.01594.x.

Ainsworth, EA; Serbin, SP; Skoneczka, JA; Townsend, PA. (2014). Using leaf optical properties to detect ozone effects on foliar biochemistry. Photosynth Res 119: 65-76. http://dx.doi.org/10.1007/s11120-013-9837-y.

Allen, EB; Temple, PJ; Bytnerowicz, A; Arbaugh, MJ; Sirulnik, AG; Rao, LE. (2007). Patterns of understory diversity in mixed coniferous forests of southern California impacted by air pollution. ScientificWorldJournal 7: 247-263. http://dx.doi.org/10.1100/tsw.2007.72.

Amundson, RG; Alscher, RG; Fellows, S; Rubin, G; Fincher, J; Van Leuken, P; Weinstein, LH. (1991). Seasonal changes in the pigments, carbohydrates and growth of red spruce as affected by exposure to ozone for two growing seasons. New Phytol 118: 127-137. http://dx.doi.org/10.1111/j.1469-8137.1991.tb00573.x.

Anav, A; De Marco, A; Sicard, P; Vitale, M; Paoletti, E. (2017). Response on 'comparing concentration-based (AOT40) and stomatal uptake (PODY) metrics for ozone risk assessment to European forests' [Comment]. Global Change Biol 23: e3-e4. http://dx.doi.org/10.1111/gcb.13511.

Anav, A; Liu, Q; De Marco, A; Proietti, C; Savi, F; Paoletti, E; Piao, S. (2018). The role of plant phenology in stomatal ozone flux modeling. Global Change Biol 24: 235-248. http://dx.doi.org/10.1111/gcb.13823.

Andersen, CP. (2003). Source-sink balance and carbon allocation below ground in plants exposed to ozone. New Phytol 157: 213-228. http://dx.doi.org/10.1046/j.1469-8137.2003.00674.x.

Andrew, C; Lilleskov, EA. (2014). Elevated CO2 and O3 effects on ectomycorrhizal fungal root tip communities in consideration of a post-agricultural soil nutrient gradient legacy. Mycorrhiza 24: 581-593. http://dx.doi.org/10.1007/s00572-014-0577-4.

Andrew, CJ; van Diepen, LTA; Miller, RM; Lilleskov, EA. (2014). Aspen-associated mycorrhizal fungal production and respiration as a function of changing CO2, O3 and climatic variables. Fungal Ecol 10: 70-80. http://dx.doi.org/10.1016/j.funeco.2013.10.005.

Arbaugh, M; Bytnerowicz, A; Grulke, N; Fenn, M; Poth, M; Temple, P; Miller, P. (2003). Photochemical smog effects in mixed conifer forests along a natural gradient of ozone and nitrogen deposition in the San Bernardino Mountains. Environ Int 29: 401-406. http://dx.doi.org/10.1016/S0160-4120(02)00176-9.

Ashmore, MR; Bell, JNB; Mimmack, A. (1988). Crop growth along a gradient of ambient air pollution. Environ Pollut 53: 99-121. http://dx.doi.org/10.1016/0269-7491(88)90028-0.

Ashmore, MR; Power, SA; Cousins, DA; Ainsworth, N. (1996). Effects of ozone on native grass and forb species: A comparison of responses of individual plants and artificial communities. In L Kärenlampi; L Skärby (Eds.), Critical levels for ozone: testing and finalizing the concepts UNECE workshop report (pp. 193-197). Kuopio, Finland: United Nations Economic Commission for Europe (UN-ECE).

Ashmore, MR; Thwaites, RH; Ainsworth, N; Cousins, DA; Power, SA; Morton, AJ. (1995). Effects of ozone on calcareous grassland communities. Water Air Soil Pollut 85: 1527-1532. http://dx.doi.org/10.1007/BF00477198.

Avnery, S; Mauzerall, DL; Liu, J; Horowitz, LW. (2011). Global crop yield reductions due to surface ozone exposure: 1. Year 2000 crop production losses and economic damage. Atmos Environ 45: 2284-2296. http://dx.doi.org/10.1016/j.atmosenv.2010.11.045.

Awmack, CS; Harrington, R; Lindroth, RL. (2004). Aphid individual performance may not predict population responses to elevated CO2 or O3. Global Change Biol Biol.10: 1414-1423. http://dx.doi.org/10.1111/j.1365-2486.2004.00800.x.

Baldantoni, D; Fagnano, M; Alfani, A. (2011). Tropospheric ozone effects on chemical composition and decomposition rate of Quercus ilex L. leaves. Sci Total Environ 409: 979-984. http://dx.doi.org/10.1016/j.scitotenv.2010.11.022.

Bao, X; Li, Q; Hua, J; Zhao, T; Liang, W. (2014). Interactive effects of elevated ozone and UV-B radiation on soil nematode diversity. Ecotoxicology 23: 11-20. http://dx.doi.org/10.1007/s10646-013-1146-x.

Bassin, S; Volk, M; Fuhrer, J. (2013). Species composition of subalpine grassland is sensitive to nitrogen deposition, but not to ozone, after seven years of treatment. Ecosystems 16: 1105-1117. http://dx.doi.org/10.1007/s10021-013-9670-3.

Bassin, S; Volk, M; Suter, M; Buchmann, N; Fuhrer, J. (2007). Nitrogen deposition but not ozone affects productivity and community composition of subalpine grassland after 3 yr of treatment. New Phytol 175: 523-534. http://dx.doi.org/10.1111/j.1469-8137.2007.02140.x.

Batty, K; Ashmore, M; Power, SA. (2001). Assessment of relative sensitivity of wetland plant species to ozone. In The ozone umbrella project (CEH project C00970): Final report. Edinburgh, Scotland: Centre for Ecology & Hydrology.

Bell, JNB; Ashmore, MR; Wilson, GB. (1991). Ecological genetics and chemical modifications of the atmosphere. In GE Taylor, Jr.; LF Pitelka; MT Clegg (Eds.), Ecological genetics and air pollution (pp. 33-59). New York, NY: Springer-Verlag.

Bender, J; Bergmann, E; Dohrmann, A; Tebbe, CC; Weigel, HJ. (2002). Impact of ozone on plant competition and structural diversity of rhizosphere microbial communities in grassland mesocosms. Phyton-Annales Rei Botanicae 42: 7-12.

Bender, J; Bergmann, E; Weigel, HJ. (2006). Responses of biomass production and reproductive development to ozone exposure differ between european wild plant species. Water Air Soil Pollut 176: 253-267. http://dx.doi.org/10.1007/s11270-006-9167-1.

Bennett, JP; Jepsen, EA; Roth, JA. (2006). Field responses of Prunus serotina and Asclepias syriaca to ozone around southern Lake Michigan. Environ Pollut 142: 354-366. http://dx.doi.org/10.1016/j.envpol.2005.09.024.

Benoit, LF; Skelly, JM; Moore, LD; Dochinger, LS. (1982). Radial growth reductions of Pinus strobus L correlated with foliar ozone sensitivity as an indicator of ozone-induced losses in eastern forests. Can J For Res 12: 673-678. http://dx.doi.org/10.1139/x82-101.

Bergmann, E; Bender, J; Weigel, HJ. (1995). Growth responses and foliar sensitivities of native herbaceous species to ozone exposures. Water Air Soil Pollut 85: 1437-1442. http://dx.doi.org/10.1007/BF00477183.

Bergmann, E; Bender, J; Weigel, HJ. (1999). Ozone threshold doses and exposure-response relationships for the development of ozone injury symptoms in wild plant species. New Phytol 144: 423-435. http://dx.doi.org/10.1046/j.1469-8137.1999.00534.x.

Bergmann, E; Bender, J; Weigel, HJ. (2017). Impact of tropospheric ozone on terrestrial biodiversity: A literature analysis to identify ozone sensitive taxa. J Appl Bot Food Qual 90: 83-105. http://dx.doi.org/10.5073/JABFQ.2017.090.012.

Bergmann, E; Bender, J; Weigel, HJ. (1996a). Effects of chronic ozone stress on growth and reproduction capacity of native herbaceous plants. In JS M. Knopflacher; G Soja (Eds.), Exceedance of Critical Loads and Levels - Spatial and Temporal Interpretation of Elements in Landscape Sensitivity to Atmospheric Pollutants (pp. 177-185). Vienna, Austria: Umweltbundesamt Österreich.

Bergmann, E; Bender, J; Weigel, HJ. (1996b). Ozone and natural vegetation: Native species sensitivity to different ozone exposure regimes. In L Kärenlampi; L Skärby (Eds.), Critical levels for ozone in Europe: Testing and finalizing the concepts, UNECE workshop report (pp. 205-209). Kuopio, Finland: University of Kuopio.

Bergweiler, CJ; Manning, WJ. (1999). Inhibition of flowering and reproductive success in spreading dogbane (Apocynum androsaemifolium) by exposure to ambient ozone. Environ Pollut 105: 333-339. http://dx.doi.org/10.1016/S0269-7491(99)00044-5.

Bergweiler, CJ; Manning, WJ; Chevone, BI. (2008). Seasonal and diurnal gas exchange differences in ozone-sensitive common milkweed (Asclepias syriaca L.) in relation to ozone uptake. Environ Pollut 152: 403-415. http://dx.doi.org/10.1016/j.envpol.2007.06.019.

Bernacchi, CJ; Leakey, AD; Kimball, BA; Ort, DR. (2011). Growth of soybean at future tropospheric ozone concentrations decreases canopy evapotranspiration and soil water depletion. Environ Pollut 159: 1464-1472. http://dx.doi.org/10.1016/j.envpol.2011.03.011.

Betzelberger, A; Yendrek, CR; Sun, J; Leisner, CP; Nelson, RL; Ort, DR; Ainsworth, EA. (2012). Ozone exposure response for U.S. soybean cultivars: Linear reductions in photosynthetic potential, biomass, and yield. Plant Physiol 160: 1827-1839. http://dx.doi.org/10.1104/pp.112.205591.

Betzelberger, AM; Gillespie, KM; Mcgrath, JM; Koester, RP; Nelson, RL; Ainsworth, EA. (2010). Effects of chronic elevated ozone concentration on antioxidant capacity, photosynthesis and seed yield of 10 soybean cultivars. Plant Cell Environ 33: 1569-1581. http://dx.doi.org/10.1111/j.1365-3040.2010.02165.x.

Beyers, JL; Riechers, GH; Temple, PJ. (1992). Effects of long-term ozone exposure and drought on the photosynthetic capacity of ponderosa pine (Pinus ponderosa Laws). New Phytol 122: 81-90. http://dx.doi.org/10.1111/j.1469-8137.1992.tb00055.x.

Black, VJ; Black, CR; Roberts, JA; Stewart, CA. (2000). Tansley Review No. 115: Impact of ozone on the reproductive development of plants. New Phytol 147: 421-447. http://dx.doi.org/10.1046/j.1469-8137.2000.00721.x.

Black, VJ; Stewart, CA; Roberts, JA; Black, CR. (2012). Timing of exposure to ozone affects reproductive sensitivity and compensatory ability in Brassica campestris. Environ Exp Bot 75: 225-234. http://dx.doi.org/10.1016/j.envexpbot.2011.07.006.

Blande, JD; Holopainen, JK; Li, T. (2010). Air pollution impedes plant-to-plant communication by volatiles. Ecol Lett 13: 1172-1181. http://dx.doi.org/10.1111/j.1461-0248.2010.01510.x.

Blande, JD; Holopainen, JK; Niinemets, U. (2014). Plant volatiles in polluted atmospheres: stress responses and signal degradation [Review]. Plant Cell Environ 37: 1892-1904. http://dx.doi.org/10.1111/pce.12352.

Blum, O; Bytnerowicz, A; Manning, W; Popovicheva, L. (1997). Ambient tropospheric ozone in the ukrainian carpathian mountains and kiev region: detection with passive samplers and bioindicator plants. Environ Pollut 98: 299-304. http://dx.doi.org/10.1016/S0269-7491(97)00158-9.

Bohler, S; Sergeant, K; Jolivet, Y; Hoffmann, L; Hausman, JF; Dizengremel, P; Renaut, J. (2013). A physiological and proteomic study of poplar leaves during ozone exposure combined with mild drought. Proteomics 13: 1737-1754. http://dx.doi.org/10.1002/pmic.201200193.

Bolsinger, M; Lier, ME; Hughes, PR. (1992). Influence of ozone air pollution on plant-herbivore interactions Part 2: effects of ozone on feeding preference, growth and consumption rates of monarch butterflies (Danaus plexippus). Environ Pollut 77: 31-37. http://dx.doi.org/10.1016/0269-7491(92)90155-4.

Bolsinger, M; Lier, ME; Lansky, DM; Hughes, PR. (1991). Influence of ozone air pollution on plant-herbivore interactions Part I: Biochemical changes in ornamental milkweed (Asclepias curassavica L; Asclepiadaceae) induced by ozone. Environ Pollut 72: 69-83. http://dx.doi.org/10.1016/0269-7491(91)90156-Q.

Bradley, KL; Pregitzer, KS. (2007). Ecosystem assembly and terrestrial carbon balance under elevated CO2. Trends Ecol Evol 22: 538-547. http://dx.doi.org/10.1016/j.tree.2007.08.005.

Broberg, MC; Feng, Z; Xin, Y; Pleijel, H. (2015). Ozone effects on wheat grain quality - a summary [Review]. Environ Pollut 197: 203-213. http://dx.doi.org/10.1016/j.envpol.2014.12.009.

Brown, VC; McNeill, S; Ashmore, MR. (1992). The effects of ozone fumigation on the performance of the black bean aphid, Aphis fabae Scop, feeding on broad beans, Vicia faba L. Agric Ecosyst Environ 38: 71-78.

Bungener, P; Balls, GR; Nussbaum, S; Geissmann, M; Grub, A; Fuhrer, J. (1999a). Leaf injury characteristics of grassland species exposed to ozone in relation to soil moisture condition and vapour pressure deficit. New Phytol 142: 271-282. http://dx.doi.org/10.1046/j.1469-8137.1999.00390.x.

Bungener, P; Nussbaum, S; Grub, A; Fuhrer, J. (1999b). Growth response of grassland species to ozone in relation to soil moisture and plant strategy. New Phytol 142: 283-293. http://dx.doi.org/10.1046/j.1469-8137.1999.00389.x.

Burkey, KO; Booker, FL; Ainsworth, EA; Nelson, RL. (2012). Field assessment of a snap bean ozone bioindicator system under elevated ozone and carbon dioxide in a free air system. Environ Pollut 166: 167-171. http://dx.doi.org/10.1016/j.envpol.2012.03.020.

Burton, A; Burkey, KO; Carter, TE, Jr; Orf, J; Cregan, PB. (2016). Phenotypic variation and identification of quantitative trait loci for ozone tolerance in a Fiskeby III x Mandarin (Ottawa) soybean population. Theor Appl Genet 129: 1113-1125. http://dx.doi.org/10.1007/s00122-016-2687-1.

Bussotti, F; Cozzi, A; Bettini, D. (2003a). Ozone-like visible foliar symptoms at the permanent monitoring plots of the CONECO-FOR programme in Italy. Ann Ist Speriment Selvicoltura 30: 99-106.

Bussotti, F; Cozzi, A; Ferretti, M. (2006). Field surveys of ozone symptoms on spontaneous vegetation. Limitations and potentialities of the European programme. Environ Monit Assess 115: 335-348. http://dx.doi.org/10.1007/s10661-006-6558-0.

Bussotti, F; Gerosa, G. (2002). Are the Mediterranean forests in Southern Europe threatened from ozone? J Mediterr Ecol 3: 23-34.

Bussotti, F; Mazzali, C; Cozzi, A; Ferretti, M; Gravano, E; Gerosa, G; Ballarin-Denti, A. (2003b). Ozone injury symptoms on vegetation in an Alpine valley, North Italy. In Developments in Environmental Science.

Byres, DP; Dean, TJ; Johnson, JD. (1992). Long-term effects of ozone and simulated acid rain on the foliage dynamics of slash pine (Pinus elliottii var elliottii Engelm). New Phytol 120: 61-67. http://dx.doi.org/10.1111/j.1469-8137.1992.tb01058.x.

Bytnerowicz, A; Olszyk, DM; Fox, CA; Dawson, PJ; Kats, G; Morrison, CL; Wolf, J. (1988). Responses of desert annual plants to ozone and water stress in an in situ experiment. J Air Waste Manag Assoc 38: 1145-1151. http://dx.doi.org/10.1080/08940630.1988.10466463.

Calatayud, V; García-Breijo, FJ; Cervero, J; Reig-Armiñana, J; Sanz, MJ. (2011). Physiological, anatomical and biomass partitioning responses to ozone in the Mediterranean endemic plant Lamottea dianae. Ecotoxicol Environ Saf 74: 1131-1138. http://dx.doi.org/10.1016/j.ecoenv.2011.02.023.

Calvete-Sogo, H; González-Fernández, I; Sanz, J; Elvira, S; Alonso, R; García-Gómez, H; Ibáñez-Ruiz, MA; Bermejo-Bermejo, V. (2016). Heterogeneous responses to ozone and nitrogen alter the species composition of Mediterranean annual pastures. Oecologia 181: 1055-1067. http://dx.doi.org/10.1007/s00442-016-3628-z.

Cannon Jr, W; Roberts, B; Barger, J. (1993). Growth and physiological response of water-stressed yellow-poplar seedlings exposed to chronic ozone fumigation and ethylenediurea. For Ecol Manage 61: 61-73. http://dx.doi.org/10.1016/0378-1127(93)90190-X.

Cannon, WN. (1990). Olfactory response of eastern spruce budworm larvae to red spruce needles exposed to acid rain and elevated levels of ozone. J Chem Ecol 16: 3255-3261. http://dx.doi.org/10.1007/BF00982096.

Cano, I; Calatayud, V; Cervero, J; Sanz, MJ. (2007). Ozone effects on three Sambucus species. Environ Monit Assess 128: 83-91. http://dx.doi.org/10.1007/s10661-006-9417-0.

Capps, SL; Driscoll, CT; Fakhraei, H; Templer, PH; Craig, KJ; Milford, JB; Lambert, KF. (2016). Estimating potential productivity cobenefits for crops and trees from reduced ozone with US coal power plant carbon standards. J Geophys Res Atmos 121: 14679-14690. http://dx.doi.org/10.1002/2016JD025141.

Ceron-Breton, JG; Ceron-Breton, RM; Guerra-Santos, JJ; Aguilar-Ucan, C; Montalvo-Romero, C; Acosta-Morales, D; Calderon-Canto, R. (2009). Ozone-exposure responses of three Mangrove species by using open top chambers. In Energy and Environmental Engineering Series.

Chapin, FS, III; Matson, PA; Mooney, HA. (2002). Principles of terrestrial ecosystem ecology. New York, NY: Springer-Verlag New York, Inc.

Chappelka, A; Renfro, J; Somers, G; Nash, B. (1997). Evaluation of ozone injury on foliage of black cherry (Prunus serotina) and tall milkweed (Asclepias exaltata) in Great Smoky Mountains National Park. Environ Pollut 95: 13-18. http://dx.doi.org/10.1016/S0269-7491(96)00120-0.

Chappelka, A; Skelly, J; Somers, G; Renfro, J; Hildebrand, E. (1999a). Mature black cherry used as a bioindicator of ozone injury. Water Air Soil Pollut 116: 261-266. http://dx.doi.org/10.1023/A:1005260422738.

Chappelka, A; Somers, G; Renfro, J. (1999b). Visible ozone injury on forest trees in Great Smoky Mountains National Park, USA. Water Air Soil Pollut 116: 255-260. http://dx.doi.org/10.1023/A:1005204305900.

Chappelka, AH. (2002). Reproductive development of blackberry (Rubus cuneifolius) as influenced by ozone. New Phytol 155: 249-255. http://dx.doi.org/10.1046/j.1469-8137.2002.00464.x.

Chappelka, AH; Kush, JS; Meldahl, RS; Lockaby, BG. (1990). An ozone-low temperature interaction in loblolly pine (Pinus taeda L). New Phytol 114: 721-726. http://dx.doi.org/10.1111/j.1469-8137.1990.tb00444.x.

Chappelka, AH; Neufeld, HS; Davison, AW; Somers, GL; Renfro, JR. (2003). Ozone injury on cutleaf coneflower (Rudbeckia laciniata) and crown-beard (Verbesina occidentalis) in Great Smoky Mountains National Park. Environ Pollut 125: 53-60. http://dx.doi.org/10.1016/S0269-7491(03)00086-1.

Chappelka, AH; Samuelson, LJ. (1998). Ambient ozone effects on forest trees of the eastern United States: A review [Review]. New Phytol 139: 91-108. http://dx.doi.org/10.1046/j.1469-8137.1998.00166.x.

Chappelka, AH; Somers, GL; Renfro, JR. (2007). Temporal patterns of foliar ozone symptoms on tall milkweed (Asclepias exaltata L) in Great Smoky Mountains National Park. Environ Pollut 149: 358-365. http://dx.doi.org/10.1016/j.envpol.2007.05.015.

Chen, Z; Wang, X; Shang, H. (2015). Structure and function of rhizosphere and non-rhizosphere soil microbial community respond differently to elevated ozone in field-planted wheat. J Environ Sci 32: 126-134. http://dx.doi.org/10.1016/j.jes.2014.12.018.

Chen, Z; Wang, XK; Yao, FF; Zheng, FX; Feng, ZZ. (2010). Elevated ozone changed soil microbial community in a rice paddy. Soil Sci Soc Am J 74: 829-837. http://dx.doi.org/10.2136/sssaj2009.0258.

Cheng, L; Booker, FL; Burkey, KO; Tu, C; Shew, HD; Rufty, TW; Fiscus, EL; Deforest, JL; Hu, S. (2011). Soil microbial responses to elevated CO2 and O3 in a nitrogen-aggrading agroecosystem. PLoS ONE 6: e21377. http://dx.doi.org/10.1371/journal.pone.0021377.

Chieppa, J; Chappelka, A; Eckhardt, L. (2015). Effects of tropospheric ozone on loblolly pine seedlings inoculated with root infecting ophiostomatoid fungi. Environ Pollut 207: 130-137. http://dx.doi.org/10.1016/j.envpol.2015.08.053.

Chung, HG; Zak, DR; Lilleskov, EA. (2006). Fungal community composition and metabolism under elevated CO2 and O3. Oecologia 147: 143-154. http://dx.doi.org/10.1007/s00442-005-0249-3.

Coleman, JS; Jones, CG. (1988). Plant stress and insect performance: cottonwood, ozone and a leaf beetle. Oecologia 76: 57-61.

Coleman, MD; Dickson, RE; Isebrands, JG; Karnosky, DF. (1996). Root growth physiology of potted and field-grown trembling aspen exposed to tropospheric ozone. Tree Physiol 16: 145-152. http://dx.doi.org/10.1093/treephys/16.1-2.145.

Cornelissen, T. (2011). Climate change and its effects on terrestrial insects and herbivory patterns [Review]. Neotrop Entomol 40: 155-163. http://dx.doi.org/10.1590/S1519-566X2011000200001.

Costa, SD; Kennedy, GG; Heagle, AS. (2001). Effect of host plant ozone stress on Colorado potato beetles. Environ Entomol 30: 824-831. http://dx.doi.org/10.1603/0046-225X-30.5.824.

Costanza, R; De Groot, R; Braat, L; Kubiszewski, I; Fioramonti, L; Sutton, P; Farber, S; Grasso, M. (2017). Twenty years of ecosystem services: How far have we come and how far do we still need to go? Ecosyst Serv 28: 1-16. http://dx.doi.org/10.1016/j.ecoser.2017.09.008.

Cotton, TEA; Fitter, AH; Miller, RM; Dumbrell, AJ; Helgason, T. (2015). Fungi in the future: interannual variation and effects of atmospheric change on arbuscular mycorrhizal fungal communities. New Phytol 205: 1598-1607. http://dx.doi.org/10.1111/nph.13224.

Couture, JJ; Lindroth, RL. (2012). Atmospheric change alters performance of an invasive forest insect. Global Change Biol 18: 3543-3557. http://dx.doi.org/10.1111/gcb.12014.

Couture, JJ; Lindroth, RL. (2014). Atmospheric change alters frass quality of forest canopy herbivores. Arthropod-Plant Inte 8: 33-47. http://dx.doi.org/10.1007/s11829-013-9286-8.

Couture, JJ; Meehan, TD; Kruger, EL; Lindroth, RL. (2015). Insect herbivory alters impact of atmospheric change on northern temperate forests. 1: 15016. http://dx.doi.org/10.1038/NPLANTS.2015.16.

Couture, JJ; Meehan, TD; Lindroth, RL. (2012). Atmospheric change alters foliar quality of host trees and performance of two outbreak insect species. Oecologia 168: 863-876. http://dx.doi.org/10.1007/s00442-011-2139-1.

Cui, H; Su, J; Wei, J; Hu, Y; Ge, F. (2014). Elevated O-3 enhances the attraction of whitefly-infested tomato plants to Encarsia formosa. Sci Rep 4: 5350. http://dx.doi.org/10.1038/srep05350.

Cui, H; Sun, Y; Chen, F; Zhang, Y; Ge, F. (2016a). Elevated O3 and TYLCV infection reduce the suitability of tomato as a host for the whitefly Bemisia tabaci. International Journal of Molecular Sciences 17: 1964. http://dx.doi.org/10.3390/ijms17121964.

Cui, H; Sun, Y; Su, J; Ren, Q, in; Li, C; Ge, F. (2012). Elevated O-3 reduces the fitness of Bemisia tabaci via enhancement of the SA-dependent defense of the tomato plant. Arthropod-Plant Inte 6: 425-437. http://dx.doi.org/10.1007/s11829-012-9189-0.

Cui, H; Wei, J; Su, J; Li, C; Ge, F. (2016b). Elevated O3 increases volatile organic compounds via jasmonic acid pathway that promote the preference of parasitoid Encarsia formosa for tomato plants. Plant Sci 253: 243-250. http://dx.doi.org/10.1016/j.plantsci.2016.09.019.

Curtis, PS; Wang, X. (1998). A meta-analysis of elevated CO 2 effects on woody plant mass, form, and physiology. Oecologia 113: 299-313. http://dx.doi.org/10.1007/s004420050381.

Danielsson, H; Gelang, J; Pleijel, H. (1999). Ozone sensitivity, growth and flower development in Phleum genotypes of different geographic origin in the Nordic countries. Environ Exp Bot 42: 41-49. http://dx.doi.org/10.1016/S0098-8472(99)00016-7.

Darbah, JNT; Kubiske, ME; Neilson, N; Oksanen, E; Vaapavuori, E; Karnosky, DF. (2007). Impacts of elevated atmospheric CO2 and O3 on paper birch (Betula papyrifera): Reproductive fitness. ScientificWorldJournal 7: 240-246. http://dx.doi.org/10.1100/tsw.2007.42.

Darbah, JNT; Kubiske, ME; Nelson, N; Oksanen, E; Vapaavuori, E; Kamosky, DF. (2008). Effects of decadal exposure to interacting elevated CO2 and/or O-3 on paper birch (Betula papyrifera) reproduction. Environ Pollut 155: 446-452. http://dx.doi.org/10.1016/j.envpol.2008.01.033.

Darrall, NM. (1989). The effect of air pollutants on physiological processes in plants. Plant Cell Environ 12: 1-30. http://dx.doi.org/10.1111/j.1365-3040.1989.tb01913.x.

Davis, DD. (2007a). Ozone-induced symptoms on vegetation within the Moosehorn National Wildlife Refuge in Maine. Northeast Nat 14: 403-414. http://dx.doi.org/10.1656/1092-6194(2007)14[403:OSOVWT]2.0.CO;2.

Davis, DD. (2007b). Ozone injury to plants within the Seney National Wildlife Refuge in northern Michigan. Northeast Nat 14: 415-424. http://dx.doi.org/10.1656/1092-6194(2007)14[415:OITPWT]2.0.CO;2.

Davis, DD. (2009). Ozone-induced stipple on plants in the Cape Romain National Wildlife Refuge, South Carolina. Southeastern Naturalist 8: 471-478. http://dx.doi.org/10.1656/058.008.0308.

Davis, DD. (2011). Ozone-induced leaf symptoms on vegetation in the Mingo National Wildlife Refuge, Missouri. Northeast Nat 18: 115-122. http://dx.doi.org/10.1656/045.018.0111.

Davis, DD; Orendovici, T. (2006). Incidence of ozone symptoms on vegetation within a National Wildlife Refuge in New Jersey, USA. Environ Pollut 143: 555-564. http://dx.doi.org/10.1016/j.envpol.2005.10.051.

Davis, DD; Skelly, JM. (1992). Growth response of four species of eastern hardwood tree seedlings exposed to ozone, acidic precipitation, and sulfur dioxide. J Air Waste Manag Assoc 42: 309-311.

Davison, AW; Neufeld, HS; Chappelka, AH; Wolff, KW; Finkelstein, PL. (2003). Interpreting spatial variation in ozone symptoms shown by cutleaf cone flower, Rudbeckia laciniata L. Environ Pollut 125: 61-70. http://dx.doi.org/10.1016/S0269-7491(03)00087-3.

De Bauer, LI; Hernandez-Tejeda, T; Skelly, JM. (2000). Air pollution problems in the forested areas of Mexico and Central America. In JL Innes; AH Haron (Eds.), Air pollution and the forests of developing and rapidly industrializing regions Report No 4 of the IUFRO Task Force on Environmental Change (pp. 35-61). Vienna, Austria: CABI Pub.; Published in association with the International Union of Forestry Research Organizations, c2000. http://dx.doi.org/10.1079/9780851994819.0035.

de Lourdes de Bauer, M; Hernandez-Tejeda, T. (2007). A review of ozone-induced effects on the forests of central Mexico [Review]. Environ Pollut 147: 446-453. http://dx.doi.org/10.1016/j.envpol.2006.12.020.

de Vries, W; Posch, M; Simpson, D; Reinds, GJ. (2017). Modelling long-term impacts of changes in climate, nitrogen deposition and ozone exposure on carbon sequestration of European forest ecosystems. Sci Total Environ 605-606: 1097-1116. http://dx.doi.org/10.1016/j.scitotenv.2017.06.132.

De Vries, W; Reinds, GJ; Vel, E. (2003). Intensive monitoring of forest ecosystems in Europe: 2: atmospheric deposition and its impacts on soil solution chemistry. For Ecol Manage 174: 97-115.

Dean, TJ; Johnson, JD. (1992). Growth response of young slash pine trees to simulated acid rain and ozone stress. Can J For Res 22: 839-848.

Decock, C; Chung, H; Venterea, R; Gray, SB; Leakey, ADB; Six, J. (2012). Elevated CO2 and O-3 modify N turnover rates, but not N2O emissions in a soybean agroecosystem. Soil Biol Biochem 51: 104-114. http://dx.doi.org/10.1016/j.soilbio.2012.04.015.

Decock, C; Six, J. (2012). Effects of elevated CO2 and O-3 on N-cycling and N2O emissions: a short-term laboratory assessment. Plant Soil 351: 277-292. http://dx.doi.org/10.1007/s11104-011-0961-1.

Diaz-De-Quijano, M; Kefauver, S; Ogaya, R; Vollenweider, P; Ribas, A; Penuelas, J. (2016). Visible ozone-like injury, defoliation, and mortality in two Pinus uncinata stands in the Catalan Pyrenees (NE Spain). European Journal of Forest Research 135: 687-696. http://dx.doi.org/10.1007/s10342-016-0964-9.

Dickson, RE; Lewin, KF; Isebrands, JG; Coleman, MD; Heilman, WE; Riemenschneider, DE; Sober, J; Host, GE; Zak, DR; Hendrey, GR; Pregitzer, KS; Karnosky, DF. (2000). Forest Atmosphere Carbon Transfer and Storage (FACTS-II) the Aspen Free-Air CO2 and O3 Enrichment (FACE) project: An overview. (General Technical Report NC-214). St. Paul, MN: U.S. Dept. of Agriculture, Forest Service. http://nrs.fs.fed.us/pubs/278.

Dietze, MC; Moorcroft, PR. (2011). Tree mortality in the eastern and central United States: Patterns and drivers. Global Change Biol 17: 3312-3326. http://dx.doi.org/10.1111/j.1365-2486.2011.02477.x.

Dohmen, GP. (1988). Indirect effects of air pollutants: changes in plant/parasite interactions. Environ Pollut 53: 197-207.

Dohrmann, AB; Tebbe, CC. (2005). Effect of elevated tropospheric ozone on the structure of bacterial communities inhabiting the rhizosphere of herbaceous plants native to Germany. Appl Environ Microbiol 71: 7750-7758. http://dx.doi.org/10.1128/AEM.71.12.7750-7758.2005.

Dumont, J; Spicher, F; Montpied, P; Dizengremel, P; Jolivet, Y; Le Thiec, D. (2013). Effects of ozone on stomatal responses to environmental parameters (blue light, red light, CO2 and vapour pressure deficit) in three Populus deltoides Populus nigra genotypes. Environ Pollut 173: 85-96. http://dx.doi.org/10.1016/j.envpol.2012.09.026.

Dunbar, J; Gallegos-Graves, L; Steven, B; Mueller, R; Hesse, C; Zak, DR; Kuske, CR. (2014). Surface soil fungal and bacterial communities in aspen stands are resilient to eleven years of elevated CO2 and O-3. Soil Biol Biochem 76: 227-234. http://dx.doi.org/10.1016/j.soilbio.2014.05.027.

Ebanyenle, E; Burton, AJ; Storer, AJ; Richter, DL; Glaeser, JA. (2016). Elevated tropospheric CO2 and O-3 may not alter initial wood decomposition rate or wood-decaying fungal community composition of Northern Hardwoods. Int Biodeterior Biodegradation 111: 74-77. http://dx.doi.org/10.1016/j.ibiod.2016.04.026.

Edwards, GS; Wullschleger, SD; Kelly, JM. (1994). Growth and physiology of northern red oak: preliminary comparisons of mature tree and seedling responses to ozone. Environ Pollut 83: 215-221. http://dx.doi.org/10.1016/0269-7491(94)90036-1.

Edwards, IP; Zak, DR. (2011). Fungal community composition and function after long-term exposure of northern forests to elevated atmospheric CO2 and tropospheric O3. Global Change Biol 17: 2184-2195. http://dx.doi.org/10.1111/j.1365-2486.2010.02376.x.

Edwards, NT; Edwards, GL; Kelly, JM; Taylor, GE, Jr. (1992). Three-year growth responses of Pinus taeda L to simulated rain chemistry, soil magnesium status, and ozone. Water Air Soil Pollut 63: 105-118. http://dx.doi.org/10.1007/BF00475625.

Emberson, L; Ashmore, MR; Cambridge, HM; Simpson, D; Tuovinen, JP. (2000a). Modelling stomatal ozone flux across Europe. Environ Pollut 109: 403-413. http://dx.doi.org/10.1016/S0269-7491(00)00043-9.

Emberson, LD; Pleijel, H; Ainsworth, EA; van Den Berg, M; Ren, W; Osborne, S; Mills, G; Pandey, D; Dentener, F; Büker, P; Ewert, F; Koebl, R; Van Dingenend, R. (2018). Ozone effects on crops and consideration in crop models. Eur J Agron 100: 19-34. http://dx.doi.org/10.1016/j.eja.2018.06.002.

Emberson, LD; Wieser, G; Ashmore, MR. (2000b). Modelling of stomatal conductance and ozone flux of Norway spruce: Comparison with field data. Environ Pollut 109: 393-402. http://dx.doi.org/10.1016/S0269-7491(00)00042-7.

Evans, LS; Fitzgerald, GA. (1993). Histological effects of ozone on slash pine (Pinus elliotti var. Densa). Environ Exp Bot 33: 505-513. http://dx.doi.org/10.1016/0098-8472(93)90024-A.

Fares, S; Vargas, R; Detto, M; Goldstein, AH; Karlik, J; Paoletti, E; Vitale, M. (2013). Tropospheric ozone reduces carbon assimilation in trees: estimates from analysis of continuous flux measurements. Global Change Biol 19: 2427-2443. http://dx.doi.org/10.1111/gcb.12222.

Farré-Armengol, G; Peñuelas, J; Li, T; Yli-Pirilä, P; Filella, I; Llusia, J; Blande, JD. (2015). Ozone degrades floral scent and reduces pollinator attraction to flowers. New Phytol 209: 152-160. http://dx.doi.org/10.1111/nph.13620.

Felzer, B; Kicklighter, D; Melillo, J; Wang, C; Xhuang, Q; Prinn, R. (2004). Effects of ozone on net primary production and carbon sequestration in the conterminous United States using a biogeochemistry model. Tellus B Chem Phys Meteorol 56: 230-248. http://dx.doi.org/10.1111/j.1600-0889.2004.00097.x.

Felzer, B; Reilly, J; Melillo, J; Kicklighter, D; Sarofim, M; Wang, C; Prinn, R; Zhuang, Q. (2005). Future effects of ozone on carbon sequestration and climate change policy using a global biogeochemical model. Clim Change 73: 345-373. http://dx.doi.org/10.1007/s10584-005-6776-4.

Felzer, BS; Cronin, TW; Melillo, JM; Kicklighter, DW; Schlosser, CA. (2009). Importance of carbon-nitrogen interactions and ozone on ecosystem hydrology during the 21st century. J Geophys Res 114: G01020. http://dx.doi.org/10.1029/2008JG000826.

Feng, Y; Lin, X; Yu, Y; Zhang, H; Chu, H; Zhu, J. (2013). Elevated ground-level O3 negatively influences paddy methanogenic archaeal community. Sci Rep 3: 3193. http://dx.doi.org/10.1038/srep03193.

Feng, Y; Lin, X; Yu, Y; Zhu, J. (2011). Elevated ground-level O3 changes the diversity of anoxygenic purple phototrophic bacteria in paddy field. Microb Ecol 62: 789-799. http://dx.doi.org/10.1007/s00248-011-9895-7.

Feng, Y; Yu, Y; Tang, H; Zu, Q; Zhu, J; Lin, X. (2015). The contrasting responses of soil microorganisms in two rice cultivars to elevated ground-level ozone. Environ Pollut 197: 195-202. http://dx.doi.org/10.1016/j.envpol.2014.11.032.

Feng, Z; Büker, P; Pleijel, H; Emberson, L; Karlsson, PE; Uddling, J. (2017). A unifying explanation for variation in ozone sensitivity among woody plants. Global Change Biol 24: 78-84. http://dx.doi.org/10.1111/gcb.13824.

Feng, ZZ; Kobayashi, K. (2009). Assessing the impacts of current and future concentrations of surface ozone on crop yield with meta-analysis. Atmos Environ 43: 1510-1519. http://dx.doi.org/10.1016/j.atmosenv.2008.11.033.

Feng, ZZ; Kobayashi, K; Ainsworth, EA. (2008). Impact of elevated ozone concentration on growth, physiology, and yield of wheat (Triticum aestivum L.): A meta-analysis. Global Change Biol 14: 2696-2708. http://dx.doi.org/10.1111/j.1365-2486.2008.01673.x.

Fenn, ME; de Bauer, LI; Hernández-Tejeda, T. (2002). Summary of air pollution impacts on forests in the Mexico City air basin. In Urban air pollution and forests. New York, NY: Springer-Verlag.

Ferreira, B; Ribeiro, H; Pereira, MS; Cruz, A; Abreu, I. (2016). Effects of ozone in Plantago lanceolata and Salix atrocinerea pollen. Aerobiologia 32: 421-430. http://dx.doi.org/10.1007/s10453-015-9415-1.

Findley, DA; Keever, GJ; Chappelka, AH; Eakes, DJ; Gilliam, CH. (1996). Sensitivity of red maple cultivars to acute and chronic exposures of ozone. J arboric 22: 264-269.

Finnveden, G. (2000). On the limitations of life cycle assessment and environmental systems analysis tools in general. Int J Life Cycle Assess 5: 229-238.

Fiscus, EL; Booker, FL; Sadok, W; Burkey, KO. (2012). Influence of atmospheric vapour pressure deficit on ozone responses of snap bean (Phaseolus vulgaris L.) genotypes. J Exp Bot 63: 2557-2564. http://dx.doi.org/10.1093/jxb/err443.

Fishman, J; Belina, KM; Encarnacion, CH. (2014). The St. Louis ozone gardens: Visualizing the impact of a changing atmosphere. Bull Am Meteorol Soc 95: 1171-1177. http://dx.doi.org/10.1175/BAMS-D-13-00009.1.

Fleischer, K; Rebel, KT; van der Molen, MK; Erisman, JW; Wassen, MJ; van Loon, EE; Montagnani, L; Gough, CM; Herbst, M; Janssens, IA; Gianelle, D; Dolman, AJ. (2013). The contribution of nitrogen deposition to the photosynthetic capacity of forests. Global Biogeochem Cycles 27: 187-199. http://dx.doi.org/10.1002/gbc.20026.

Flowers, MD; Fiscus, EL; Burkey, KO; Booker, FL; Dubois, JJB. (2007). Photosynthesis, chlorophyll fluorescence, and yield of snap bean (Phaseolus vulgaris L.) genotypes differing in sensitivity to ozone. Environ Exp Bot 61: 190-198. http://dx.doi.org/10.1016/j.envexpbot.2007.05.009.

Foot, JP; Caporn, SJM; Lee, JA; Ashenden, TW. (1996). The effect of long-term ozone fumigation on the growth, physiology and frost sensitivity of Calluna vulgaris. New Phytol 133: 503-511. http://dx.doi.org/10.1111/j.1469-8137.1996.tb01918.x.

Fowler, D; Flechard, C; Skiba, U; Coyle, M; Cape, JN. (1998). The atmospheric budget of oxidized nitrogen and its role in ozone formation and deposition. New Phytol 139: 11-23. http://dx.doi.org/10.1046/j.1469-8137.1998.00167.x.

Franzaring, J; Tonneijck, AEG; Kooijman, AWN; Dueck, TA. (2000). Growth responses to ozone in plant species from wetlands. Environ Exp Bot 44: 39-48. http://dx.doi.org/10.1016/S0098-8472(00)00052-6.

Fuentes, JD; Chamecki, M; Roulston, T; Chen, B; Pratt, KR. (2016). Air pollutants degrade floral scents and increase insect foraging times. Atmos Environ 141: 361-374. http://dx.doi.org/10.1016/j.atmosenv.2016.07.002.

Fuentes, JD; Roulston, TH; Zenker, J. (2013). Ozone impedes the ability of a herbivore to find its host. Environ Res Lett 8. http://dx.doi.org/10.1088/1748-9326/8/1/014048.

Fuhrer, J. (1994). Effects of ozone on managed pasture: 1. Effects of open-top chambers on microclimate, ozone flux, and plant growth. Environ Pollut 86: 297-305. http://dx.doi.org/10.1016/0269-7491(94)90170-8.

Gao, F; Catalayud, V; Paoletti, E; Hoshika, Y; Feng, Z. (2017). Water stress mitigates the negative effects of ozone on photosynthesis and biomass in poplar plants. Environ Pollut 230: 268-279. http://dx.doi.org/10.1016/j.envpol.2017.06.044.

Gate, IM; Mcneill, S; Ashmore, MR. (1995). Effects of air pollution on the searching behaviour of an insect parasitoid. Water Air Soil Pollut 85: 1425-1430. http://dx.doi.org/10.1007/BF00477181.

Gaucher, C; Dizengremel, P; Mauffette, Y; Chevrier, N. (2005). Response of Acer saccharum seedlings to elevated O-3 and CO2 concentrations. Phytoprotection 86: 7-17.

Gerosa, G; Ballarin-Denti, A. (2003). Regional scale risk assessment of ozone and forests. In DF Karnosky; KE Percy; AH Chappelka; C Simpson; J Pikkarainen (Eds.), Air pollution, global change and forests in the new millennium (pp. 119-139). Amsterdam, Netherlands: Elsevier.

Geyh, AS; Xue, J; Ozkaynak, H; Spengler, JD. (2000). The Harvard Southern California chronic ozone exposure study: Assessing ozone exposure of grade-school-age children in two southern California communities. Environ Health Perspect 108: 265-270. http://dx.doi.org/10.1289/ehp.00108265.

Ghimire, RP; Kivimaenpaa, M; Kasurinen, A; Haikio, E; Holopainen, T; Holopainen, JK. (2017). Herbivore-induced BVOC emissions of Scots pine under warming, elevated ozone and increased nitrogen availability in an open-field exposure. Agr Forest Meteorol 242: 21-32. http://dx.doi.org/10.1016/j.agrformet.2017.04.008.

Gillespie, C; Stabler, D; Tallentire, E; Goumenaki, E; Barnes, J. (2015). Exposure to environmentally-relevant levels of ozone negatively influence pollen and fruit development. Environ Pollut 206: 494-501. http://dx.doi.org/10.1016/j.envpol.2015.08.003.

Gilliland, NJ; Chappelka, AH; Muntifering, RB; Booker, FL; Ditchkoff, SS. (2012). Digestive utilization of ozone-exposed forage by rabbits (Oryctolagus cuniculus). Environ Pollut 163: 281-286. http://dx.doi.org/10.1016/j.envpol.2012.01.003.

Gilliland, NJ; Chappelka, AH; Muntifering, RB; Ditchkoff, SS. (2016). Changes in southern Piedmont grassland community structure and nutritive quality with future climate scenarios of elevated tropospheric ozone and altered rainfall patterns. Plant Biol (Stuttg) 18: 47-55. http://dx.doi.org/10.1111/plb.12324.

Gimeno, BS; Bermejo, V; Sanz, J; De La Torre, D; Elvira, S. (2004). Growth response to ozone of annual species from Mediterranean pastures. Environ Pollut 132: 297-306. http://dx.doi.org/10.1016/j.envpol.2004.04.022.

Giron-Calva, PS; Li, T; Mande, JD. (2016). Plant-plant interactions affect the susceptibility of plants to oviposition by pests but are disrupted by ozone pollution. Agric Ecosyst Environ 233: 352-360. http://dx.doi.org/10.1016/j.agee.2016.09.028.

Godzik, B; Grodzinska, K. (2002). Ground level ozone concentration and its effect on plants of Polish national parks. Kosmos 5: 427.

Grantz, DA; Gunn, S; Vu, HB. (2006). O3 impacts on plant development: A meta-analysis of root/shoot allocation and growth. Plant Cell Environ 29: 1193-1209. http://dx.doi.org/10.1111/j.1365-3040.2006.01521.x.

Grantz, DA; Jackson, A; Vu, HB; Burkey, KO; Mcgrath, MT; Harvey, G. (2014). High ozone increases soil perchlorate but does not affect foliar perchlorate content. J Environ Qual 43: 1460-1466. http://dx.doi.org/10.2134/jeq2013.11.0464.

Grantz, DA; Paudel, R; Vu, HB; Shrestha, A; Grulke, N. (2016). Diel trends in stomatal response to ozone and water deficit: a unique relationship of midday values to growth and allometry in Pima cotton? Plant Biol (Stuttg) 18: 37-46. http://dx.doi.org/10.1111/plb.12355.

Grantz, DA; Shrestha, A; Vu, HB. (2008). Early vigor and ozone response in horseweed (Conyza canadensis) biotypes differing in glyphosate resistance. Weed Sci 56: 224-230. http://dx.doi.org/10.1614/ws-07-130.1.

Grantz, DA; Vu, HB. (2009). O3 sensitivity in a potential C4 bioenergy crop: Sugarcane in California. Crop Sci 49: 643-650. http://dx.doi.org/10.2135/cropsci2008.04.0210.

Grantz, DA; Vu, HB; Heath, RL; Burkey, KO. (2013). Demonstration of a diel trend in sensitivity of Gossypium to ozone: a step toward relating O-3 injury to exposure or flux. J Exp Bot 64: 1703-1713. http://dx.doi.org/10.1093/jxb/ert032.

Grantz, DA; Vu, HB; Tew, TL; Veremis, JC. (2012). Sensitivity of gas exchange parameters to ozone in diverse C-4 sugarcane hybrids. Crop Sci 52: 1270-1280. http://dx.doi.org/10.2135/cropsci2011.08.0413.

Grantz, DA; Zhang, XJ; Massman, W; Delany, A; Pederson, R. (1997). Ozone deposition to a cotton (Gossypium hirsutum L) field: Stomatal and surface wetness effects during the California Ozone Deposition experiment. Agr Forest Meteorol 85: 19-31. http://dx.doi.org/10.1016/S0168-1923(96)02396-9.

Grantz, DA; Zhang, XJ; Massman, WJ; Den Hartog, G; Neumann, HH; Pederson, J. R. (1995). Effects of stomatal conductance and surface wetness on ozone deposition in field-grown grape. Atmos Environ 29: 3189-3198. http://dx.doi.org/10.1016/1352-2310(95)00129-M.

Greaver, TL; Sullivan, TJ; Herrick, JD; Barber, MC; Baron, JS; Cosby, BJ; Deerhake, ME; Dennis, RL; Dubois, J, -JB; Goodale, CL; Herlihy, AT; Lawrence, GB; Liu, L; Lynch, JA; Novak, KJ. (2012). Ecological effects of nitrogen and sulfur air pollution in the US: What do we know? Front Ecol Environ 10: 365-372. http://dx.doi.org/10.1890/110049.

Grebenc, T; Kraigher, H. (2007). Changes in the community of ectomycorrhizal fungi and increased fine root number under adult beech trees chronically fumigated with double ambient ozone concentration. Plant Biol (Stuttg) 9: 279-287. http://dx.doi.org/10.1055/s-2006-924489.

Gregg, JW; Jones, CG; Dawson, TE. (2003). Urbanization effects on tree growth in the vicinity of New York City [Letter]. Nature 424: 183-187. http://dx.doi.org/10.1038/nature01728.

Gregg, JW; Jones, CG; Dawson, TE. (2006). Physiological and developmental effects of O3 on cottonwood growth in urban and rural sites. Ecol Appl 16: 2368-2381. http://dx.doi.org/10.1890/1051-0761(2006)016[2368:PADEOO]2.0.CO;2.

Grulke, NE. (1999). Physiological responses of ponderosa pine to gradients of environmental stressors. In PR Miller; JR McBride (Eds.), Oxidant air pollution impacts in the montane forests of southern California: A case study of the San Bernardino Mountains (pp. 126-163). New York, NY: Springer. http://dx.doi.org/10.1007/978-1-4612-1436-6_7.

Grulke, NE; Alonso, R; Nguyen, T; Cascio, C; Dobrowolski, W. (2004). Stomata open at night in pole-sized and mature ponderosa pine: Implications for O3 exposure metrics. Tree Physiol 24: 1001-1010. http://dx.doi.org/10.1093/treephys/24.9.1001.

Grulke, NE; Andersen, CP; Fenn, ME; Miller, PR. (1998). Ozone exposure and nitrogen deposition lowers root biomass of ponderosa pine in the San Bernardino Mountains, California. Environ Pollut 103: 63-73. http://dx.doi.org/10.1016/S0269-7491(98)00130-4.

Grulke, NE; Johnson, R; Esperanza, A; Jones, D; Nguyen, T; Posch, S; Tausz, M. (2003). Canopy transpiration of Jeffrey pine in mesic and xeric microsites: O3 uptake and injury response. Trees Struct Funct 17: 292-298. http://dx.doi.org/10.1007/s00468-002-0237-8.

Gundel, PE; Sorzoli, N; Ueno, AC; Ghersa, CM; Seal, CE; Bastias, DA; Martinez-Ghersa, MA. (2015). Impact of ozone on the viability and antioxidant content of grass seeds is affected by a vertically transmitted symbiotic fungus. Environ Exp Bot 113: 40-46. http://dx.doi.org/10.1016/j.envexpbot.2015.01.001.

Gunthardt-Goerg, MS; Maurer, S; Bolliger, J; Clark, AJ; Landolt, W; Bucher, JB. (1999). Responses of young trees (five species in a chamber exposure) to near-ambient ozone concentrations. Water Air Soil Pollut 116: 323-332.

Gustafson, EJ; Kubiske, ME; Sturtevant, BR; Miranda, BR. (2013). Scaling Aspen-FACE experimental results to century and landscape scales. Landsc Ecol 28: 1785-1800. http://dx.doi.org/10.1007/s10980-013-9921-x.

Habeck, CW; Lindroth, RL. (2013). Influence of global atmospheric change on the feeding behavior and growth performance of a mammalian herbivore, Microtus ochrogaster. PLoS ONE 8: e72717. http://dx.doi.org/10.1371/journal.pone.0072717.

Haesler, F; Hagn, A; Engel, M; Schloter, M. (2014). Impact of elevated atmospheric O-3 on the actinobacterial community structure and function in the rhizosphere of European beech (Fagus sylvatica L.). FMICB 5: 36. http://dx.doi.org/10.3389/fmicb.2014.00036.

Handley, T; Grulke, NE. (2008). Interactive effects of O3 exposure on California black oak (Quercus kelloggii Newb.) seedlings with and without N amendment. Environ Pollut 156: 53-60. http://dx.doi.org/10.1016/j.envpol.2008.01.002.

Hanson, PJ; Wullschleger, SD; Norby, RJ; Tschaplinski, TJ; Gunderson, CA. (2005). Importance of changing CO2, temperature, precipitation, and ozone on carbon and water cycles of an upland-oak forest: incorporating experimental results into model simulations. Global Change Biol 11: 1402-1423. http://dx.doi.org/10.1111/j.1365-2486.2005.00991.x.

Hayes, F; Jones, MLM; Mills, G; Ashmore, M. (2007). Meta-analysis of the relative sensitivity of semi-natural vegetation species to ozone. Environ Pollut 146: 754-762. http://dx.doi.org/10.1016/j.envpol.2006.06.011.

Hayes, F; Mills, G; Ashmore, M. (2009). Effects of ozone on inter- and intra-species competition and photosynthesis in mesocosms of Lolium perenne and Trifolium repens. Environ Pollut 157: 208-214. http://dx.doi.org/10.1016/j.envpol.2008.07.002.

Hayes, F; Mills, G; Harmens, H; Wyness, K. (2011). Within season and carry-over effects following exposure of grassland species mixtures to increasing background ozone. Environ Pollut 159: 2420-2426. http://dx.doi.org/10.1016/j.envpol.2011.06.034.

Hayes, F; Mills, G; Jones, L; Abbott, J; Ashmore, M; Barnes, J; Cape, JN; Coyle, M; Peacock, S; Rintoul, N; Toet, S; Wedlich, K; Wyness, K. (2016). Consistent ozone-induced decreases in pasture forage quality across several grassland types and consequences for UK lamb production. Sci Total Environ 543: 336-346. http://dx.doi.org/10.1016/j.scitotenv.2015.10.128.

Hayes, F; Mills, G; Jones, L; Ashmore, M. (2010). Does a simulated upland grassland community respond to increasing background, peak or accumulated exposure of ozone? Atmos Environ 44: 4155-4164. http://dx.doi.org/10.1016/j.atmosenv.2010.07.037.

Hayes, F; Mills, G; Williams, P; Harmens, H; Buker, P. (2006). Impacts of summer ozone exposure on the growth and overwintering of UK upland vegetation. Atmos Environ 40: 4088-4097. http://dx.doi.org/10.1016/j.atmosenv.2006.03.012.

Hayes, F; Wagg, S; Mills, G; Wilkinson, S; Davies, W. (2012a). Ozone effects in a drier climate: implications for stomatal fluxes of reduced stomatal sensitivity to soil drying in a typical grassland species. Global Change Biol 18: 948-959. http://dx.doi.org/10.1111/j.1365-2486.2011.02613.x.

Hayes, F; Williamson, J; Mills, G. (2012b). Ozone pollution affects flower numbers and timing in a simulated BAP priority calcareous grassland community. Environ Pollut 163: 40-47. http://dx.doi.org/10.1016/j.envpol.2011.12.032.

He, Z; Xiong, J; Kent, AD; Deng, Y; Xue, K; Wang, G; Wu, L; Van Nostrand, J; Zhou, J. (2014). Distinct responses of soil microbial communities to elevated CO2 and O3 in a soybean agro-ecosystem. ISME J 8: 714-726. http://dx.doi.org/10.1038/ismej.2013.177.

Heagle, AS; Body, DE; Heck, WW. (1973). An open-top field chamber to assess the impact of air pollution on plants. J Environ Qual 2: 365-368. http://dx.doi.org/10.2134/jeq1973.00472425000200030014x.

Heagle, AS; Brandenburg, RL; Burns, JC; Miller, JE. (1994). Ozone and carbon dioxide effects on spider mites in white clover and peanut. J Environ Qual 23: 1168-1176. http://dx.doi.org/10.2134/jeq1994.00472425002300060006x.

Heck, WW; Heagle, AS; Miller, JE; Rawlings, JO. (1991). A national program (NCLAN) to assess the impact of ozone on agricultural resources. In RL Berglund; DR Lawson; DJ McKee (Eds.), Tropospheric ozone and the environment: papers from an international conference; March 1990; Los Angeles, CA (pp. 225-254). Pittsburgh, PA: Air & Waste Management Association.

Heck, WW; Philbeck, RB; Dunning, JA. (1978). A continuous stirred tank reactor (CSTR) system for exposing plants to gaseous air contaminants: Principles, specifications, construction, and operation. (ARS-S-181). New Orleans, LA: U.S. Department of Agriculture, Agricultural Research Service.

Heck, WW; Taylor, OC; Adams, R; Bingham, G; Miller, J; Preston, E; Weinstein, L. (1982). Assessment of crop loss from ozone. J Air Pollut Control Assoc 32: 353-361. http://dx.doi.org/10.1080/00022470.1982.10465408.

Hendrey, GR; Ellsworth, DS; Lewin, KF; Nagy, J. (1999). A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO2. Global Change Biol 5: 293-309. http://dx.doi.org/10.1046/j.1365-2486.1999.00228.x.

Hendrey, GR; Kimball, BA. (1994). The FACE program. Agr Forest Meteorol 70: 3-14. http://dx.doi.org/10.1016/0168-1923(94)90044-2.

Hildebrand, E; Skelly, JM; Fredericksen, TS. (1996). Foliar response of ozone-sensitive hardwood tree species from 1991 to 1993 in the Shenandoah National Park, Virginia. Can J For Res 26: 658-669. http://dx.doi.org/10.1139/x26-076.

Hillstrom, ML; Couture, JJ; Lindroth, RL. (2014). Elevated carbon dioxide and ozone have weak, idiosyncratic effects on herbivorous forest insect abundance, species richness, and community composition. Insect Conservation and Diversity 7: 553-562. http://dx.doi.org/10.1111/icad.12078.

Hillstrom, ML; Lindroth, RL. (2008). Elevated atmospheric carbon dioxide and ozone alter forest insect abundance and community composition. Insect Conservation and Diversity 1: 233-241. http://dx.doi.org/10.1111/j.1752-4598.2008.00031.x.

Hofmockel, KS; Zak, DR; Moran, KK; Jastrow, JD. (2011). Changes in forest soil organic matter pools after a decade of elevated CO2 and O3. Soil Biol Biochem 43: 1518-1527. http://dx.doi.org/10.1016/j.soilbio.2011.03.030.

Hogg, A; Uddling, J; Ellsworth, D; Carroll, MA; Pressley, S; Lamb, B; Vogel, C. (2007). Stomatal and non-stomatal fluxes of ozone to a northern mixed hardwood forest. Tellus B Chem Phys Meteorol 59: 514-525. http://dx.doi.org/10.1111/j.1600-0889.2007.00269.x.

Hogsett, WE; Olszyk, D; Ormrod, DP; Taylor, GE, Jr; Tingey, DT. (1987a). Air pollution exposure systems and experimental protocols: Volume 2: Description of facilities. (EPA/600/3-87/037b). Corvallis, OR: U.S. Environmental Protection Agency, Environmental Research Laboratory. http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=30000KQH.txt.

Hogsett, WE; Olszyk, D; Ormrod, DP; Taylor, GE, Jr; Tingey, DT. (1987b). Air pollution exposure systems and experimental protocols: Volume I: A review and evaluation of performance. (EPA/600/3-87/037a). Corvallis, OR: U.S. Environmental Protection Agency.

Hogsett, WE; Tingey, DT; Holman, SR. (1985). A programmable exposure control system for determination of the effects of pollutant exposure regimes on plant growth. Atmos Environ 19: 1135-1145. http://dx.doi.org/10.1016/0004-6981(85)90198-2.

Holmes, CD. (2014). Air pollution and forest water use [Comment]. Nature 507: E1-E2. http://dx.doi.org/10.1038/nature13113.

Holmes, WE; Zak, DR; Pregitzer, KS; King, JS. (2006). Elevated CO2 and O3 alter soil nitrogen transformations beneath trembling aspen, paper birch, and sugar maple. Ecosystems 9: 1354-1363. http://dx.doi.org/10.1007/s10021-006-0163-5.

Hong, B; Weinstein, D; Swaney, D. (2006). Assessment of ozone effects on nitrate export from Hubbard Brook Watershed 6. Environ Pollut 141: 8-21. http://dx.doi.org/10.1016/j.envpol.2005.08.030.

Hong, Y; Yi, T; Tan, X; Zhao, Z; Ge, F. (2016). High ozone (O3) affects the fitness associated with the microbial composition and abundance of Q biotype Bemisia tabaci. FMICB 7: 1593. http://dx.doi.org/10.3389/fmicb.2016.01593.

Horn, KJ; Thomas, RQ; Clark, CM; Pardo, LH; Fenn, ME; Lawrence, GB; Perakis, SS; Smithwick, EAH; Baldwin, D; Braun, S; Nordin, A; Perry, CH; Phelan, JN; Schaberg, PG; St Clair, SB; Warby, R; Watmough, S. (2018). Growth and survival relationships of 71 tree species with nitrogen and sulfur deposition across the conterminous. PLoS ONE 13: Article #e0205296. http://dx.doi.org/10.1371/journal.pone.0205296.

Hoshika, Y; Carriero, G; Feng, Z; Zhang, Y; Paoletti, E. (2014). Determinants of stomatal sluggishness in ozone-exposed deciduous tree species. Sci Total Environ 481: 453-458. http://dx.doi.org/10.1016/j.scitotenv.2014.02.080.

Hoshika, Y; De Marco, A; Materassi, A; Paoletti, E. (2016). Light intensity affects ozone-induced stomatal sluggishness in snapbean. Water Air Soil Pollut 227. http://dx.doi.org/10.1007/s11270-016-3127-1.

Hoshika, Y; Katata, G; Deushi, M; Watanabe, M; Koike, T; Paoletti, E. (2015). Ozone-induced stomatal sluggishness changes carbon and water balance of temperate deciduous forests. Sci Rep 5: 9871. http://dx.doi.org/10.1038/srep09871.

Hoshika, Y; Omasa, K; Paoletti, E. (2011). Both ozone exposure and soil water stress are able to induce stomatal sluggishness. Environ Exp Bot 88: 19-23. http://dx.doi.org/10.1016/j.envexpbot.2011.12.004.

Hoshika, Y; Watanabe, M; Carrari, E; Paoletti, E; Koike, T. (2018). Ozone-induced stomatal sluggishness changes stomatal parameters of Jarvis-type model in white birch and deciduous oak. Plant Biol (Stuttg) 20: 20-28. http://dx.doi.org/10.1111/plb.12632.

Hoshika, Y; Watanabe, M; Inada, N; Koike, T. (2012a). Modeling of stomatal conductance for estimating ozone uptake of Fagus crenata under experimentally enhanced free-air ozone exposure. Water Air Soil Pollut 223: 3893-3901. http://dx.doi.org/10.1007/s11270-012-1158-9.

Hoshika, Y; Watanabe, M; Inada, N; Koike, T. (2012b). Ozone-induced stomatal sluggishness develops progressively in Siebold's beech (Fagus crenata). Environ Pollut 166: 152-156. http://dx.doi.org/10.1016/j.envpol.2012.03.013.

Huang, YZ; Zhong, M. (2015). Influence of elevated ozone concentration on methanotrophic bacterial communities in soil under field condition. Atmos Environ 108: 59-66. http://dx.doi.org/10.1016/j.atmosenv.2015.02.075.

Hunova, I; Matouskova, L; Srnensky, R; Kozelkova, K. (2011). Ozone influence on native vegetation in the Jizerske hory Mts. of the Czech Republic: Results based on ozone exposure and ozone-induced visible symptoms. Environ Monit Assess 183: 501-515. http://dx.doi.org/10.1007/s10661-011-1935-8.

Iriti, M; Faoro, F. (2009). Chemical diversity and defence metabolism: How plants cope with pathogens and ozone pollution [Review]. International Journal of Molecular Sciences 10: 3371-3399. http://dx.doi.org/10.3390/ijms10083371.

Jackson, DM; Heagle, AS; Eckel, RVW. (1999). Ovipositional response of tobacco hornworm moths (Lepidoptera: Sphyngidae) to tobacco plants grown under elevated levels of ozone. Environ Entomol 28: 566-571. http://dx.doi.org/10.1093/ee/28.4.566.

Jackson, DM; Rufty, TW; Heagle, AS; Severson, RF; Eckel, RWV. (2000). Survival and development of tobacco hornworm larvae on tobacco plants grown under elevated levels of ozone. J Chem Ecol 26: 1-18.

Jacob, DJ; Winner, DA. (2009). Effect of climate change on air quality. Atmos Environ 43: 51-63. http://dx.doi.org/10.1016/j.atmosenv.2008.09.051.

Jamieson, MA; Burkle, LA; Manson, JS; Runyon, JB; Trowbridge, AM; Zientek, J. (2017). Global change effects on plant-insect interactions: the role of phytochemistry [Review]. Curr Opin Insect Sci 23: 70-80. http://dx.doi.org/10.1016/j.cois.2017.07.009.

Jones, ME; Paine, TD. (2006). Detecting changes in insect herbivore communities along a pollution gradient. Environ Pollut 143: 377-387. http://dx.doi.org/10.1016/j.envpol.2005.12.013.

Jones, MLM; Hodges, G; Mills, G. (2010). Nitrogen mediates above-ground effects of ozone but not below-ground effects in a rhizomatous sedge. Environ Pollut 158: 559-565. http://dx.doi.org/10.1016/j.envpol.2009.08.002.

Jones, TG; Freeman, C; Lloyd, A; Mills, G. (2009). Impacts of elevated atmospheric ozone on peatland below-ground doc characteristics. Ecol Eng 35: 971-977. http://dx.doi.org/10.1016/j.ecoleng.2008.08.009.

Juergens, A; Bischoff, M. (2017). Changing odour landscapes: the effect of anthropogenic volatile pollutants on plant-pollinator olfactory communication. Funct Ecol 31: 56-64. http://dx.doi.org/10.1111/1365-2435.12774.

Kanerva, T; Palojarvi, A; Ramo, K; Manninen, S. (2008). Changes in soil microbial community structure under elevated tropospheric O3 and CO2. Soil Biol Biochem 40: 2502-2510. http://dx.doi.org/10.1016/j.soilbio.2008.06.007.

Kanerva, T; Palojarvi, A; Ramo, K; Ojanpera, K; Esala, M; Manninen, S. (2006). A 3-year exposure to CO2 and O3 induced minor changes in soil N cycling in a meadow ecosystem. Plant Soil 286: 61-73. http://dx.doi.org/10.1007/s11104-006-9026-2.

Kanerva, T; Regina, K; Ramo, K; Ojanpera, K; Manninen, S. (2007). Fluxes of N2O, CH4 and CO2 in a meadow ecosystem exposed to elevated ozone and carbon dioxide for three years. Environ Pollut 145: 818-828. http://dx.doi.org/10.1016/j.envpol.2006.03.055.

Karnosky, DF; Gagnon, ZE; Dickson, RE; Coleman, MD; Lee, EH; Isebrands, JG. (1996). Changes in growth, leaf abscission, biomass associated with seasonal tropospheric ozone exposures of Populus tremuloides clones and seedlings. Can J For Res 26: 23-37.

Karnosky, DF; Mankovska, B; Percy, K; Dickson, RE; Podila, GK; Sober, J; Noormets, A; Hendrey, G; Coleman, MD; Kubiske, M; Pregitzer, KS; Isebrands, JG. (1999). Effects of tropospheric ozone on trembling aspen and interaction with CO2: Results from an O3-gradient and a FACE experiment. Water Air Soil Pollut 116: 311-322. http://dx.doi.org/10.1023/A:1005276824459.

Kasurinen, A; Biasi, C; Holopainen, T; Rousi, M; Maenpaa, M; Oksanen, E. (2012). Interactive effects of elevated ozone and temperature on carbon allocation of silver birch (Betula pendula) genotypes in an open-air field exposure. Tree Physiol 32: 737-751. http://dx.doi.org/10.1093/treephys/tps005.

Kasurinen, A; Keinanen, MM; Kaipainen, S; Nilsson, LO; Vapaavuori, E; Kontro, MH; Holopainen, T. (2005). Below-ground responses of silver birch trees exposed to elevated CO2 and O3 levels during three growing seasons. Global Change Biol 11: 1167-1179. http://dx.doi.org/10.1111/j.1365-2486.2005.00970.x.

Kasurinen, A; Riikonen, J; Oksanen, E; Vapaavuori, E; Holopainen, T. (2006). Chemical composition and decomposition of silver birch leaf litter produced under elevated CO2 and O3. Plant Soil 282: 261-280. http://dx.doi.org/10.1007/s11104-005-6026-6.

Kasurinen, A; Silfver, T; Rousi, M; Mikola, J. (2017). Warming and ozone exposure effects on silver birch (Betula pendula Roth) leaf litter quality, microbial growth and decomposition. Plant Soil 414: 127-142. http://dx.doi.org/10.1007/s11104-016-3122-8.

Kats, G; Olszyk, DM; Thompson, CR. (1985). Open top experimental chambers for trees. J Air Waste Manag Assoc 35: 1298-1301. http://dx.doi.org/10.1080/00022470.1985.10466033.

Kats, G; Thompson, CR; Kuby, WC. (1976). Improved ventilation of open top greenhouses. J Air Pollut Control Assoc 26: 1089-1090. http://dx.doi.org/10.1080/00022470.1976.10470367.

Kefauver, SC; Penuelas, J; Ustin, S. (2013). Using topographic and remotely sensed variables to assess ozone injury to conifers in the Sierra Nevada (USA) and Catalonia (Spain). Rem Sens Environ 139: 138-148. http://dx.doi.org/10.1016/j.rse.2013.07.037.

Kefauver, SC; Penuelas, J; Ustin, SL. (2012a). Improving assessments of tropospheric ozone injury to Mediterranean montane conifer forests in California (USA) and Catalonia (Spain) with GIS models related to plant water relations. Atmos Environ 62: 41-49. http://dx.doi.org/10.1016/j.atmosenv.2012.08.013.

Kefauver, SC; Peñuelas, J; Ustin, SL. (2012b). Applications of hyperspectral remote sensing and GIS for assessing forest health and air pollution. In 2012 IEEE International Geoscience and Remote Sensing Symposium (IGARSS). Piscataway, NJ: IEEE. http://dx.doi.org/10.1109/IGARSS.2012.6350696.

Keller, T; Häsler, R. (1984). The influence of a fall fumigation with ozone on the stomatal behavior of spruce and fir. Oecologia 64: 284-286. http://dx.doi.org/10.1007/BF00376884.

Kelting, DL; Burger, JA; Edwards, GS. (1995). The effects of ozone on the root dynamics of seedlings and mature red oak (Quercus rubra L). For Ecol Manage 79: 197-206. http://dx.doi.org/10.1016/0378-1127(95)03603-2.

Khaling, E; Li, T, ao; Holopainen, JK; Blande, JD. (2016). Elevated Ozone Modulates Herbivore-Induced Volatile Emissions of Brassica nigra and Alters a Tritrophic Interaction. J Chem Ecol 42: 368-381. http://dx.doi.org/10.1007/s10886-016-0697-8.

Khaling, E; Papazian, S; Poelman, EH; Holopainen, JK; Albrectsen, BR; Blande, JD. (2015). Ozone affects growth and development of Pieris brassicae on the wild host plant Brassica nigra. Environ Pollut 199: 119-129. http://dx.doi.org/10.1016/j.envpol.2015.01.019.

King, JS; Kubiske, ME; Pregitzer, KS; Hendrey, GR; Mcdonald, EP; Giardina, CP; Quinn, VS; Karnosky, DF. (2005). Tropospheric O3 compromises net primary production in young stands of trembling aspen, paper birch and sugar maple in response to elevated atmospheric CO2. New Phytol 168: 623-635. http://dx.doi.org/10.1111/j.1469-8137.2005.01557.x.

King, JS; Pregitzer, KS; Zak, DR; Sober, J; Isebrands, JG; Dickson, RE; Hendrey, GR; Karnosky, DF. (2001). Fine-root biomass and fluxes of soil carbon in young stands of paper birch and trembling aspen as affected by elevated atmospheric CO2 and tropospheric O3. Oecologia 128: 237-250.

Kline, LJ; Davis, DD; Skelly, JM; Decoteau, DR. (2009). Variation in ozone sensitivity within Indian hemp and common milkweed selections from the Midwest. Northeast Nat 16: 307-313. http://dx.doi.org/10.1656/045.016.0210.

Kline, LJ; Davis, DD; Skelly, JM; Savage, JE; Ferdinand, J. (2008). Ozone sensitivity of 28 plant selections exposed to ozone under controlled conditions. Northeast Nat 15: 57-66. http://dx.doi.org/10.1656/1092-6194(2008)15[57:OSOPSE]2.0.CO;2.

Koerner, C. (2006). Plant CO2 responses: an issue of definition, time and resource supply. New Phytol 172: 393-411. http://dx.doi.org/10.1111/j.1469-8137.2006.01886.x.

Kohut, R. (2007). Assessing the risk of foliar injury from ozone on vegetation in parks in the US National Park Service's Vital Signs Network. Environ Pollut 149: 348-357. http://dx.doi.org/10.1016/j.envpol.2007.04.022.

Kohut, R; Flanagan, C; Cheatham, J; Porter, E. (2012). Foliar ozone injury on cutleaf coneflower at Rocky Mountain National Park, Colorado. West N Am Nat 72: 32-42. http://dx.doi.org/10.3398/064.072.0104.

Kohut, RJ; Laurence, JA; Amundson, RG. (1988). Effects of ozone and sulfur dioxide on yield of red clover and timothy. J Environ Qual 17: 580-585. http://dx.doi.org/10.2134/jeq1988.00472425001700040010x.

Kostiainen, K; Saranpaa, P; Lundqvist, SO; Kubiske, ME; Vapaavuori, E. (2014). Wood properties of Populus and Betula in long-term exposure to elevated CO2 and O-3. Plant Cell Environ 37: 1452-1463. http://dx.doi.org/10.1111/pce.12261.

Kozovits, AR; Matyssek, R; Blaschke, H; Gottlein, A; Grams, TEE. (2005). Competition increasingly dominates the responsiveness of juvenile beech and spruce to elevated CO2 and/or O3 concentrations throughout two subsequent growing seasons. Global Change Biol 11: 1387-1401. http://dx.doi.org/10.1111/j.1365-2486.2005.00993.x.

Kress, LW; Skelly, JM. (1982). Response of several eastern forest tree species to chronic doses of ozone and nitrogen dioxide. Plant Dis 66: 1149-1152. http://dx.doi.org/10.1094/PD-66-1149.

Krupa, SV; Grunhage, L; Jager, HJ; Nosal, M; Manning, WJ; Legge, AH; Hanewald, K. (1995). Ambient ozone (O3) and adverse crop response: A unified view of cause and effect. Environ Pollut 87: 119-126. http://dx.doi.org/10.1016/S0269-7491(99)80014-1.

Kubiske, ME; Foss, AR. (2015). Carbon dioxide and ozone data from the Aspen FACE Experiment, 1998-2009, and Phase II Experiment, 2010 [Database]. Fort Collins, CO: Forest Service Research Data Archive. Retrieved from https://www.fs.usda.gov/rds/archive/Product/RDS-2015-0001

Kubiske, ME; Quinn, VS; Marquardt, PE; Karnosky, DF. (2007). Effects of elevated atmospheric CO2 and/or O3 on intra- and interspecific competitive ability of aspen. Plant Biol (Stuttg) 9: 342-355. http://dx.doi.org/10.1055/s-2006-924760.

Kvalevåg, MM; Myhre, G. (2013). The effect of carbon-nitrogen coupling on the reduced land carbon sink caused by tropospheric ozone. Geophys Res Lett 40: 3227-3231. http://dx.doi.org/10.1002/grl.50572.

Landesmann, JB; Gundel, PE; Martínez-Ghersa, MA; Ghersa, CM. (2013). Ozone exposure of a weed community produces adaptive changes in seed populations of Spergula arvensis. PLoS ONE 8: e75820. http://dx.doi.org/10.1371/journal.pone.0075820.

Lapina, K; Henze, DK; Milford, JB; Travis, K. (2016). Impacts of Foreign, Domestic, and State-Level Emissions on Ozone-Induced Vegetation Loss in the United States. Environ Sci Technol 50: 806-813. http://dx.doi.org/10.1021/acs.est.5b04887.

Laurence, JA; Amundson, RG; Kohut, RJ; Weinstein, DA. (1997). Growth and water use of red spruce (Picea rubens Sarg) exposed to ozone and simulated acidic precipitation for four growing seasons. Forest Sci 43: 355-361.

Laurence, JA; Kohut, RJ; Amundson, RG; Weinstein, DA; MacLean, DC. (1996). Response of sugar maple to multiple year exposure to ozone and simulated acidic precipitation. Environ Pollut 92: 119-126. http://dx.doi.org/10.1016/0269-7491(95)00105-0.

Lee, EH; Hogsett, WE. (1996). Methodology for calculating inputs for ozone secondary standard benefits anaylsis: Part II. Research Triangle Park, NC: U.S. Environmental Protection Agency.

Lee, EH; Tingey, DT; Waschmann, RS; Phillips, DL; Olszyk, DM; Johnson, MG; Hogsett, WE. (2009). Seasonal and long-term effects of CO2 and O3 on water loss in ponderosa pine and their interaction with climate and soil moisture. Tree Physiol 29: 1381-1393. http://dx.doi.org/10.1093/treephys/tpp071.

Lefohn, AS; Laurence, JA; Kohut, RJ. (1988). A comparison of indices that describe the relationship between exposure to ozone and reduction in the yield of agricultural crops. Atmos Environ 22: 1229-1240. http://dx.doi.org/10.1016/0004-6981(88)90353-8.

Lefohn, AS; Runeckles, VC. (1987). Establishing standards to protect vegetation - ozone exposure/dose considerations. Atmos Environ 21: 561-568. http://dx.doi.org/10.1016/0004-6981(87)90038-2.

Legge, AH; Grunhage, L; Nosal, M; Jager, HJ; Krupa, SV. (1995). Ambient ozone and adverse crop response: An evaluation of North American and European data as they relate to exposure indices and critical levels. J Appl Bot Food Qual 69: 192-205.

Leisner, CP; Ainsworth, EA. (2012). Quantifying the effects of ozone on plant reproductive growth and development. Global Change Biol 18: 606-616. http://dx.doi.org/10.1111/j.1365-2486.2011.02535.x.

Leisner, CP; Yendrek, CR; Ainsworth, EA. (2017). Physiological and transcriptomic responses in the seed coat of field-grown soybean (Glycine max L. Merr.) to abiotic stress. BMC Plant Biol 17: 242. http://dx.doi.org/10.1186/s12870-017-1188-y.

Lewis, JS; Ditchkoff, SS; Lin, JC; Muntifering, RB; Chappelka, AH. (2006). Nutritive quality of big bluestem (Andropogon gerardii) and eastern gamagrass (Tripsacum dactyloides) exposed to tropospheric ozone. Rangeland Ecol Manag 59: 267-274. http://dx.doi.org/10.2111/05-025R2.1.

Lewis, SL; Maslin, MA. (2015). Defining the anthropocene. Nature 519: 171-180. http://dx.doi.org/10.1038/nature14258.

Li, CH; Wang, TZ; Li, Y; Zheng, YH; Jiang, GM. (2013a). Flixweed is more competitive than winter wheat under ozone pollution: evidences from membrane lipid peroxidation, antioxidant enzymes and biomass. PLoS ONE 8: e60109. http://dx.doi.org/10.1371/journal.pone.0060109.

Li, L; Manning, WJ; Tong, L; Wang, X. (2015). Chronic drought stress reduced but not protected Shantung maple (Acer truncatum Bunge) from adverse effects of ozone (O3) on growth and physiology in the suburb of Beijing, China. Environ Pollut 201: 34-41. http://dx.doi.org/10.1016/j.envpol.2015.02.023.

Li, Q; Yang, Y; Bao, X; Zhu, J; Liang, W; Bezemer, TM. (2016a). Cultivar specific plant-soil feedback overrules soil legacy effects of elevated ozone in a rice-wheat rotation system. Agric Ecosyst Environ 232: 85-92. http://dx.doi.org/10.1016/j.agee.2016.07.025.

Li, T; Blande, JD. (2015). Associational susceptibility in broccoli: mediated by plant volatiles, impeded by ozone. Global Change Biol 21: 1993-2004. http://dx.doi.org/10.1111/gcb.12835.

Li, T; Blande, JD; Holopainen, JK. (2016b). Atmospheric transformation of plant volatiles disrupts host plant finding. Sci Rep 6: 33851. http://dx.doi.org/10.1038/srep33851.

Li, X; Deng, Y; Li, Q; Lu, C; Wang, J; Zhang, H; Zhu, J; Zhou, J; He, Z. (2013b). Shifts of functional gene representation in wheat rhizosphere microbial communities under elevated ozone. ISME J 7: 660-671. http://dx.doi.org/10.1038/ismej.2012.120.

Lindroth, RL; Reich, PB; Tjoelker, MG; Volin, JC; Oleksyn, J. (1993). Light environment alters response to ozone stress in seedlings of Acer saccharum March, and hybrid Populus L III Consequences for performance of gypsy moth. New Phytol 124: 647-654. http://dx.doi.org/10.1111/j.1469-8137.1993.tb03854.x.

Liu, L; King, JS; Giardina, CP. (2007). Effects of elevated atmospheric CO2 and tropospheric O3 on nutrient dynamics: Decomposition of leaf litter in trembling aspen and paper birch communities. Plant Soil 299: 65-82. http://dx.doi.org/10.1007/s11104-007-9361-y.

Lloyd, KL; Davis, DD; Marini, RP; Decoteau, DR. (2018). Effects of nighttime ozone treatment at ambient concentrations on sensitive and resistant snap bean genotypes. J Am Soc Hortic Sci 143: 23-33. http://dx.doi.org/10.21273/JASHS04253-17.

Loats, KV; Rebbeck, J. (1999). Interactive effects of ozone and elevated carbon dioxide on the growth and physiology of black cherry, green ash, and yellow poplar seedlings. Environ Pollut 106: 237-248. http://dx.doi.org/10.1016/S0269-7491(99)00069-X.

Lombardozzi, D; Levis, S; Bonan, G; Hess, PG; Sparks, JP. (2015). The Influence of Chronic Ozone Exposure on Global Carbon and Water Cycles. J Clim 28: 292-305. http://dx.doi.org/10.1175/JCLI-D-14-00223.1.

Lorenz, M; Becher, G; Mues, V; Fischer, R; Becker, R; Catalayud, V; Dise, N; Krause, GHM; Sanz, M; Ulrich, E. (2005). Forest Condition in Europe, 2005 Technical Report of IOCP Forests. Hamburg, Germany: Federal Research Centre for Forestry and Forest Products (BFH) and Department of Wood Science University of Hamburg.

Lucas, PW; Cottam, DA; Sheppard, LJ; Francis, BJ. (1988). Growth responses and delayed winter hardening in Sitka spruce following summer exposure to ozone. New Phytol 108: 495-504. http://dx.doi.org/10.1111/j.1469-8137.1988.tb04192.x.

Lucas, PW; Rantanen, L; Mehlhorn, H. (1993). Needle chlorosis in Sitka spruce following a three-year exposure to low concentrations of ozone: changes in mineral content, pigmentation and ascorbic acid. New Phytol 124: 265-275. http://dx.doi.org/10.1111/j.1469-8137.1993.tb03816.x.

Mahmud, A; Tyree, M; Cayan, D; Motallebi, N; Kleeman, MJ. (2008). Statistical downscaling of climate change impacts on ozone concentrations in California. J Geophys Res 113: D21103. http://dx.doi.org/10.1029/2007JD009534.

Mandl, RH; Laurence, JA; Kohut, RJ. (1989). Development and testing of open-top chambers for exposing large, perennial plants to air pollutants. J Environ Qual 18: 534-540. http://dx.doi.org/10.2134/jeq1989.00472425001800040026x.

Mandl, RH; Weinstein, LH; McCune, DC; Keveny, M. (1973). A cylindrical, open-top chamber for the exposure of plants to air pollutants in the field. J Environ Qual 2: 371-376.

Maňkovská, B; Percy, KE; Karnosky, DF. (2005). Impacts of greenhouse gases on epicuticular waxes of Populus tremuloides Michx.: Results from an open-air exposure and a natural O3 gradient. Environ Pollut 137: 580-586. http://dx.doi.org/10.1016/j.envpol.2005.01.043.

Manninen, AM; Holopainen, T; Lyytikainen-Saarenmaa, P; Holopainen, JK. (2000). The role of low-level ozone exposure and mycorrhizas in chemical quality and insect herbivore performance on Scots pine seedlings. Global Change Biol 6: 111-121. http://dx.doi.org/10.1046/j.1365-2486.2000.00290.x.

Manning, WJ; Godzik, B. (2004). Bioindicator plants for ambient ozone in Central and Eastern Europe. Environ Pollut 130: 33-39. http://dx.doi.org/10.1016/j.envpol.2003.10.033.

Manning, WJ; Godzik, B; Musselman, R. (2002). Potential bioindicator plant species for ambient ozone in forested mountain areas of central Europe. Environ Pollut 119: 283-290. http://dx.doi.org/10.1016/S0269-7491(02)00102-1.

Manning, WJ; Krupa, SV. (1992). Experimental methodology for studying the effects of ozone on crops and trees. In AS Lefohn (Ed.), Surface level ozone exposures and their effects on vegetation (pp. 93-156). Chelsea, MI: Lewis Publishers.

Martínez-Ghersa, MA; Menéndez, AI; Gundel, PE; Folcia, AM; Romero, AM; Landesmann, JB; Ventura, L; Ghersa, CM. (2017). Legacy of historic ozone exposure on plant community and food web structure. PLoS ONE 12: e0182796. http://dx.doi.org/10.1371/journal.pone.0182796.

Matyssek, R; Le Thiec, D; Low, M; Dizengremel, P; Nunn, AJ; Haberle, KH. (2006). Interactions between drought and O3 stress in forest trees [Review]. Plant Biol (Stuttg) 8: 11-17. http://dx.doi.org/10.1055/s-2005-873025.

Matyssek, R; Sandermann, H; Wieser, G; Booker, F; Cieslik, S; Musselman, R; Ernst, D. (2008). The challenge of making ozone risk assessment for forest trees more mechanistic [Review]. Environ Pollut 156: 567-582. http://dx.doi.org/10.1016/j.envpol.2008.04.017.

Matyssek, R; Wieser, G; Ceulemans, R; Rennenberg, H; Pretzsch, H; Haberer, K; Low, M; Nunn, AJ; Werner, H; Wipfler, P; Obwald, W; Nikolova, P; Hanke, DE; Kraigher, H; Tausz, M; Bahnweg, G; Kitao, M; Dieler, J; Sandermann, H; Herbinger, K; Grebenc, T; Blumenrother, M; Deckmyn, G; Grams, TEE; Heerdt, C; Leuchner, M; Fabian, P; Haberle, KH. (2010). Enhanced ozone strongly reduces carbon sink strength of adult beech (Fagus sylvatica): Resume from the free-air fumigation study at Kranzberg Forest. Environ Pollut 158: 2527-2532. http://dx.doi.org/10.1016/j.envpol.2010.05.009.

Mavity, E; Berrang, P. (1993). Effects of ozone on Solidago albopilosa, and endangered species of goldenrod from central Kentucky. Bull Ecol Soc Am 75: 146.

McBride, JR; Laven, RD. (1999). Impact of oxidant air pollutants on forest succession in the mixed conifer forests of the San Bernardino Mountains. In PR Miller; JR McBride (Eds.), Oxidant air pollution impacts in the montane forests of southern California: A case study of the San Bernardino Mountains (pp. 338-352). New York, NY: Springer. http://dx.doi.org/10.1007/978-1-4612-1436-6_16.

Mcfrederick, QS; Kathilankal, JC; Fuentes, JD. (2008). Air pollution modifies floral scent trails. Atmos Environ 42: 2336-2348. http://dx.doi.org/10.1016/j.atmosenv.2007.12.033.

Mcgrath, JM; Betzelberger, AM; Wang, S; Shook, E; Zhu, XG; Long, SP; Ainsworth, EA. (2015). An analysis of ozone damage to historical maize and soybean yields in the United States. Proc Natl Acad Sci USA 112: 14390-14395. http://dx.doi.org/10.1073/pnas.1509777112.

Mclaughlin, SB; Nosal, M; Wullschleger, SD; Sun, G. (2007a). Interactive effects of ozone and climate on tree growth and water use in a southern Appalachian forest in the USA. New Phytol 174: 109-124. http://dx.doi.org/10.1111/j.1469-8137.2007.02018.x.

Mclaughlin, SB; Wullschleger, SD; Sun, G; Nosal, M. (2007b). Interactive effects of ozone and climate on water use, soil moisture content and streamflow in a southern Appalachian forest in the USA. New Phytol 174: 125-136. http://dx.doi.org/10.1111/j.1469-8137.2007.01970.x.

Meehan, TD; Couture, JJ; Bennett, AE; Lindroth, RL. (2014). Herbivore-mediated material fluxes in a northern deciduous forest under elevated carbon dioxide and ozone concentrations. New Phytol 204: 397-407. http://dx.doi.org/10.1111/nph.12947.

Menendez, AI; Gundel, PE; Lores, LM; Alejandra Martinez-Ghersa, M. (2017). Assessing the impacts of intra- and interspecific competition between Triticum aestivum and Trifolium repens on the species' responses to ozone. Botany 95: 923-932. http://dx.doi.org/10.1139/cjb-2016-0275.

Menendez, AI; Romero, AM; Folcia, AM; Martinez-Ghersa, MA. (2009). Getting the interactions right: Will higher O3 levels interfere with induced defenses to aphid feeding? Basic Appl Ecol 10: 255-264. http://dx.doi.org/10.1016/j.baae.2008.03.010.

Menendez, AI; Romero, AM; Folcia, AM; Martinez-Ghersa, MA. (2010). Aphid and episodic O3 injury in arugula plants (Eruca sativa Mill) grown in open-top field chambers. Agric Ecosyst Environ 135: 10-14. http://dx.doi.org/10.1016/j.agee.2009.08.005.

Miglietta, F; Peressotti, A; Vaccari, FP; Zaldei, A; Deangelis, P; Scarascia-Mugnozza, G. (2001). Free-air CO2 enrichment (FACE) of a poplar plantation: the POPFACE fumigation system. New Phytol 150: 465-476. http://dx.doi.org/10.1046/j.1469-8137.2001.00115.x.

Miller, P; Guthrey, R; Schilling, S; Carroll, J. (1998). Ozone injury responses of ponderosa and Jeffrey pine in the Sierra Nevada and San Bernardino Mountains in California. General Technical Report - Pacific Southwest Research Station. U.S. Department of Agriculture, Forest Service :: USDA.

Miller, PL. (1973). Oxidant-induced community change in a mixed conifer forest. In JA Naegele (Ed.), Air pollution damage to vegetation (pp. 101-117). Washington, DC: American Chemical Society.

Mills, G; Harmens, H; Wagg, S; Sharps, K; Hayes, F; Fowler, D; Sutton, M; Davies, B. (2016). Ozone impacts on vegetation in a nitrogen enriched and changing climate. Environ Pollut 208: 898-908. http://dx.doi.org/10.1016/j.envpol.2015.09.038.

Mills, G; Hayes, F; Jones, MLM; Cinderby, S. (2007). Identifying ozone-sensitive communities of (semi-)natural vegetation suitable for mapping exceedance of critical levels. Environ Pollut 146: 736-743. http://dx.doi.org/10.1016/j.envpol.2006.04.005.

Mills, G; Pleijel, H; Braun, S; Büker, P; Bermejo, V; Calvo, E; Danielsson, H; Emberson, L; Fernández, IG; Grünhage, L; Harmens, H; Hayes, F; Karlsson, P, erE; Simpson, D. (2011). New stomatal flux-based critical levels for ozone effects on vegetation. Atmos Environ 45: 5064-5068. http://dx.doi.org/10.1016/j.atmosenv.2011.06.009.

Mills, G; Pleijel, H; Malley, CS; Sinha, B; Cooper, OR; Schultz, MG; Neufeld, HS; Simpson, D; Sharps, K; Feng, Z; Gerosa, G; Harmens, H; Kobayashi, K; Saxena, P; Paoletti, E; Sinha, V; Xu, X. (2018). Tropospheric Ozone Assessment Report: Present-day tropospheric ozone distribution and trends relevant to vegetation. Elementa: Science of the Anthropocene 6. http://dx.doi.org/10.1525/elementa.302.

Mills, G; Wagg, S; Harmens, H. (2013). Ozone pollution: Impacts on ecosystem services and biodiversity. Bangor, UK: NERC/Centre for Ecology & Hydrology. http://nora.nerc.ac.uk/id/eprint/502675/.

Mina, U; Fuloria, A; Aggarwal, R. (2016). Effect of ozone and antioxidants on wheat and its pathogen - Bipolaris sorokininana. Cereal Research Communications 44: 594-604. http://dx.doi.org/10.1556/0806.44.2016.039.

Mofikoya, AO; Kim, TH; Abd El-Raheem, AM; Blande, JD; Kivimäenpää, M; Holopainen, JK. (2017). Passive adsorption of volatile monoterpene in pest control: Aided by proximity and disrupted by ozone. J Agric Food Chem 65: 9579-9586. http://dx.doi.org/10.1021/acs.jafc.7b03251.

Mondor, EB; Tremblay, MN; Awmack, CS; Lindroth, RL. (2005). Altered genotypic and phenotypic frequencies of aphid populations under enriched CO2 and O3 atmospheres. Global Change Biol 11: 1990-1996. http://dx.doi.org/10.1111/j.1365-2486.2005.01054.x.

Moran, EV; Kubiske, ME. (2013). Can elevated CO2 and ozone shift the genetic composition of aspen (Populus tremuloides) stands? New Phytol 198: 466-475. http://dx.doi.org/10.1111/nph.12153.

Morgan, PB; Ainsworth, EA; Long, SP. (2003). How does elevated ozone impact soybean? A meta-analysis of photosynthesis, growth and yield. Plant Cell Environ 26: 1317-1328. http://dx.doi.org/10.1046/j.0016-8025.2003.01056.x.

Morgan, PB; Bernacchi, CJ; Ort, DR; Long, SP. (2004). An in vivo analysis of the effect of season-long open-air elevation of ozone to anticipated 2050 levels on photosynthesis in soybean. J Plant Physiol 135: 2348-2357. http://dx.doi.org/10.1104/pp.104.043968.

Morgan, PB; Mies, TA; Bollero, GA; Nelson, RL; Long, SP. (2006). Season-long elevation of ozone concentration to projected 2050 levels under fully open-air conditions substantially decreases the growth and production of soybean. New Phytol 170: 333-343. http://dx.doi.org/10.1111/j.1469-8137.2006.01679.x.

Morsky, SK; Haapala, JK; Rinnan, R; Tiiva, P; Saarnio, S; Silvola, J; Holopainen, T; Martikainen, PJ. (2008). Long-term ozone effects on vegetation, microbial community and methane dynamics of boreal peatland microcosms in open-field conditions. Global Change Biol 14: 1891-1903. http://dx.doi.org/10.1111/j.1365-2486.2008.01615.x.

Mortensen, L. (1994a). The influence of carbon dioxide or ozone concentration on growth and assimilate partitioning in seedlings of nine conifers. Acta Agric Scand B Soil Plant Sci 44: 157-163. http://dx.doi.org/10.1080/09064719409410239.

Mortensen, LM. (1992). Effects of ozone on growth of seven grass and one clover species. Acta Agric Scand B Soil Plant Sci 42: 235-239. http://dx.doi.org/10.1080/09064719209410218.

Mortensen, LM. (1993). Effects of ozone on growth of several subalpine plant species. Norweg J Agr Sci 7: 129-138.

Mortensen, LM. (1994b). Further studies on the effects of ozone concentration on growth of subalpine plant species. Norweg J Agr Sci 8: 91-97.

Mortensen, LM; Nilsen, J. (1992). Effects of ozone and temperature on growth of several wild plant species. Norweg J Agr Sci 6: 195-204.

Mortensen, LM; Skre, O. (1990). Effects of low ozone concentrations on growth of Betula pubescens Ehrh, Betula verrucosa Ehrh and Alnus incana (L) Moench. New Phytol 115: 165-170. http://dx.doi.org/10.1111/j.1469-8137.1990.tb00934.x.

Muntifering, RB; Crosby, DD; Powell, MC; Chappelka, AH. (2000). Yield and quality characteristics of bahiagrass (Paspalum notatum) exposed to ground-level ozone. Anim Feed Sci Technol 84: 243-256. http://dx.doi.org/10.1016/S0377-8401(00)00124-3.

Musselman, RC; Massman, WJ. (1999). Ozone flux to vegetation and its relationship to plant response and ambient air quality standards. Atmos Environ 33: 65-73.

NAPCA (National Air Pollution Control Administration). (1970). Air quality criteria for photochemical oxidants [EPA Report]. (AP-63). Washington, DC: U.S. Department of Health, Education, and Welfare. http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=9100E2Z7.txt.

Neufeld, HS; Johnson, J; Kohut, R. (2018). Comparative ozone responses of cutleaf coneflowers (Rudbeckia laciniata var. digitata, var. ampla) from Rocky Mountain and Great Smoky Mountains National Parks, USA. Sci Total Environ 610-611: 591-601. http://dx.doi.org/10.1016/j.scitotenv.2017.08.046.

Neufeld, HS; Lee, EH; Renfro, JR; Hacker, WD. (2000). Seedling insensitivity to ozone for three conifer species native to Great Smoky Mountains National Park. Environ Pollut 108: 141-151. http://dx.doi.org/10.1016/S0269-7491(99)00247-X.

Neufeld, HS; Lee, EH; Renfro, JR; Hacker, WD; Yu, BH. (1995). Sensitivity of seedlings of black cherry (Prunus serotina Ehrh) to ozone in Great Smoky Mountains National Park I Exposure-response curves for biomass. New Phytol 130: 447-459. http://dx.doi.org/10.1111/j.1469-8137.1995.tb01839.x.

Noble, R; Jensen, KF; Ruff, BS; Loats, K. (1992). Response of Acer saccharum seedlings to elevated carbon dioxide and ozone. Ohio J Sci 92: 60-62.

Norby, RJ; Zak, DR. (2011). Ecological Lessons from Free-Air CO2 Enrichment (FACE) Experiments. Annu Rev Ecol Evol Systemat 42: 181-203. http://dx.doi.org/10.1146/annurev-ecolsys-102209-144647.

Novak, K; Skelly, JM; Schaub, M; Krauchi, N; Hug, C; Landolt, W; Bleuler, P. (2003). Ozone air pollution and foliar injury development on native plants of Switzerland. Environ Pollut 125: 41-52. http://dx.doi.org/10.1016/S0269-7491(03)00085-X.

Nunn, AJ; Reiter, IM; Häberle, KH; Werner, H; Langebartels, C; Sandermann, H; Heerdt, C; Fabian, P; Matyssek, R. (2002). "Free-air" ozone canopy fumigation in an old-growth mixed forest: Concept and observations in beech. Phyton-Annales Rei Botanicae 42: 105-119.

Oikawa, S; Ainsworth, EA. (2016). Changes in leaf area, nitrogen content and canopy photosynthesis in soybean exposed to an ozone concentration gradient. Environ Pollut 215: 347-355. http://dx.doi.org/10.1016/j.envpol.2016.05.005.

Oksanen, E. (2003). Responses of selected birch (Betula pendula Roth) clones to ozone change over time. Plant Cell Environ 26: 875-886. http://dx.doi.org/10.1046/j.1365-3040.2003.01020.x.

Orendovici, T; Skelly, JM; Ferdinand, JA; Savage, JE; Sanz, MJ; Smith, GC. (2003). Response of native plants of northeastern United States and southern Spain to ozone exposures; determining exposure/response relationships. Environ Pollut 125: 31-40. http://dx.doi.org/10.1016/S0269-7491(03)00089-7.

Ormrod, DP; Marie, BA; Allen, OB. (1988). Research approaches to pollutant crop loss functions. In WW Heck; OC Taylor; DT Tingey (Eds.), Assessment of crop loss from air pollutants: proceedings of an international conference; October 1987; Raleigh, NC (pp. 27-44). Essex, United Kingdom: Elsevier Science Publishers, Ltd.

Osborne, SA; Mills, G; Hayes, F; Ainsworth, EA; Büker, P; Emberson, L. (2016). Has the sensitivity of soybean cultivars to ozone pollution increased with time? An analysis of published dose-response data. Global Change Biol 22: 3097-3111. http://dx.doi.org/10.1111/gcb.13318.

Oshima, RJ; Braegelmann, PK; Baldwin, DW; Van Way, V; Taylor, OC. (1977). Reduction of tomato fruit size and yield by ozone. J Am Soc Hortic Sci 102: 289-293.

Oshima, RJ; Poe, MP; Braegelmann, PK; Baldwin, DW; Van Way, V. (1976). Ozone dosage-crop loss function for alfalfa: A standardized method for assessing crop losses from air pollutants. J Air Pollut Control Assoc 26: 861-865. http://dx.doi.org/10.1080/00022470.1976.10470330.

Ozolincius, R; Serafinaviciute, B. (2003). Ozone-induced visible foliar injuries in Lithuania. Baltic Forestry 9: 51-57.

Panek, JA. (2004). Ozone uptake, water loss and carbon exchange dynamics in annually drought-stressed Pinus ponderosa forests: Measured trends and parameters for uptake modeling. Tree Physiol 24: 277-290. http://dx.doi.org/10.1093/treephys/24.3.277.

Panek, JA; Goldstein, AH. (2001). Responses of stomatal conductance to drought in ponderosa pine: Implications for carbon and ozone uptake. Tree Physiol 21: 337-344. http://dx.doi.org/10.1093/treephys/21.5.337.

Paoletti, E; Grulke, NE. (2010). Ozone exposure and stomatal sluggishness in different plant physiognomic classes. Environ Pollut 158: 2664-2671. http://dx.doi.org/10.1016/j.envpol.2010.04.024.

Paoletti, E; Manning, WJ. (2007). Toward a biologically significant and usable standard for ozone that will also protect plants [Review]. Environ Pollut 150: 85-95. http://dx.doi.org/10.1016/j.envpol.2007.06.037.

Parsons, WFJ; Bockheim, JG; Lindroth, RL. (2008). Independent, interactive, and species-specific responses of leaf litter decomposition to elevated CO2 and O3 in a northern hardwood forest. Ecosystems 11: 505-519. http://dx.doi.org/10.1007/s10021-008-9148-x.

Paudel, R; Grantz, DA; Vu, HB; Shrestha, A. (2016). Tolerance of elevated ozone and water stress in a California population of Palmer amaranth (Amaranthus palmeri). Weed Sci 64: 276-284. http://dx.doi.org/10.1614/WS-D-15-00146.1.

Payne, RJ; Stevens, CJ; Dise, NB; Gowing, DJ; Pilkington, MG; Phoenix, GK; Emmett, BA; Ashmore, MR. (2011). Impacts of atmospheric pollution on the plant communities of British acid grasslands. Environ Pollut 159: 2602-2608. http://dx.doi.org/10.1016/j.envpol.2011.06.009.

Payne, RJ; Toet, S; Ashmore, M; Jassey, VEJ; Gilbert, D. (2017). Impacts of tropospheric ozone exposure on peatland microbial consumers. Soil Biol Biochem 115: 124-128. http://dx.doi.org/10.1016/j.soilbio.2017.08.012.

Pell, EJ; Sinn, JP; Brendley, BW; Samuelson, L; Vinten-Johansen, C; Tien, M; Skillman, J. (1999). Differential response of four tree species to ozone-induced acceleration of foliar senescence. Plant Cell Environ 22: 779-790. http://dx.doi.org/10.1046/j.1365-3040.1999.00449.x.

Peltonen, PA; Vapaavuori, E; Heinonen, J; Julkunen-Tiitto, R; Holopainen, JK. (2010). Do elevated atmospheric CO2 and O-3 affect food quality and performance of folivorous insects on silver birch? Global Change Biol 16: 918-935. http://dx.doi.org/10.1111/j.1365-2486.2009.02073.x.

Percy, KE; Awmack, CS; Lindroth, RL; Kubiske, ME; Kopper, BJ; Isebrands, JG; Pregitzer, KS; Hendry, GR; Dickson, RE; Zak, DR; Oksanen, E; Sober, J; Harrington, R; Karnosky, DF. (2002). Altered performance of forest pests under atmospheres enriched with CO2 and O3. Nature 420: 403-407. http://dx.doi.org/10.1038/nature01028.

Peterson, DL; Arbaugh, MJ; Wakefield, VA; Miller, PR. (1987). Evidence of growth reduction in ozone-injured Jeffrey pine (Pinus jeffreyi Grev and Balf) in Sequoia and Kings Canyon National Parks. J Air Waste Manag Assoc 37: 906-912. http://dx.doi.org/10.1080/08940630.1987.10466283.

Pfleeger, TG; Plocher, M; Bichel, P. (2010). Response of pioneer plant communities to elevated ozone exposure. Agric Ecosyst Environ 138: 116-126. http://dx.doi.org/10.1016/j.agee.2010.04.009.

Piikki, K; De Temmerman, L; Hogy, P; Pleijel, H. (2008). The open-top chamber impact on vapour pressure deficit and its consequences for stomatal ozone uptake. Atmos Environ 42: 6513-6522. http://dx.doi.org/10.1016/j.atmosenv.2008.04.014.

Pinto, DM; Blande, JD; Nykanen, R; Dong, WX; Nerg, AM; Holopainen, JK. (2007). Ozone degrades common herbivore-induced plant volatiles: Does this affect herbivore prey location by predators and parasitoids? J Chem Ecol 33: 683-694. http://dx.doi.org/10.1007/s10886-007-9255-8.

Pinto, DM; Blande, JD; Souza, SR; Nerg, AM; Holopainen, JK. (2010). Plant volatile organic compounds (VOCs) in ozone (O3) polluted atmospheres: The ecological effects [Review]. J Chem Ecol 36: 22-34. http://dx.doi.org/10.1007/s10886-009-9732-3.

Pinto, DM; Himanen, SJ; Nissinen, A; Nerg, AM; Holopainen, JK. (2008). Host location behavior of Cotesia plutellae Kurdjumov (Hymenoptera: Braconidae) in ambient and moderately elevated ozone in field conditions. Environ Pollut 156: 227-231. http://dx.doi.org/10.1016/j.envpol.2007.12.009.

Pleijel, H; Broberg, MC; Uddling, J; Mills, G. (2018). Current surface ozone concentrations significantly decrease wheat growth, yield and quality. Sci Total Environ 613-614: 687-692. http://dx.doi.org/10.1016/j.scitotenv.2017.09.111.

Pleijel, H; Danielsson, H. (1997). Growth of 27 herbs and grasses in relation to ozone exposure and plant strategy. New Phytol 135: 361-367. http://dx.doi.org/10.1046/j.1469-8137.1997.00648.x.

Pleijel, H; Danielsson, H; Simpson, D; Mills, G. (2014). Have ozone effects on carbon sequestration been overestimated? A new biomass response function for wheat. Biogeosciences 11: 4521-4528. http://dx.doi.org/10.5194/bg-11-4521-2014.

Powell, MC; Muntifering, RB; Lin, JC; Chappelka, AH. (2003). Yield and nutritive quality of sericea lespedeza (Lespedeza cuneata) and little bluestem (Schizachyrium scoparium) exposured to ground-level ozone. Environ Pollut 122: 313-322. http://dx.doi.org/10.1016/S0269-7491(02)00331-7.

Power, SA; Ashmore, MR. (2002). Responses of fen and fen-meadow communities to ozone. New Phytol 156: 399-408. http://dx.doi.org/10.1046/j.1469-8137.2002.00540.x.

Pregitzer, K; Loya, W; Kubiske, M; Zak, D. (2006). Soil respiration in northern forests exposed to elevated atmospheric carbon dioxide and ozone. Oecologia 148: 503-516. http://dx.doi.org/10.1007/s00442-006-0381-8.

Pregitzer, KS; Burton, AJ; King, JS; Zak, DR. (2008). Soil respiration, root biomass, and root turnover following long-term exposure of northern forests to elevated atmospheric Co-2 and tropospheric O-3. New Phytol 180: 153-161. http://dx.doi.org/10.1111/j.1469-8137.2008.02564.x.

Pregitzer, KS; Euskirchen, ES. (2004). Carbon cycling and storage in world forests: biome patterns related to forest age. Global Change Biol 10: 2052-2077. http://dx.doi.org/10.1111/j.1365-2486.2004.00866.x.

Pregitzer, KS; Talhelm, AF. (2013). Belowground carbon cycling at Aspen FACE: Dynamic responses to CO2 and O3 in developing forests. In R Matyssek; N Clarke; P Cudlin; TN Mikkelsen; JP Tuovinen; G Wieser; E Paoletti (Eds.), Climate change, air pollution and global challenges: Understanding and perspectives from forest research (pp. 209-226). Amsterdam, Netherlands: Elsevier. http://dx.doi.org/10.1016/B978-0-08-098349-3.00010-4.

Pritsch, K; Esperschuetz, J; Haesler, F; Raidl, S; Winkler, B; Schloter, M. (2009). Structure and activities of ectomycorrhizal and microbial communities in the rhizosphere of Fagus sylvatica under ozone and pathogen stress in a lysimeter study. Plant Soil 323: 97-109. http://dx.doi.org/10.1007/s11104-009-9972-6.

Pujol Pereira, EI; Chung, H; Scow, K; Sadowsky, MJ; van Kessel, C; Six, J. (2011). Soil nitrogen transformations under elevated atmospheric CO and O during the soybean growing season. Environ Pollut 159: 401-407. http://dx.doi.org/10.1016/j.envpol.2010.10.033.

Qiu, Z; Chappelka, AH; Somers, GL; Lockaby, BG; Meldahl, RS. (1992). Effects of ozone and simulated acidic precipitation on above- and below-ground growth of loblolly pine (Pinus taeda). Can J For Res 22: 582-587.

Rao, MV; Hale, BA; Ormrod, DP. (1995). Amelioration of ozone-induced oxidative damage in wheat plants grown under high carbon dioxide: Role of antioxidant enzymes. J Plant Physiol 109: 421-432.

Rasheed, MU; Kasurinen, A; Kivimaenpaa, M; Ghimire, R; Haikio, E; Mpamah, P; Holopainen, JK; Holopainen, T. (2017). The responses of shoot-root-rhizosphere continuum to simultaneous fertilizer addition, warming, ozone and herbivory in young Scots pine seedlings in a high latitude field experiment. Soil Biol Biochem 114: 279-294. http://dx.doi.org/10.1016/j.soilbio.2017.07.024.

Rebbeck, J. (1996a). Chronic ozone effects on three northeastern hardwood species: growth and biomass. Can J For Res 26: 1788-1798. http://dx.doi.org/10.1139/x26-203.

Rebbeck, J. (1996b). The chronic response of yellow-poplar and eastern white pine to ozone and elevated carbon dioxide: three-year summary (pp. 23-30). U.S. Department of Agriculture, Forest Service :: USDA. https://www.fs.usda.gov/treesearch/pubs/13377.

Reiling, K; Davison, AW. (1992). The response of native, herbaceous species to ozone: growth and fluorescence screening. New Phytol 120: 29-37. http://dx.doi.org/10.1111/j.1469-8137.1992.tb01055.x.

Reinert, RA; Eason, G; Barton, J. (1997). Growth and fruiting of tomato as influenced by elevated carbon dioxide and ozone. New Phytol 137: 411-420. http://dx.doi.org/10.1046/j.1469-8137.1997.00846.x.

Reinert, RA; Ho, MC. (1995). Vegetative growth of soybean as affected by elevated carbon dioxide and ozone. Environ Pollut 89: 89-96. http://dx.doi.org/10.1016/0269-7491(94)00039-G.

Ren, W; Tian, H; Tao, B; Huang, Y; Pan, S. (2012). China's crop productivity and soil carbon storage as influenced by multifactor global change. Global Change Biol 18: 2945-2957. http://dx.doi.org/10.1111/j.1365-2486.2012.02741.x.

Resco de Dios, V; Mereed, TE; Ferrio, JP; Tissue, DT; Voltas, J. (2016). Intraspecific variation in juvenile tree growth under elevated CO2 alone and with O3: a meta-analysis. Tree Physiol 36: 682-693. http://dx.doi.org/10.1093/treephys/tpw026.

Rhea, LK; King, JS. (2012). Depth-dependency of trembling aspen and paper birch small-root responses to eCO(2) and eO(3). Plant Soil 355: 215-229. http://dx.doi.org/10.1007/s11104-011-1094-2.

Richards, BL; Middleton, JT; Hewitt, WB. (1958). Air pollution with relation to agronomic crops: V: Oxidant stipple of grape. J Am Soc Agron 50: 559-561. http://dx.doi.org/10.2134/agronj1958.00021962005000090019x.

Riddell, J; Padgett, PE; Nash, TH, III. (2012). Physiological responses of lichens to factorial fumigations with nitric acid and ozone. Environ Pollut 170: 202-210. http://dx.doi.org/10.1016/j.envpol.2012.06.014.

Ritter, W; Andersen, CP; Matyssek, R; Grams, TEE. (2011). Carbon flux to woody tissues in a beech/spruce forest during summer and in response to chronic O-3 exposure. Biogeosciences 8: 3127-3138. http://dx.doi.org/10.5194/bg-8-3127-2011.

Rodenkirchen, H; Gottlein, A; Kozovits, AR; Matyssek, R; Grams, TEE. (2009). Nutrient contents and efficiencies of beech and spruce saplings as influenced by competition and O3/CO2 regime. European Journal of Forest Research 128: 117-128. http://dx.doi.org/10.1007/s10342-008-0221-y.

Rogers, A; Allen, DJ; Davey, PA; Morgan, PB; Ainsworth, EA; Bernacchi, CJ; Cornic, G; Dermody, OC; Dohleman, FG; Heaton, EA; Mahoney, J; Zhu, XG; Delucia, EH; Ort, DR; Long, SP. (2004). Leaf photosynthesis and carbohydrate dynamics of soybean grown throughout their life-cycle under free-air carbon dioxide enrichment. Plant Cell Environ 27: 449-458.

Romaneckiene, R; Pilipavicius, V; Romaneckas, K. (2008). The influence of ozone and uv-b radiation on fat-hen growth in different temperature conditions. Zemdirbyste-Agriculture 95: 122-132.

Runeckles, VC; Wright, EF. (1996). Delayed impact of chronic ozone stress on young Douglas-fir grown under field conditions. Can J For Res 26: 629-638. http://dx.doi.org/10.1139/x26-073.

Samuelson, LJ; Kelly, JM; Mays, PA; Edwards, GS. (1996). Growth and nutrition of Quercus rubra L seedlings and mature trees after three seasons of ozone exposure. Environ Pollut 91: 317-323. http://dx.doi.org/10.1016/0269-7491(95)00067-4.

Sanz, J; Bermejo, V; Muntifering, R; González-Fernández, I; Gimeno, BS; Elvira, S; Alonso, R. (2011). Plant phenology, growth and nutritive quality of Briza maxima: responses induced by enhanced ozone atmospheric levels and nitrogen enrichment. Environ Pollut 159: 423-430. http://dx.doi.org/10.1016/j.envpol.2010.10.026.

Sanz, J; González-Fernández, I; Elvira, S; Muntifering, R; Alonso, R; Bermejo-Bermejo, V. (2016). Setting ozone critical levels for annual Mediterranean pasture species: Combined analysis of open-top chamber experiments. Sci Total Environ 571: 670-679. http://dx.doi.org/10.1016/j.scitotenv.2016.07.035.

Saviranta, NMM; Julkunen-Tiitto, R; Oksanen, E; Karjalainen, RO. (2010). Leaf phenolic compounds in red clover (Trifolium pratense L.) induced by exposure to moderately elevated ozone. Environ Pollut 158: 440-446. http://dx.doi.org/10.1016/j.envpol.2009.08.029.

Schmidt, MWI; Torn, MS; Abiven, S; Dittmar, T; Guggenberger, G; Janssens, IA; Kleber, M; Köegel-Knabner, I; Lehmann, J; Manning, DAC; Nannipieri, P; Rasse, DP; Weiner, S; Trumbore, SE. (2011). Persistence of soil organic matter as an ecosystem property. Nature 478: 49-56. http://dx.doi.org/10.1038/nature10386.

Seiler, LK; Decoteau, DR; Davis, DD. (2014). Evaluation of Ailanthus altissima as a bioindicator to detect phytotoxic levels of ozone. Northeast Nat 21: 541-553. http://dx.doi.org/10.1656/045.021.0405.

Shafer, SR; Reinert, RA; Eason, G; Spruill, SE. (1993). Analysis of ozone concentration - biomass response relationships among open-pollinated families of loblolly pine. Can J For Res 23: 706-715.

Shelburne, VB; Reardon, JC; Paynter, VA. (1993). The effects of acid rain and ozone on biomass and leaf area parameters of shortleaf pine (Pinus echinata Mill). Tree Physiol 12: 163-172.

Simini, M; Skelly, JM; Davis, DD; Savage, JE; Comrie, AC. (1992). Sensitivity of four hardwood species to ambient ozone in north central Pennsylvania. Can J For Res 22: 1789-1799. http://dx.doi.org/10.1139/x92-234.

Sitch, S; Cox, PM; Collins, WJ; Huntingford, C. (2007). Indirect radiative forcing of climate change through ozone effects on the land-carbon sink. Nature 448: 791-794. http://dx.doi.org/10.1038/nature06059.

Skelly, JM; Innes, JL; Savage, JS; Snyder, KR; Vanderheyden, DZ; Sanz, MJ. (1999). Observation and confirmation of foliar injury ozone symptoms of native plant species of Switzerland and southern Spain. Water Air Soil Pollut 116: 227-234. http://dx.doi.org/10.1023/A:1005275431399.

Smith, G. (2012). Ambient ozone injury to forest plants in Northeast and North Central USA: 16 years of biomonitoring. Environ Monit Assess 184: 4049-4065. http://dx.doi.org/10.1007/s10661-011-2243-z.

Smith, G; Coulston, J; Jepsen, E; Prichard, T. (2003). A national ozone biomonitoring program: Results from field surveys of ozone sensitive plants in northeastern forests (1994-2000). Environ Monit Assess 87: 271-291. http://dx.doi.org/10.1023/A:1024879527764.

Smith, GC; Coulston, JW; O'Connell, BM. (2008). Ozone bioindicators and forest health: a guide to the evaluation, analysis, and interpretation of the ozone injury data in the Forest Inventory and Analysis Program. (NRS-34). Newtown Square, PA: U.S. Department of Agriculture, Forest Service. http://dx.doi.org/10.2737/NRS-GTR-34.

Somers, GL; Chappelka, AH; Rosseau, P; Renfro, JR. (1998). Empirical evidence of growth decline related to visible ozone injury. For Ecol Manage 104: 129-137. http://dx.doi.org/10.1016/S0378-1127(97)00252-1.

Souza, L; Neufeld, HS; Chappelka, AH; Burkey, KO; Davison, AW. (2006). Seasonal development of ozone-induced foliar injury on tall milkweed (Asclepias exaltata) in Great Smoky Mountains National Park. Environ Pollut 141: 175-183. http://dx.doi.org/10.1016/j.envpol.2005.07.022.

Spence, RD; Rykiel, EJ, Jr; Sharpe, PJH. (1990). Ozone alters carbon allocation in loblolly pine: assessment with carbon-11 labeling. Environ Pollut 64: 93-106. http://dx.doi.org/10.1016/0269-7491(90)90107-N.

Stampfli, A; Fuhrer, J. (2010). Spatial heterogeneity confounded ozone-exposure experiment in semi-natural grassland. Oecologia 162: 515-522. http://dx.doi.org/10.1007/s00442-009-1462-2.

Stoelken, G; Pritsch, K; Simon, J; Mueller, CW; Grams, TEE; Esperschuetz, J; Gayler, S; Buegger, F; Brueggemann, N; Meier, R; Zeller, B; Winkler, JB; Rennenberg, H. (2010). Enhanced ozone exposure of European beech (Fagus sylvatica) stimulates nitrogen mobilization from leaf litter and nitrogen accumulation in the soil. Plant Biosyst 144: 537-546. http://dx.doi.org/10.1080/11263500903429346.

Subramanian, N; Karlsson, PE; Bergh, J; Nilsson, U. (2015). Impact of ozone on sequestration of carbon by Swedish forests under a changing climate: A modeling study. Forest Sci 61: 445-457. http://dx.doi.org/10.5849/forsci.14-026.

Suding, KN; Collins, SL; Gough, L; Clark, C; Cleland, EE; Gross, KL; Milchunas, DG; Pennings, S. (2005). Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. Proc Natl Acad Sci USA 102: 4387-4392. http://dx.doi.org/10.1073/pnas.0408648102.

Sun, G; Mclaughlin, SB; Porter, JH; Uddling, J; Mulholland, PJ; Adams, MB; Pederson, N. (2012). Interactive influences of ozone and climate on streamflow of forested watersheds. Global Change Biol 18: 3395-3409. http://dx.doi.org/10.1111/j.1365-2486.2012.02787.x.

Sun, J; Feng, Z; Ort, DR. (2014). Impacts of rising tropospheric ozone on photosynthesis and metabolite levels on field grown soybean. Plant Sci 226: 147-161. http://dx.doi.org/10.1016/j.plantsci.2014.06.012.

Super, I; de Arellano, JVG; Krol, MC. (2015). Cumulative ozone effect on canopy stomatal resistance and the impact on boundary layer dynamics and CO2 assimilation at the diurnal scale: A case study for grassland in the Netherlands. Jour Geo Res: Biog 120: 1348-1365. http://dx.doi.org/10.1002/2015JG002996.

Szantoi, Z; Chappelka, AH; Muntifering, RB; Somers, GL. (2007). Use of ethylenediurea (EDU) to ameliorate ozone effects on purple coneflower (Echinacea purpurea). Environ Pollut 150: 200-208. http://dx.doi.org/10.1016/j.envpol.2007.01.020.

Szantoi, Z; Chappelka, AH; Muntifering, RB; Somers, GL. (2009). Cutleaf coneflower (Rudbeckia laciniata L.) response to ozone and ethylenediurea (EDU). Environ Pollut 157: 840-846. http://dx.doi.org/10.1016/j.envpol.2008.11.014.

Tai, APK; Martin, MV. (2017). Impacts of ozone air pollution and temperature extremes on crop yields: Spatial variability, adaptation and implications for future food security. Atmos Environ 169: 11-21. http://dx.doi.org/10.1016/j.atmosenv.2017.09.002.

Taia, W; Basahi, J; Hassan, I. (2013). Impact of ambient air on physiology, pollen tube growth, pollen germination and yield in pepper (Capsicum annuum L.). Pakistan J Bot 45: 921-926.

Takemoto, BK; Bytnerowicz, A; Dawson, PJ; Morrison, CL; Temple, PJ. (1997). Effects of ozone on Pinus ponderosa seedlings: comparison of responses in the first and second growing seasons of exposure. Can J For Res 27: 23-30. http://dx.doi.org/10.1139/cjfr-27-1-23.

Takemoto, BK; Bytnerowicz, A; Fenn, ME. (2001). Current and future effects of ozone and atmospheric nitrogen deposition on California's mixed conifer forests. For Ecol Manage 144: 159-173. http://dx.doi.org/10.1016/S0378-1127(00)00368-6.

Talhelm, AF; Pregitzer, KS; Giardina, CP. (2012). Long-term leaf production response to elevated atmospheric carbon dioxide and tropospheric ozone. Ecosystems 15: 71-82. http://dx.doi.org/10.1007/s10021-011-9493-z.

Talhelm, AF; Pregitzer, KS; Kubiske, ME; Zak, DR; Campany, CE; Burton, AJ; Dickson, RE; Hendrey, GR; Isebrands, JG; Lewin, KF; Nagy, J; Karnosky, DF. (2014). Elevated carbon dioxide and ozone alter productivity and ecosystem carbon content in northern temperate forests. Global Change Biol 20: 2492-2504. http://dx.doi.org/10.1111/gcb.12564.

Taylor, M. (2002). The impact of ozone on a salt marsh cordgrass (Spartina alterniflora). Environ Pollut 120: 701-705. http://dx.doi.org/10.1016/S0269-7491(02)00178-1.

Telesnicki, MC; Alejandra Martinez-Ghersa, M; Arneodo, JD; Ghersa, CM. (2015). Direct effect of ozone pollution on aphids: Revisiting the evidence at individual and population scales. Entomol Exp Appl 155: 71-79. http://dx.doi.org/10.1111/eea.12288.

Temple, P; Bytnerowicz, A; Fenn, M; Poth, M. (2005). Air pollution impacts in the mixed conifer forests of southern California. USDA Forest Service General Technical Report (pp. 145-164). (PSW-GTR-195). U.S. Department of Agriculture, Forest Service :: USDA.

Temple, PJ. (1999). Effects of ozone on understory vegetation in the mixed conifer forest. In PR Miller; JR McBride (Eds.), Oxidant air pollution impacts in the montane forests of southern California: A case study of the San Bernardino Mountains (pp. 208-222). New York, NY: Springer. http://dx.doi.org/10.1007/978-1-4612-1436-6_10.

Temple, PJ; Kupper, RS; Lennox, RW; Rohr, K. (1988). Injury and yield responses of differentially irrigated cotton to ozone. Agron J 80: 751-755. http://dx.doi.org/10.2134/agronj1988.00021962008000050011x.

Temple, PJ; Miller, PR. (1994). Foliar ozone injury and radial growth of ponderosa pine. Can J For Res 24: 1877-1882.

Temple, PJ; Riechers, GH; Miller, PR. (1992). Foliar injury responses of ponderosa pine seedlings to ozone, wet and dry acidic deposition, and drought. Environ Exp Bot 32: 101-113. http://dx.doi.org/10.1016/0098-8472(92)90035-Z.

Temple, PJ; Riechers, GH; Miller, PR; Lennox, RW. (1993). Growth responses of ponderosa pine to long-term exposure to ozone, wet and dry acidic deposition, and drought. Can J For Res 23: 59-66. http://dx.doi.org/10.1139/x93-010.

Terrer, C; Vicca, S; Stocker, BD; Hungate, BA; Phillips, RP; Reich, PB; Finzi, AC; Prentice, IC. (2018). Ecosystem responses to elevated CO2 governed by plant-soil interactions and the cost of nitrogen acquisition [Review]. New Phytol 217: 507-522. http://dx.doi.org/10.1111/nph.14872.

Tetteh, R; Yamaguchi, M; Wada, Y; Funada, R; Izuta, T. (2015). Effects of ozone on growth, net photosynthesis and yield of two African varieties of Vigna unguiculata. Environ Pollut 196: 230-238. http://dx.doi.org/10.1016/j.envpol.2014.10.008.

Thomas, RQ; Canham, CD; Weathers, KC; Goodale, CL. (2010). Increased tree carbon storage in response to nitrogen deposition in the US. Nat Geosci 3: 13-17. http://dx.doi.org/10.1038/NGEO721.

Thompson, CR; Olszyk, DM; Kats, G; Bytnerowicz, A; Dawson, PJ; Wolf, JW. (1984). Effects of ozone or sulfur-dioxide on annual plants of the Mojave Desert. J Air Pollut Control Assoc 34: 1017-1022.

Tian, H; Chen, G; Zhang, C; Liu, M; Sun, G; Chappelka, A; Ren, W; Xu, X; Lu, C; Pan, S; Chen, H; Hui, D; McNulty, S; Lockaby, G; Vance, E. (2012). Century-scale responses of ecosystem carbon storage and flux to multiple environmental changes in the southern United States. Ecosystems 15: 674-694. http://dx.doi.org/10.1007/s10021-012-9539-x.

Tjoelker, MG; Volin, JC; Oleksyn, J; Reich, PB. (1993). Light environment alters response to ozone stress in seedlings of Acer saccharum Marsh and hybrid Populus L I In situ net photosynthesis, dark respiration and growth. New Phytol 124: 627-636. http://dx.doi.org/10.1111/j.1469-8137.1993.tb03852.x.

Toet, S; Ineson, P; Peacock, S; Ashmore, M. (2011). Elevated ozone reduces methane emissions from peatland mesocosms. Global Change Biol 17: 288-296. http://dx.doi.org/10.1111/j.1365-2486.2010.02267.x.

Tong, D; Mathur, R; Schere, K; Kang, D; Yu, S. (2007). The use of air quality forecasts to assess impacts of air pollution on crops: Methodology and case study. Atmos Environ 41: 8772-8784. http://dx.doi.org/10.1016/j.atmosenv.2007.07.060.

Tonneijck, AEG; Franzaring, J; Brouwer, G; Metselaar, K; Dueck, TA. (2004). Does interspecific competition alter effects of early season ozone exposure on plants from wet grasslands? Results of a three-year experiment in open-top chambers. Environ Pollut 131: 205-213. http://dx.doi.org/10.1016/j.envpol.2004.02.005.

Turnipseed, AA; Burns, SP; Moore, DJP; Hu, J; Guenther, AB; Monson, RK. (2009). Controls over ozone deposition to a high elevation subalpine forest. Agr Forest Meteorol 149: 1447-1459. http://dx.doi.org/10.1016/j.agrformet.2009.04.001.

U.S. EPA (U.S. Environmental Protection Agency). (1977). Photochemical oxidant air pollutant effects on a mixed conifer forest ecosystem: A progress report [EPA Report]. (EPA-600/3-77-104). Corvallis, OR. http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=20015IYP.txt.

U.S. EPA (U.S. Environmental Protection Agency). (1978). Air quality criteria for ozone and other photochemical oxidants [EPA Report]. (EPA/600/8-78/004). Washington, DC. http://nepis.epa.gov/exe/ZyPURL.cgi?Dockey=200089CW.txt.

U.S. EPA (U.S. Environmental Protection Agency). (1980). Photochemical oxidant air pollution effects on a mixed conifer forest ecosystem: Final report, 1977 [EPA Report]. (EPA-600/3-80-002). Corvallis, OR. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=PB80176779.

U.S. EPA (U.S. Environmental Protection Agency). (1986). Air quality criteria for ozone and other photochemical oxidants [EPA Report]. (EPA-600/8-84-020aF - EPA-600/8-84-020eF). Research Triangle Park, NC. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=PB87142949.

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

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

U.S. EPA (U.S. Environmental Protection Agency). (2008). HERO.

U.S. EPA (U.S. Environmental Protection Agency). (2009). HERO, the Database. Available online at http://www.epa.gov/hero (accessed

U.S. EPA (U.S. Environmental Protection Agency). (2013). Integrated science assessment for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-10/076F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment-RTP Division. http://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=247492.

U.S. EPA (U.S. Environmental Protection Agency). (2014). Welfare Risk and Exposure Assessment for Ozone, Final. (EPA-452/P-14-005a). Research Triangle Park, NC: Office of Air Quality Planning and Standards. https://www3.epa.gov/ttn/naaqs/standards/ozone/data/20141021welfarerea.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2015). Preamble to the integrated science assessments [EPA Report]. (EPA/600/R-15/067). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, RTP Division. https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=310244.

U.S. EPA (U.S. Environmental Protection Agency). (2018). Integrated science assessment for oxides of nitrogen, oxides of sulfur and particulate matter -Ecological criteria (2nd external review draft). (EPA/600/R-18/097). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment. http://cfint.rtpnc.epa.gov/ncea/prod/recordisplay.cfm?deid=340671.

Uddling, J; Teclaw, RM; Pregitzer, KS; Ellsworth, DS. (2009). Leaf and canopy conductance in aspen and aspen-birch forests under free-air enrichment of carbon dioxide and ozone. Tree Physiol 29: 1367-1380. http://dx.doi.org/10.1093/treephys/tpp070.

Ueda, Y; Frindte, K; Knief, C; Ashrafuzzaman, M; Frei, M. (2016). Effects of elevated tropospheric ozone concentration on the bacterial community in the phyllosphere and rhizoplane of rice. PLoS ONE 11: e0163178. http://dx.doi.org/10.1371/journal.pone.0163178.

Ueno, AC; Gundel, PE; Omacini, M; Ghersa, CM; Bush, LP; Alejandra Martinez-Ghersa, M. (2016). Mutualism effectiveness of a fungal endophyte in an annual grass is impaired by ozone. Funct Ecol 30: 226-234. http://dx.doi.org/10.1111/1365-2435.12519.

Unsworth, MH; Heagle, AS; Heck, WW. (1984a). Gas exchange in open-top field chambers: I. Measurement and analysis of atmospheric resistances to gas exchange. Atmos Environ 18: 373-380. http://dx.doi.org/10.1016/0004-6981(84)90111-2.

Unsworth, MH; Heagle, AS; Heck, WW. (1984b). Gas exchange in open-top field chambers: II. Resistances to ozone uptake by soybeans. Atmos Environ 18: 381-385. http://dx.doi.org/10.1016/0004-6981(84)90112-4.

USDA (U.S. Department of Agriculture). (2015). Natural Resources Conservation Service PLANTS database. Available online at http://plants.usda.gov/java/ (accessed

Valkama, E; Koricheva, J; Oksanen, E. (2007). Effects of elevated O3, alone and in combination with elevated CO2, on tree leaf chemistry and insect herbivore performance: A meta-analysis. Global Change Biol 13: 184-201. http://dx.doi.org/10.1111/j.1365-2486.01284.x.

Van Dingenen, R; Dentener, FJ; Raes, F; Krol, MC; Emberson, L; Cofala, J. (2009). The global impact of ozone on agricultural crop yields under current and future air quality legislation. Atmos Environ 43: 604-618. http://dx.doi.org/10.1016/j.atmosenv.2008.10.033.

van Goethem, TM; Azevedo, LB; van Zelm, R; Hayes, F; Ashmore, MR; Huijbregts, MA. (2013). Plant species sensitivity distributions for ozone exposure. Environ Pollut 178: 1-6. http://dx.doi.org/10.1016/j.envpol.2013.02.023.

van Groenigen, KJ; Qi, X; Osenberg, CW; Luo, Y; Hungate, BA. (2014). Faster decomposition under increased atmospheric CO2 limits soil carbon storage. Science 344: 508-509. http://dx.doi.org/10.1126/science.1249534.

Vanderheyden, D; Skelly, J; Innes, J; Hug, C; Zhang, J; Landolt, W; Bleuler, P. (2001). Ozone exposure thresholds and foliar injury on forest plants in Switzerland. Environ Pollut 111: 321-331. http://dx.doi.org/10.1016/S0269-7491(00)00060-9.

Vanloocke, A; Betzelberger, A; Ainsworth, EA; Bernacchi, CJ. (2012). Rising ozone concentrations decrease soybean evapotranspiration and water use efficiency whilst increasing canopy temperature. New Phytol 195: 164-171. http://dx.doi.org/10.1111/j.1469-8137.2012.04152.x.

Vigue, LM; Lindroth, RL. (2010). Effects of genotype, elevated CO2 and elevated O3 on aspen phytochemistry and aspen leaf beetle Chrysomela crotchi performance. Agr Forest Entomol 12: 267-276. http://dx.doi.org/10.1111/j.1461-9563.2010.00475.x.

Volin, JC; Reich, PB; Givnish, TJ. (1998). Elevated carbon dioxide ameliorates the effects of ozone on photosynthesis and growth: species respond similarly regardless of photosynthetic pathway or plant functional group. New Phytol 138: 315-325. http://dx.doi.org/10.1046/j.1469-8137.1998.00100.x.

Volk, M; Bungener, P; Contat, F; Montani, M; Fuhrer, J. (2006). Grassland yield declined by a quarter in 5 years of free-air ozone fumigation. Global Change Biol 12: 74-83. http://dx.doi.org/10.1111/j.1365-2486.2005.01083.x.

Volk, M; Geissmann, M; Blatter, A; Contat, F; Fuhrer, J. (2003). Design and performance of a free-air exposure system to study long-term effects of ozone on grasslands. Atmos Environ 37: 1341-1350. http://dx.doi.org/10.1016/S1352-2310(02)01012-9.

Vuorinen, T; Nerg, AM; Holopainen, JK. (2004). Ozone exposure triggers the emission of herbivore-induced plant volatiles, but does not disturb tritrophic signalling. Environ Pollut 131: 305-311. http://dx.doi.org/10.1016/j.envpol.2004.02.027.

Wagg, S; Mills, G; Hayes, F; Wilkinson, S; Davies, WJ. (2013). Stomata are less responsive to environmental stimuli in high background ozone in Dactylis glomerata and Ranunculus acris. Environ Pollut 175: 82-91. http://dx.doi.org/10.1016/j.envpol.2012.11.027.

Wahid, A; Ahmad, SS; Butt, ZA; Ahmad, M. (2011). Exploring the hidden threat of gaseous Pollutants using rice (Oryza sativa l.) Plants in Pakistan. Pakistan J Bot 43: 365-382.

Wan, W; Manning, WJ; Wang, X; Zhang, H; Sun, X; Zhang, Q. (2014). Ozone and ozone injury on plants in and around Beijing, China. Environ Pollut 191: 215-222. http://dx.doi.org/10.1016/j.envpol.2014.02.035.

Wan, W; Xia, Y; Zhang, H; Wang, J; Wang, X. (2013). [The ambient ozone pollution and foliar injury of the sensitive woody plants in Beijing exurban region]. Acta Ecol Sin 33: 1098-1105.

Wang, B, in; Shugart, HH; Shuman, JK; Lerdau, MT. (2016). Forests and ozone: productivity, carbon storage, and feedbacks. Sci Rep 6: 22133. http://dx.doi.org/10.1038/srep22133.

Wang, D; Bormann, FH; Karnosky, DF. (1986). Regional tree growth reductions due to ambient ozone: Evidence from field experiments. Environ Sci Technol 20: 1122-1125. http://dx.doi.org/10.1021/es00153a007.

Wang, H; Zhang, L; Ma, X; Zou, J; Siemann, E. (2018). The effects of elevated ozone and CO2 on growth and defense of native, exotic and invader trees. Journal of Plant Ecology 11: 266-272. http://dx.doi.org/10.1093/jpe/rtw142.

Wang, P; Baines, A; Lavine, M; Smith, G. (2012). Modelling ozone injury to US forests. Environ Ecol Stat 19: 461-472. http://dx.doi.org/10.1007/s10651-012-0195-2.

Wang, P; Marsh, EL; Ainsworth, EA; Leakey, ADB; Sheflin, AM; Schachtman, DP. (2017). Shifts in microbial communities in soil, rhizosphere and roots of two major crop systems under elevated CO2and O3. Sci Rep 7: 15019. http://dx.doi.org/10.1038/s41598-017-14936-2.

Wang, S; Wang, F; Diao, X; He, L. (2014). Effects of elevated O3 on microbes in the rhizosphere of mycorrhizal snap bean with different O3 sensitivity. Can J Microbiol 60: 93-103. http://dx.doi.org/10.1139/cjm-2013-0851.

Wang, X; Taub, DR; Jablonski, LM. (2015). Reproductive allocation in plants as affected by elevated carbon dioxide and other environmental changes: a synthesis using meta-analysis and graphical vector analysis. Oecologia 177: 1075-1087. http://dx.doi.org/10.1007/s00442-014-3191-4.

Warwick, KR; Taylor, G. (1995). Contrasting effects of tropospheric ozone on five native herbs which coexist in calcareous grassland. Global Change Biol 1: 143-151. http://dx.doi.org/10.1111/j.1365-2486.1995.tb00014.x.

Wedlich, KV; Rintoul, N; Peacock, S; Cape, JN; Coyle, M; Toet, S; Barnes, J; Ashmore, M. (2012). Effects of ozone on species composition in an upland grassland. Oecologia 168: 1137-1146. http://dx.doi.org/10.1007/s00442-011-2154-2.

Werner, H; Fabian, P. (2002). Free-air fumigation of mature trees: A novel system for controlled ozone enrichment in grown-up beech and spruce canopies. Environ Sci Pollut Res Int 9: 117-121. http://dx.doi.org/10.1007/BF02987458.

Westerling, AL; Bryant, BP; Preisler, HK; Holmes, TP; Hidalgo, HG; Das, T; Shrestha, SR, SR. (2011). Climate change and growth scenarios for California wildfire. Clim Change 109: 445-463. http://dx.doi.org/10.1007/s10584-011-0329-9.

Wieser, G; Manning, WJ; Tausz, M; Bytnerowicz, A. (2006). Evidence for potential impacts of ozone on Pinus cembra L. at mountain sites in Europe: An overview. Environ Pollut 139: 53-58. http://dx.doi.org/10.1016/j.envpol.2005.04.037.

Williams, W; Macgregor, N. (1975). Oxidant induced air pollution damage to forest trees in the Southern Sierra-Sevada Moun-tains of California. Proc Am Phytopathol Soc 2: 120.

Williams, WT; Brady, M; Willison, SC. (1977). Air pollution damage to the forests of the Sierra Nevada Mountains of California. J Air Waste Manag Assoc 27: 230-234.

Winner, WE; Lefohn, AS; Cotter, IS; Greitner, CS; Nellessen, J; Mcevoy, LR, Jr; Olson, RL; Atkinson, CJ; Moore, LD. (1989). Plant responses to elevational gradients of O3 exposures in Virginia. Proc Natl Acad Sci USA 86: 8828-8832. http://dx.doi.org/10.1073/pnas.86.22.8828.

Wiselogel, AE; Bailey, JK; Newton, RJ; Fong, F. (1991). Growth response of loblolly pine (Pinus taeda L) seedlings to ozone fumigation. Environ Pollut 71: 43-56. http://dx.doi.org/10.1016/0269-7491(91)90043-V.

Wittig, VE; Ainsworth, EA; Long, SP. (2007). To what extent do current and projected increases in surface ozone affect photosynthesis and stomatal conductance of trees? A meta-analytic review of the last 3 decades of experiments [Review]. Plant Cell Environ 30: 1150-1162. http://dx.doi.org/10.1111/j.1365-3040.2007.01717.x.

Wittig, VE; Ainsworth, EA; Naidu, SL; Karnosky, DF; Long, SP. (2009). Quantifying the impact of current and future tropospheric ozone on tree biomass, growth, physiology and biochemistry: A quantitative meta-analysis. Global Change Biol 15: 396-424. http://dx.doi.org/10.1111/j.1365-2486.2008.01774.x.

Wright, GA; Lutmerding, A; Dudareva, N; Smith, BH. (2005). Intensity and the ratios of compounds in the scent of snapdragon flowers affect scent discrimination by honeybees (Apis mellifera). J Comp Physiol A Neuroethol Sens Neural Behav Physiol 191: 105-114.

Wright, IJ; Reich, PB; Westoby, M; Ackerly, DD; Baruch, Z; Bongers, F; Cavender-Bares, J; Chapin, T; Cornelissen, JHC; Diemer, M; Flexas, J; Garnier, E; Groom, PK; Gulias, J; Hikosaka, K; Lamont, BB; Lee, T; Lee, W; Lusk, C; Midgley, JJ; Navas, ML; Niinemets, U; Oleksyn, J; Osada, N; Poorter, H; Poot, P; Prior, L; Pyankov, VI; Roumet, C; Thomas, SC; Tjoelker, MG; Veneklaas, EJ; Villar, R. (2004). The worldwide leaf economics spectrum. Nature 428: 821-827. http://dx.doi.org/10.1038/nature02403.

Wyness, K; Mills, G; Jones, L; Barnes, JD; Jones, DL. (2011). Enhanced nitrogen deposition exacerbates the negative effect of increasing background ozone in Dactylis glomerata, but not Ranunculus acris. Environ Pollut 159: 2493-2499. http://dx.doi.org/10.1016/j.envpol.2011.06.022.

Xu, S; Fu, W; He, X; Chen, W; Zhang, W; Li, B; Huang, Y. (2017). Drought alleviated the negative effects of elevated O3 on Lonicera maackii in urban area. Bull Environ Contam Toxicol 99: 648-653. http://dx.doi.org/10.1007/s00128-017-2179-2.

Yang, N; Wang, X; Zheng, F; Chen, Y. (2017). The impact of elevated ozone on the ornamental features of two flowering plants (Tagetes erecta linn. Petunia hybrida Vilm.). Int J Environ Pollut 61: 29-45. http://dx.doi.org/10.1504/IJEP.2017.10003698.

Yendrek, CR; Koester, RP; Ainsworth, EA. (2015). A comparative analysis of transcriptomic, biochemical, and physiological responses to elevated ozone identifies species-specific mechanisms of resilience in legume crops. J Exp Bot 66: 7101-7112. http://dx.doi.org/10.1093/jxb/erv404.

Yuan, JS; Himanen, SJ; Holopainen, JK; Chen, F; Stewart, CN, Jr. (2009). Smelling global climate change: Mitigation of function for plant volatile organic compounds [Review]. Trends Ecol Evol 24: 323-331. http://dx.doi.org/10.1016/j.tree.2009.01.012.

Yue, K; Peng, Y; Peng, C; Yang, W; Peng, X; Wu, F. (2016). Stimulation of terrestrial ecosystem carbon storage by nitrogen addition: A meta-analysis. Sci Rep 6: 19895. http://dx.doi.org/10.1038/srep19895.

Yue, X; Unger, N. (2014). Ozone vegetation damage effects on gross primary productivity in the United States. Atmos Chem Phys 14: 9137-9153. http://dx.doi.org/10.5194/acp-14-9137-2014.

Yun, SC; Laurence, JA. (1999). The response of clones of Populus tremuloides differing in sensitivity to ozone in the field. New Phytol 141: 411-421. http://dx.doi.org/10.1046/j.1469-8137.1999.00359.x.

Yuska, DE; Skelly, JM; Ferdinand, JA; Stevenson, RE; Savage, JE; Mulki, J; Hines, A. (2003). Use of bioindicators and passive sampling devices to evaluate ambient ozone concentrations in north central Pennsylvania. Environ Pollut 125: 71-80. http://dx.doi.org/10.1016/S0269-7491(03)00096-4.

Zak, DR; Kubiske, ME; Pregitzer, KS; Burton, AJ. (2012). Atmospheric CO2 and O3 alter competition for soil nitrogen in developing forests. Global Change Biol 18: 1480-1488. http://dx.doi.org/10.1111/j.1365-2486.2011.02596.x.

Zak, DR; Pregitzer, KS; Kubiske, ME; Burton, AJ. (2011). Forest productivity under elevated CO2 and O3: positive feedbacks to soil N cycling sustain decade-long net primary productivity enhancement by CO2. Ecol Lett 14: 1220-1226. http://dx.doi.org/10.1111/j.1461-0248.2011.01692.x.

Zhang, J; Tang, H; Zhu, J; Lin, X; Feng, Y. (2016). Divergent responses of methanogenic archaeal communities in two rice cultivars to elevated ground-level O3. Environ Pollut 213: 127-134. http://dx.doi.org/10.1016/j.envpol.2016.01.062.

Zheng, F; Wang, X; Lu, F; Hou, P; Zhang, W; Duan, X; Zhou, X; Ai, Y; Zheng, H; Ouyang, Z; Feng, Z. (2011). Effects of elevated ozone concentration on methane emission from a rice paddy in Yangtze River Delta, China. Global Change Biol 17: 898-910. http://dx.doi.org/10.1111/j.1365-2486.2010.02258.x.

APPENDIX 9 THE ROLE OF TROPOSPHERIC OZONE IN CLIMATE EFFECTS

Summary of Causality Determinations

Recent evidence continues to support a causal relationship between tropospheric ozone and radiative forcing, and a likely-to-be-causal relationship, via radiative forcing, between tropospheric ozone and temperature, precipitation, and related climate variables (referred to as "climate change" in the 2013 Ozone ISA; the revised title for this causal statement provides a more accurate reflection of the available evidence). The new evidence comes from the Intergovernmental Panel on Climate Change (IPCC) Fifth Assessment Report (AR5) (Myhre et al., 2013) and its supporting references—in addition to a few more recent studies—and builds on evidence presented in the 2013 Ozone ISA. None of the new studies indicate a change to either causality determination included in the 2013 Ozone ISA.

Effects	Relationship
Tropospheric ozone and climate change	
Radiative forcing	Causal
Temperature, precipitation, and related climate variables	Likely-to-be-causal

9.1 Introduction

9.1.1 Summary from the 2013 Ozone ISA

Changes in the abundance of tropospheric ozone perturb the radiative balance of the atmosphere by interacting with incoming solar radiation and outgoing longwave radiation. This effect is quantified by the radiative forcing (RF) metric. Through this effect on the Earth's radiation balance, tropospheric ozone plays a major role in the climate system, and increases in ozone abundance contribute to climate change (Forster et al., 2007).

- Increases in tropospheric ozone are tied to the rise in emissions of ozone precursors from human activity, mainly from fossil fuel consumption and agricultural processes. Models estimate that the global average tropospheric ozone concentration has increased 30−70% since preindustrial times (Gauss et al., 2006), and observations indicate that tropospheric ozone concentrations may have increased by factors of 4 or 5 in some regions (Marenco et al., 1994; Staehelin et al., 1994). In the 21st century, as the Earth's population continues to grow and energy technology spreads to

developing countries, a further rise in the global concentration of tropospheric ozone is likely (Forster et al., 2007).

- The 2007 IPCC report estimated RF from tropospheric ozone since preindustrial times (1750 to 2005) to be 0.35 W/m^2 (average) from multimodel studies, with 95th percentile error bars ranging from 0.25 to 0.65 W/m^2 (Forster et al., 2007).
- The transport of ozone to the Arctic from the midlatitudes leads to RF estimates greater than 1.0 W/m^2 in this region, especially in summer (Shindell et al., 2006; Liao et al., 2004; Mickley et al., 1999).
- Based on RF, increasing tropospheric ozone concentration since preindustrial times is estimated to contribute about 25−40% of the total warming effects of anthropogenic carbon dioxide (CO_2) and about 75% of the effects of anthropogenic methane (CH_4) (Forster et al., 2007), ranking ozone third in importance for affecting global climate behind these two major greenhouse gases.
- The Earth's land-atmosphere-ocean system responds to the RF with a change in climate, typically expressed as a change in near-surface air temperature (Forster et al., 2007). The effect of tropospheric ozone on global surface temperature is approximately proportional to the change in tropospheric ozone concentration. The Earth's surface temperatures are most sensitive to ozone abundance perturbations in the mid to upper troposphere (Forster et al., 2007).
- The increase of tropospheric ozone abundance has contributed an estimated 0.1−0.3ºC warming to the global climate since 1750 (Hansen et al., 2005; Mickley et al., 2004).
- Tropospheric ozone also has the potential to contribute to UV-B shielding, with further implications for human health and ecosystem effects. Almost no studies were found in the 2013 Ozone ISA examining the incremental effects of changes in tropospheric ozone concentrations on UV-B—and, of those, such effects of tropospheric ozone concentrations on UV-B were found to be small (Madronich et al., 2011). No studies were found that adequately examined the incremental health or welfare effects attributable specifically to changes in UV-B exposure resulting from perturbations in tropospheric ozone concentrations.

9.1.2 Scope for the Current Review

The scope of this section is defined by a scoping tool that generally describes the relevant Population, Exposure, Comparison, Outcome, and Study Design (PECOS). The PECOS tool defines the parameters and provides a framework to help identify the relevant literature to inform the draft 2019 Ozone ISA. The current review builds on the findings from the 2013 Ozone ISA and draws on peer-reviewed research, including the Intergovernmental Panel on Climate Change (IPCC) Fifth Assessment Report (AR5) (Myhre et al., 2013), to integrate evidence on how changing tropospheric ozone concentrations might affect climate. None of the new studies indicate a change to either climate-related causality determination included in the 2013 Ozone ISA. The studies evaluated and subsequently discussed within this section were included if they satisfied all of the components of the following PECOS tool summarized in Table 9-1.

Table 9-1 Population, exposure, comparison, outcome, and study design (PECOS) tool for radiative forcing and climate change.

	Radiative Forcing	Temperature, Precipitation, and Related Climate Variables
Population/geographical scope (P)	Regional, continental, and/or global scale	Regional, continental and/or global scale
Exposure/environmental variable (E)	Tropospheric ozone concentration distributions in 3D (observed/modeled)	Tropospheric ozone concentration distributions in 3D (observed/modeled)
Comparison (C)	Relevant baseline or nonperturbed scenarios/conditions	Relevant baseline or nonperturbed scenarios/conditions
Outcome (O)	Changes in RF resulting from change in tropospheric ozone	Subsequent climate effects (via RF) (e.g., global surface temperature) resulting from change in tropospheric ozone
Study design (S)	Observations or modeling studies	Observations or modeling studies

The following sections of this Appendix provide a brief background on the Earth's climate system and the pathways through which ozone influences it (Section 9.1.3) and on the new scientific evidence contributing to the causality determinations for RF (Section 9.2) and temperature, precipitation, and related climate variables (Section 9.3). In addition, this Appendix provides a brief background on the issue of UV-B shielding and reports on the results of a literature screening carried out to determine if any new evidence on incremental effects of tropospheric ozone concentrations on UV-B has emerged since the 2013 Ozone ISA (Section 9.1.3.4).

9.1.3 Introduction to Climate, Ozone Chemistry, and Radiative Forcing

9.1.3.1 Climate Change

Human activity has led to observable increases of greenhouse gases (GHGs) in the atmosphere since the start of the Industrial Revolution, mainly through fossil fuel combustion. Over the last century, global-average surface air temperature has increased by approximately 1.0ºC, and emissions of greenhouse gases are the main cause (Wuebbles et al., 2017; IPCC, 2013). There are many other aspects of the global climate system that are changing in addition to this warming, including melting glaciers, reductions in snow cover and sea ice, sea level rise, ocean acidification, and increases in the frequency or intensity of many types of extreme weather events (Wuebbles et al., 2017). The magnitude of future

climate change, globally and regionally, and in terms of both temperature increases and these other types of associated effects, will depend primarily on the amount of greenhouse gases emitted globally (Wuebbles et al., 2017; IPCC, 2013).

Comprehensively assessing the role of anthropogenic activity in past and future climate change, including the influence of changing tropospheric concentrations of ozone, is the mandate of the IPCC, an initiative begun in 1988 by the World Meteorological Organization (WMO) and the United Nations Environment Programme (UNEP). The IPCC supports the work of the Conference of Parties to the United Nations Framework Convention on Climate Change (UNFCCC). New IPCC reports are issued every 5 to 7 years, and the climate discussion in the 2013 Ozone ISA relied heavily on the IPCC Fourth Assessment Report (AR4), published in 2007. The next iteration, AR5 (Wuebbles et al., 2017; IPCC, 2013), reports on the key scientific advances in understanding the climate effects of ozone since AR4. This Appendix draws substantially upon AR5 in summarizing these effects, in particular Chapter 8 of AR5, on RF (Myhre et al., 2013), as well as more recent literature published subsequent to AR5.

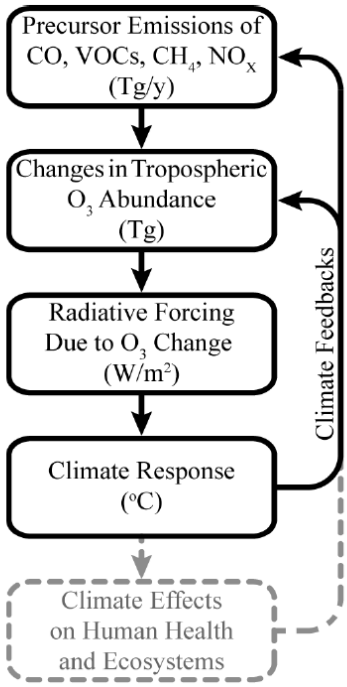
9.1.3.2 Ozone Chemistry and Role in Climate

The 2013 Ozone ISA described how tropospheric ozone differs in important ways from other greenhouse gases, in that it is not emitted directly, but is produced through photochemical oxidation of carbon monoxide (CO), CH_4, and nonmethane volatile organic compounds (VOCs) in the presence of nitrogen oxides ($NO_X = NO + NO_2$). It is also supplied by vertical transport from the stratosphere, where it is formed naturally by other chemical processes. In addition, because the lifetime of ozone in the troposphere is typically a few weeks, it is not distributed uniformly like the well-mixed GHGs (e.g., CO_2 and CH_4), but instead has an inhomogeneous distribution that also varies seasonally. See Appendix 1 for additional context.

Ozone influences the Earth's radiation budget by its longwave absorption, mainly in the 9.6 micron band, where absorption by the long-lived greenhouse gases and water vapor is relatively weak. In addition, unlike other major greenhouse gases, ozone absorbs in the shortwave as well as in the longwave part of the spectrum. This absorption leads to RF and the consequences described below.

Figure 9-1 depicts the influence of tropospheric ozone on climate. Emissions of ozone precursors including CO, VOCs, CH_4, and NO_X lead to the production of tropospheric ozone. A change in the abundance of tropospheric ozone perturbs the radiative balance of the atmosphere, an effect quantified by RF (defined in more detail in the next subsection). As discussed in the 2013 Ozone ISA, the Earth's land-atmosphere-ocean system responds to this RF with a change in climate, including a change in near-surface air temperature with associated effects on precipitation and atmospheric circulation patterns. This climate response causes downstream climate-related health and ecosystem effects. Feedbacks from both the direct climate response and such downstream effects can, in turn, affect the abundance of tropospheric ozone and ozone precursors through multiple mechanisms. This Appendix provides a brief

discussion of some of the most direct feedbacks, but the downstream effects and their longer term feedbacks are extremely complex and outside the scope of this assessment.



Note: Climate effects and their feedbacks are de-emphasized in this figure since these downstream effects are extremely complex and outside the scope of this assessment.

Figure 9-1 Schematic diagram of the effects of tropospheric ozone on climate.

9.1.3.3 Radiative Forcing

RF is a perturbation in net radiative flux at the tropopause (or top of the atmosphere) caused by a change in radiatively active forcing agent(s) after stratospheric temperatures have readjusted to radiative equilibrium (stratospherically adjusted RF) (Fiore et al., 2015; Myhre et al., 2013). It is commonly expressed in units of W/m^2. All else being equal, a positive RF results in net warming of the Earth's surface, while negative RF leads to a net cooling. Effective radiative forcing (ERF) accounts for both the effects of the forcing agent and the rapid adjustments to that forcing agent [i.e., atmospheric temperature, cloud cover, water vapor; (Myhre et al., 2013)]. While global mean RF and ERF are both important measures of climate response to radiative effects, this assessment focuses on ozone RF as an endpoint because ozone ERF estimates in the published literature are more limited, and differences between RF and

ERF for ozone tend to be small compared to existing uncertainties in RF and ERF (Myhre et al., 2013). The nonuniform distribution of ozone (spatially and temporally) also makes quantifying global and regional ozone RF challenging. Unlike RF estimates for well-mixed GHGs, which can be and are determined from observations at a few surface sites (Myhre et al., 2013), ozone RF estimates are generally calculated using a combination of general circulation model (GCM) radiative transfer parameterization schemes and more detailed line-by-line radiative transfer models. As noted above, tropospheric ozone RF is estimated to be about 25−40% of the total warming effects of anthropogenic carbon dioxide (CO_2) and about 75% of the effects of anthropogenic methane (CH_4), ranking ozone third in importance for global climate behind these two major greenhouse gases (Forster et al., 2007).

9.1.3.4 Tropospheric Ozone and Ultraviolet-B (UV-B) Shielding

UV radiation from the sun contains sufficient energy when it reaches the Earth to photolyze chemical bonds in molecules, thereby leading to damaging effects on living organisms and materials. It is well understood that stratospheric ozone plays a crucial role in reducing exposure to solar UV radiation at the Earth's surface. The question of whether tropospheric ozone has a supplemental UV-B shielding effect with significance for human health and ecosystems was considered in the 2013 Ozone ISA (as part of Chapter 10, in addition to the discussion of climate effects). The 2013 Ozone ISA concluded:

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

For the current review, a literature screening on tropospheric ozone and UV-B was conducted. This screening determined that there was no new evidence since the 2013 Ozone ISA relevant to the question of UV-B shielding by tropospheric ozone, including the incremental effects of tropospheric ozone concentration changes on UV-B. While the literature screening identified a small number of studies that examined tropospheric ozone and UV-B together in some way, these studies addressed different scientific and technical issues, such as the impact of UV-B on tropospheric ozone production [e.g., (Jasaitis et al., 2016)], or the development of improved model parameterizations for better capturing the influence of total column ozone, in combination with other input parameters, on surface UV radiation [e.g., (Lamy et al., 2018)]. Based on this review of the literature, evidence is inadequate to determine if a causal relationship exists between changes in tropospheric ozone concentrations and UV-B effects.

9.2 Ozone Impacts on Radiative Forcing

Highlights of Recent Evidence for Radiative Forcing

Changes in the abundance of tropospheric ozone affect RF. The 2013 Ozone ISA reports a RF of 0.35 W/m^2 from tropospheric ozone since preindustrial times (1750 to 2005) based on multimodel studies (Forster et al., 2007). The most recent IPCC assessment, AR5, reports tropospheric ozone RF as 0.40 (0.20 to 0.60) W/m^2 (Myhre et al., 2013), which is within range of previous assessments (i.e., AR4). There have also been a few studies since AR5, including the study of tropospheric ozone RF based on the Coupled Model Intercomparison Project Phase 6 (CMIP6) data set and the Atmospheric Chemistry and Climate Model Intercomparison Project (ACCMIP) multimodel study of tropospheric chemistry, both of which reinforce the AR5 estimates and the causal relationship between tropospheric ozone and RF.

9.2.1 Recent Evidence for Historical Period

- The AR5 best estimate of tropospheric ozone RF is 0.40 (0.20 to 0.60) W/m^2 [from 1750 to 2011; Table 9-2 (Shindell et al., 2013; Sovde et al., 2012; Skeie et al., 2011)]. There are uncertainties from inter-model spread across atmospheric models (−0.11 to 0.11 W/m^2) and differences between standalone radiative transfer models (−0.07 to 0.07 W/m^2), where all ranges represent the 95% confidence intervals (Myhre et al., 2013). One of the largest uncertainties in calculating ozone RF is estimating ozone concentrations in preindustrial times. Trends in free tropospheric ozone and upper tropospheric ozone (where RF is particularly sensitive to changes in ozone) are not captured well by models (Hu et al., 2017; Sherwen et al., 2017; Parrish et al., 2014). Uncertainties also remain in preindustrial emissions and the representation of chemical and physical processes beyond those already included in the current models. These additional model uncertainties include those associated with specific rate constants for important reactions (e.g., $NO_2 + OH \rightarrow HNO_3$ and $O_3 + NO \rightarrow NO_2 + O_2$) (Newsome and Evans, 2017) and halogen chemistry (Sherwen et al., 2017). Despite this, the IPCC AR5 overall confidence in the tropospheric ozone RF is high (Table 9-3, Figure 9-2). AR5 concluded there were no major changes in understanding of this confidence level since AR4.
- Additionally, there have been a few studies since AR5, including the study of tropospheric ozone RF based on the Coupled Model Intercomparison Project Phase 6 (CMIP6) data set (Checa-Garcia et al., 2018) and the Atmospheric Chemistry and Climate Model Intercomparison Project (ACCMIP) multimodel study of tropospheric chemistry (Conley et al., 2013; Stevenson et al., 2013), both of which reinforce the AR5 estimates. The latest individual estimates of tropospheric ozone RF, based on the CMIP6 data set for ozone, give a present-day (2000−2014 relative to 1850−1860) tropospheric ozone RF of 0.33 ± 0.17 W/m^2 (Checa-Garcia et al., 2018).Myhre et al. (2017) also recently estimated ozone RF for the 1990−2015 time period with a multimodel mean of 0.06 W/m^2, which is ~50% greater than the AR5 estimate for this same time period. The difference is likely due to an increase in NO_X [in Myhre et al. (2017)] that is more than twice that in the AR5 emission data.

Table 9-2 Contributions of tropospheric ozone changes to radiative forcing (W/m^2) from 1750 to 2011.[a]

	Troposphere Total
AR5[a]	0.40 (0.20 to 0.60)
ACCMIP (multimodel results)[b]	0.41 (0.21 to 0.61)
Shindell et al. (2013)	0.33 (0.31 to 0.35)
(Sovde et al., 2012)[c]	0.45 0.38
Skeie et al. (2011)	0.41 (0.21 to 0.61)
AR4[c]	0.35 (0.25 to 0.65)

[a]Table 9-2 is adapted from IPCC AR5 Table 8.3.

[b]Stevenson et al. (2013).

[c]0.45 based on REF chemistry, 0.38 based on R^2 chemistry, see Sovde et al. (2012).

[d]Forster et al. (2007).

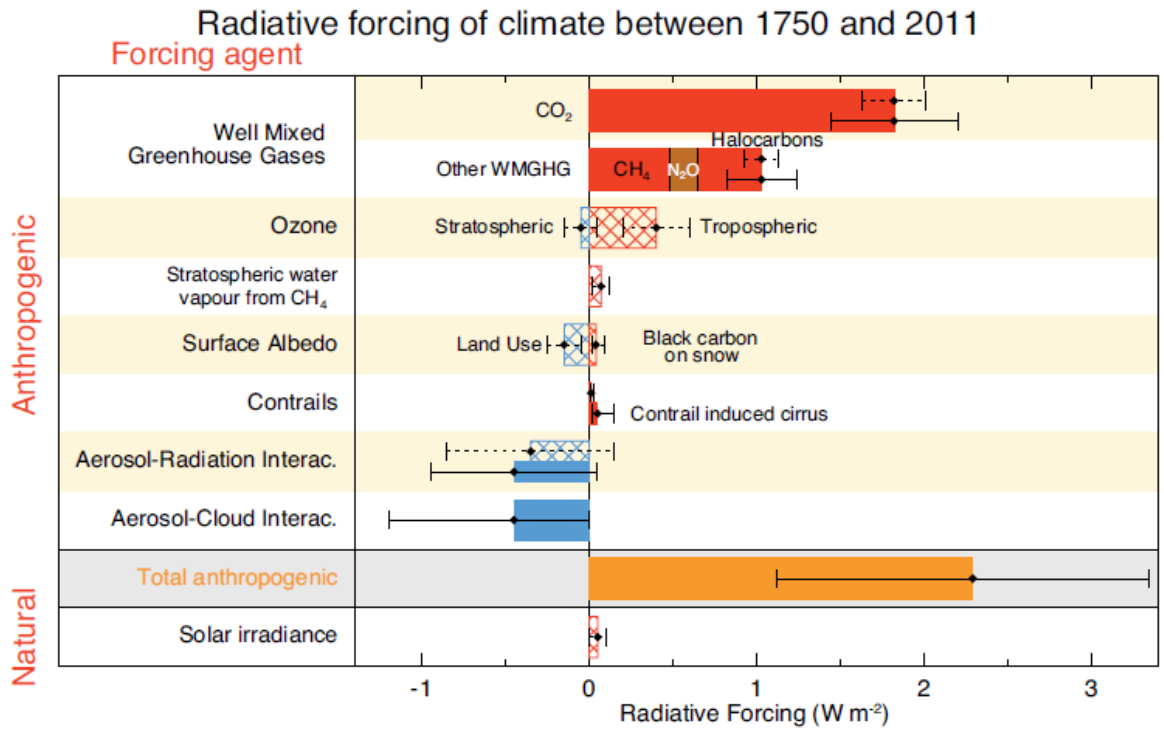
Source: Myhre et al. (2013).

Table 9-3 Confidence level for ozone forcing for the 1750−2011 period.[a]

	Evidence	Agreement	Confidence Level	Basis for Uncertainty Estimates (More Certain/Less Certain)	Change in Understanding Since AR4
Tropospheric ozone	Robust	Medium	High	Observed trends of ozone in the troposphere and model results for the industrial era/differences between model estimates of RF	No major change

[a]Table 9-3 is an abbreviated version of IPCC AR5 Table 8.5.

Source: Myhre et al. (2013).



Note: Figure 9-2 is taken from IPCC AR5 Figure 8.15.

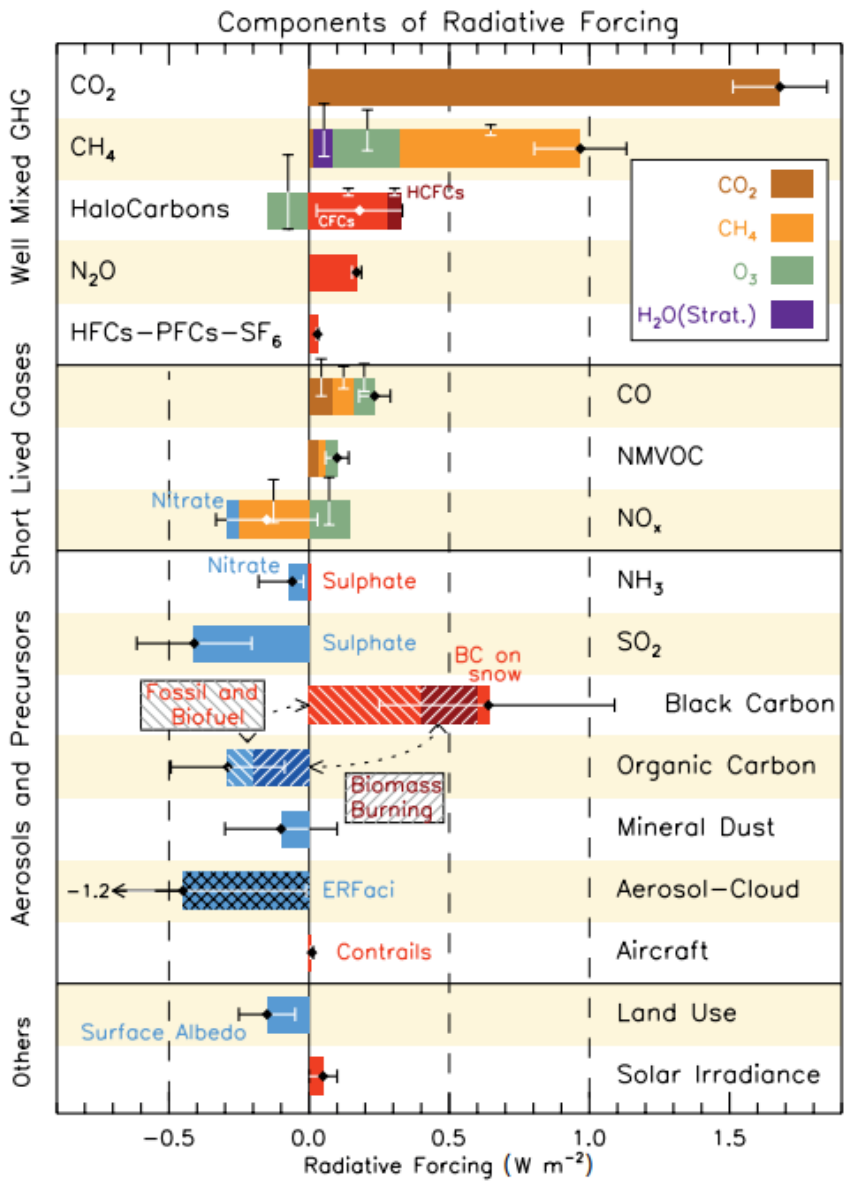
Uncertainties (5 to 95% confidence range) are given for RF (dotted lines) and ERF (solid lines). For ozone, differences between RF and ERF tend to be small compared to existing uncertainties in RF and ERF, so only RF is reported.

Source: Myhre et al. (2013).

Figure 9-2 Bar chart for radiative forcing (RF; hatched) and effective radiative forcing (ERF; solid) for the period 1750−2011.

- Ozone-depleting substances (ODSs) also affect tropospheric ozone. Recent simulations with the NCAR-CAM3.5 model show that ODSs contribute an ozone RF of −0.15 (−0.3 to 0.0) W/m^2, some of which is in the troposphere, and tropospheric ozone precursors contribute an ozone RF of 0.50 (0.30 to 0.70) W/m^2, some of which is in the stratosphere (Myhre et al., 2013; Shindell et al., 2013).
- Instantaneous radiative kernels (IRKs), which represent the sensitivity of top-of-the-atmosphere (TOA) radiative flux to each observed (satellite) ozone profile, have been used to estimate all-sky global average TOA longwave radiative effect (LWRE) of tropospheric ozone as 0.33 ± 0.02 W/m^2 (Worden et al., 2011). The LWRE due to ozone is computed with respect to the TOA radiative flux as observed from space by the Aura-Tropospheric Emission Spectrometer (TES); this is distinguished from the IPCC AR5 RF definition, which represents the difference in total irradiance at the tropopause due to changes between preindustrial and present tropospheric ozone concentrations. More recently, Bowman et al. (2013) applied IRKs and the ACCMIP model results to estimate a multimodel mean tropospheric ozone RF of 0.39 ± 0.042 W/m^2 (1 standard deviation).
- Tropospheric ozone concentrations and RF are also sensitive to changes in ozone precursor emissions, which can alter the radiative balance of the atmosphere—sometimes in competing

ways. Ozone and its precursors exert a strong control on the oxidizing capacity of the troposphere, thereby affecting the lifetime of CH_4 and other gases (Derwent et al., 2001), with further implications for RF and climate. CO and VOC emissions exert an overall positive RF (warming) by increasing tropospheric ozone and CH_4 concentrations. NO_X emissions contribute a positive RF by increasing tropospheric ozone, but exert a negative RF by lowering global CH_4 (via hydroxyl radical [OH] increases) and increasing nitrate aerosols. VOC emissions also contribute to organic particulate matter production, which influences RF. CH_4 is itself a powerful greenhouse gas and a precursor to ozone, leading to further warming (Fiore et al., 2015). Short-lived ozone precursors (CO, VOCs, and NO_X) influence ozone RF globally and regionally (Fry et al., 2012) and additionally can affect its seasonality (Bellouin et al., 2016). Figure 9-3, from the IPCC AR5, summarizes the magnitude of direct RF over the industrial era associated with ozone precursor species themselves, as well as their indirect RF due to their influence on ozone concentrations (Myhre et al., 2013).



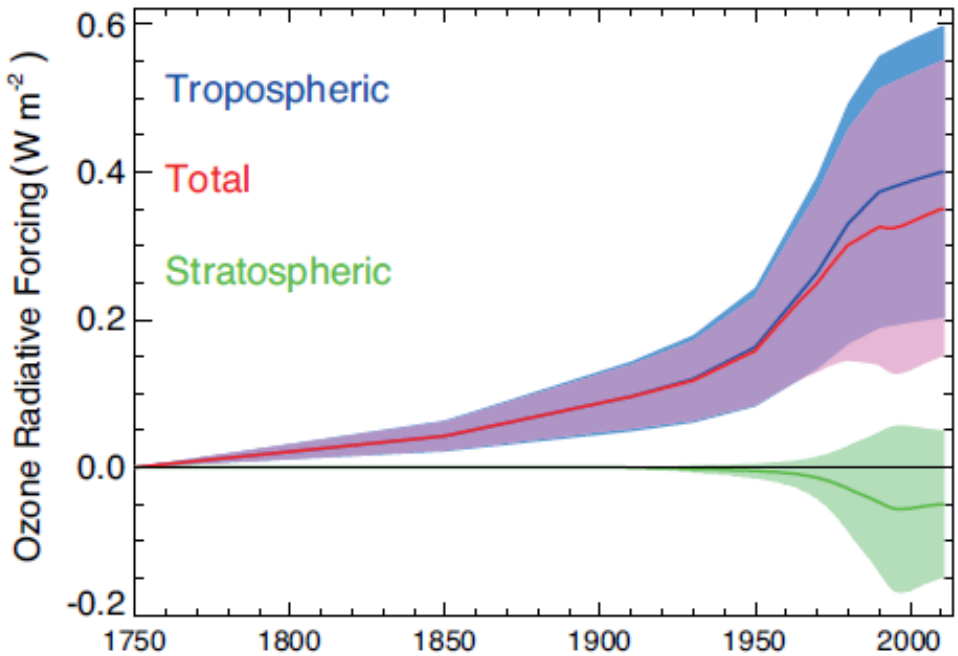
Note: Figure 9-3 is taken from IPCC AR5 Figure 8.17.

Source: Myhre et al. (2013).

Figure 9-3 Radiative forcing (RF) over the industrial era associated with emitted compounds, including ozone (green bars) and its precursors.

9.2.2 Recent Evidence of Radiative Forcing Temporal and Spatial Trends

- Recent evidence from AR5 also provides the temporal trends of ozone RF. Tropospheric ozone RF increased slowly before 1950, grew rapidly from 1950 to 1990, and increased slowly again after 1990, matching the trends in anthropogenic ozone precursor emissions (Figure 9-4). Changes in NO_X emissions related to traffic and industry, for example, contributed to increasing ozone RF trends over recent decades (Dahlmann et al., 2011). In general, changes in NO_X, CO, and VOC emissions affect tropospheric ozone on short time scales (days to months), while CH_4-induced changes in tropospheric ozone (via CH_4 oxidation) occur on decadal timescales (Fiore et al., 2015).

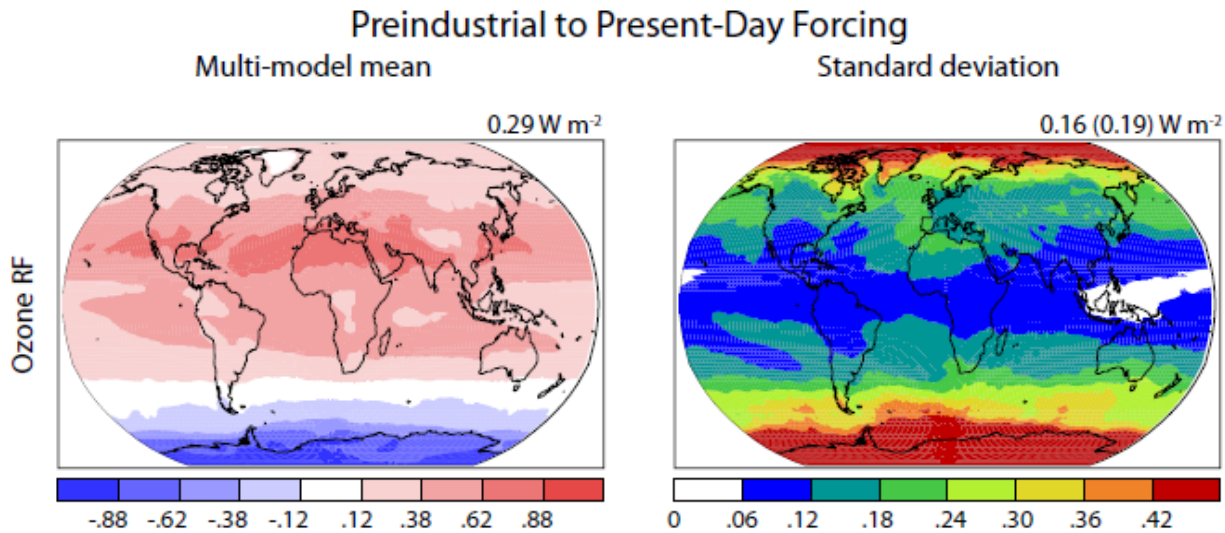


Note: Figure 9-4 is taken from IPCC AR5 Figure 8.7.
Note: Stratospheric ozone radiative forcing is also shown but is not discussed as part of this Appendix.
Source: Myhre et al. (2013).

Figure 9-4 Time evolution of the radiative forcing (RF) from tropospheric ozone from 1750 to 2010.

- IPCC has also examined future RF trends using multimodel estimates, with the magnitude of future ozone RF effects consistent with our general understanding of the relationship between RF and ozone concentrations described above. As shown in AR5, future ozone RF projections are contingent on the specific emissions scenarios chosen for the model simulations (Myhre et al., 2013).

- The spatial distribution of net ozone RF (1850 to 2000) is depicted in Figure 9-5 based on the ACCMIP models, with the greatest ozone RF occurring in subtropical latitudes. The positive tropospheric ozone forcing in the Northern Hemisphere is associated with increases in tropospheric ozone, while the negative forcing in the Southern Hemisphere's polar region is related to stratospheric ozone loss. The ACCMIP models also show the largest standard deviation in the polar regions, where lower stratosphere/upper troposphere changes vary among models (Young et al., 2013). Shifts in global tropospheric ozone concentrations may be driven most strongly by the spatial distribution of anthropogenic emissions (1980 to 2010), compared to changes in the overall magnitude of emissions and global CH_4 concentrations (Zhang et al., 2016).
- AR5 rates the confidence in the spatial distribution of ozone RF as medium (lower than global mean ozone RF) because of the large regional standard deviations between models.



Note: Figure 9-5 is taken from IPCC AR5 Figure 8.23, fourth row.

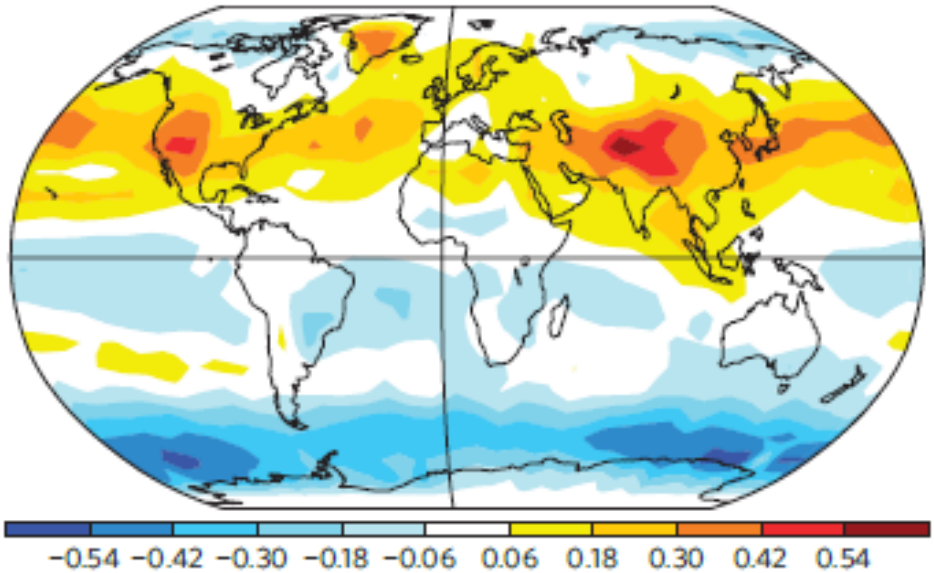
Note: Values are the average of the area-weighted global means (11 models), with the area-weighted mean of the standard deviation of models at each point provided in parenthesis.

Source: Myhre et al. (2013).

Figure 9-5 Radiative forcing (RF) spatial distribution of 1850 to 2000 ozone RF among the atmospheric chemistry and Climate Model Intercomparison Project models, mean values (left) and standard deviation (right).

- To address present-day model biases and improve spatial distribution estimates, modeled ozone RF distributions can be adjusted by vertical information retrieved from the Tropospheric Emission Spectrometer (TES) (Shindell et al., 2013). For example, Figure 9-6 shows the difference in the annual average RF between modeled (GISS-E2-R) and observed TES present-day (2005−2009) total natural plus anthropogenic ozone throughout the atmosphere. The global mean tropospheric ozone RF difference is 0.016 W/m^2. Shindell et al. (2013) found good

agreement between the modeled and observed global mean RFs, in part due to a cancellation of positive biases in the Northern Hemisphere and negative biases in the Southern Hemisphere.



Note: Figure 9-6 is taken from Shindell et al. (2013), Figure 3.
Source: Shindell et al. (2013).

Figure 9-6 Difference in annual average radiative forcing (W/m^2) between modeled (GISS-E2-R) and observed Tropospheric Emission Spectrometer present-day (2005−2009) total natural plus anthropogenic ozone throughout the atmosphere.

9.2.3 Summary and Causality Determination

Recent evidence continues to indicate a **causal relationship between tropospheric ozone and RF** as concluded in the 2013 Ozone ISA (Table 9-4). The new evidence comes from the IPCC AR5 (Myhre et al., 2013) and its supporting references—in addition to a few more recent studies—and builds on evidence presented in the 2013 Ozone ISA. None of the new studies indicate a change to the causality determination included in the 2013 Ozone ISA.

- The AR5 best estimate of tropospheric ozone RF is 0.40 (0.20 to 0.60) W/m^2 (from 1750 to 2011) [Table 9-2; (Myhre et al., 2013)]. The overall confidence in the tropospheric ozone RF is high (Table 9-3, Figure 9-2). Additionally, there have been a few studies since AR5, including the study of tropospheric ozone RF based on the CMIP6 data set (Checa-Garcia et al., 2018) and the

ACCMIP multimodel study of tropospheric chemistry (Conley et al., 2013; Stevenson et al., 2013), both of which reinforce the AR5 estimates.

- Tropospheric ozone concentrations and RF are sensitive to changes in ozone precursor emissions. Ozone precursors themselves exert a strong control on the oxidizing capacity of the troposphere and can alter the radiative balance of the atmosphere—sometimes in competing ways. Figure 9-3 from the IPCC AR5 summarizes the magnitude of RF over the industrial era associated with ozone precursor species (Myhre et al., 2013).
- Evidence from AR5 and more recent literature addresses temporal trends and spatial patterns of ozone RF. Tropospheric ozone RF increased slowly before 1950, grew rapidly from 1950 to 1990, and increased slowly again after 1990, matching the trends in anthropogenic ozone precursor emissions (Figure 9-4). Spatially, ozone RF is estimated to be highest in the Northern Hemisphere, in association with spatial emissions patterns.
- One of the largest contributors to uncertainty in ozone RF is estimating preindustrial ozone concentrations. In addition, trends in free tropospheric ozone (above atmospheric boundary layer) and upper tropospheric ozone (where RF is particularly sensitive to changes in ozone) are not captured well by models. Uncertainties also remain in preindustrial emissions and the representation of chemical and physical processes beyond those included in the current models, such as specific rate constants and halogen chemistry. Despite these uncertainties, the overall confidence in current estimates of tropospheric ozone RF is high (Table 9-3).
- Further research in the following areas can help in address remaining uncertainties. These areas include improving (1) the quantification of observed trends in ozone concentrations in the free troposphere, upper troposphere, and remote regions; (2) the understanding of the CH_4 budget and of ozone coupling with temperature, water vapor, and clouds (with implications for the height- and latitude-dependence of ozone RF); and (3) the estimates of ozone spatio-temporal structure developed using global models constrained by observations.

Table 9-4 Summary of evidence for a causal relationship between tropospheric ozone and radiative forcing.

Rationale for Causality Determination	Key Evidence	Key References
Consistent evidence from multiple, high-quality studies	Multidecadal, global chemistry-climate modeling ensemble studies constrained by historical observations of ozone concentrations (e.g., IPCC AR5; ACCMIP; CMIP6)	Myhre et al. (2013); Section 9.2.1
Robust physical understanding	Robust, well-understood relationship between tropospheric ozone concentration and RF	Myhre et al. (2013); Section 9.2.1; Section 9.1.3.3
Spatial/temporal effect correspondence	Temporal trends in ozone RF match the historical trends in anthropogenic ozone precursor emissions; spatially, largest effects seen in the subtropics, again consistent with observed emissions patterns	Myhre et al. (2013); Section 9.2.2

9.3 Ozone Impacts on Temperature, Precipitation, and Related Climate Variables

Highlights of Recent Evidence for Impacts on Temperature, Precipitation, and Related Climate Variables

Consistent with previous estimates, the effect of tropospheric ozone on global surface temperature, through its impact on RF, continues to be estimated at roughly 0.1−0.3°C since preindustrial times (Xie et al., 2016; Myhre et al., 2013), with larger effects regionally. In addition to temperature, tropospheric ozone changes have impacts on other climate metrics such as precipitation and atmospheric circulation patterns (Macintosh et al., 2016; Allen et al., 2012; Shindell et al., 2012a). While the warming effect of tropospheric ozone in the climate system is well established in general, precisely quantifying changes in surface temperature due to tropospheric ozone changes, along with related climate effects, requires complex climate simulations, including important feedbacks and interactions. Current limitations in climate modeling tools, variation across models, and the need for more comprehensive observational data on these effects represent sources of uncertainty in quantifying the precise magnitude of climate responses to ozone changes, particularly at regional scales (Myhre et al., 2013). All of this evidence reinforces the likely-to-be-causal relationship between tropospheric ozone and temperature, precipitation, and related climate variables (referred to as "climate change" in the 2013 Ozone ISA).

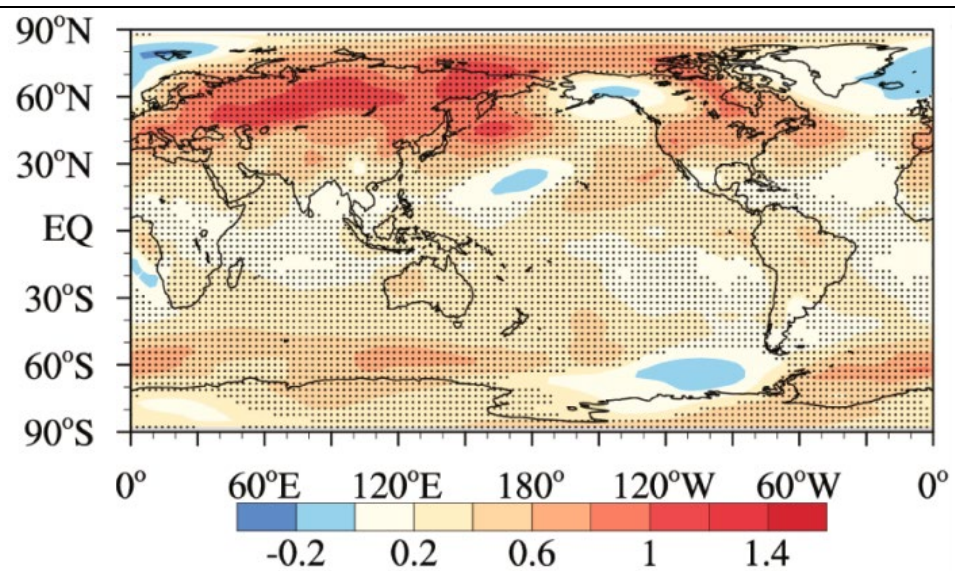
As described above, estimates of RF provide a first-order metric of the effect of tropospheric ozone on climate. The Earth's land-atmosphere-ocean system then responds to this RF with a change in climate, beginning with changes in near-surface air temperature, and/or atmospheric radiative heating/cooling, followed by "downstream" impacts on other climate variables, including changes in precipitation and shifts in atmospheric circulation patterns. Even further downstream effects resulting from these changes in climatic conditions occur as well, for example, on ecosystems, and may in turn create complex climate system feedbacks, including those with potential impacts on tropospheric ozone concentrations, as summarized in the 2013 Ozone ISA (Figure 9-1). A comprehensive accounting of all possible Earth system impacts of ozone-induced climate change and associated potential feedback loops, however, is beyond the scope of this review.

9.3.1 Recent Evidence for Effects on Temperature

- Literature cited in IPCC AR4, and referenced in the 2013 Ozone ISA, estimated that the increase of tropospheric ozone abundance since 1750 has likely contributed roughly 0.1−0.3°C warming to the global climate (in the context of the roughly 1.0°C total warming from preindustrial times to the present).

- Literature cited in IPCC AR5 (Myhre et al., 2013) continues to be consistent with these earlier estimates of the effect of tropospheric ozone on global surface temperature. Xie et al. (2016), in a more recent modeling study consisting of a series of 15-year simulations with a global coupled chemistry-climate model, found a similar increase in global- and annual-mean surface temperature of 0.36°C (averaged over the last 10 years of each simulation) from preindustrial times to the present (i.e., calculated as the difference in tropospheric ozone concentration between 1850 and 2013).

- Regional temperature effects may be larger. For example, earlier modeling studies indicated that increased tropospheric ozone over the second half of the 20th century has caused proportionally more warming in the Northern Hemisphere than globally, particularly in the Arctic and in continental interiors (Chang et al., 2009; Shindell and Faluvegi, 2009; Shindell et al., 2006; Mickley et al., 2004). More recent evidence from Xie et al. (2016) found stronger surface temperature increases over the high latitudes in both hemispheres, with the maximum increase exceeding 1.4°C in Siberia (Figure 9-7). This type of regional warming pattern (e.g., Arctic amplification) is broadly similar to that associated with other radiative forcing agents, including well-mixed GHGs.



Note: Figure 9-7 is taken from Xie et al. (2016), Figure 4a.
Source: Xie et al. (2016).

Figure 9-7 Mean annual change in surface temperature (°C) resulting from tropospheric ozone concentration changes from 1850−2013.

- Idealized modeling studies also support this basic magnitude of the impact of ozone RF on global and regional temperatures (Huszar et al., 2012; Yang et al., 2012).

- New evidence from recent modeling studies find that the uneven spatial distribution of RF from historical changes in both aerosols and tropospheric ozone leads to stronger climate response per

unit of global-mean RF than for the well-mixed GHGs [globally and in the Northern Hemisphere extratropics; (Shindell et al., 2015; Shindell, 2014)]. This enhanced sensitivity occurs because most of this RF is located in Northern Hemisphere extratropical latitudes where it triggers more rapid land responses and stronger feedbacks.

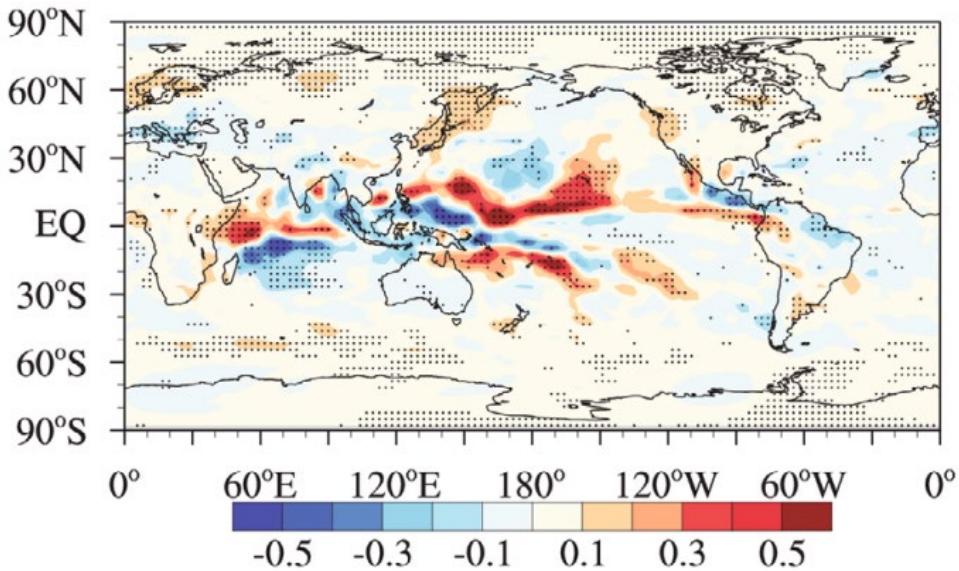
- As described in detail in the 2013 Ozone ISA and the IPCC assessments, however, the heterogeneous distribution of ozone in the troposphere complicates the direct attribution of spatial patterns of temperature change to ozone-induced RF (e.g., horizontal gradients in ozone RF and resulting induced heat transport may weaken the correlation between local RF and local temperature response). Such effects may also create ozone-climate feedbacks that further alter the relationship between ozone RF and temperature (and other climate variables) in complex ways (Fiore et al., 2015). In addition, the vertical distribution of ozone in the troposphere is also nonuniform, which may influence atmospheric stability and convection and cloud processes, leading to further effects on climate and the potential for additional feedbacks.
- Quantifying the climate effects of tropospheric ozone requires complex climate simulations that include the important feedbacks and interactions discussed above. Current limitations in climate modeling tools, variation across models, and the need for more comprehensive observational data on these effects represent sources of uncertainty in quantifying the precise magnitude of climate responses to ozone changes, particularly at regional scales (Myhre et al., 2013). These are in addition to the key sources of uncertainty in quantifying ozone RF changes, such as emissions over the time period of interest and baseline ozone concentrations during preindustrial times.
- As discussed in the 2013 Ozone ISA, future trends in tropospheric ozone concentrations, and therefore, effects on RF and climate, depend largely on what emissions pathway the world follows in the coming decades, as discussed in the IPCC AR5 (Myhre et al., 2013). Such ozone effects trends will also depend on changes in a suite of climate-sensitive factors, such as the water vapor content of the atmosphere. From the 2013 Ozone ISA: "*Several studies have included tropospheric ozone in their investigations of the response in the future atmosphere to a suite of short-lived species [e.g., (*Levy et al., 2008*;* Shindell et al., 2008*;* Shindell et al., 2007*)]. Few studies, however, have calculated the climate response to changes in tropospheric ozone concentrations alone in the future atmosphere.*" This conclusion remains the case with the current literature (Myhre et al., 2013).

9.3.2 Recent Evidence for Other Climate Effects

Some new research has explored certain additional aspects of the climate response to ozone RF beyond global and regional temperature change. Specifically, ozone changes are understood to have impacts on other climate metrics such as precipitation and atmospheric circulation patterns, and new evidence has continued to support and further quantify this understanding. This new evidence is limited to a relatively small number of studies, however, leaving various uncertainties still to be resolved. While less work has been done on ozone, recent work on understanding impacts on precipitation from other heterogeneously distributed radiative forcers, such as aerosols (Liu et al., 2018; Westervelt et al., 2018), could improve estimates of ozone RF effects on precipitation going forward.

9.3.2.1 Precipitation

- The Xie et al. (2016) study, cited above for temperature, also examined precipitation. The study authors found in their model simulations that the difference in tropospheric ozone concentration between 1850 and 2013 caused an increase of 0.02 mm/day in global-average precipitation, with regional shifts in precipitation patterns near the equator (Figure 9-8).



Note: Figure 9-8 is taken from Xie et al. (2016), Figure 4c.
Source: Xie et al. (2016).

Figure 9-8 Mean annual change in precipitation (mm/day) resulting from tropospheric ozone concentration changes from 1850−2013.

- Additional modeling studies have examined the relative influence of ozone on precipitation compared with well-mixed GHGs. Using simple model calculations of the time-dependent precipitation change due to an RF perturbation, Macintosh et al. (2016) found that the contribution of ozone change from 1765−2011 to precipitation change over that period could exceed 50% of that due to CO_2 change (including both stratospheric and tropospheric ozone, though the bulk of the RF change is from tropospheric ozone). Shindell et al. (2012b) also found that both ozone and aerosol RF typically induce larger precipitation responses than the equivalent CO_2 forcing, and that these spatially heterogeneous forcers are therefore potentially disruptive to the hydrologic cycle at regional scales.

9.3.2.2 Atmospheric Circulation

- The climate modeling study of Allen et al. (2012) concluded that RF due to increases in black carbon and tropospheric ozone were the most likely causes of an observed increase in the width of the tropical belt and associated poleward shift in the Northern Hemisphere extratropical storm tracks over the last few decades. This result is uncertain, however, because other studies have found that increases in well-mixed GHGs alone can account for this widening of the tropical belt and storm track shift (Lau and Kim, 2015).

9.3.3 Summary and Causality Determination

Recent evidence is sufficient to conclude that there is a **likely to be causal relationship between tropospheric ozone and temperature, precipitation, and related climate variables** concluded in the 2013 Ozone ISA (Table 9-5) (referred to as "climate change" in the 2013 Ozone ISA). The new evidence comes from the IPCC AR5 (Myhre et al., 2013) and its supporting references—in addition to a few more recent studies—and builds on evidence presented in the 2013 Ozone ISA. None of the new studies indicate a change to the causality determination included in the 2013 Ozone ISA.

- Consistent with previous estimates, the effect of tropospheric ozone on global surface temperature continues to be estimated at roughly 0.1−0.3°C since preindustrial times (Xie et al., 2016; Myhre et al., 2013), with larger effects regionally. In addition to temperature, ozone changes have impacts on other climate metrics such as precipitation and atmospheric circulation patterns (Macintosh et al., 2016; Allen et al., 2012; Shindell et al., 2012a).
- Various uncertainties render the precise magnitude of the overall effect of tropospheric ozone on climate more uncertain than that of the well-mixed GHGs (Myhre et al., 2013). These include the remaining uncertainties in the magnitude of RF estimated to be attributed to tropospheric ozone. In addition, precisely quantifying the change in surface temperature (and other climate variables) due to tropospheric ozone requires complex climate simulations that include relevant feedbacks and interactions. Current limitations in climate modeling tools, variation across models, and the need for more comprehensive observational data on these effects represent sources of uncertainty in quantifying the precise magnitude of climate responses to ozone changes, particularly at regional scales (Myhre et al., 2013).
- Even with these uncertainties, however, evidence from climate models indicates that tropospheric ozone changes have likely contributed to observed increases in global mean and regional surface temperatures.

Further research in the following areas can help address these remaining uncertainties, which include quantifying a more precise relationship between regional ozone RF and regional climate change; improving understanding of the impacts of heterogeneously distributed RF, including from ozone, aerosols, and other short-lived climate forcers, on the hydrologic cycle, precipitation, and atmospheric circulation patterns; improving understanding of, and ability to model, critical ozone-climate feedbacks; and continuing exploration of links between precursor pollutant control strategies, climate, and ozone concentrations.

Table 9-5 Summary of evidence for a likely to be causal relationship between ozone and temperature, precipitation, and related climate variables.

Rationale for Causality Determination	Key Evidence	Key References
Consistent evidence from multiple, high-quality studies	Temperature: Multidecadal, global chemistry-climate modeling ensemble studies constrained by historical observations of ozone concentrations; not as many such studies as for RF	Xie et al. (2016); Myhre et al. (2013)
	Other climate effects (precipitation, atmospheric circulation): Multidecadal, global chemistry-climate modeling ensemble studies constrained by historical observations of ozone concentrations; only a limited number of such modeling studies for these other climate effects	Xie et al. (2016); Allen et al. (2012)
Robust physical understanding	Temperature: Robust, well-understood relationship between RF and atmospheric and surface temperatures	Huszar et al. (2012); Yang et al. (2012); Xie et al. (2016); Myhre et al. (2013); Fiore et al. (2015)
	Other climate effects (precipitation, atmospheric circulation): Good understanding of the potential ways ozone RF and temperature effects further influence atmospheric thermodynamics and dynamics; however, multiple complex interactions and feedbacks confound precise quantification of the magnitude of the ozone effect	Xie et al. (2016); Macintosh et al. (2016); Allen et al. (2012); Shindell et al. (2012a)
Spatial/temporal effect correspondence	Temperature: Largest effects seen in the Northern Hemisphere's middle and high latitudes, consistent with observed patterns of pollutant emissions combined with climate dynamical processes that lead to Arctic amplification of temperature responses to RF	Xie et al. (2016); Myhre et al. (2013); Shindell and Faluvegi (2009); Shindell et al. (2015); Shindell (2014)
	Other climate effects (precipitation, atmospheric circulation): Spatial correspondence not as strong with these other climate effects, due to complex interactions and feedbacks in the climate system at multiple space and time scales; more limited studies to date	Xie et al. (2016); Allen et al. (2012)

9.4 References

Allen, RJ; Sherwood, SC; Norris, JR; Zender, CS. (2012). Recent Northern Hemisphere tropical expansion primarily driven by black carbon and tropospheric ozone. Nature 485: 350-354. http://dx.doi.org/10.1038/nature11097.

Bellouin, N; Baker, L; Hodnebrog, O; Olivie, D; Cherian, R; Macintosh, C; Samset, B; Esteve, A; Aamaas, B; Quaas, J; Myhre, G. (2016). Regional and seasonal radiative forcing by perturbations to aerosol and ozone precursor emissions. Atmos Chem Phys 16: 13885-13910. http://dx.doi.org/10.5194/acp-16-13885-2016.

Bowman, KW; Shindell, DT; Worden, HM; Lamarque, JF; Young, PJ; Stevenson, DS; Qu, Z; De La torre, M; Bergmann, D; Cameron-Smith, PJ; Collins, WJ; Doherty, R; Dalsøren, SB; Faluvegi, G; Folberth, G; Horowitz, LW; Josse, BM; Lee, YH; Mackenzie, IA; Myhre, G; Nagashima, T; Naik, V; Plummer, DA; Rumbold, ST; Skeie, RB; Strode, SA; Sudo, K; Szopa, S; Voulgarakis, A; Zeng, G; Kulawik, SS; Aghedo, AM; Worden, J. R. (2013). Evaluation of ACCMIP outgoing longwave radiation from tropospheric ozone using TES satellite observations. Atmos Chem Phys 13: 4057-4072. http://dx.doi.org/10.5194/acp-13-4057-2013.

Chang, W; Liao, H; Wang, H. (2009). Climate responses to direct radiative forcing of anthropogenic aerosols, tropospheric ozone, and long-lived greenhouse gases in eastern China over 1951-2000. Adv Atmos Sci 26: 748-762. http://dx.doi.org/10.1007/s00376-009-9032-4.

Checa-Garcia, R; Hegglin, MI; Kinnison, D; Plummer, DA; Shine, KP. (2018). Historical tropospheric and stratospheric ozone radiative forcing using the CMIP6 database. Geophys Res Lett 45: 3264-3273. http://dx.doi.org/10.1002/2017GL076770.

Conley, AJ; Lamarque, JF; Vitt, F; Collins, WD; Kiehl, J. (2013). PORT, a CESM tool for the diagnosis of radiative forcing. GMD 6: 469-476. http://dx.doi.org/10.5194/gmd-6-469-2013.

Dahlmann, K; Grewe, V; Ponater, M; Matthes, S. (2011). Quantifying the contributions of individual NO(x) sources to the trend in ozone radiative forcing. Atmos Environ 45: 2860-2868. http://dx.doi.org/10.1016/j.atmosenv.2011.02.071.

Derwent, RG; Collins, WJ; Johnson, CE; Stevenson, DS. (2001). Transient behaviour of tropospheric ozone precursors in a global 3-D CTM and their indirect greenhouse effects. Clim Change 49: 463-487. http://dx.doi.org/10.1023/A:1010648913655.

Fiore, AM; Naik, V; Leibensperger, EM. (2015). Air quality and climate connections [Review]. J Air Waste Manag Assoc 65: 645-685. http://dx.doi.org/10.1080/10962247.2015.1040526.

Forster, P; Ramaswamy, V; Artaxo, P; Berntsen, T; Betts, R; Fahey, DW; Haywood, J; Lean, J; Lowe, DC; Myhre, G; Nganga, J; Prinn, R; Raga, G; Schultz, M; Van Dorland, R. (2007). Changes in atmospheric constituents and in radiative forcing. In Climate change 2007: The physical science basis Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (pp. 129-234). Cambridge, United Kingdom: Cambridge University Press. http://www.ipcc.ch/pdf/assessment-report/ar4/wg1/ar4-wg1-chapter2.pdf.

Fry, MM; Naik, V; West, JJ; Schwarzkopf, MD; Fiore, AM; Collins, WJ; Dentener, FJ; Shindell, DT; Atherton, C; Bergmann, D; Duncan, BN; Hess, P; Mackenzie, I; Marmer, E; Schultz, MG; Szopa, S; Wild, O; Zeng, G. (2012). The influence of ozone precursor emissions from four world regions on tropospheric composition and radiative climate forcing. J Geophys Res 117: n/a-n/a. http://dx.doi.org/10.1029/2011JD017134.

Gauss, M; Myhre, G; Isaksen, ISA; Grewe, V; Pitari, G; Wild, O; Collins, WJ; Dentener, FJ; Ellingsen, K; Gohar, LK; Hauglustaine, DA; Iachetti, D; Lamarque, JF; Mancini, E; Mickley, LJ; Prather, MJ; Pyle, JA; Sanderson, MG; Shine, KP; Stevenson, DS; Sudo, K; Szopa, S; Zeng, G. (2006). Radiative forcing since preindustrial times due to ozone change in the troposphere and the lower stratosphere. Atmos Chem Phys 6: 575-599. http://dx.doi.org/10.5194/acp-6-575-2006.

Hansen, J; Sato, M; Ruedy, R; Nazarenko, L; Lacis, A; Schmidt, GA; Russell, G; Aleinov, I; Bauer, M; Bauer, S; Bell, N; Cairns, B; Canuto, V; Chandler, M; Cheng, Y; Del Genio, A; Faluvegi, G; Fleming, E; Friend, A; Hall, T; Jackman, C; Kelley, M; Kiang, N; Koch, D; Lean, J; Lerner, J; Lo, K; Menon, S; Miller, R; Minnis, P; Novakov, T; Oinas, V; Perlwitz, J; Perlwitz, J; Rind, D; Romanou, A; Shindell, D; Stone, P; Sun, S; Tausnev, N; Thresher, D; Wielicki, B; Wong, T; Yao, M; Zhang, S. (2005). Efficacy of climate forcings. J Geophys Res 110: D18104. http://dx.doi.org/10.1029/2005JD005776.

Hu, L; Jacob, DJ; Liu, X; Zhang, Y; Zhang, L; Kim, PS; Sulprizio, MP; Yantosca, RM. (2017). Global budget of tropospheric ozone: Evaluating recent model advances with satellite (OMI), aircraft (IAGOS), and ozonesonde observations. Atmos Environ 167: 323-334. http://dx.doi.org/10.1016/j.atmosenv.2017.08.036.

Huszar, P; Miksovsky, J; Pisoft, P; Belda, M; Halenka, T. (2012). Interactive coupling of a regional climate model and a chemical transport model: evaluation and preliminary results on ozone and aerosol feedback. Clim Res 51: 59-88. http://dx.doi.org/10.3354/cr01054.

IPCC (Intergovernmental Panel on Climate Change). (2013). Climate change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change. In TF Stocker; D Qin; GK Plattner; MMB Tignor; SK Allen; J Boschung; A Nauels; Y Xia; V Bex; PM Midgley (Eds.). Cambridge, UK: Cambridge University Press. http://www.ipcc.ch/report/ar5/wg1/.

Jasaitis, D; Vasiliauskiene, V; Chadysiene, R; Peciuliene, M. (2016). Surface Ozone Concentration and Its Relationship with UV Radiation, Meteorological Parameters and Radon on the Eastern Coast of the Baltic Sea. Atmosphere (Basel) 7. http://dx.doi.org/10.3390/atmos7020027.

Lamy, K; Portafaix, T; Brogniez, C; Godin-Beekmann, S; Bencherif, H; Morel, B; Pazmino, A; Metzger, JM; Auriol, F; Deroo, C; Duflot, V; Goloub, P; Long, CN. (2018). Ultraviolet radiation modelling from ground-based and satellite measurements on Reunion Island, southern tropics. Atmos Chem Phys 18: 227-246. http://dx.doi.org/10.5194/acp-18-227-2018.

Lau, WK; Kim, KM. (2015). Robust Hadley Circulation changes and increasing global dryness due to CO2 warming from CMIP5 model projections. Proc Natl Acad Sci USA 112: 3630-3635. http://dx.doi.org/10.1073/pnas.1418682112.

Levy, H; Schwaarzkopf, MD; Horowitz, L; Ramaswamy, V; Findell, KL. (2008). Strong sensitivity of late 21st century climate to projected changes in short-lived air pollutants. J Geophys Res 113: D06102. http://dx.doi.org/10.1029/2007JD009176.

Liao, H; Seinfeld, JH; Adams, PJ; Mickley, LJ. (2004). Global radiative forcing of coupled tropospheric ozone and aerosols in a unified general circulation model. J Geophys Res 109: D16207. http://dx.doi.org/10.1029/2003JD004456.

Liu, L; Shawki, D; Voulgarakis, A; Kasoar, M; Samset, BH; Myhre, G; Forster, PM; Hodnebrog, O; Sillmann, J; Aalbergsjo, SG; Boucher, O; Faluvegi, G; Iversen, T; Kirkevag, A; Lamarque, JF; Olivie, D; Richardson, T; Shindell, D; Takemura, T. (2018). A PDRMIP multimodel study on the impacts of regional aerosol forcings on global and regional precipitation. J Clim 31: 4429-4447. http://dx.doi.org/10.1175/JCLI-D-17-0439.1.

Macintosh, CR; Allan, RP; Baker, LH; Bellouin, N; Collins, W; Mousavi, Z; Shine, KP. (2016). Contrasting fast precipitation responses to tropospheric and stratospheric ozone forcing. Geophys Res Lett 43: 1263-1271. http://dx.doi.org/10.1002/2015GL067231.

Madronich, S; Wagner, M; Groth, P. (2011). Influence of tropospheric ozone control on exposure to ultraviolet radiation at the surface. Environ Sci Technol 45: 6919-6923. http://dx.doi.org/10.1021/es200701q.

Marenco, A; Gouget, H; Nédélec, P; Pagés, JP; Karcher, F. (1994). Evidence of a long-term increase in tropospheric ozone from Pic du Midi data series: Consequences: Positive radiative forcing. J Geophys Res 99: 16617-16632. http://dx.doi.org/10.1029/94JD00021.

Mickley, LJ; Jacob, DJ; Field, BD; Rind, D. (2004). Climate response to the increase in tropospheric ozone since preindustrial times: A comparison between ozone and equivalent CO2 forcings. J Geophys Res 109: D05106. http://dx.doi.org/10.1029/2003JD003653.

Mickley, LJ; Murti, PP; Jacob, DJ; Logan, JA; Koch, DM; Rind, D. (1999). Radiative forcing from tropospheric ozone calculated with a unified chemistry-climate model. J Geophys Res 104: 30153-30172. http://dx.doi.org/10.1029/1999JD900439.

Myhre, G; Aas, W; Cherian, R; Collins, W; Faluvegi, G; Flanner, M; Forster, P; Hodnebrog, O; Klimont, Z; Lund, MT; Muelmenstaedt, J; Myhre, CL; Olivie, D; Prather, M; Quaas, J; Samset, BH; Schnell, JL; Schulz, M; Shindell, D; Skeie, RB; Takemura, T; Tsyro, S. (2017). Multi-model simulations of aerosol and ozone radiative forcing due to anthropogenic emission changes during the period 1990-2015. Atmos Chem Phys 17: 2709-2720. http://dx.doi.org/10.5194/acp-17-2709-2017.

Myhre, G; Shindell, D; Bréon, FM; Collins, W; Fuglestvedt, J; Huang, J; Koch, D; Lamarque, JF; Lee, D; Mendoza, B; Nakajima, T; Robock, A; Stephens, G; Takemura, T; Zhang, H. (2013). Anthropogenic and natural radiative forcing. In TF Stocker; D Qin; GK Plattner; MMB Tignor; SK Allen; J Boschung; A Nauels; Y Xia; V Bex; PM Midgley (Eds.), Climate change 2013: The physical science basis Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change (pp. 659-740). Cambridge, UK: Cambridge University Press. http://www.ipcc.ch/report/ar5/wg1/.

Newsome, B; Evans, M. (2017). Impact of uncertainties in inorganic chemical rate constants on tropospheric composition and ozone radiative forcing. Atmos Chem Phys 17: 14333-14352. http://dx.doi.org/10.5194/acp-17-14333-2017.

Parrish, DD; Lamarque, JF; Naik, V; Horowitz, L; Shindell, DT; Staehelin, J; Derwent, R; Cooper, OR; Tanimoto, H; Volz-Thomas, A; Gilge, S; Scheel, HE; Steinbacher, M; Froehlich, M. (2014). Long-term changes in lower tropospheric baseline ozone concentrations: Comparing chemistry-climate models and observations at northern midlatitudes. J Geophys Res Atmos 119: 5719-5736. http://dx.doi.org/10.1002/2013JD021435.

Sherwen, T; Evans, MJ; Carpenter, LJ; Schmidt, JA; Mickley, LJ. (2017). Halogen chemistry reduces tropospheric O3 radiative forcing. Atmos Chem Phys 17: 1557-1569. http://dx.doi.org/10.5194/acp-17-1557-2017.

Shindell, D; Faluvegi, G. (2009). Climate response to regional radiative forcing during the twentieth century. Nat Geosci 2: 294-300. http://dx.doi.org/10.1038/ngeo473.

Shindell, D; Faluvegi, G; Lacis, A; Hansen, J; Ruedy, R; Aguilar, E. (2006). Role of tropospheric ozone increases in 20th-century climate change. J Geophys Res 111: D08302. http://dx.doi.org/10.1029/2005JD006348.

Shindell, D; Faluvegi, G; Nazarenko, L; Bowman, K; Lamarque, JF; Voulgarakis, A; Schmidt, GA; Pechony, O; Ruedy, R. (2013). Attribution of historical ozone forcing to anthropogenic emissions. Nat Clim Chang 3: 567-570. http://dx.doi.org/10.1038/nclimate1835.

Shindell, D; Kuylenstierna, JC; Vignati, E; van Dingenen, R; Amann, M; Klimont, Z; Anenberg, SC; Muller, N; Janssens-Maenhout, G; Raes, F; Schwartz, J; Faluvegi, G; Pozzoli, L; Kupiainen, K; Höglund-Isaksson, L; Emberson, L; Streets, D; Ramanathan, V; Hicks, K; Oanh, NT; Milly, G; Williams, M; Demkine, V; Fowler, D. (2012a). Simultaneously mitigating near-term climate change and improving human health and food security. Science 335: 183-189. http://dx.doi.org/10.1126/science.1210026.

Shindell, DT. (2014). Inhomogeneous forcing and transient climate sensitivity. Nat Clim Chang 4: 274-277. http://dx.doi.org/10.1038/NCLIMATE2136.

Shindell, DT; Faluvegi, G; Bauer, SE; Koch, DM; Unger, N; Menon, S; Miller, RL; Schmidt, GA; Streets, DG. (2007). Climate response to projected changes in short-lived species under an A1B scenario from 2000-2050 in the GISS climate model. J Geophys Res 112: D20103. http://dx.doi.org/10.1029/2007jd008753.

Shindell, DT; Faluvegi, G; Rotstayn, L; Milly, G. (2015). Spatial patterns of radiative forcing and surface temperature response. J Geophys Res Atmos 120: 5385-5403. http://dx.doi.org/10.1002/2014JD022752.

Shindell, DT; Levy H, II; Schwarzkopf, MD; Horowitz, LW; Lamarque, JF; Faluvegi, G. (2008). Multimodel projections of climate change from short-lived emissions due to human activities. J Geophys Res 113: D11109. http://dx.doi.org/10.1029/2007JD009152.

Shindell, DT; Voulgarakis, A; Faluvegi, G; Milly, G. (2012b). Precipitation response to regional radiative forcing. Atmos Chem Phys 12: 6969-6982. http://dx.doi.org/10.5194/acp-12-6969-2012.

Skeie, RB; Berntsen, TK; Myhre, G; Tanaka, K; Kvalevåg, MM; Hoyle, CR. (2011). Anthropogenic radiative forcing time series from pre-industrial times until 2010. Atmos Chem Phys 11: 11827-11857. http://dx.doi.org/10.5194/acp-11-11827-2011.

Sovde, OA; Hoyle, CR; Myhre, G; Isaksen, ISA. (2012). The HNO3 forming branch of the HO2 + NO reaction: pre-industrial-to-present trends in atmospheric species and radiative forcings (vol 11, pg 8929, 2011). Atmos Chem Phys 12: 7725-7725. http://dx.doi.org/10.5194/acp-12-7725-2012.

Staehelin, J; Thudium, J; Buehler, R; Volz-Thomas, A; Graber, W. (1994). Trends in surface ozone concentrations at Arosa (Switzerland). Atmos Environ 28: 75-87. http://dx.doi.org/10.1016/1352-2310(94)90024-8.

Stevenson, DS; Young, PJ; Naik, V; Lamarque, JF; Shindell, DT; Voulgarakis, A; Skeie, RB; Dalsoren, SB; Myhre, G; Berntsen, TK; Folberth, GA; Rumbold, ST; Collins, WJ; Mackenzie, IA; Doherty, RM; Zeng, G; van Noije, TPC; Strunk, A; Bergmann, D; Cameron-Smith, P; Plummer, DA; Strode, SA; Horowitz, L; Lee, YH; Szopa, S; Sudo, K; Nagashima, T; Josse, B; Cionni, I; Righi, M; Eyring, V; Conley, A; Bowman, KW; Wild, O; Archibald, A. (2013). Tropospheric ozone changes, radiative forcing and attribution to emissions in the Atmospheric Chemistry and Climate Model Intercomparison Project (ACCMIP). Atmos Chem Phys 13: 3063-3085. http://dx.doi.org/10.5194/acp-13-3063-2013.

Westervelt, DM; Conley, AJ; Fiore, AM; Lamarque, JF; Shindell, DT; Previdi, M; Mascioli, NR; Faluvegi, G; Correa, G; Horowitz, LW. (2018). Connecting regional aerosol emissions reductions to local and remote precipitation responses. Atmos Chem Phys 18: 12461-12475. http://dx.doi.org/10.5194/acp-18-12461-2018.

Worden, HM; Bowman, KW; Kulawik, SS; Aghedo, AM. (2011). Sensitivity of outgoing longwave radiative flux to the global vertical distribution of ozone characterized by instantaneous radiative kernels from Aura-TES. J Geophys Res 116: D14115. http://dx.doi.org/10.1029/2010JD015101.

Wuebbles, DJ; Fahey, DW; Hibbard, KA; Dokken, DJ; Stewart, BC; Maycock, TK. (2017). USGCRP, 2017: Climate science special report: Fourth national climate assessment, Volume I. Washington, DC: U.S. Global Change Research Program. https://science2017.globalchange.gov/.

Xie, B; Zhang, H; Wang, Z; Zhao, S; Fu, Q. (2016). A modeling study of effective radiative forcing and climate response due to tropospheric ozone. Adv Atmos Sci 33: 819-828. http://dx.doi.org/10.1007/s00376-016-5193-0.

Yang, J; Hu, Y; Peltier, WR. (2012). Radiative effects of ozone on the climate of a Snowball Earth. Climate of the Past 8: 2019-2029. http://dx.doi.org/10.5194/cp-8-2019-2012.

Young, PJ; Archibald, AT; Bowman, KW; Lamarque, JF; Naik, V; Stevenson, DS; Tilmes, S; Voulgarakis, A; Wild, O; Bergmann, D; Cameron-Smith, P; Cionni, I; Collins, WJ; Dalsoren, SB; Doherty, RM; Eyring, V; Faluvegi, G; Horowitz, LW; Josse, B; Lee, YH; Mackenzie, IA; Nagashima, T; Plummer, DA; Righi, M; Rumbold, ST; Skeie, RB; Shindell, DT; Strode, SA; Sudo, K; Szopa, S; Zeng, G. (2013). Pre-industrial to end 21st century projections of tropospheric ozone from the Atmospheric Chemistry and Climate Model Intercomparison Project (ACCMIP). Atmos Chem Phys 13: 2063-2090. http://dx.doi.org/10.5194/acp-13-2063-2013.

Zhang, Y; Cooper, OR; Gaudel, A; Thompson, AM; Nedelec, P; Ogino, SY; West, JJ. (2016). Tropospheric ozone change from 1980 to 2010 dominated by equatorward redistribution of emissions. Nat Geosci 9: 875-+. http://dx.doi.org/10.1038/NGEO2827.

APPENDIX 10 DEVELOPMENT OF THE INTEGRATED SCIENCE ASSESSMENT—PROCESS

10.1 Introduction

The Integrated Science Assessment (ISA) provides the scientific foundation for the review of the National Ambient Air Quality Standards (NAAQS). The ISA contains a synthesis and evaluation of the most policy-relevant science using methods and approaches described in the Preamble to the Integrated Science Assessments (U.S. EPA, 2015a), hereafter "Preamble," which provides an overview of the ISA development process. Early in the review, U.S. EPA releases an Integrated Review Plan (IRP) to summarize the entire process for the NAAQS review, including a plan for developing the ISA (https://www.epa.gov/naaqs/ozone-o3-standards-planning-documents-current-review). The ISA is developed by U.S. EPA scientists in the Office of Research and Development (ORD) with extensive knowledge in their respective fields, other U.S. EPA scientists with relevant expertise, and extramural scientists who are solicited by the U.S. EPA for their subject matter expertise. The general development steps are presented in Figure 10-1, but the specific details can vary from assessment to assessment. This Appendix builds off the Preamble and the IRP to describe the process for this current ISA. Appendix 10 was developed, in part to describe the methods for literature review, study quality evaluation, and quality assurance and quality control. Details related to specific quantitative analyses are not described in this Appendix, but have been included in those specific appendices where the analyses are presented.

10.2 Literature Search and Initial Screen

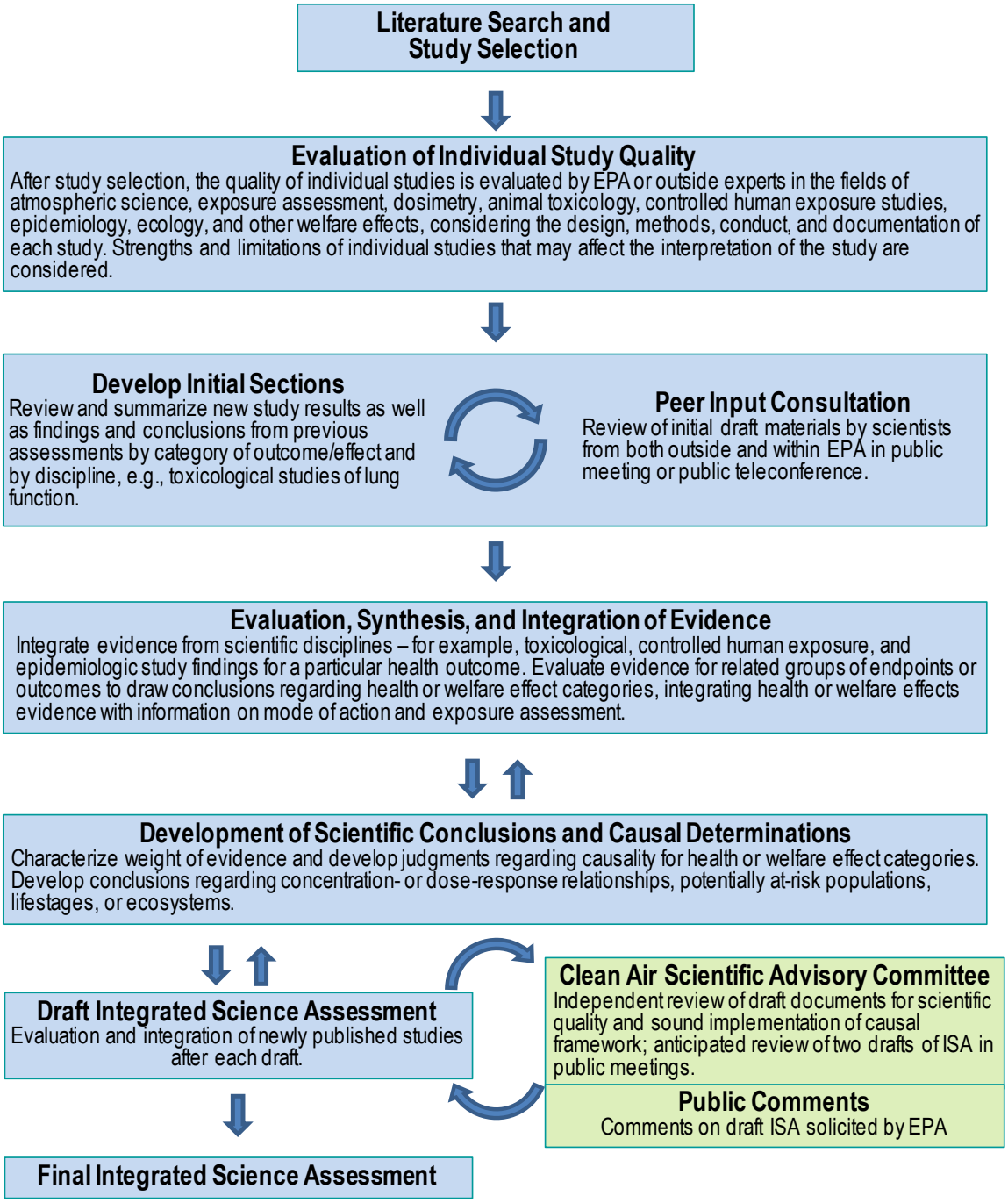
Development of the Ozone ISA began with an initial literature search and screening process. For this step, the ISA authors applied systematic review methodologies to identify relevant scientific findings that have emerged since the previous ISA for ozone, which was published in 2013 (U.S. EPA, 2013) and included peer-reviewed literature published through July 2011. Search techniques for the current ISA identified and evaluated studies and reports that have undergone scientific peer review and were published or accepted for publication between January 1, 2011 (providing some overlap with the cutoff date from the last review) and March 30, 2018. Studies published after the literature cutoff date for this review were also considered if they were submitted in response to the Call for Information or identified in subsequent phases of ISA development (e.g., peer-input consultation, see Figure 10-1), particularly to the extent that they provide new information that affects key scientific conclusions.

Peer-reviewed literature was identified and evaluated to provide a better understanding of the following issues: (1) the natural and anthropogenic sources of ozone precursors in the ambient air;

(2) formation, transport, and fate of ozone in the environment; (3) measurement methods and ambient concentrations of ozone; (4) how exposure assessment methods used in epidemiologic studies can influence inferences drawn about ozone health effects; (5) the independent effect of ozone exposure on health and welfare[1] effects; (6) the potential influence of other factors (e.g., other pollutants in the ambient air, ambient air temperature) shown to be correlated with ozone and health or welfare effects; (7) the shape of the concentration-response relationship at ozone concentrations at the low end of the distribution; and (8) populations and lifestages at increased risk of ozone-related health effects.

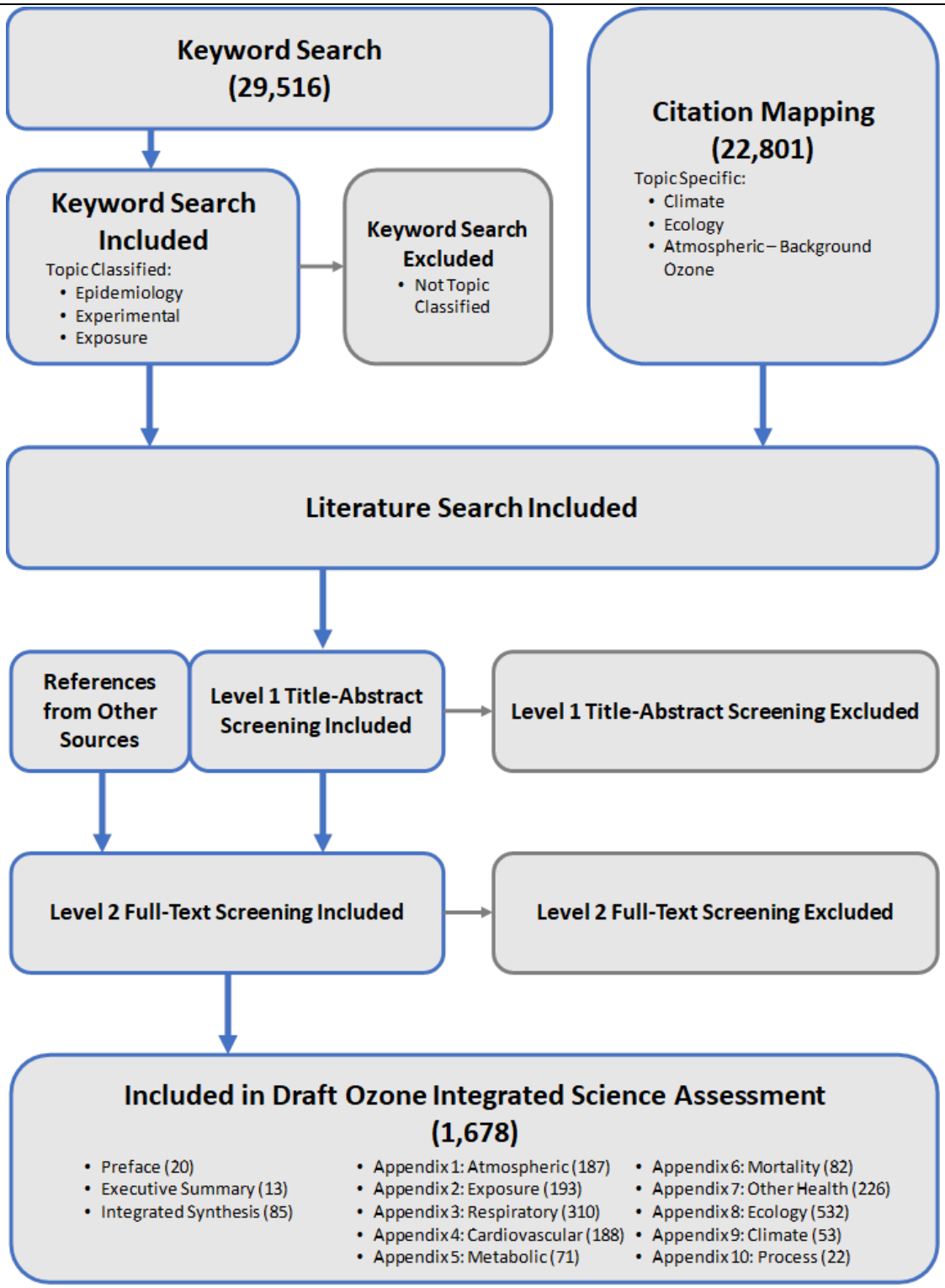
The literature search and screening process are described in the sections below and are summarized in the Literature Flow Diagram shown below (Figure 10-2).

[1] Section 302(h) of the Act [42 U.S.C. 7602(h)] provides that all language referring to effects on welfare includes, but is not limited to, "effects on soils, water, crops, vegetation, man-made materials, animals, wildlife, weather, visibility and climate, damage to and deterioration of property, and hazards to transportation, as well as effects on economic values and on personal comfort and well-being…" (CAA, 2005).



Source: Modified from Figure II of the Preamble to the Integrated Science Assessments U.S. EPA (2015a).

Figure 10-1 General process for development of Integrated Science Assessments.



Notes: These references are tagged in the HERO database to provide greater transparency in the approach used to identify references for inclusion in the ISA.

Figure 10-2 Literature Flow Diagram for the Ozone Integrated Science Assessment.

10.2.1 Literature Search

The U.S. EPA uses a structured approach to identify relevant studies for consideration and inclusion in the ISAs. The search for relevant literature in this review began with publication of the *Federal Register* notice announcing the initiation of this ozone NAAQS review and requesting information from the public including relevant literature (83 FR 29785, June 26, 2018). In addition, the U.S. EPA identified publications by conducting multitiered systematic literature searches that included extensive mining of literature databases on specific topics in a variety of disciplines. The search strategies were designed a priori to optimize identification of pertinent published papers.

For this ISA, discipline-specific approaches were used to identify literature. In each case, careful consideration was given to literature search strategies used in the development of previous assessments and to the methods that would result in the best precision[1] and recall[2] for each of the disciplines. The literature identification approaches included broad keyword searches in routinely used databases with automatic topic classification and topic-specific citation mapping (see Section 10.2.2 for specific approaches used for each discipline).

As has been done for past ISAs, a broad keyword search was developed as a starting point to capture literature pertinent to the pollutant of interest. In this case, the main keyword string used was "ozone OR O_3," which is sufficiently broad to capture ozone-relevant literature in each database (i.e., PubMed, Web of Science, TOXLINE). Following the broad keyword search for ozone, automatic topic classification was used to categorize references by discipline (e.g., epidemiology, toxicology, etc.). This step employs machine learning where positive and negative seed references[3] for a particular discipline are used to train an algorithm to identify discipline-specific references based on word use and frequency in titles and abstracts. This method, used in several previous ISAs, varies in effectiveness across disciplines due to the broad range of topics and the variability in term usage in some evidence bases.

Another approach used in past ISAs that was employed in this review is topic-specific citation mapping, also known as relational reference searching. In this approach, a set of relevant published references are identified as a seed set and then more recent literature that has cited any of the references in the seed set are collected. Topic-specific references from the 2013 Ozone ISA comprised the seed set for this draft ISA. Because the seed set is highly relevant to the topic of interest, this targeted approach to

[1] Precision refers to the number of relevant references identified divided by the total number of references identified.

[2] Recall is the number of relevant references identified divided by the total number of relevant references that exist.

[3] Positive seed references are those that are examples of references that are relevant (i.e., the references would be selected for full-text screening). Negative seed references are those that are examples of references that are not relevant (i.e., they would not be selected for full-text screening). For ISAs, the positive seed set includes references from the prior ISA for the discipline of interest. The negative seed set includes the references from all of the other disciplines in the previous ISA.

reference identification is more precise than keyword searches, and it further allows for relevance ranking based on the number of references in a bibliography that match references in the seed set.

References were also identified for consideration in this ISA in other ways, including identification of relevant literature by U.S. EPA expert scientists, recommendations received in response to the Call for Information and the external review process for the ISA (see Section 10.4.3), and by review of citations included in previous assessments.

10.2.2 Study Selection: Initial Screening (Level 1) of Studies from the Literature Search

Once studies were identified, ISA authors (U.S. EPA staff and extramural scientists) reviewed and screened the studies for a further refined list of references to be considered for inclusion in the ISA. References for each discipline (i.e., atmospheric science, exposure assessment, experimental health studies, epidemiology, ecology, and climate-related science) first went through title and abstract screening using SWIFT-ActiveScreener (SWIFT-AS), which is referred to as Level 1 screening. Level 1 screening criteria for inclusion were intended to be broad and err on the side of inclusion. For each discipline, title and abstracts were selected for consideration if there was indication of ozone and a quantifiable effect relevant to an identified discipline. SWIFT-AS is a software application that employs machine learning in real time to identify relevant literature. The machine learning feature builds a model to predict relevant references based on inclusion/exclusion screening decisions in real time as scientists screen each reference. As title/abstract screening is conducted, references are queued based on the predicted relevance and SWIFT-AS estimates when a 95% recall threshold has been reached,[1] a level often used to evaluate the performance of machine learning applications and considered comparable to human error rates (Howard et al., 2016; Cohen et al., 2006).

The application of SWIFT-AS was tailored for each discipline. This included using a specific seed set of 50−100 relevant references from the 2013 Ozone ISA to train the SWIFT-AS algorithm and developing specific screening questions for each discipline to allow for the categorization of references based on the information available in the title and abstract. Specific details about inclusion/exclusion criteria and the screening questions for each discipline are described in more detail below. The references identified for inclusion after Level 1 screening were then reviewed in Level 2 full-text screening. Figure 10-3 demonstrates the efficiency gained by using SWIFT-AS for each discipline. Figure 10-4 is another example of the efficiency of SWIFT-AS demonstrating for toxicological references the rate at which 95% recall was achieved with the machine learning algorithm relative to that of manual screening.

[1] A 95% recall threshold represents the point at which 95% of the potentially relevant references have been identified.

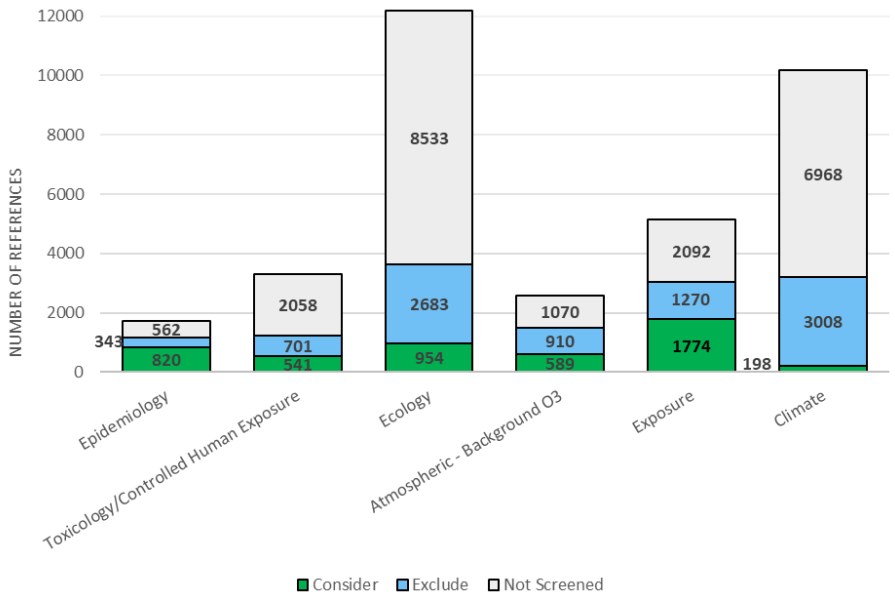
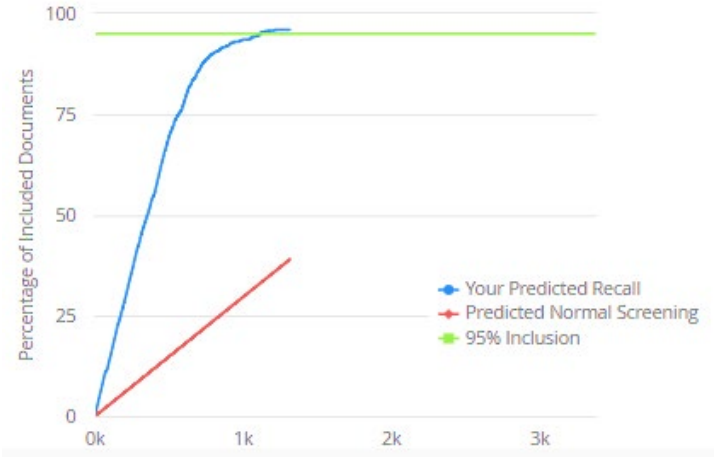


Figure 10-3 Summary of title/abstract screening in SWIFT-ActiveScreener.



Notes: The green line represents the predicted 95% recall threshold, while the blue line represents the screening progression to achieve 95% recall using the machine learning algorithm. The red line represents the approximate rate of manual screening all references.

Figure 10-4 Example of screening efficiency using SWIFT-ActiveScreener.

10.2.2.1 Atmospheric Science

Literature related to the atmospheric science topics discussed in Appendix 1 was identified by topic-specific citation mapping methods that relied upon references cited in the 2013 Ozone ISA. More specifically, references were collected from the atmospheric science sections of the 2013 Ozone ISA, including subtopics on physical and chemical processes, atmospheric modeling, monitoring, and background ozone concentrations. Consistent with the ozone IRP, the primary focus for evaluation of the recent literature was on studies estimating U.S. background (USB) ozone and its sources. Studies selected from citation mapping were systematically title/abstract screened using SWIFT-AS. A recent critical review of the science in which the U.S. EPA played an active role (Jaffe et al., 2018) was selected to serve as the primary reference for most of the Appendix 1 discussion concerning USB ozone. In addition to Jaffe et al. (2018), papers published as part of the International Global Atmospheric Chemistry (IGAC) Project Tropospheric Ozone Assessment Report [TOAR; Gaudel et al. (2018)] were reviewed and evaluated. Articles found with citation mapping and subsequent screening that were not cited and discussed in either the critical review by Jaffe et al. (2018) or the IGAC TOAR report Gaudel et al. (2018) were individually reviewed. Additional topics, including newly described photochemical mechanisms and the role of variability and change in large-scale meteorological patterns, were also included in Appendix 1. Literature concerning these topics was identified by separate keyword searches conducted in Web of Science.

10.2.2.2 Exposure Assessment

Exposure literature relevant to ozone was identified using the broad keyword search and automatic topic classification, as described in Section 10.2.1. Automatic topic classification for exposure references included a sufficiently large set of positive and negative seeds from previous ISAs. Positive seeds included references from the exposure chapter from the 2016 ISA for Oxides of Nitrogen-Health Criteria[1] and the 2013 Ozone ISA; the negative seeds included nonrelevant references (i.e., those from other disciplines in these two ISAs). Following identification and sorting of the literature by topic, SWIFT-AS was used for Level 1 screening. Positive seeds to train the SWIFT-AS algorithm included a subset of the exposure references cited in the 2013 Ozone ISA. Additionally, references were categorized in Level 1 screening in SWIFT-AS by study type, study location, and exposure duration.

[1] The 2016 ISA for Oxides of Nitrogen-Health Criteria is the most recent ISA using automatic topic classification. Since automatic topic classification is not pollutant specific, including references from this literature set increased the number of seed references to improve precision of the algorithm for each discipline.

10.2.2.3 Health—Experimental Studies

Experimental (i.e., controlled human exposure and animal toxicology) studies examining the health effects of ozone exposure were identified using the broad keyword search and automatic topic classification, as described in Section 10.2.1. The automatic topic classification for experimental references encompassed a sufficiently large set of positive seeds, including controlled human exposure and animal toxicology references cited in the 2016 ISA for Oxides of Nitrogen-Health Criteria and the 2013 Ozone ISA, and a sufficiently large set of negative seeds, including nonexperimental references cited in these two ISAs. Following identification of the literature, SWIFT-AS was used for Level 1 screening. The SWIFT-AS algorithm was trained using a set of positive seed references from a selection of controlled human exposure and animal toxicological studies cited in the 2013 Ozone ISA. Additionally, references were categorized in Level 1 screening in SWIFT-AS by health outcome category (e.g., respiratory, cardiovascular, metabolic, etc.), exposure duration (e.g., short-term, long-term), and study type (e.g., controlled human exposure, animal toxicology, etc.).

10.2.2.4 Health—Epidemiologic Studies

Identification of recent epidemiologic studies examining a health effect and ambient exposure to ozone was identified using the broad keyword search and automatic topic classification, as described in Section 10.2.1. The approach for automatic topic classification to identify epidemiologic studies from the broad literature search results paralleled the approach described in Section 10.2.2.3 for the experimental studies on health effects. A sufficiently large set of seed references cited in the 2016 ISA for Oxides of Nitrogen-Health Criteria and the 2013 Ozone ISA was used, with positive seeds consisting of epidemiologic references in those ISAs and negative seeds consisting of all references other than epidemiologic references. Following identification of the literature, SWIFT-AS was used for Level 1 screening. Positive seeds were also used to train the SWIFT-AS algorithm and included select epidemiologic references cited in the 2013 Ozone ISA. Additionally, references were categorized in Level 1 SWIFT-AS screening by health outcome category (e.g., mortality, respiratory, cardiovascular, etc.), exposure duration (e.g., short term, long term), and study location (e.g., U.S., Canada, Europe, etc.).

10.2.2.5 Welfare—Ecological Studies

Studies relevant to the ecological effects of ozone exposure were primarily identified by topic-specific citation mapping in Web of Science, based on ecological studies cited in the 2013 Ozone ISA. The broad keyword searches and automatic topic classification have traditionally resulted in a poorly targeted set of references for Level 1 screening in past ISAs for ecological endpoints. Following citation mapping, Level 1 screening of the identified references was conducted in SWIFT-AS, including the use of a positive seed set of ecological references from the 2013 Ozone ISA. Screening questions to facilitate

organization of the literature included effect category (e.g., foliar injury, plant growth, biodiversity, etc.), exposure conditions, location, and ecosystem type (e.g., wetland, crop, etc.).

10.2.2.6 Welfare—Effects on Climate

Studies examining the effect of tropospheric ozone on climate were identified in two ways. First, references were identified by topic-specific citation mapping in Web of Science using references cited in the 2013 Ozone ISA. In addition, relevant references were identified from recent national and international climate assessments, such as the National Climate Assessment (Wuebbles et al., 2017), the Intergovernmental Panel on Climate Change Fifth Assessment Report (IPCC, 2013), and other recent, more focused reports relevant to ozone climate forcing. Level 1 screening of the identified references was conducted in SWIFT-AS aided by a seed set of selected references from the climate section of the 2013 Ozone ISA. Screening questions to aid in organizing the literature included radiative forcing, effects on climate, precursor and copollutant effects, and factors and feedbacks.

10.2.3 Documentation

To improve transparency, all studies identified in the literature search for ozone are documented in the Health and Environmental Research Online (HERO) database. The HERO project page for this ISA (https://hero.epa.gov/hero/index.cfm/project/page/project_id/2737) contains the references that were considered for inclusion in the ISA and provides bibliographic information and abstracts. It is accessible to the public.

References that were identified by topic classification from the keyword literature search are tagged in the HERO database, as described in Figure 10-2. References from topic-specific citation mapping are also tagged for each discipline. Finally, the references that passed through Level 1 screening are tagged in HERO as Title-Abstract Screening Included. All inclusion and exclusion decisions are documented in the HERO database, as well as discipline tags from automatic topic classification.

10.3 Study Selection: Full-Text Evaluation of Studies (Level 2)

Following Level 1 screening, NCEA subject matter experts reviewed the group of references during full-text Level 2 screening. This level of screening evaluated studies by relevance (Section 10.3.1) and quality (Section 10.3.2).

10.3.1 Relevance

Relevance was evaluated in two stages. The first stage looked at the extent to which a study was potentially policy-relevant and informative. Such studies included those that describe or provide a basis for the relationship between ozone exposure and health or welfare effects. Also pertinent are studies that provide insights concerning the sources of background ozone and its concentration patterns, those that describe innovation in measurement methods or study design, or those that present novel information on previously unidentified effects or issues. Informative studies were not limited to specific study designs, model systems, or outcomes.

The second stage of the relevancy review included a determination of the specific scope for the health and welfare portions of the ISA. This stage relied on scoping tools that explicitly define the relevant Population, Exposure, Comparison, Outcome, and Study design (PECOS) serving as criteria for inclusion/exclusion decisions. The PECOS tool characterizes the parameters and provides a framework to help identify literature relevant to the ISA. Discipline-specific PECOS tools were developed for experimental studies, epidemiologic studies, ecological studies, and for studies on the effects of tropospheric ozone on climate. PECOS tools differ by study area depending on the types of questions to be answered and by *a priori* knowledge related to that question. The use of PECOS tools is widely accepted and increasingly applied for systematic review in risk assessment. The use of these tools is consistent with recommendations by the National Academy of Sciences for improving the design of risk assessment through planning, scoping, and problem formulation to better meet the needs of decision makers (NASEM, 2018). PECOS tools are identified in each of the relevant appendices in this ISA (Appendix 3-Appendix 9). Specific details about scope and the screening questions for each discipline, including atmospheric science and exposure assessment, are described in more detail below.

While useful for research studies that evaluate a specific health or welfare outcome, PECOS tools were less informative for the atmospheric science and exposure assessment portions of the ISA. Full-text (Level 2) screening of references is further described for these disciplines in the sections below.

10.3.1.1 Atmospheric Sciences

The role of the atmospheric sciences discussion in the ISA is to provide necessary context and insight into the processes that produce ambient ozone and its variable concentration patterns for the assessment of human and ecosystem exposure and subsequent effects. The available information generally falls into four categories: (1) emissions measurements and inventories, (2) ambient air measurements, (3) discussions of insights gained through laboratory chemistry studies, and (4) discussions of ozone photochemical models and their process, and concentration estimates. The relevance criteria applied to the body of title/abstract screened peer-reviewed literature included:

1. The study addresses ozone and its precursors;

2. The study addresses the human activities, natural processes, atmospheric chemistry and dynamics that are responsible for formation in or introduction of ozone into the lower troposphere (planetary boundary layer) where exposure to ozone may lead to damaging effects to human health, ecosystems, the climate system, and other elements of the environment relevant to human welfare. The study is also relevant if it describes spatial and temporal patterns in lower tropospheric ozone concentrations;
3. The study addresses the origins and concentrations of ozone in the U.S.; and
4. The study provides insight into the sources, causes, and concentrations of USB ozone, the methodologies used to estimate USB ozone, and any technical issues that must be considered in order to correctly interpret the scientific findings relevant to USB ozone.

The studies cited in Appendix 1 met all four criteria. Also meeting these criteria were supplemental studies that were included as necessary supporting material or studies identified outside of topic-specific citation mapping.

10.3.1.2 Exposure Assessment

The ISA describes the exposure assessment methods employed in epidemiologic studies discussed in the health appendices. For these studies, an additional screening step with added search terms was applied to further identify potentially relevant references. The additional step was needed because the Level 1 title/abstract screening was insufficient to gauge whether each paper covered the methods discussed in the epidemiology literature. It was also needed because of the disparate nature of exposure assessment references and the large number of references relative to other disciplines. The search terms correspond to the sections of Appendix 2. Each of the references obtained through this search was then evaluated in the Level 2 full-text screening for relevance.

Relevance was based on whether an exposure assessment study was representative of the population and conditions addressed in the epidemiology literature. If there was sufficient evidence that a method could provide an adequate representation of exposure from the U.S., there was no need to consider studies conducted outside of the U.S. or Canada. If there was not sufficient evidence that a method could provide an adequate representation of exposure from the U.S., then there was a need to consider western European and Australian studies, which were the next most similar to studies conducted in the U.S. If there was not sufficient evidence that a method could provide an adequate representation of exposure from the U.S., Canada, western Europe, or Australia, then it was necessary to consider all studies regardless of geographic location.

10.3.1.3 Health—Experimental Studies

For experimental studies, specifically, controlled human or animal exposure studies, the relevance evaluation focused on those studies with appropriate study designs and relevant exposure concentrations, as well as those that address key uncertainties and limitations in the evidence identified in the previous review (Table 10-1). The scope of the experimental evidence encompassed studies of short-term (i.e., hours to weeks) and long-term (i.e., months to years) exposures conducted at concentrations of ozone that are relevant to the range of human exposures to ambient air (up to 2 ppm, which is one to two orders of magnitude above ambient concentrations).

Table 10-1 Population, Exposure, Comparison, Outcome, and Study design (PECOS) tool to define the parameters and provide a framework for identifying relevant experimental studies.

Exposure Duration and Health Effect	Population, Exposure, Comparison, Outcome, and Study Design (PECOS)
Short-term exposure and respiratory, cardiovascular, metabolic, nervous system, reproductive or developmental effects	**Population:** Study populations of any controlled human exposure or animal toxicological study of mammals at any lifestage **Exposure:** Short-term (in the order of minutes to weeks) inhalation exposure to relevant ozone concentrations (i.e., 0.4 ppm or below for humans, 2 ppm or below for other mammals) **Comparison:** Human subjects that serve as their own controls with an appropriate washout period or when comparison to a reference population exposed to lower levels is available, or, in toxicological studies of mammals, an appropriate comparison group that is exposed to a negative control (i.e., clean air or filtered air control) **Outcome:** Respiratory, cardiovascular, metabolic, or nervous system; reproductive or developmental effects **Study Design:** Controlled human exposure (i.e., chamber) studies; in vivo acute, subacute, or repeated-dose toxicity studies in mammals; reproductive toxicity or immunotoxicity studies
Long-term exposure and respiratory, cardiovascular, metabolic, nervous system, carcinogenic, reproductive or developmental effects	**Population:** Study population of any animal toxicological study of mammals at any lifestage **Exposure:** Long-term (in the order of months to years) inhalation exposure to relevant ozone concentrations (i.e., 2 ppm or below) **Comparison:** Appropriate comparison group exposed to a negative control (i.e., clean air or filtered air control) **Outcome:** Respiratory, cardiovascular, metabolic or nervous system; carcinogenic, reproductive, or developmental effects **Study Design:** In vivo chronic, subchronic, or repeated-dose toxicity studies in mammals; reproductive toxicity or immunotoxicity studies; genotoxicity/mutagenicity studies

Population: In controlled human exposure studies, generally healthy adults approved for study participation by the appropriate IRB or ethics committee; for toxicological studies, well-defined/well-characterized strains of mammals at any lifestage.

Exposure: Ozone concentrations deliberately delivered to subjects for a predefined duration.

Comparator: In controlled human exposure studies, subjects serve as their own controls with an appropriate washout period, or a reference population exposed to lower ozone concentrations, or, in toxicological studies, an appropriate comparison group that is exposed to a negative control (i.e., clean air or filtered air control).

Outcome: Clearly measurable health endpoint.

Study design: Controlled human exposure (i.e., chamber) studies; in vivo acute, subacute, subchronic, chronic or repeated-dose toxicity studies in mammals; reproductive toxicity or immunotoxicity studies; genotoxicity/mutagenicity studies.

10.3.1.4 Health—Epidemiologic Studies

The evaluation of epidemiologic studies focused on the associations between short- and long-term exposure to ozone and a range of health effects, including respiratory, cardiovascular, reproductive and developmental, metabolic, and nervous system outcomes (Table 10-2). In instances when a "causal" or "likely to be a causal" relationship was concluded in the 2013 Ozone ISA (i.e., short-term ozone exposure and respiratory and cardiovascular effects and total mortality, and long-term ozone exposure and respiratory effects), the epidemiologic studies evaluated for those outcomes were more limited in scope and targeted towards study locations that include U.S. airsheds or airsheds that are similar to those found in the U.S., as reflected in the PECOS tool. For outcomes for which the 2013 Ozone ISA concluded that evidence was "suggestive of" or "inadequate to infer" a causal relationship (i.e., short-term ozone exposure and nervous system effects and long-term ozone exposure and cardiovascular, nervous system, reproductive or developmental effects, cancer, or mortality), the epidemiologic studies evaluated were not limited geographically or by airshed characteristics, as reflected in the PECOS tool.

Table 10-2 Population, Exposure, Comparison, Outcome, and Study design (PECOS) tool to define the parameters and provide a framework for identifying relevant epidemiologic studies.

Exposure Duration and Health Effect	Population, Exposure, Comparison, Outcome, and Study Design (PECOS)
Short-term exposure and respiratory effects	**Population:** Any U.S. or Canadian population, including populations or lifestages that might be at increased risk **Exposure:** Short-term (on the order of one to several days) ambient concentration of ozone **Comparison:** Per unit increase (in ppb) **Outcome:** Change in risk (incidence/prevalence) of respiratory effects **Study Design:** Epidemiologic studies consisting of panel, case-crossover, time-series, and case-control studies; cross-sectional studies with appropriate timing of exposure for the health endpoint of interest
Short-term exposure and mortality	**Population:** Any U.S. or Canadian population, including populations or lifestages that might be at increased risk **Exposure:** Short-term exposure (on the order of one to several days) to ambient concentrations of ozone **Comparison:** Per unit increase (in ppb) **Outcome:** Change in risk (incidence) of mortality **Study Design:** Epidemiologic studies consisting of case-crossover or time-series studies with appropriate timing of exposure for the health endpoint of interest

Table 10 2 (Continued): Population, Exposure, Comparison, Outcome, and Study Design (PECOS) tool to define the parameters and provide a framework for identifying relevant epidemiologic studies.

Exposure Duration and Health Effect	Population, Exposure, Comparison, Outcome, and Study Design (PECOS)
Long-term exposure and respiratory effects	**Population:** Any U.S. or Canadian population, including populations or lifestages that might be at increased risk **Exposure:** Long-term (on the order of months to years) ambient concentration of ozone **Comparison:** Per unit increase (in ppb) **Outcome:** Change in risk (incidence/prevalence) of respiratory effects **Study Design:** Epidemiologic studies consisting of cohort and case-control studies; time-series, case-crossover, and cross-sectional studies with appropriate timing of exposure for the health endpoint of interest
Short-term exposure and cardiovascular effects	**Population:** Any U.S., Canadian, European, or Australian population, including populations or lifestages that might be at increased risk **Exposure:** Short-term (on the order of one to several days) ambient concentration of ozone **Comparison:** Per unit increase (in ppb) **Outcome:** Change in risk (incidence/prevalence) of cardiovascular effects **Study Design:** Epidemiologic studies consisting of panel, case-crossover, time-series, and case-control studies; cross-sectional studies with appropriate timing of exposure for the health endpoint of interest
Short-term exposure and nervous system effects	**Population:** Any population, including populations or lifestages that might be at increased risk **Exposure:** Short-term (on the order of one to several days) ambient concentration of ozone **Comparison:** Per unit increase (in ppb) **Outcome:** Change in risk (incidence/prevalence) of a nervous system effect **Study Design:** Epidemiologic studies consisting of panel, case-crossover, time-series, and case-control studies; cross-sectional studies with appropriate timing of exposure for the health endpoint of interest
Long-term exposure and cardiovascular, nervous system, reproductive, or developmental effects; cancer, or mortality	**Population:** Any population, including populations or lifestages that might be at increased risk **Exposure:** Long-term (on the order of months to years) ambient concentration of ozone **Comparison:** Per unit increase (in ppb) **Outcome:** Change in risk (incidence/prevalence) of a cardiovascular, nervous system, reproductive, or developmental effect; cancer or mortality **Study Design:** Epidemiologic studies consisting of cohort and case-control studies; time-series, case-crossover, and cross-sectional studies with appropriate timing of exposure for the health endpoint of interest

Table 10 2 (Continued): Population, Exposure, Comparison, Outcome, and Study Design (PECOS) tool to define the parameters and provide a framework for identifying relevant epidemiologic studies.

Exposure Duration and Health Effect	Population, Exposure, Comparison, Outcome, and Study Design (PECOS)
	Population: The general population, all age groups, living both in urban and in rural areas exposed on a daily basis to ozone through outdoor (ambient) air, and not exclusively in occupational settings or as a result of indoor exposure. Populations and lifestages at increased risk are included, such as those with specific pre-existing health conditions (e.g., respiratory or cardiovascular diseases), children, or older adults. **Exposure:** Ambient ozone from any source measured as short-term (minutes to weeks) or long-term (months to years). **Comparator:** The health effect observed by unit increase in concentration of ozone in the same or in a control population. **Outcome:** Clearly measurable health endpoint. **Study design:** Epidemiologic studies on health effects of ozone consisting of cross-sectional, case-control, case-crossover, cohort, panel, and time-series studies.

10.3.1.5 Welfare—Ecological Studies

Similar to health effects, this ISA builds on information available during the last review (i.e., effects of ozone exposure on vegetation and ecosystems). For research evaluating ecological effects, emphasis was placed on recent studies that: (1) evaluated effects at ozone concentrations likely to occur in North American airsheds and (2) investigated effects on any individual, population (in the sense of a group of individuals of the same species), community, or ecosystem in North America (Table 10-3). In instances when a "causal relationship" was concluded in the 2013 Ozone ISA (i.e., visible foliar injury, vegetation growth, reduced yield/quality of agricultural crops, reduced productivity, alteration of belowground biogeochemical cycles) the current review only evaluated studies conducted in North America. For all other ecological endpoints in Table 10-3 (terrestrial water cycling, carbon sequestration, terrestrial community composition, plant reproduction, phenology, or mortality, insects, other wildlife, plant-animal signaling) there are no geographic constraints and all available evidence was considered.

Table 10-3 Population, Exposure, Comparison, Outcome, and Study design (PECOS) tool to define the parameters and provide a framework for identifying relevant ecological studies.

Ecological Endpoint	Population, Exposure, Comparison, Outcome, and Study Design (PECOS) Tool
Visible foliar injury, vegetation growth, yield/quality of agricultural crops, productivity, belowground biogeochemical cycling	**Population:** For any species, an individual, population (in the sense of a group of individuals of the same species), community, or ecosystem in North America **Exposure:** Concentrations occurring in the environment or experimental ozone concentrations within an order of magnitude of recent concentrations (as described in Appendix 1) **Comparison:** Relevant control sites, treatments, or parameters **Outcome:** Visible foliar injury, alteration of vegetative growth, yield/quality of agricultural crops, productivity, belowground biogeochemical cycles **Study Design:** Laboratory, greenhouse, OTC, FACE, field, gradient, or modeling studies
Terrestrial water cycling; carbon sequestration; terrestrial community composition; plant reproduction, phenology, or mortality; insects, other wildlife, plant-animal signaling	**Population:** For any species, an individual, population (in the sense of a group of individuals of the same species), community, or ecosystem in any continent[a] **Exposure:** Concentrations occurring in the environment or experimental ozone concentrations within an order of magnitude of recent concentrations (as described in Appendix 1) **Comparison:** Relevant control sites, treatments, or parameters **Outcome:** Alteration of terrestrial water cycling; carbon sequestration; terrestrial community composition; plant reproduction, phenology, mortality; growth, reproduction, and survival of insects and other wildlife; plant-animal signaling **Study Design:** Laboratory, greenhouse, OTC, FACE, field, gradient, or modeling studies

Comparator = change in endpoint observed by unit increase in concentration of ozone in the same or in a control population; exposure = environmental variable to which population is exposed; outcome = measurable endpoint resulting from exposure; population = unit of study; study design = laboratory, field, gradient, open top chamber (OTC), free-air carbon dioxide enrichment (FACE), greenhouse, and modeling studies.

Notes: This definition of population is for the purpose of applying PECOS to ecology. Ecological populations are defined as a group of individuals of the same species.

[a]In cases where a comprehensive list of affected species was available, non-agricultural North American species were separated out from the larger data sets and the evidence was evaluated (e.g., foliar injury, biomass).

10.3.1.6 Welfare—Effects on Climate

For effects on climate, the ISA focused on effects of tropospheric ozone on climate, consistent with the inclusion of "climate" in the list of effects on welfare in Section 302(h) of the Clean Air Act. The ISA does not focus on downstream ecosystem effects from changes in climate, climate-related human health effects, or future air quality projections resulting from changes in climate. In addition, the ISA

assessed the available evidence on the effects of tropospheric ozone as an absorber of UV-B radiation. Studies that assess the independent role of ozone in climate forcing as well as its effects on U.S. national and regional climate are within the scope of the literature considered in the review (Table 10-4).

Table 10-4 Population, Exposure, Comparison, Outcome, and Study design (PECOS) tool to define the parameters and provide a framework for identifying relevant studies on the effects of tropospheric ozone on climate.

Effect on Climate	Population, Exposure, Comparison, Outcome, and Study Design (PECOS)
Changes in radiative forcing (RF)	**Population/Geographical Scope:** Evaluations of radiative forcing at the regional, continental, and/or global scale **Exposure:** Tropospheric ozone concentration distributions in 3D (observed/modeled) **Comparison:** Relevant baseline or unperturbed scenarios/conditions **Outcome:** Changes in RF resulting from change in tropospheric ozone **Study Design:** Observations or modeling studies
Changes in climate (e.g., surface temperature, hydrological cycle)	**Population/Geographical Scope:** Evaluations of climate effects at the regional, continental, and/or global scale **Exposure:** Tropospheric ozone concentration distributions in 3D (observed/modeled) **Comparison:** Relevant baseline or unperturbed scenarios/conditions **Outcome:** Subsequent climate effects (via radiative forcing) (e.g., global surface temperature) resulting from change in tropospheric ozone **Study Design:** Observational or modeling studies

Population/geographical scope: Spatial extent of study
Exposure: Environmental variable (tropospheric O_3 concentrations)
Comparator: Radiative forcing or climate effects observed from unit change in tropospheric O_3 concentration
Outcome: Relevant radiative forcing or climate outcomes resulting from change in tropospheric O_3
Study design: Observations/satellite, modeling

10.3.2 Individual Study Quality

After selecting studies for inclusion based on relevance, individual study quality was evaluated by considering the design, methods, conduct, and documentation of each study, but not the study results. For ISAs, the overall individual study quality process is described in the Preamble to the ISA (U.S. EPA, 2015a).

The process for individual study quality criteria has been refined by discipline with each ISA. Prior to evaluating the evidence for the health disciplines (i.e., experimental and epidemiology), study quality criteria tables were developed to define important aspects to consider in evaluating if a study addresses issues specific to ozone in a proper manner. Study quality criteria tables have been developed for each of the most recent ISAs (U.S. EPA, 2018, 2017, 2016) and are pollutant specific. Study quality criteria tables mirror the process described in the Preamble and serve as the foundation for review of health studies. Ecological studies used a broad set of questions from the Preamble to review study quality, while other disciplines (e.g., atmospheric sciences) used similar approaches appropriate for and consistent with the fields of study. The processes used are described in the sections below.

Study quality was a final step in Level 2 screening before deciding whether to include a study in the ISA. Any references that did not pass the study quality review, and deemed critically deficient, were excluded from the ISA. Any study that passed both the relevance screening and the study quality evaluation was included in the ISA. The combination of approaches described above are intended to produce a comprehensive collection of pertinent studies needed to address the key scientific issues that form the basis of the ISA.

10.3.2.1 Atmospheric Science

The topics relevant to the atmospheric science of ground-level ozone formation include estimated emissions of ozone precursors by anthropogenic, natural (i.e., nonanthropogenic) and international precursor sources, photochemical formation mechanisms, atmospheric dynamics, photochemical modeling, ambient ozone measurement methods, and the temporal and spatial concentration patterns of anthropogenic and USB ozone. Study quality in this context is established using the following criteria: (1) quality assurance and control standards were applied in the development of the data sets used to create figures showing national precursor emissions and ground-level ozone concentrations and (2) studies describing new understanding of the relevant precursor emissions, atmospheric processes, and photochemical modeling of the formation of ozone were subject to peer review. Peer review represents the current standard for establishing study quality in the atmospheric sciences.

10.3.2.2 Exposure Assessment

Exposure assessment studies that passed the PECOS relevance screening were then evaluated for study quality. For exposure assessment methodology studies, study quality was deemed adequate if the exposure assessment methods were clearly described, the selected exposure assessment methods were appropriate for the research question evaluated, the assumptions of the method(s) were clearly stated, the uncertainties and limitations of the methods were clearly stated, and if quality assurance testing had been performed. These requirements were based on studies in the literature that provided good examples of

study quality evaluation for exposure assessment methods (Zou et al., 2009; Ryan and Lemasters, 2007; Nieuwenhuijsen et al., 2006; Gilliland et al., 2005; Jerrett et al., 2005). Validation techniques for quality assurance testing varied from study to study but often included some form of comparison with Federal Reference Method monitoring data.

10.3.2.3 Health Approach

Consistent with the Preamble, conclusions across the body of evidence are made after independently evaluating the overall quality of each study. This uniform approach considers the strengths, limitations, and possible roles of chance, confounding, and other biases that may influence the results of the study.

The evaluation of individual study quality in ISAs has evolved over the past several ISAs. U.S. EPA developed the Preamble in 2015 based on input and feedback from numerous reviews by the CASAC over several years. In recent ISAs (U.S. EPA, 2018, 2017, 2016), study quality tables for human health were developed to provide greater clarity on important aspects of study quality. These tables describe the characteristics of multiple study domains (e.g., study design, exposure assessment, potential confounding) that can increase or decrease confidence in the study results.

For this ISA, the study quality tables were tailored to address factors specific to health studies of ozone exposure (Table Annex 3-1). For example, the study quality table for ozone describes the potential confounding factors, copollutants, lags, averaging times, and seasonal considerations that are relevant to the analysis of ozone and health effects.

To further facilitate the study quality evaluation of health studies, the study quality tables were used to develop prompting questions for each of the study domains included in the study quality table. These prompting questions were designed to assist in the narrative documentation of study quality for each of the individual domains, ensuring consistent information is included across reviewers. The specific prompting questions are different for epidemiology and experimental (i.e., animal toxicology and controlled human exposure) studies, reflecting differences in relevant study quality factors between the two disciplines. The goal of the narrative approach is to provide nuanced and transparent documentation of the strengths and limitations that support expert judgement on the inclusion/exclusion decisions for individual studies. Narrative reviews were completed for the most policy-relevant studies,[1] and the completed reviews for each of the selected studies were recorded in the Health Assessment Workspace Collaborative (HAWC) database and can be accessed from the HERO project page (see Section 10.3.3). The HAWC project page also contains that guidance text and prompting questions (EPA, 2019).

[1] Studies reviewed included those related to health effects for which there was a "causal" or "likely to be a causal" relationship in the 2013 Ozone ISA, or for which the determination about the causal relationship changed from the determination made in the 2013 Ozone ISA.

10.3.2.4 Ecological Approach

Worldwide, the field of study quality evaluation is much more robust for human health research than for ecological research. Study quality is still very important for ecological research, and U.S. EPA staff have relied on the Preamble as criteria for reviewing the quality of individual ecological studies within this ISA. The Preamble provides a base set of questions for consideration when evaluating the scientific quality of studies, intended for use in both human health and ecological studies:

- Were the study designs, study groups, methods, data, and results clearly presented in relation to the study objectives to allow for study evaluation? Were limitations and any underlying assumptions of the design and other aspects of the study stated?
- Were the ecosystems, study site(s), study populations, subjects, or organism models adequately selected, and are they adequately defined to allow for meaningful comparisons between study or exposure groups?
- Are the air quality, exposure, or dose metrics of adequate quality and are they sufficiently representative of or pertinent to ambient air?
- Are the welfare effect measurements meaningful, valid, and reliable?
- Were likely covariates or modifying factors adequately controlled or taken into account in the study design and statistical analysis?
- Do the analytical methods provide adequate sensitivity and precision to support conclusions?
- Were the statistical analyses appropriate, properly performed, and properly interpreted?

U.S. EPA also relied on discussions in previous ISAs about the strengths and weaknesses of various ecological study designs (see Section 8.1.2.1). A limited number of studies were excluded based on consideration of these study quality questions and application of the PECOS tool. The main reasons that studies were eliminated were due to insufficient or low replication, ozone exposures were unable to be determined, lack of statistical testing for endpoints of interest, methods were inadequately described, selective reporting of data, flaws in study design, or a model was based on flawed data or incorrect assumptions.

10.3.3 Documentation

During the review of references for Level 2 screening, inclusion and exclusion decisions were carefully documented. The inclusion/exclusion results are recorded in HERO, while specific data about concentrations, experimental design, and results are reported within the appendices. Reference-specific information about study quality are documented in HAWC for select studies and can be accessed via the HERO project page associated with this ISA (EPA, 2019). All decisions about Level 2 screening, including both relevance and quality, are documented in the HERO database and on the publicly available HERO project page for this ISA.

10.4 Peer Review and Public Participation

Peer review is an important component of any scientific assessment. U.S. EPA has formal guidance about peer review in the Peer Review Handbook (U.S. EPA, 2015b), and this ISA follows all the policies and procedures identified therein. Additionally, this ISA follows all the guidelines of the Information Quality Guidelines (U.S. EPA, 2002).

U.S. EPA has designated this ISA as a Highly Influential Scientific Assessment, which is defined by The Office of Management and Budget's *Final Information Quality Bulletin for Peer Review* (hereafter, "Peer Review Bulletin") as:

> A subset of Influential Scientific Information that is a scientific assessment (i.e., an evaluation of a body of scientific or technical knowledge, which typically synthesizes multiple factual inputs, data, models, assumptions and/or applies best professional judgment to bridge uncertainties in the available information) that "could have a potential impact of more than $500 million in any year on either the public or private sector" or "is novel, controversial, or precedent-setting, or has significant interagency interest." (https://obamawhitehouse.archives.gov/omb/memoranda_fy2005_m05-03/).

As such, there are additional review and transparency steps required in the release of this information. These steps are described below. CASAC serves an important role in reviewing this ISA (see Section 10.4.5).

10.4.1 Call for Information

Consistent with the Preamble, a Call for Information was published in the Federal Register on June 26, 2018 (83 FR 29785). The purpose of this Call for Information was announcing the beginning of the review cycle of the air quality criteria and the ozone NAAQS. Specifically, the Call for Information stated that U.S. EPA would be preparing an Integrated Review Plan and Integrated Science Assessment. The public was given 30 days "…to assist the U.S. EPA by submitting information regarding significant new ozone research and policy-relevant issues for consideration in this review of the primary (health-based) and secondary (welfare-based) ozone standards." U.S. EPA received 14 comments via the Federal eRulemaking Portal (http://www.regulations.gov).

In previous assessments, U.S. EPA has held a kick-off workshop to begin a review cycle. The workshop brought together subject area experts and the public to highlight significant new and emerging research and make recommendations to the U.S. EPA regarding the design and scope of the review. However, process efficiencies were discussed in the Administrator's May 9, 2018 memorandum, "Back-to-Basics Process for Reviewing National Ambient Air Quality Standards."

(https://www.epa.gov/sites/production/files/2018-05/documents/image2018-05-09-173219.pdf). In particular, the Administrator called for "…efficiencies and opportunities to streamline the NAAQS review process to ensure they finish within a 5-year interval. For the next review of the ozone NAAQS, U.S. EPA shall seek efficiencies through replacing the kick-off workshop with a more robust request for information…." As a result, no kick-off workshop was held for this review cycle and the Call for Information served as the formal initiation of the NAAQS review process.

10.4.2 Integrated Review Plan

Following the Call for Information, U.S. EPA prepared an Integrated Review Plan (IRP) that summarizes the current plan for this NAAQS review, a projected timeline, and the process for conducting the review. The IRP also identifies key policy-relevant issues or questions intended to guide the review. The draft IRP for this review cycle was announced in the Federal Register on November 2, 2018 (83 FR 55163) for consultation with the CASAC and for public comment. The public was given 30 days to respond, and U.S. EPA received 13 comments via the Federal eRulemaking Portal (http://www.regulations.gov).

A CASAC consultation was held in a public meeting on November 29, 2018, and documentation of that occurrence along with written comments from individual CASAC members were sent to the U.S. EPA Administrator in a letter dated December 10, 2018 (https://yosemite.epa.gov/sab/sabproduct.nsf/A286A0F0151DC8238525835F007D348A/$File/EPA-CASAC-19-001.pdf). The final IRP was prepared in consideration of CASAC and public comments and released in August 2019.

10.4.3 Peer Input

The role of peer input is described in the Preamble, as well as the Peer Review Handbook (U.S. EPA, 2015a, b). After a thorough literature search and screening process, U.S. EPA staff developed preliminary drafts of all appendices for initial peer input. Peer input is a process that allows U.S. EPA to solicit feedback from subject-matter experts to ensure that the ISA is up-to-date and focused on the most policy-relevant findings. This review also assists U.S. EPA with integration of evidence within and across disciplines. Peer input serves as a supplement to other peer-review mechanisms and does not take the place of a thorough external peer review.

For this ISA, U.S. EPA worked with ICF International to run the peer input process. Following the guidelines in the peer-review handbook, U.S. EPA prepared a list of expertise needed for a first review of the ISA material. ICF was solely responsible for inviting 24 experts across four discipline areas (link to Front Matter page with list of reviewers) to participate in the peer input workshops and for coordinating all communication for this consultation. A Federal Register Notice was issued on October

23, 2018 announcing the workshops and included information for public access to the discussions (83 FR 53472). No formal public comment was held at this early stage.

U.S. EPA developed a charge for the reviewers that included questions specific to each discipline, as well as three overarching prompts:

- Please comment on the extent to which the initial draft materials capture the key studies from the peer-reviewed literature that have been published since the completion of the 2013 Ozone ISA. As noted in the following Session areas, please identify additional studies published since the 2013 Ozone ISA that should be included.
- The review of evidence is intended to be concise and coherent, not encyclopedic. To that end, please comment on the extent to which the scientific information is accurately characterized and with the appropriate level of detail in draft materials. Are there any additional topics, endpoints, factors, etc. that should be included?
- Are there specific issues that should be considered or highlighted that will be important for integrating evidence across disciplines?

Reviewers provided written responses to U.S. EPA both prior to the workshop discussion and a revised version afterwards. Webinar workshops were held during October and November 2018. Following these consultation workshops, U.S. EPA considered reviewer comments and literature suggestions to revise the document.

10.4.4 Internal Technical Review

The U.S. EPA Office of Research and Development guidelines require an internal technical review process prior to any external dissemination of scientific information. Consistent with this policy, the draft ISA was reviewed by U.S. EPA subject-matter experts, both those who had been involved in developing the draft document and those who had not. Following the technical review, U.S. EPA used the reviewers' comments to revise the document.

10.4.5 Clean Air Scientific Advisory Committee (CASAC) Peer Review

The Clean Air Act governs the NAAQS review process, and also includes instruction about review of science and policy documents developed by U.S. EPA (CAA, 1990). Section 109(d)(2) addresses the appointment and advisory functions of an independent scientific review committee. Section 109(d)(2)(A) requires the Administrator to appoint this committee, which is to be composed of "seven members including at least one member of the National Academy of Sciences, one physician, and one person representing State air pollution control agencies." Section 109(d)(2)(B) provides that the independent scientific review committee "shall complete a review of the criteria…and the national primary and secondary ambient air quality standards…and shall recommend to the Administrator any

new…standards and revisions of existing criteria and standards as may be appropriate….” Since the early 1980s, this independent review function has been performed by the CASAC of the U.S. EPA’s Science Advisory Board.

The CASAC serves as the official peer review mechanism for this ISA. As a Highly Influential Scientific Assessment, the review process is also governed by the Peer Review Bulletin. All requirements in the Peer Review Bulletin about selection of reviewers, information access, opportunity for public participation, transparency, and management of peer-review process and reviewer selection have been met.

Release of this draft ISA has been announced in the Federal Register. This also begins a period of time for the public to provide comment on this draft. Additionally, the CASAC will hold a public meeting to discuss the draft ISA and provide an independent scientific peer review of the document.

10.5 Quality Assurance

The use of quality assurance (QA) helps ensure that the U.S. EPA conducts high-quality science that can be used to inform policymakers, industry, and the public. Agency-wide, the U.S. EPA Quality System provides the framework for planning, implementing, documenting, and assessing work performed by the Agency, and for carrying out required quality assurance and quality control activities. Additionally, the Quality System covers the implementation of the U.S. EPA Information Quality Guidelines (U.S. EPA, 2002). This ISA follows all Agency guidelines to ensure a high-quality document.

Within the U.S. EPA, Quality Assurance Project Plans (QAPPs) are developed to ensure that all Agency materials meet a high standard for quality. U.S. EPA has developed a Program-level QAPP (PQAPP) for the ISA Program to describe the technical approach and associated QA/QC procedures associated with the ISA Program. All QA objectives and measurement criteria detailed in the PQAPP have been employed in developing this draft ISA. More specifically, QA was conducted on all appendices, and the numbers from every tenth reference, or in some instances more, were checked for accuracy. Furthermore, publicly available databases (e.g., National Emissions Inventory, Air Quality System database) from which data was used in analyses were verified to have QA processes in place.

Additionally, U.S. EPA QA staff are responsible for the review and approval of all quality-related documentation. Because this is a Highly Influential Scientific Assessment (see Section 10.4), U.S. EPA QA staff will perform a Technical System Audit on this draft before final release. This audit verifies that the appropriate QA procedures, criteria, reviews, and data verification are adequately performed and documented.

10.6 Conclusion

Overall, U.S. EPA has a robust set of policies and procedures in place to ensure the highest-quality products. In developing this ISA, the U.S. EPA has followed all current processes and endeavors to add additional steps as needed. This Appendix will be updated before final release to include information about the CASAC review, QA audit, and any other process developments.

10.7 References

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

Cohen, AM; Hersh, WR; Peterson, K; Yen, PY. (2006). Reducing workload in systematic review preparation using automated citation classification. J Am Med Inform Assoc 13: 206-219. http://dx.doi.org/10.1197/jamia.M1929.

EPA (Environmental Protection Agency). (2019). Ozone ISA Study Quality Evaluations - Health.

Gaudel, A; Cooper, OR; Ancellet, G; Barret, B; Boynard, A; Burrows, JP; Clerbaux, C; Coheur, PF; Cuesta, J; Cuevas, E; Doniki, S; Dufour, G; Ebojie, F; Foret, G; Garcia, O; Granados Muños, MJ; Hannigan, J; Hase, F; Huang, G; Hassler, B; Hurtmans, D; Jaffe, D; Jones, N; Kalabokas, P; Kerridge, B; Kulawik, SS; Latter, B; Leblanc, T; Le Flochmoën, E; Lin, W; Liu, J; Liu, X; Mahieu, E; McClure-Begley, A; Neu, JL; Osman, M; Palm, M; Petetin, H; Petropavlovskikh, I; Querel, R; Rahpoe, N; Rozanov, A; Schultz, MG; Schwab, J; Siddans, R; Smale, D; Steinbacher, M; Tanimoto, H; Tarasick, D; Thouret, V; Thompson, AM; Trickl, T; Weatherhead, E; Wespes, C; Worden, H; Vigouroux, C; Xu, X; Zeng, G; Ziemke, J. (2018). Tropospheric ozone assessment report: Present-day distribution and trends of tropospheric ozone relevant to climate and global atmospheric chemistry model evaluation. Elementa: Science of the Anthropocene 6: 39. http://dx.doi.org/10.1525/elementa.291.

Gilliland, F; Avol, E; Kinney, P; Jerrett, M; Dvonch, T; Lurmann, F; Buckley, T; Breysse, P; Keeler, G; de Villiers, T; Mcconnell, R. (2005). Air pollution exposure assessment for epidemiologic studies of pregnant women and children: Lessons learned from the Centers for Children's Environmental Health and Disease Prevention Research. Environ Health Perspect 113: 1447-1454. http://dx.doi.org/10.1289/ehp.7673.

Howard, BE; Phillips, J; Miller, K; Tandon, A; Mav, D; Shah, MR; Holmgren, S; Pelch, KE; Walker, V; Rooney, AA; Macleod, M; Shah, RR; Thayer, K. (2016). SWIFT-Review: a text-mining workbench for systematic review. Syst Rev 5: 87. http://dx.doi.org/10.1186/s13643-016-0263-z.

IPCC (Intergovernmental Panel on Climate Change). (2013). Climate change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change. In TF Stocker; D Qin; GK Plattner; MMB Tignor; SK Allen; J Boschung; A Nauels; Y Xia; V Bex; PM Midgley (Eds.). Cambridge, UK: Cambridge University Press. http://www.ipcc.ch/report/ar5/wg1/.

Jaffe, DA; Cooper, OR; Fiore, AM; Henderson, BH; Tonnesen, GS; Russell, AG; Henze, DK; Langford, AO; Lin, M; Moore, T. (2018). Scientific assessment of background ozone over the US: Implications for air quality management. 6. http://dx.doi.org/10.1525/elementa.309.

Jerrett, M; Arain, A; Kanaroglou, P; Beckerman, B; Potoglou, D; Sahsuvaroglu, T; Morrison, J; Giovis, C. (2005). A review and evaluation of intraurban air pollution exposure models [Review]. J Expo Anal Environ Epidemiol 15: 185-204. http://dx.doi.org/10.1038/sj.jea.7500388.

NASEM (National Academies of Sciences, Engineering, and Medicine). (2018). Progress toward transforming the Integrated Risk Information System (IRIS) program. A 2018 evaluation. Washington, DC: The National Academies Press. http://dx.doi.org/10.17226/25086.

Nieuwenhuijsen, M; Paustenbach, D; Duarte-Davidson, R. (2006). New developments in exposure assessment: The impact on the practice of health risk assessment and epidemiological studies [Review]. Environ Int 32: 996-1009. http://dx.doi.org/10.1016/j.envint.2006.06.015.

Ryan, PH; Lemasters, GK. (2007). A review of land-use regression models for characterizing intraurban air pollution exposure [Review]. Inhal Toxicol 19: 127. http://dx.doi.org/10.1080/08958370701495998.

U.S. EPA (U.S. Environmental Protection Agency). (2002). Guidelines for ensuring and maximizing the quality, objectivity, utility, and integrity of information disseminated by the Environmental Protection Agency. (EPA/260/R-02/008). Washington, DC: U.S. Environmental Protection Agency, Office of Environmental Information. https://www.epa.gov/sites/production/files/2017-03/documents/epa-info-quality-guidelines.pdf.

U.S. EPA (U.S. Environmental Protection Agency). (2013). Integrated science assessment for ozone and related photochemical oxidants [EPA Report]. (EPA/600/R-10/076F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment-RTP Division. http://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=247492.

U.S. EPA (U.S. Environmental Protection Agency). (2015a). Preamble to the integrated science assessments [EPA Report]. (EPA/600/R-15/067). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, RTP Division. https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=310244.

U.S. EPA (U.S. Environmental Protection Agency). (2015b). Science policy council peer review handbook [EPA Report] (4th ed.). (EPA/100/B-15/001). Washington, DC: U.S. Environmental Protection Agency, Science Policy Council. https://www.epa.gov/osa/peer-review-handbook-4th-edition-2015.

U.S. EPA (U.S. Environmental Protection Agency). (2016). Integrated science assessment for oxides of nitrogen-Health Criteria (final report) [EPA Report]. (EPA/600/R-15/068). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment. http://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=526855.

U.S. EPA (U.S. Environmental Protection Agency). (2017). Integrated science assessment for sulfur oxides: Health criteria [EPA Report]. (EPA/600/R-17/451). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment-RTP. https://www.epa.gov/isa/integrated-science-assessment-isa-sulfur-oxides-health-criteria.

U.S. EPA (U.S. Environmental Protection Agency). (2018). U.S. EPA. Integrated Science Assessment (ISA) for Particulate Matter (External Review Draft). (EPA/600/R-18/179). Washington, DC. https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=341593.

Wuebbles, DJ; Fahey, DW; Hibbard, KA; Dokken, DJ; Stewart, BC; Maycock, TK. (2017). USGCRP, 2017: Climate science special report: Fourth national climate assessment, Volume I. Washington, DC: U.S. Global Change Research Program. https://science2017.globalchange.gov/.

Zou, B; Wilson, JG; Zhan, FB; Zeng, Y. (2009). Air pollution exposure assessment methods utilized in epidemiological studies [Review]. J Environ Monit 11: 475-490. http://dx.doi.org/10.1039/b813889c.