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Integrated Science Assessment for Oxides of Nitrogen– Health Criteria

(Second External Review Draft)

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

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ACRONYMS AND ABBREVIATIONS

Acronym/Abbreviation	Meaning
α	alpha, exposure factor
α-ATD	alpha 1-antitrypsin deficiency
A4	not classifiable for humans or animals
AADT	annual average daily traffic
ABI	ankle brachial index
Abs	absorbance coefficient
$ABTS^{\bullet -}$	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical
$ABTS^{2-}$	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
ACS	American Cancer Society
ADRB2	beta-2-adrenergic receptor
AERMOD	American Meteorological Society/Environmental Protection Agency Regulatory Model
AHR	airway hyperresponsiveness
AHSMOG	California Seventh-Day Adventists cohort
a_j	air exchange rate
AIRES	Aerosol Research Inhalation Epidemiology Study
ARIES	Atlanta Aerosol Research Inhalation Epidemiology Study
AK	Alaska
AKR/J	mice strain with short life-span; often used as model for aging
AL	Alabama; alpine
ALKP	alkaline phosphatase
ALRI	acute lower respiratory infection
a.m.	ante meridiem (before noon)
AM	alveolar macrophages
AMF	air mass factor
ANN	artificial neural networks
ANPR	advanced notice of public rulemaking
APEX	Air Pollution Exposure model
APHEA	Air Pollution and Health: A European Approach study
APHENA	Air Pollution and Health: A European and North American Approach study
$ApoE^{-/-}$	apolipoprotein E knockout
AQCD	air quality criteria document
AQI	air quality index
AQM	air quality model
AQS	air quality system
AR	Arkansas; airway responsiveness
ARIMA	autoregressive integrated moving average
AT	atascadero
ATS	American Thoracic Society
AUSTAL2000	Ausbreitungsmodell gemäß der Technischen Anleitung zur Reinhaltung der Luft
avg	average
AW	area wide
AZ	Arizona
β	beta
β_0	model intercept
β_1	effect estimate for the ambient exposure
β_Z	vector of slope related to each covariate
BAL	bronchoalveolar lavage
BALF	bronchoalveolar lavage fluid
BAMSE	Children, Allergy, Milieu, Stockholm, Epidemiology Survey
BC	black carbon
BD	bronchodilator
BEIS	Biogenic Emission Inventory System
BHPN	N-bis (2-hydroxy-propyl) nitrosamine
BIR	birch

Acronym/Abbreviation	Meaning
BL	bronchial lavage
BMI	body mass index
BP	blood pressure
BR^-	bromide
BS	black smoke
BSA	body surface area
BTEX	sum of the VOCs benzene, toluene, ethybenzene, xylene
BW	body weight
BWHS	Black Women's Health Study
C_6H_6	benzene
C	degrees Celsius; the product of microenvironmental concentration; carbon;
C'	average concentration normalized by the standard deviation of concentration
C&RT	classification and regression tree
Ca^{2+}	calcium
CA	California; cat allergen
C_a	ambient NO_2 concentration
$C_{a,csm}$	ambient concentration at a central site monitor
CAA	Clean Air Act
CAMP	Childhood Asthma Management Program
CAP	concentrated ambient particle
CAPES	China Air Pollution and Health Effects Study
CAPS	cavity attenuated phase shift
CARB	carachol
CASAC	Clean Air Scientific Advisory Committee
CASNET	Clean Air Status and Trends Network
C_b	NO_2 concentration contribution away from the influence of the road
CBSA	Core Based Statistical Area
CBVD	cerebrovascular disease
CC16	club cell protein
CDC	Centers for Disease Control and Prevention
C_{far}	farthest concentration
CFD	computational fluid dynamics
CFR	Code of Federal Regulations
cGMP	cyclic guanosine monophosphate
CH_4	methane
CHD	coronary heart disease
CHF	congestive heart failure
CHS	Children's Health Study
CHIMERE	regional chemistry transport model
C_i	average NO_2 concentration in the *i*th microenvironment; substrate concentrations
CI(s)	confidence interval(s)
cIMT	carotid intima-media thickness
C_j	average NO_2 concentration in the jth microenvironment
CJ-A	Ciudad Juarez–site A
CJ-B	Ciudad Juarez–site B
Cl^-	chloride
CL/MC	chemiluminescence analyzer with a MoO_X catalytic converter
CL/PC	chemiluminescence analyzer with measurements from a photolytic converter
ClNO	nitrosyl chloride
$ClNO_2$	nitryl chloride
CMAQ	Community Multiscale Air Quality
C_{near}	nearest concentration
CO	carbon monoxide; Colorado
$C_{o,j}$	ambient exposure to NO_2 outdoor concentration
CO_2	carbon dioxide
COD	coefficient of divergence
COLD	cold-dry air
COMET	single cell gel electrophoresis
Cong.	congress
COPD	chronic obstructive pulmonary disease
C-R	concentration-response (relationship)

Acronym/Abbreviation	Meaning
CRDS	cavity ring down spectroscopy
CRP	C-reactive protein
CT	Connecticut
CTM	chemical transport models
CTS	California Teachers Study
Cu	copper
C_V	NO_2 concentration contribution from vehicles on a roadway
CV	coefficient of variation
CVD	cardiovascular disease
C_X	NO_2 concentration at a distance x from a road
$Cys^{\bullet}$	cysteine radical
D	molecular diffusion coefficient of NO_2
d	distance
D	distance in kilometers
D.C. Cir	Distric of Columbia Circuit
DBP	diastolic blood pressure
DC	District of Columbia
DE	Deleware
DEARS	Detroit Exposure and Aerosol Research Study
DEP	diesel exhaust particles
df	degrees of freedom
DHA	dehydroascorbate
DL	distributed lag
DM	diabetes mellitus
DNA	deoxyribonucleic acid
DOAS	differential optical absorption spectroscopy
DOCs	diesel oxidation catalysts
dPD	change in provocative dose
DPF	diesel particulate filter
DPPC	dipalmitoyl phosphatidylcholine
Dt/dx	change in x with respect to time as the limit of x approaches zero
DVT	deep vein thrombosis
e.g.	exempli gratia (for example)

Acronym/Abbreviation	Meaning
E_a	the sum of an individual's ambient NO_2 exposure
E_{anj}	indoor exposures from nonambient sources
EBC	expired (exhaled) breath condensate
EC	elemental carbon
ECG	electrocardiographic
ECP	eosinophil cationic protein
ECRHS	European Community Respiratory Health Survey
ED	emergency department
EGR	exhaust gas recirculation
EGU	electric power generating unit
E_{ij}	indoor NO_2 exposures in the jth microenvironment
ELF	epithelial lining fluid
E_{na}	the sum of an individual's nonambient NO_2 exposure
eNO	exhaled nitric oxide
eNOS	endothelial nitric oxide synthase
E_o	outdoor microenvironmental NO_2 exposures
$E_{o,j}$	outdoor NO_2 exposures in the jth microenvironment
EP	entire pregnancy
EPA	U.S. Environmental Protection Agency
EP-A	El Paso–site A
EP-B	El Paso–site B
ESCAPE	European Study of Cohorts for Air Pollution Effects
ESR	erythrocyte sedimentation rate
E_T	total personal exposure
ET-1	vasoconstrictor endothelin-1
ETS	environmental tobacco smoke
EXPOLIS	exposure in polis or cities
f	female
FEF	forced epiratory flow
$FEF_{25-75\%}$	forced expiratory flow at 25-75% of exhaled volume

Acronym/Abbreviation	Meaning
$FEF_{50\%}$	forced expiratory flow at 50% of forced vital capacity
FEM	federal equivalent method
FEV	forced expiratory volume
FEV_1	forced expiratory volume in 1 second
FL	Florida
FR	Federal Register
FRM	federal reference method
FVC	forced vital capacity
γ	gamma; uptake coefficients
γ’	semivariogram
g	gram
g/bhp-h	grams per brake horsepower-hour
GA	Georgia
GAM	generalized additive models
GASPII	Gene and Environmental Prospective Study in Italy
GCLC	gene that encodes the catalytic subunit for the human enzyme glutamate-cysteine ligase
GCLM	gene that encodes the regulatory subunit for the human enzyme glutamate-cysteine ligase
GD	gestation day
GEE	generalized estimating equations
GEOS	Goddard Earth Observing System
GINI	German Infant Nutritional Intervention
GINI SOUTH	German Infant Nutritional Intervention covers the urban city of Munich, Germany, and its surrounding areas (approximately 28,000 km^2)
GINIplus	German Infant Nutritional Intervention plus environmental and genetic influences
GIS	geographic information systems
GLM	generalized linear model
GLMM	generalized linear mixed model
GM-CSF	granulocyte macrophage-colony stimulating factor
GP	general practice
GPS	global positioning system
GPx	glutathione peroxidase
$GS^{\bullet}$	glutathione radical
GSD	geometric standard deviation
GSH	glutathione
GSNOR	nitrosoglutathione reductase
GSR	glutathione reductase
GSS	glutathione synthetase
GST	glutathione S-transferase
GSTM1	glutathione S-transferase Mu 1
GSTP1	glutathione S-transferase Pi 1
GSTT1	glutathione S-transferase theta 1
GW	gestational week
h	hour(s)
H^+	hydrogen ion
H_2SO_4	sulfuric acid
HC	hydrocarbon(s)
hCAEC	human coronary artery endothelial cell
HCl	hydrochloric acid
HDL	high-density lipoprotein
HDM	house dust mite; house dust mite allergen
HERO	Health and Environmental Research Online
HEV	hold-out evaluation
HF	high frequency; high frequency component of HRV
HFE	human hemochromatosis protein
HFn	high frequency domain normalized for heart rate
HGF	hepatocyte growth factor
HI	Hawaii
HIST	histamine
HMOX	heme oxygenase
HNO_2	nitrous acid
HNO_3	nitric acid
HNO_4	peroxynitric acid
HO-1	heme oxygenase-1

Acronym/Abbreviation	Meaning
HO_2	hydroperoxyl radical
HO_2NO_2	peroxynitric acid
HONO	nitrous acid
HOONO	pernitrous acid
HR	hazard ratio(s); heart rate
HRV	heart rate variability
HS	hemorrhagic stroke
HSC	Harvard Six Cities
hs-CRP	high sensitivity C-reactive protein
IA	Iowa
I ACID	inorganic acid
i.e.	id est (that is)
I.V.	intravenously
ICAM-1	intercellular adhesion molecule 1
ICAS	Inner-City Asthma Study
ICD	International Classification of Diseases; implantable cardioverter defibrillators
ICS	inhaled corticosteroids
ID	Idaho
IDW	inverse distance weighting
IFN-γ	interferon gamma
IgE	immunoglobulin E
IGM	impaired glucose metabolism
IHD	ischemic heart disease
IL	interleukin; Illinois
IL-6	interleukin-6
IL-8	interleukin-8
Ile	isoleucine
IM	immediately after exposure
IMSI	Integrated Mobile Source Indicator
IMT6seg	intima-media thickness of the left and rifht common carotid arteries, internal carotid arteries, and carotid bulbs
IMTcca	intima-media thickness of the common carotid artery
IN	Indiana; isoprene nitrate

Acronym/Abbreviation	Meaning
INDAIR	probabilistic model for indoor pollution exposures
INF_j	infiltration of outdoor NO_2
iNOS	inducible nitric oxide synthase
IOM	Institute of Medicine
IQR	interquartile range
IRP	Integrated Review Plan
IRR	incidence rate ratios
IS	ischemic stroke
ISA	Integrated Science Assessment
IT	intratracheal
IUGR	intrauterine growth restriction
IVF	in vitro fertilization
j	microenvironment
JE	joint model estimate
k	reaction rate; decay constant derived from empirical data
kcal	kilocalorie(s)
kg	kilogram(s)
k_i	second-order rate constant(s)
k_j	decay rate
km	kilometer(s)
kPa	kilopascal(s)
KS	Kansas
KY	Kentucky
L	liter(s)
LA	Louisiana; Los Angeles; Lake Arrowhead
LAT	L-type amino acid transporter
LB	Long Beach
LBW	low birth weight
LDH	lactate dehydrogenase
LE	Lake Elsinore
LETO	Long-Evans Tokushima
LF	low-frequency component of HRV
LF/HF	ratio of LF and HF components of HRV
LIE	Long Island Expressway
LIF	laser induced fluorescence

Acronym/Abbreviation	Meaning
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
LM	Lompoc
LN	Lancaster
LOESS	locally weighted scatterplot smoothing
LOOCV	Leave-one-out cross-validation
LOPAP	long path absorption photometer
LOX-1	lectin-like oxLDL receptor
Lp-PLA_2	lipoprotein-associated phospholipase A2
LRTI	lower respiratory tract infection
LUR	land use regression
µ	mu; micro
$\mu g/m^3$	micrograms per cubic meter
m	meter
M	male
MA	Massachusetts
M1	Month 1
M2	Month 2
M3	Month 3
M4	Month 4
MAAS	Manchester Asthma and Allergy Study
max	maximum
MCP-1	monocyte chemoattractant protein-1
MD	Maryland
MDA	malondialdehyde
ME	Maine
MESA-Air	Multi-Ethnic Study of Atherosclerosis and Air Pollution
MET	MET receptor tyrosine kinase gene
METH	methacholine
METS	metabolic equivalents
MI	myocardial infarction ("heart attack"); myocardial ischemia; Michigan
min	minimum
ML	Mira Loma
mL	milliliter(s)
MLI	mean linear intercept
MMEF	maximum (or maximal) midexpiratory flow
mmHg	millimeters of mercury
MMP	matrix metalloproteinase
MMP-3	matrix metalloproteinase-3
MMP-7	matrix metalloproteinase-7
MMP-9	matrix metalloproteinase-9
MN	Minnesota
mo	month(s)
MO	Missouri
MOA	mode(s) of action
mol	mole
MoO_X	molybdenum oxide
MPO	myeloperoxidase
mRNA	messenger ribonucleic acid
MS	Mississippi
MT	Montana
n	sample size; total number of microenvironments that the individual has encountered
N	nitrogen; population number
N_2O_3	dinitrogen trioxide
N_2O_4	dinitrogen tetroxide
N_2O_5	dinitrogen pentoxide
NA	not available
Na^+	sodium ion
NAAQS	National Ambient Air Quality Standards
NAB	North American Background
NaCl	sodium chloride
NADPH	reduced nicotinamide adenine dinucleotide phosphate

Acronym/Abbreviation	Meaning
NAL	nasal lavage
NAMS	National Air Monitoring Stations
NAS	National Academy of Sciences
NC	North Carolina
NCEA	National Center for Environmental Assessment
NCICAS	National Cooperative Inner-City Asthma Study
NCore	National Core network
ND	North Dakota
NDMA	N-nitrosodimethylamine
NE	Nebraska
NEI	National Emissions Inventory
NFκB	nuclear factor kappa‑light-chain-enhancer of activated B cells
NH	New Hampshire
NH_3	ammonia
$(NH_4)_2SO_4$	ammonium sulfate
NHAPS	National Human Activity Pattern Survey
NHS	Nurses Health Study
NJ	New Jersey
NLCS	Netherlands Cohort Study on Diet and Cancer
nm	nanometer
NM	New Mexico
NMMAPS	The National Morbidity Mortality Air Pollution Study
NMOR	N-nitrosomorpholine
NO	nitric oxide
NO_2	nitrogen dioxide
NO_2^-	nitrite
NO_3^-	nitrate
NO_3	nitrate radical
non-HS	non-hemhorragic stroke
NOS	nitric oxide synthase
NO_X	the sum of NO and NO_2
NO_Y	oxides of nitrogen
NO_Z	reactive oxides of nitrogen (e.g., HNO_3, HONO, PAN, particulate nitrates)
NQO1	NADPH-quinone oxidoreductase (genotype)
NR	not reported; no quantitative results reported; near road
NS	not statistically significant
NV	Nevada
NY	New York
O ACID	organic acid
O_3	ozone
OAQPS	Office of Air Quality Planning & Standards
OC	organic carbon
OH	hydroxide; Ohio
8-OHdG	8-hydroxy-29-deoxyguanosine
OK	Oklahoma
OLETF	Otsuka Long-evans Tokushima Fatty
OLM	ozone limiting method
OMI	Ozone Monitoring Instrument
OR	odds ratio(s); Oregon
OVA	ovalbumin
p	probability
P	Pearson correlation
Pa	pascal(s)
PA	policy assessment; Pennsylvania
PAARC	Air Pollution and Chronic Respiratory Diseases
PAH(s)	polycyclic aromatic hydrocarbon(s)
PAMS	Photochemical Monitoring Stations
PAN	peroxyacetyl nitrate; peroxyacl nitrate
PAPA	Public Health and Air Pollution in Asia
Pb	lead
PBL	planetary boundary layer
PC	provocative concentration
PCA	principal component analysis
PCO	protein carbonyl
PD	provocative dose

Acronym/Abbreviation	Meaning
PE	pulmonary embolism
PEF	peak expiratory flow
PFK	phosphofructokinase
PIAMA	Prevention and Incidence of Asthma and Mite Allergy
PiZZ	severe alpha-1 antitrypsin deficiency
P_j	air pollutant penetration
PK	pyruvate kinase
p.m.	post meridiem (after noon)
PM	particulate matter
PM_{10}	In general terms, particulate matter with a nominal aerodynamic diameter less than or equal to 10 μm; a measurement of thoracic particles (i.e., that subset of inhalable particles thought small enough to penetrate beyond the larynx into the thoracic region of the respiratory tract). In regulatory terms, particles with an upper 50% cut-point of 10 ± 0.5 μm aerodynamic diameter (the 50% cut point diameter is the diameter at which the sampler collects 50% of the particles and rejects 50% of the particles) and a penetration curve as measured by a reference method based on Appendix J of 40 CFR Part 50 and designated in accordance with 40 CFR Part 53 or by an equivalent method designated in accordance with 40 CFR Part 53.
$PM_{10-2.5}$	In general terms, particulate matter with a nominal aerodynamic diameter less than or equal to 10 μm and greater than a nominal 2.5 μm; a measurement of thoracic coarse particulate matter or the coarse fraction of PM_{10}. In regulatory terms, particles with an upper 50% cut-point of 10 μm aerodynamic diameter and a lower 50% cut-point of 2.5 μm aerodynamic diameter (the 50% cut point diameter is the diameter at which the sampler collects 50% of the particles and rejects 50% of the particles) as measured by a reference method based on Appendix O of 40 CFR Part 50 and designated in accordance with 40 CFR Part 53 or by an equivalent method designated in accordance with 40 CFR Part 53.
$PM_{2.5}$	In general terms, particulate matter with a nominal aerodynamic diameter less than or equal to 2.5 μm; a measurement of fine particles. In regulatory terms, particles with an upper 50% cut-point of 2.5 μm aerodynamic diameter (the 50% cut point diameter is the diameter at which the sampler collects 50% of the particles and rejects 50% of the particles) and a penetration curve as measured by a reference method based on Appendix L of 40 CFR Part 50 and designated in accordance with 40 CFR Part 53, by an equivalent method designated in accordance with 40 CFR Part 53, or by an approved regional method designated in accordance with Appendix C of 40 CFR Part 58.
PMA	phorbol myristate acetate
PMN(s)	polymorphonuclear cell(s), polymorphonuclear leukocyte
PNC	particle number concentration
PND	postnatal day

Acronym/Abbreviation	Meaning
pNN50	Proportion of pairs of successive normal simus intervals exceeds 50 milliseconds divided by the total number of successive pairs of normal simus intervals
pNO	particulate nitrogen species
pNO_3	particulate nitrate
PPARγ	peroxisome proliferator activated receptor gamma
ppb	parts per billion
ppm	parts per million
PROtEuS	Prostate Cancer and Environment Study
PTB	preterm birth
PVMRM	plume volume molar ratio method
Q1	1st quartile or quintile
Q2	2nd quartile or quintile
Q3	3rd quartile or quintile
Q4	4th quartile or quintile
Q5	5th quintile
QC-TILDAS	quantum cascade – tunable infrared laser differential absorption spectrometer
QT interval	time between start of Q wave and end of T wave in ECG
QTc	corrected QT interval
QTVI	QT variable index
QUIC	Quick Urban and Industrial Commplex
r	Pearson correlation coefficient; Spearman correlation coefficient
R	Pearson correlation coefficient
R^2	square of the correlation coefficient
RAG	ragweed
RANCH	Road Traffic and Aircraft Noise Exposure and Children's Cognition and Health
RBC	red blood cells
RC(=O)	acyl group
$RC(=O)OONO_2$	peroxyacylnitrates
REA	Risk and Exposure Assessment
REGICOR	Registre Gironi del Cor
RH	relative humidity
RI	Rhode Island
RIVM	National Air Quality Monitoring Network of the National Institute of Public Health and the Environment
rMSSD	root mean square of successive differences; a measure of HRV
RNS	reactive nitrogen species
$RONO_2$	organic nitrates
ROS	reactive oxygen species
RR	risk ratio(s), relative risk
RSNO	S-nitrosothiols
RSV	respiratory syncytial virus
RV	Riverside
s	second(s)
S. Rep	Senate Report
s/L	seconds per liter
S/N	Signal-to-noise ratio
SALIA	Study on the Influence of Air Pollution on Lung, Inflammation, and Aging
SA-LUR	source-area land use regression
SAPALDIA	Swiss Study on Air Pollution and Lung Disease in Adults
SAT	switching attention test
SBP	systolic blood pressure
SC	South Carolina
SCR	selective catalytic reduction
SD	standard deviation; South Dakota; San Dimas
SDNN	standard deviation of all normal-to-normal intervals, an index of total HRV
SE	standard error
Se	selenium
SEARCH	Southeast Aerosol Research Characterization
sec	second(s)
SEI	socio-economic index
Se-L	low selenium

Acronym/Abbreviation	Meaning
SES	socioeconomic status
Se-S	supplemented selenium
Sess.	session
SF_6	sulfur hexafluoride
SGA	small for gestational age
sGaw	specific airway conductance
SHARP	Study of Houston Atmospheric Radical Precursors
SHEDS	Stochastic Human Exposure and Dose Simulation
SHEEP	Stockholm Heart Epidemiology Program
sICAM-1	soluble intercellular adhesion molecule-1
SLAMS	state and local air monitoring stations
SM	Santa Maria
SNP	single nucleotide polymorphism
SO_2	sulfur dioxide
SO_4	sulfate
SOA	secondary organic aerosols
SOD	superoxide dismutase
SP-D	surfactant protein D
SPE	single-pollutant model estimate
sRaw	specific airway resistance
SRTT	simple reaction time test
ST segment	segment of the electrocardiograph between the end of the S wave and beginning of the T wave
sVCAM-1	soluble vascular adhesion molecule-1
t	fraction of time spent in a microenvironment across an individual's microenvironmental exposures, time
TBARS	thiobabituric acid reactive substances (species)
T1	first trimester
T2	second trimester
T3	third trimester
TBARS	thiobarbituric acid reactive substances
TCHS	Taiwan Children Health Study
TEA	triethanolamine
Th17	T helper cell 17
Th2	T-derived lymphocyte helper 2
TIA	transient ischemic attack
TIM	timothy
TIMP-2	tissue inhibitor of matrix metalloproteinase-2
t_j	fraction of total time spent in the jth microenvironment
TLR	Toll-like receptor
TN	Tennessee
TNF	tumor necrosis factor
TNF-α	tumor necrosis factor alpha
TSP	total suspended solids
TWA	time-weighted average
TX	Texas
U.S.C.	U.S. Code
UCD	University of California, Davis
UF1	ultrafine particle number beginning at 3 nanometers
UF2	ultrafine particle number beginning at 15 nanometers
UFP	ultrafine particle(s)
UK	universal kriging
U.K.	United Kingdom
ULTRA	The Exposure and Risk Assessment for Fine and Ultrafine Particles in Ambient Air Study conducted in Europe
UP	Upland
URI	upper respiratory infection
U.S.	United States of America
UT	Utah
VA	Virginia
Val	valine
VCAM-1	vascular adhesion molecule-1
$\dot{V}_E$	minute volume
VEGF	vascular endothelial growth factor
VOC	volatile organic compound

Acronym/Abbreviation	Meaning
VPTB	very preterm birth
V_T	tital volume
VT	ventricular tachyarrhythmias; Vermont
vWF	von Willbrand factor
WBC	white blood cell
WHI	Women's Health Initiative
WHO	World Health Organization
WI	Wisconsin
WV	West Virginia
WY	Wyoming
X	distance from the road
Y	health effect of interest
y_i	fraction of time spent indoors
$y_{i,j}$	fraction of a day spent in each indoor microenvironment
y_o	fraction of all time spent outdoors
$y_{o,j}$	fraction of a day spent in each outdoor microenvironment
yr	year(s)
Z	covariate vector; the measured concentration; standard normal deviate
Z*	the true concentration
Zn	zinc
ε	random error
τ	half-time
●	radical species

PREAMBLE

1. Process of Integrated Science Assessment Development

This Preamble outlines the general process for developing an Integrated Science Assessment (ISA) including the framework for evaluating weight of evidence and drawing scientific conclusions and causal judgments. The ISA provides a concise review, synthesis, and evaluation of the most policy-relevant science to serve as a scientific foundation for the review of the National Ambient Air Quality Standards (NAAQS).[1] As described in the Preface, the NAAQS are established based on consideration of the air quality criteria (represented by the ISA) for the pollutants identified by the Administrator using Section 108 of the Clean Air Act (CAA). The pollutants currently identified are carbon monoxide (CO), lead (Pb), oxides of nitrogen, photochemical oxidants, particulate matter (PM), and sulfur oxides (CAA, 1990a, b). Figure I depicts the general NAAQS review process. Information for individual NAAQS reviews is available online.[2]

In the process for each review, the development of the ISA is preceded by the release of an Integrated Review Plan (IRP) that discusses the planned scope of the NAAQS review; the planned approaches for developing the key assessment documents [e.g., ISA, Risk and Exposure Assessment (if warranted), Policy Assessment]; and the schedule for release and review of the documents and subsequent rulemaking notices. The key policy-relevant questions included in the IRP serve to clarify and focus the NAAQS review on the critical scientific and policy issues, including addressing uncertainties discussed during the previous review and newly emerging literature. The IRP is informed by a U.S. Environmental Protection Agency (EPA)-hosted public "kick-off workshop" that seeks input on the current state of the science and engages stakeholders and experts in discussion of the policy-relevant questions that will frame the review.

[1] The general process for NAAQS reviews is described at http://www.epa.gov/ttn/naaqs/review.html.
[2] Information for individual NAAQS reviews is available at www.epa.gov/ttn/naaqs.

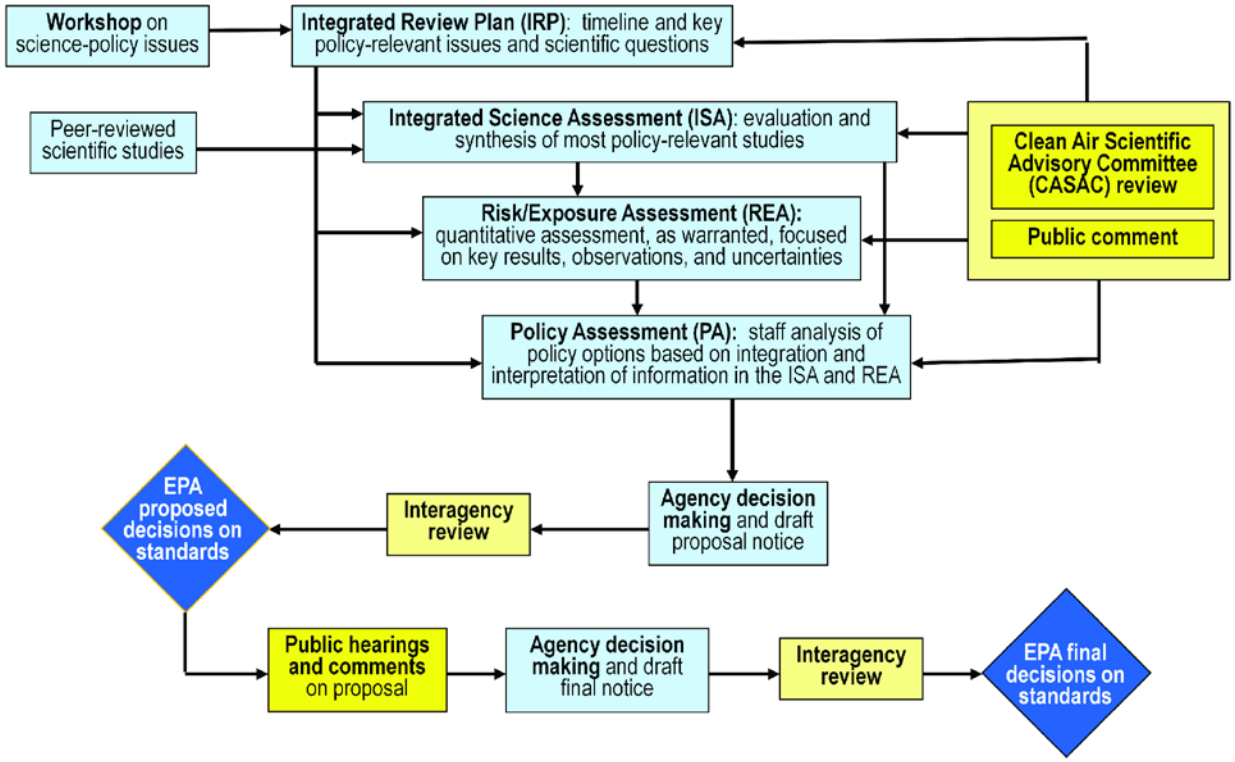

Figure I **Schematic of the key steps in the process of the review of National Ambient Air Quality Standards.**

This Preamble is a general discussion of the basic steps and criteria used in developing an ISA. Details and considerations specific to an individual ISA are included in the Preface and introductory section for that assessment. The general process for ISA development is illustrated in Figure II An initial step (not shown) is publication of a call for information in the *Federal Register* that invites the public to provide information relevant to the assessment, such as new or recent publications on health or welfare effects of the pollutant, or from atmospheric and exposure sciences fields.

The fundamental process for developing an ISA includes:

- literature searches;
- study selection;
- evaluation of individual study quality;
- evaluation, synthesis, and integration of the evidence; and
- development of scientific conclusions and causal determinations.

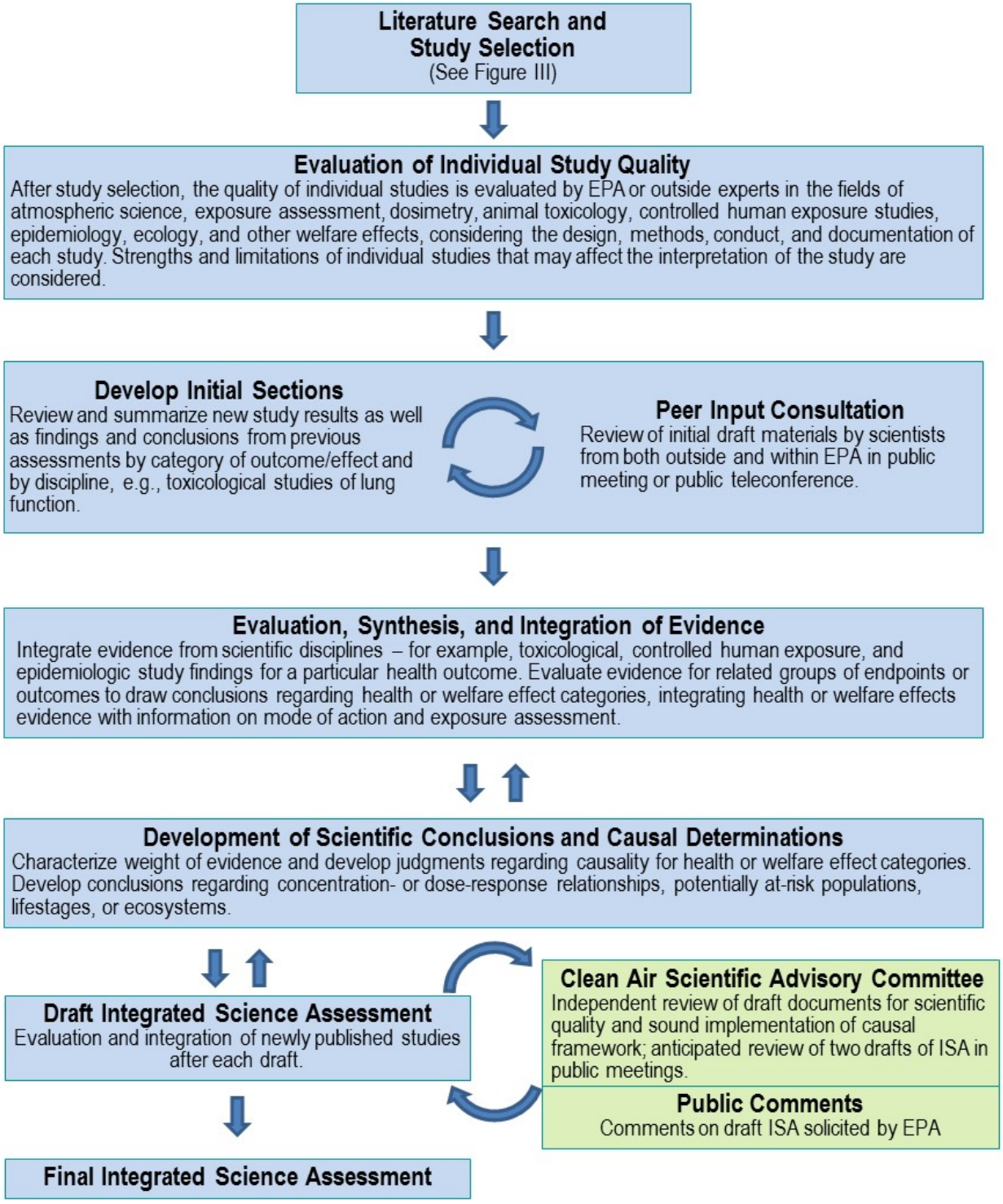

Figure II **Characterization of the general process of Integrated Science Assessment (ISA) development.**

In developing an ISA, EPA reviews and summarizes the evidence from studies on atmospheric sciences, human exposure, animal toxicology, controlled human exposure, epidemiology, and/or ecology and other welfare[1] effects. In the process of developing the first draft ISA, EPA may convene a peer input meeting in which the scientific content of preliminary draft materials is reviewed to ensure that the ISA is up-to-date and is focused on the most policy-relevant findings, and to assist EPA with integration of evidence within and across disciplines.

EPA integrates the evidence from across scientific disciplines or study types and characterizes the weight of evidence for relationships between the pollutant and various outcomes. The integration of evidence on human health or welfare effects involves collaboration between scientists from various disciplines. As an example, an evaluation of human health effects evidence would generally include the integration of the results from epidemiologic, controlled human exposure, and toxicological studies, consideration of exposure assessment, and application of the causal framework (described below) to draw conclusions.

Integration of results on human health or welfare effects that are logically or mechanistically connected (e.g., respiratory symptoms; asthma exacerbations) informs judgments of causality on the broader health effect category (e.g., effects on the respiratory system). Using the causal framework described in this Preamble, EPA scientists consider aspects such as strength, consistency, coherence, and biological plausibility of the evidence and develop causality determinations on the nature of the relationships. Causality determinations often entail an iterative process of review and evaluation of the evidence. Two drafts of the ISA are typically released for review by the Clean Air Scientific Advisory Committee (CASAC) and the public, and comments received on the characterization of the science as well as the implementation of the causal framework are carefully considered in revising the draft ISA and completing the final ISA.

2. Literature Search

In addition to the call for information in the *Federal Register* referenced above, EPA maintains an ongoing literature search process to identify relevant scientific studies published since the last ISA for a given criteria pollutant. Search strategies are designed a priori for pollutants and scientific disciplines and iteratively modified to optimize

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

identification of pertinent publications. In addition, papers are identified for inclusion in several ways: specialized searches on specific topics, identification of new publications by relational searches conducted using citations from previous assessments, review of tables of contents for journals in which relevant papers may be published, identification of relevant literature by expert scientists, review of citations in previous assessments, and recommendations by the public and CASAC during the call for information and external review processes. References identified through the multipronged search strategy are screened by title and abstract. Those references that are potentially relevant after reading the title are "considered" for inclusion in the ISA and are added to the Health and Environmental Research Online (HERO) database developed by EPA (http://hero.epa.gov/).[1] Additional review steps (described in Section 3 below) precede a decision to include a study in the ISA. The references cited in the ISA include a hyperlink to the HERO database. This literature search and study selection process is depicted in Figure III.

Studies and reports that have undergone scientific peer review and been published (or accepted for publication) are considered for inclusion in the ISA. This includes only studies that have been ethically conducted (e.g., approval by an Institutional Review Board or Institutional Animal Care and Use Committee). All relevant epidemiologic, controlled human exposure, toxicological, ecological, and welfare effects studies published since the last review are considered, including those related to exposure-response relationships, mode(s) of action (MOA), and populations and lifestages at increased risk of air pollution-related health effects. Studies and data analyses on atmospheric chemistry, air quality and emissions, environmental fate and transport, dosimetry, toxicokinetics, and exposure are also considered for inclusion in the document. References considered for inclusion in a specific ISA can be found using the HERO website (http://hero.epa.gov).

[1] The list of considered references and bibliographic information is accessible to the public through HERO.

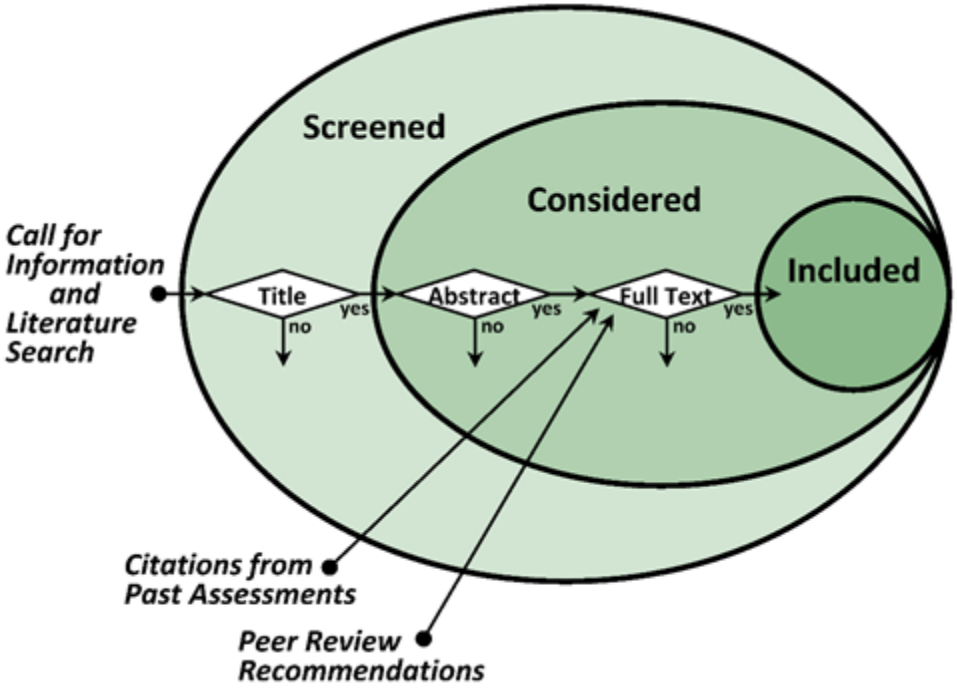

Figure III **Illustration of processes for literature search and study selection used for development of Integrated Science Assessments.**

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

3. Study Selection

References considered for inclusion in the ISA undergo abstract and full-text review to determine whether they will be included in the ISA. The selection process is based on the extent to which the study is informative, pertinent, and policy relevant. Informative, pertinent, and policy-relevant studies include those that provide a basis for or describe the relationship between the criteria pollutant and effects, including studies that offer innovation in method or design and studies that reduce uncertainty on critical issues. Emphasis is placed on studies that examine effects associated with pollutant

concentrations and exposure conditions relevant to current human population and ecosystem exposures, and particularly those pertaining to concentrations currently found in ambient air. Other studies are included if they contain unique data, such as a previously unreported effect or MOA for an observed effect, or examine multiple concentrations to elucidate exposure-response relationships.

4. Evaluation of Individual Study Quality

After selecting studies for inclusion, the individual study quality is evaluated by considering the design, methods, conduct, and documentation of each study, but not the study results. This uniform approach aims to consider the strengths, limitations, and possible roles of chance, confounding, and other biases that may affect the interpretation of individual studies and the strength of inference from their results.

These criteria provide standards for evaluating various studies and for focusing on the policy-relevant studies in assessing the body of human health, ecological, and other welfare effects evidence. Particular aspects or the absence of some of these features in a study do not necessarily define a less informative study or exclude a study from consideration in an ISA. As stated initially, the intent of the ISA is to provide a concise review, synthesis, and evaluation of the most policy-relevant science to serve as a scientific foundation for the review of the NAAQS, not extensive summaries of all human health, ecological, and other welfare effects studies for a pollutant. Of most relevance for inclusion of studies is whether they provide useful qualitative or quantitative information on exposure-response relationships for effects associated with pollutant exposures at doses or concentrations relevant to ambient conditions that can inform decisions on whether to retain or revise the standards.

In general, in assessing the scientific quality of studies on health and welfare effects, the following considerations have been taken into account.

- Were study design, 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 stated?
- Were the ecosystems, study site(s), study populations, subjects, or organism models adequately selected, and are they sufficiently well defined to allow for meaningful comparisons between study or exposure groups?
- Are the air quality data, exposure, or dose metrics of adequate quality and sufficiently representative of information regarding ambient conditions?
- Are the health, ecological, or other 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?

Additional considerations specific to particular disciplines are discussed below.

a. Atmospheric Science and Exposure Assessment

Atmospheric science and exposure assessment studies that are considered for inclusion in the ISA focus on measurement of, behavior of, and exposure to ambient air pollution using quality-assured field, experimental, and/or modeling techniques. The most informative measurement-based studies will include detailed descriptive statistics for measurements taken at varying spatial and temporal scales. These studies will also include a clear and comprehensive description of measurement techniques and quality-control procedures used. Quality-control metrics (e.g., method detection limits) and quantitative relationships between and within pollutant measurements (e.g., regression slopes, intercepts, and fit statistics) should be provided when appropriate. Measurements that include contrasting conditions for various time periods (e.g., weekday/weekend, season), populations, regions, and categories (e.g., urban/rural) are particularly useful. The most informative modeling-based studies will incorporate appropriate chemistry, transport, dispersion, and/or exposure modeling techniques with a clear and comprehensive description of model evaluation procedures, metrics, and technique strengths and limitations. The ISA also may include analyses of data pertinent to characterizing air quality or exposure such as emissions sources and ambient air pollutant concentrations. Sources of monitoring and modeling data should be clearly referenced and described to foster transparency and reproducibility of any analyses. In general, atmospheric science studies and data analyses focusing on locations pertinent to the U.S. will have maximum value in informing review of the NAAQS.

Exposure measurement error, which refers to inaccuracies in the characterization of the exposures of study participants, can be an important contributor to uncertainty in air pollution epidemiologic study results. Exposure measurement error can influence observed epidemiologic associations between ambient pollutant concentrations and health outcomes by biasing effect estimates toward or away from the null and widening confidence intervals around those estimates (Zeger et al., 2000). Factors that could influence exposure estimates include, but are not limited to: choice of exposure metric, spatial variability of the pollutant concentration, nonambient sources of exposure,

topography of the natural and built environment, meteorology, instrument errors, time-activity patterns, and differential infiltration of air pollutants into indoor environments. The influence of these factors on effect estimates also depends on epidemiologic study design. For example, when longitudinal studies depend on spatial contrasts in exposure estimates, it is important that the exposure estimates correspond in space to the population of interest. Likewise for time-series studies, the temporal variability of the exposure estimate must correspond temporally to the true exposures of the study population.

b. Epidemiology

In evaluating individual study quality for inference about health effects in epidemiologic studies, EPA considers, in addition to the general quality considerations discussed previously, whether a given study: (1) presents information on associations with short- or long-term pollutant exposures at or near conditions relevant to ambient exposures; (2) addresses potential confounding, particularly by other pollutants; (3) assesses potential effect modifiers; (4) evaluates health endpoints and populations not previously extensively researched; and (5) evaluates important methodological issues related to interpretation of the health evidence (e.g., lag or time period between exposure and effects, model specifications, thresholds).

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

Several statistical methods are available to detect and control for potential confounders; however, none of these methods is completely satisfactory. Multivariable regression models constitute one tool for estimating the association between exposure and outcome after adjusting for characteristics of participants that might confound the results. As much of the uncertainty in inferring causality may be due to potential confounding by

copollutants, this topic is of particular importance when evaluating individual studies. The use of copollutant regression models has been the prevailing approach for controlling potential confounding by copollutants in air pollution health effects studies. Trying to determine whether an individual pollutant is independently associated with the health outcome of interest from copollutant regression models is made difficult by the possibility that one or more air pollutants is acting as a surrogate for an unmeasured or poorly measured pollutant or for a particular mixture of pollutants. In addition, pollutants may independently exert effects on the same system; for example, several pollutants may be associated with a respiratory effect through either the same or different MOAs. Despite these limitations, the use of copollutant models is still the prevailing approach employed in most air pollution epidemiologic studies and provides some insight into the potential for confounding or interaction among pollutants.

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

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

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

When a risk factor is a confounder, it is the true cause of the association observed between the exposure and the outcome; when a risk factor is an effect modifier, it changes the magnitude of the association between the exposure and the outcome in stratified analyses. For example, the presence of a pre-existing disease or indicator of low socioeconomic status (SES) may act as effect modifiers if they are associated with increased risk of effects related to air pollution exposure. It is often possible to stratify the relationship between health outcome and exposure by one or more of these potential

effect modifiers. For variables that modify the association, effect estimates in each stratum will be different from one another and different from the overall estimate, indicating a different exposure-response relationship may exist in populations represented by these variables.

c. Controlled Human Exposure and Animal Toxicology

Controlled human exposure and animal toxicological studies experimentally evaluate the health effects of administered exposures in human volunteers and animal models under highly controlled laboratory conditions. Controlled human exposure studies are also referred to as human clinical studies. In these experiments, investigators expose subjects to known concentrations of air pollutants under carefully regulated environmental conditions and activity levels. In addition to the general quality considerations discussed previously, evaluation of controlled human exposure and animal toxicological studies includes assessing the design and methodology of each study with focus on (1) characterization of the intake dose, dosing regimen, and exposure route; (2) characterization of the pollutant(s); (3) sample size and statistical power to detect differences; and (4) control of other variables that could influence the occurrence of effects. The evaluation of study design generally includes consideration of factors that minimize bias in results such as randomization, blinding, and allocation concealment of study subjects, investigators, and research staff, and unexplained loss of animals or withdrawal/exclusion of subjects. Additionally, studies must include appropriate control groups and exposures to allow for accurate interpretation of results relative to exposure. Emphasis is placed on studies that address concentration-dependent responses or time-course of responses and studies that investigate potentially at-risk populations (e.g., age or pre-existing disease).

Controlled human exposure or animal toxicological studies that approximate expected human exposures in terms of concentration, duration, and route of exposure are of particular interest. Relevant pollutant exposures are considered to be those generally within two orders of magnitude of recent ambient concentrations. This range in relevant exposures is to account for differences in dosimetry, toxicokinetics, and biological sensitivity of various species, strains, or potentially at-risk populations. Studies using higher concentration exposures or doses will be considered to the extent that they provide information relevant to understanding MOA or mechanisms, interspecies variation, or at-risk human populations. In vitro studies may provide mechanistic insight for effects examined in vivo or in epidemiologic studies.

d. Ecological and Other Welfare Effects

Ecological effects considered in the ISAs typically include several of the topics given as examples by the Clean Air Act definition of welfare including soils, water, vegetation, animals, and wildlife. Additional topic areas, often referred to as "other welfare effects" that may be evaluated by an ISA include visibility, weather, and climate, as well as materials damage, economic values, and impacts to personal comfort and well-being. In evaluating studies that consider welfare effects, in addition to assessing the general quality considerations discussed previously, emphasis is placed on studies that evaluate effects at or near concentrations of the ambient air pollutants. Studies conducted in any country that contribute significantly to the general understanding of air pollutant effects may be evaluated for relevancy to U.S. air quality considerations and inclusion in the ISA.

For ecological effects, studies at higher concentrations are used to evaluate ecological effects only when they are part of a range of concentrations that also included more typical values, or when they inform understanding of MOAs and illustrate the wide range of sensitivity to air pollutants across taxa or across biomes and ecoregions. In evaluating quantitative exposure-response relationships, emphasis is placed on findings from studies conducted in the U.S. and Canada as having ecological and climatic conditions most relevant for review of the NAAQS. The type of experimental approach used in the study (e.g., controlled laboratory exposure, growth chamber, open-top chamber, mesocosm, gradient, field study, etc.) is also evaluated when considering the applicability of the results to the review of criteria air pollutant effects.

In evaluating studies on climate and visibility, emphasis is placed on studies that use well-established measurement and modeling techniques, especially those that report uncertainty or compare results from an ensemble of techniques. Novel methods may also be informative in addressing knowledge gaps not well characterized by existing techniques. Relevant climate studies include those evaluating direct and indirect climate impacts of criteria air pollutants at a global scale, while for visibility, studies conducted in the U.S. and Canada provide information more applicable for review of the NAAQS. In both cases, studies that evaluate effects by source sector or region, such as regional climate modeling studies, are particularly informative. Studies that report impacts of multiple PM components and, for climate, multiple criteria pollutants are useful in evaluating interactions and the relative contributions of atmospheric constituents. For example, ozone (O_3) and hence its climate forcing depends on atmospheric chemistry involving CO and NO_X (the sum of nitric oxide and nitrogen dioxide). Visibility preference and valuation studies that explicitly separate preferences for visibility from

concerns about health risks of air pollution are particularly relevant in considering a welfare-based secondary NAAQS.

5. Evaluation, Synthesis, and Integration of Evidence across Disciplines and Development of Scientific Conclusions and Causal Determinations

EPA has developed an approach for integrating the scientific evidence gained from the array of studies discussed above in order to draw conclusions regarding the causal nature of ambient air pollutant-related health or welfare effects. Evidence from all disciplines is integrated to evaluate consistency and inconsistency in the pattern of effects as well as strengths and limitations of the evidence across disciplines. Part of this approach includes a framework for making determinations with regard to the existence of a causal relationship between the pollutant in ambient air and health or welfare effects (described in Section 5b). This framework establishes uniform language concerning causality and brings specificity to the conclusions.

a. Evaluation, Synthesis, and Integration of Evidence across Disciplines

The ISA focuses on evaluation of the findings from the body of evidence across disciplines, drawing upon the results of all studies judged of adequate quality and relevance per the criteria described previously. Evidence from across scientific disciplines for related and similar health or welfare effects is evaluated, synthesized, and integrated to develop conclusions and causality determinations. This includes the evaluation of strengths and weaknesses in the overall collection of studies across disciplines. Confidence in the collective body of evidence is based on evaluation of study design and quality. The roles of different types of evidence in drawing the conclusions varies by pollutant or assessment, as does the availability of different types of evidence for causality determination. Consideration of human health effects are informed by controlled human exposure, epidemiologic, and toxicological studies. Evidence on ecological and other welfare effects may be drawn from a variety of experimental approaches (e.g., greenhouse, laboratory, field) and numerous disciplines (e.g., community ecology, biogeochemistry, paleontological/historical reconstructions). Other evidence including mechanistic, toxicokinetics, and exposure assessment may be highlighted if it is relevant to the evaluation of health and welfare effects and is of sufficient importance to affect the overall evaluation. Causal inference can be strengthened by the integration of evidence across disciplines. A weak inference from

one line of evidence can be addressed by other lines of evidence, and coherence of these lines of evidence can add support to a cause-effect interpretation of the association. Interpretation of the body of epidemiologic associations as evidence of causal relationships involves assessment of the full evidence base with regard to elimination of alternative explanations for the association.

Evaluation and integration of evidence must also include consideration of uncertainty, which is inherent in scientific findings. “Uncertainty” can be defined as a deficit of knowledge to describe the existing state or future outcome with accuracy and precision (e.g., the lack of knowledge about the correct value for a specific measure or estimate). Uncertainty analysis may be qualitative or quantitative in nature. In many cases, the analysis is qualitative and can include professional judgment or inferences based on analogy with similar situations. Quantitative uncertainty analysis may include use of simple measures (e.g., ranges) and analytical techniques. Quantitative uncertainty analysis might progress to more complex measures and techniques, if needed for decision support. Various approaches to evaluating uncertainty include classical statistical methods, sensitivity analysis, or probabilistic uncertainty analysis, in order of increasing complexity and data requirements. However, data may not be available for all aspects of an assessment, and those data that are available may be of questionable or unknown quality. Ultimately, the assessment is based on a number of assumptions with varying degrees of uncertainty. While the ISA may include quantitative analysis approaches such as meta-regression in some situations, generally qualitative evaluation of uncertainties is used in assessing the evidence from across studies.

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

Potential strengths and limitations of the body of studies can vary across disciplines and are evaluated during data synthesis and integration. Direct evidence of a relationship between pollutant exposures and human health effects may come from controlled human exposure studies. These studies can also provide important information on the biological plausibility of associations observed in epidemiologic studies and inform determinations of factors that may increase or decrease the risk of health effects in certain populations. In some instances, controlled human exposure studies can be used to characterize

concentration-response relationships at pollutant concentrations relevant to ambient conditions. Controlled human exposures are typically conducted using a randomized crossover design, with subjects exposed both to the pollutant and a clean air control. In this way, subjects serve as their own experimental controls, effectively limiting the variance associated with potential inter-individual confounders. Limitations that must be considered in evaluating controlled human study findings include the generally small sample size and short exposure time used in experimental studies, and that severe health outcomes are not assessed. By experimental design, controlled human exposure studies are structured to evaluate physiological or biomolecular outcomes in response to exposure to a specific air pollutant and/or combination of pollutants. In addition, the study design generally precludes inclusion of subjects with serious health conditions, and therefore the results often cannot be generalized to an entire population, which includes populations or lifestages at potentially increased risk of air pollutant-induced effects. Although some controlled human exposure studies have included health-compromised individuals such as those with mild or moderate respiratory or cardiovascular disease, these individuals may also be relatively healthy and may not represent the most sensitive individuals in the population. Thus, observed effects in these studies may underestimate the response in certain populations. In addition, the study design is limited to exposures and endpoints that are not expected to result in severe health outcomes.

Epidemiologic studies provide important information on the associations between health effects and exposure of human populations to ambient air pollution. In epidemiologic or observational studies of humans, the investigator does not control exposures or intervene with the study population. Broadly, observational studies can describe associations between exposures and effects. These studies fall into several categories; for example, cross-sectional, prospective cohort, time-series, and panel studies. Each type of study has various strengths and limitations. Cross-sectional ecologic studies use health outcome, exposure, and covariate data available at the community level (e.g., annual mortality rates and pollutant concentrations), but do not have individual-level data. Prospective cohort studies include some data collected at the individual level, typically health outcome data, and in some cases, individual-level data on exposure and covariates are collected. Time-series and case-crossover studies are often used to evaluate the relationship between day-to-day changes in air pollution exposures and a specific health outcome at the population-level (i.e., mortality, hospital admissions or emergency department visits). Panel studies include repeated measurements of health outcomes, such as respiratory symptoms or heart rate variability, at the individual level. “Natural experiments” offer the opportunity to investigate changes in health related to a change in exposure, such as closure of a pollution source.

When evaluating the collective body of epidemiologic studies, consideration of many study design factors and limitations must be taken into account to properly inform their interpretation. One key consideration is the evaluation of the potential independent contribution of the pollutant to a health outcome when it is a component of a complex air pollutant mixture. Reported effect estimates in epidemiologic studies may reflect (1) independent effects on health outcomes; (2) effects of the pollutant acting as an indicator of a copollutant or a complex ambient air pollution mixture; and (3) effects resulting from interactions between that pollutant and copollutants.

The third main type of health effects evidence, animal toxicological studies, provides information on the pollutant's biological action under controlled and monitored exposure circumstances. Taking into account biological differences among species, these studies contribute to our understanding of potential health effects, exposure-response relationships, and MOAs. Further, animal models can inform determinations of factors that may increase or decrease the risk of health effects in certain populations. These studies evaluate the effects of exposures to a variety of pollutants in a highly controlled laboratory setting and allow exploration of toxicological pathways or mechanisms by which a pollutant may cause effects. Understanding the biological mechanisms underlying various health outcomes can prove crucial in establishing or negating causality. In the absence of human studies data, extensive, well-conducted animal toxicological studies can support determinations of causality, if the evidence base indicates that similar responses are expected in humans under ambient exposure conditions.

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

For ecological effects assessment, both laboratory and field studies (including field experiments and observational studies) can provide useful data for causal determination. Because conditions can be controlled in laboratory studies, responses may be less

variable and smaller effects may be easier to detect. However, the control conditions may limit the range of responses (e.g., animals may not be able to seek alternative food sources) or incompletely reflect pollutant bioavailability, so the responses under controlled conditions may not reflect responses that would occur in the natural environment. In addition, larger-scale processes are difficult to reproduce in the laboratory.

Field observational studies measure biological changes in uncontrolled situations with high natural variability (in organismal genetics or in abiotic seasonal, climatic, or soil-related factors) and describe an association between a disturbance and an ecological effect. Field data can provide important information to assess multiple stressors or circumstances where site-specific factors significantly influence exposure. They are also often useful for analyses of pollutant effects at larger geographic scales and higher levels of biological organization. However, because conditions are not controlled, variability of the response is expected to be higher and may mask effects. Field surveys are most useful for linking stressors with effects when stressor and effect levels are measured concurrently. The presence of confounding factors can make it difficult to attribute observed effects to specific stressors.

Ecological impacts of pollutants are also evaluated in studies "intermediate" between the lower variability typically associated with laboratory exposures and high natural variability usually found in field studies. Some use environmental media collected from the field to examine the biological responses under controlled laboratory conditions. Others are experiments that are performed in the natural environment while controlling for some, but not all, of the environmental or genetic variability (e.g., mesocosm studies). This type of study in manipulated natural environments can be considered a hybrid between a field experiment and laboratory study because some sources of response variation are removed through use of control conditions, while others are included to mimic natural variation. Such studies make it possible to observe community and/or ecosystem dynamics and provide strong evidence for causality when combined with findings of studies that have been made under more controlled conditions.

b. Considerations in Developing Scientific Conclusions and Causal Determinations

In its evaluation and integration of the scientific evidence on health or welfare effects of criteria pollutants, EPA determines the weight of evidence in support of causation and characterizes the strength of any resulting causal classification. EPA also evaluates the quantitative evidence and draws scientific conclusions, to the extent possible, regarding

the concentration-response relationships and the loads to ecosystems, exposures, doses or concentrations, exposure duration, and pattern of exposures at which effects are observed.

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

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

Many of the health and environmental outcomes reported in these studies have complex etiologies. Diseases such as asthma, coronary heart disease, or cancer are typically initiated by multiple agents. Outcomes depend on a variety of factors, such as age, genetic background, nutritional status, immune competence, and social factors (IOM, 2008; Gee and Payne-Sturges, 2004). Effects on ecosystems are also often multifactorial with a complex web of causation. Further, exposure to a combination of agents could cause synergistic or antagonistic effects. Thus, the observed risk may represent the net effect of many actions and counteractions.

To aid judgment, various "aspects"[1] of causality have been discussed by many philosophers and scientists. The 1964 Surgeon General's report on tobacco smoking discussed criteria for the evaluation of epidemiologic studies, focusing on consistency, strength, specificity, temporal relationship, and coherence (HEW, 1964). Sir Austin Bradford Hill (Hill, 1965) articulated aspects of causality in epidemiology and public health that have been widely used (IOM, 2008; IARC, 2006; U.S. EPA, 2005; CDC,

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

2004). These aspects (Hill, 1965) have been modified (Table I) for use in causal determinations specific to health and welfare effects for pollutant exposures (U.S. EPA, 2009).[1] Although these aspects provide a framework for assessing the evidence, they do not lend themselves to being considered in terms of simple formulas or fixed rules of evidence leading to conclusions about causality (Hill, 1965). For example, one cannot simply count the number of studies reporting statistically significant results or statistically nonsignificant results and reach credible conclusions about the relative weight of evidence and the likelihood of causality. Rather, these aspects provide a framework for systematic appraisal of the body of evidence, informed by peer and public comment and advice, which includes weighing alternative views on controversial issues. In addition, it is important to note that the aspects in Table I cannot be used as a strict checklist, but rather to determine the weight of evidence for inferring causality. In particular, not meeting one or more of the principles does not automatically preclude a determination of causality [see discussion in (CDC, 2004)].

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

Table I Aspects to aid in judging causality.

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

Consistency of findings across studies is informed by the repeated observation of effects or associations across multiple independent studies. Further strength is provided by

reproducibility of findings in different populations under different circumstances. However, discordant results among independent investigations may be explained by differences in study methods, random errors, exposure, confounding factors, or study power, and thus may not be used to rule out a causal connection.

In evaluating the consistency of findings across studies, EPA emphasizes the importance of examining the pattern of results across various studies, and has not focused solely on statistical significance or the magnitude of the direction of the association as criteria of study reliability. 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. Statistical significance is just one of the means of evaluating confidence in the observed relationship and assessing the probability of chance as an explanation. Other indicia of reliability such as the consistency and coherence of a body of studies as well as other confirming data may be used to justify reliance on the results of a body of epidemiologic studies, even if individual studies may lack statistical significance. Traditionally, statistical significance is used to a larger extent to evaluate the findings of controlled human exposure and animal toxicology studies. Understanding that statistical inferences may result in both false positives and false negatives, consideration is given to both trends in data and reproducibility of results. Thus, in drawing judgments regarding causality, EPA emphasizes statistically significant findings from experimental studies, but does not limit its focus or consideration to statistically significant results in epidemiologic studies.

In evaluating the strength of the observed association, EPA considers both the magnitude and statistical precision (i.e., width of confidence interval) of the association in epidemiologic studies. In a large study that accounts for several potential confounding factors, a strong association can serve to increase confidence that a finding is not due to a weak unmeasured confounder, chance, or other biases. However, in a study that accounts for several potential confounding factors and other sources of bias, a weak association does not rule out a causal connection. The health effects evaluated in the ISAs tend to have multiple risk factors that likely vary in strength of effect, and the magnitude of effect of air pollution exposure will depend on the prevalence of other risk factors in the study population. Further, a small effect size can be important from a public health impact perspective. The air pollution-related change in a health effect observed in a study represents a shift in the distribution of responses in the study population and potentially an increase in the proportion of individuals with clinically important effects.

In making judgments regarding causality, the biological plausibility of effects resulting from air pollutant exposure is considered. Experimental results from in vivo studies involving animal models and humans, as well as from in vitro studies when appropriate,

may be used to establish biological plausibility and to interpret other lines of evidence (e.g., health effects from epidemiologic studies). Biological plausibility is often provided from understanding the MOA by which exposure to a pollutant leads to health effects. This understanding may encompass several different levels of biological organization including, but not limited to, molecular and cellular events in the pathways leading to disease. While a complete understanding of the MOA is not considered necessary for making causal determinations within the ISA, biological plausibility plays a key role.

c. Framework for Causal Determinations

In the ISA, EPA assesses the body of relevant literature, building upon evidence available during previous NAAQS reviews, to draw conclusions on the causal relationships between relevant pollutant exposures and health or environmental effects. ISAs use a five-level hierarchy that classifies the weight of evidence for causation.[1] This weight-of-evidence evaluation is based on integration of findings from various lines of evidence from across health and environmental effect disciplines that are integrated into a qualitative statement about the overall weight of the evidence and causality. The five descriptors for causal determination are described in Table II.

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

Table II Weight of evidence for causal determination.

	Health Effects	Ecological and Welfare Effects
Causal relationship	Evidence is sufficient to conclude that there is a causal relationship with relevant pollutant exposures (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 (e.g., doses or exposures generally within one to two orders of magnitude of current levels). That is, the pollutant has been shown to result in effects in studies in which chance, 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, 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.
Inadequate to infer a causal relationship	Evidence is inadequate to determine that a causal relationship exists with relevant pollutant exposures. The available studies are of insufficient quantity, quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an effect.	The available studies are of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an effect.
Not likely to be a causal relationship	Evidence 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.	Several adequate studies examining relationships with relevant exposures are consistent in failing to show an effect at any level of exposure.

This standardized language was drawn from sources across the federal government and wider scientific community, especially the EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), U.S. Surgeon General's report, *The Health Consequences of Smoking* (CDC, 2004). and NAS IOM document, *Improving the Presumptive Disability Decision-Making Process for Veterans* (IOM, 2008), a comprehensive report on evaluating causality.

This framework:

- describes the kinds of scientific evidence used in making determinations on causal relationships between exposure and health or welfare effects,
- summarizes the key aspects of the evaluation of evidence necessary to reach a conclusion about the existence of a causal relationship,
- identifies issues and approaches related to uncertainty, and
- classifies and characterizes the weight of evidence in support of a general causal relationship.

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

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

from exposures or doses generally ranging from recent ambient concentrations to one or two orders of magnitude above recent ambient concentrations.

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

6. Public Health Impact

Once a determination is made regarding the causality of relationship between the pollutant and outcome category, the public health impact of exposure to the pollutant is evaluated. Important questions regarding the public health impact include:

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

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

a. Approach to Identifying, Evaluating, and Characterizing At-Risk Factors

A critical part of assessing the public health impact of an air pollutant is the identification, evaluation, and characterization of populations potentially at greater risk of an air pollutant-related health effect. Under the Clean Air Act, the NAAQS are intended to protect public health with an adequate margin of safety. In doing so, protection is provided for both the population as a whole and those groups potentially at increased risk for health effects from exposure to a criteria air pollutant. To inform decisions under the NAAQS, the ISA evaluates the currently available information regarding those factors (e.g., lifestage, pre-existing disease, etc.) that contribute to portions of the population being at greater risk for an air pollutant-related health effect.

Studies often use a variety of terms to classify factors and subsequently populations that may be at increased risk of an air pollutant-related health effect, including "susceptible," "vulnerable," "sensitive," and "at-risk," with recent literature introducing the term "response-modifying factor" (Vinikoor-Imler et al., 2014; Sacks et al., 2011; U.S. EPA, 2010, 2009). The inconsistency in the definitions for each of these terms across the scientific literature has shifted the focus away from answering the key questions: Which populations are at increased risk and what evidence forms the basis of this conclusion? (Vinikoor-Imler et al., 2014). Due to the lack of a consensus on terminology in the scientific community, the term "susceptible populations" was used in reviews and previous ISAs (Sacks et al., 2011; U.S. EPA, 2010, 2009) to encompass these various factors. However, it was recognized that even using the term "susceptible populations" was problematic because it often refers to populations at increased risk specifically due to biological or intrinsic factors such as pre-existing disease or lifestage. As such, starting with the ISA for Ozone and Related Photochemical Oxidants (U.S. EPA, 2013), the terminology "at-risk" was introduced to define populations and lifestages potentially at increased risk of an air pollutant-related health effect. In assessing the overall public health impact of an air pollutant, the ISA focuses on identifying, evaluating, and characterizing "at-risk" factors to address the main question of what populations and lifestages are at increased risk of an air pollutant-related health effect. Each "at-risk" factor is evaluated with a focus on identifying whether the factor contributes to a population at increased risk of an air pollutant-related health effect. It is recognized that some factors may lead to a reduction in risk, and these are recognized during the evaluation process, but for the purposes of identifying those populations or lifestages at increased risk to inform decisions on the NAAQS, the focus of this ISA is on characterizing those factors that may increase risk.

It is recognized that factors may be intrinsic and increase risk for an effect through a biological mechanism. Intrinsic factors include genetic or developmental factors, race, sex, lifestage, or the presence of pre-existing diseases. In general, people in this category would have a steeper concentration-risk relationship compared to those not in the category. Additionally, increased risk may be attributable to an extrinsic, nonbiological factor, such as SES (e.g., educational attainment, income, access to healthcare), activity pattern, and exercise level. Some groups may be at increased risk because of increased internal dose at a given exposure concentration. This category includes individuals that have a greater dose of delivered pollutant because of breathing patterns such as children who are typically more active outdoors. In addition, some groups could have greater exposure (concentration × time) regardless of the delivered dose, such as outdoor workers. Finally, there are those who might be at increased risk for experiencing a greater exposure by being exposed at a higher concentration, such as populations that live near roadways. Some factors described above are multifaceted and may influence the risk of an air pollutant-related health effect through a combination of ways (e.g., SES). Additionally, it is recognized that some portions of the population may be at increased risk of an air pollutant-related health effect because they experience insults from a combination of factors. The emphasis is to identify and understand the factors that potentially increase the risk of air pollutant-related health effects, regardless of whether the increased risk is due to intrinsic factors, extrinsic factors, increased dose/exposure, or a combination due to the often interconnectedness of factors.

To identify at-risk factors that potentially lead to some portions of the population being at increased risk of air pollution-related health effects, the evidence is systematically evaluated across relevant scientific disciplines (i.e., exposure sciences, dosimetry, toxicology, and epidemiology). The evaluation process first consists of evaluating studies that conduct stratified analyses (i.e., epidemiologic, controlled human exposure) to compare populations or lifestages exposed to similar air pollutant concentrations within the same study design. Experimental studies also provide an important line of evidence in evaluating factors that can lead to increased risk of an air pollutant-related health effect. Specifically, toxicological studies conducted using animal models of disease and controlled human exposure studies that examine individuals with underlying disease or genetic polymorphisms can provide support for coherence with the health effects observed in epidemiologic studies as well as an understanding of biological plausibility. The potential increased risk of an air pollutant-related health effect may also be determined from studies that examined factors that result in differential air pollutant exposures. Building on the causal framework discussed in detail in Table II, the characterization of each at-risk factor consists of evaluating the strength of evidence across scientific disciplines and assessing whether the factor can contribute to increased risk of an air pollutant-related health effect. The conclusions drawn consider the “aspects

to aid in judging causality" discussed in Table I. The categories considered for evaluating the potential increased risk of an air pollutant-related health effect are "adequate evidence," "suggestive evidence," "inadequate evidence," and "evidence of no effect." They are described in more detail in Table III.

Table III Characterization of evidence for potential at-risk factors.

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.

b. Evaluating Adversity of Human Health Effects

In evaluating health evidence, a number of factors can be considered in delineating between adverse and nonadverse health effects resulting from exposure to air pollution. Some health outcomes, such as hospitalization for respiratory or cardiovascular diseases, are clearly considered adverse. It is more difficult to determine the extent of change that constitutes adversity in more subtle health measures. These more subtle health effects include a wide variety of responses, such as alterations in markers of inflammation or oxidative stress, changes in pulmonary function or heart rate variability, or alterations in neurocognitive function measures. The challenge is determining the magnitude of change in these measures when there is no clear point at which a change becomes adverse. The extent to which a change in health measure constitutes an adverse health effect may vary

between populations. Some changes that may not be considered adverse in healthy individuals would be potentially adverse in more at-risk individuals.

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

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

c. Concentration-Response Relationships

An important consideration in characterizing the public health impacts associated with exposure to a pollutant is whether the concentration-response relationship is linear across the range of concentrations or if nonlinear relationships exist along any part of this range. The shape of the concentration-response curve at and below the level of the current standards is of particular interest. Various sources of variability and uncertainty, such as

low data density in the lower concentration range, possible influence of exposure measurement error, and variability among individuals with respect to air pollution health effects, tend to smooth and "linearize" the concentration-response function and thus can obscure the existence of a threshold or nonlinear relationship. Because individual thresholds vary from person-to-person due to individual differences such as genetic differences or pre-existing disease conditions (and even can vary from one time to another for a given person), it can be difficult to demonstrate that a threshold exists in a population study. These sources of variability and uncertainty may explain why the available human data at ambient concentrations for some environmental pollutants (e.g., PM, O_3, Pb, environmental tobacco smoke, radiation) do not exhibit population-level thresholds for cancer or noncancer health effects, even though likely mechanisms include nonlinear processes for some key events.

7. Public Welfare Impact

Once a determination is made regarding the causality of relationships between the pollutant and outcome category, important questions regarding the public welfare impact include:

- What endpoints or services appear to be differentially affected (i.e., at greater or less risk of experiencing effects)? What elements of the ecosystem (e.g., types, regions, taxonomic groups, populations, functions, etc.) appear to be affected, or are more sensitive to effects? Are there differences between locations or materials in welfare effects responses, such as impaired visibility or materials damage?
- What is concluded from the evidence with regard to other types of welfare effects?
- Under what exposure conditions (amount deposited or concentration, duration, and pattern) are effects seen?
- What is the shape of the concentration-response, exposure-response, or dose-response relationship?

To address these questions, the entirety of quantitative evidence is evaluated to characterize pollutant concentrations and exposure durations at which effects were observed. To accomplish this, evidence is considered from multiple and diverse types of studies, and a study or set of studies that best approximates the concentration-response relationships between welfare outcomes and the pollutant may be identified. Controlled experimental studies provide the most direct and quantifiable exposure-response data on the effects of pollutant exposures. To the extent available, the ISA also evaluates results from less controlled field studies that characterize the shape of the relationship between a pollutant and an outcome. Other types of data may also inform evaluation of

concentration-response relationships, particularly relative to MOAs and characteristics of at-risk ecosystems.

a. Evaluating Adversity of Ecological and Other Welfare Effects

The final step in assessing the public welfare impact of an air pollutant is the evaluation of the level considered to be adverse. A secondary standard, as defined in Section 109(b)(2) of the CAA 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 such air pollutant in the ambient air." In setting standards that are "requisite" to protect public health and welfare, as provided in Section 109(b), EPA's task is to establish standards that are neither more nor less stringent than necessary for these purposes.

Adversity of ecological effects can be understood in terms ranging in biological level of organization from the cellular level to the individual organism and to the population, community, and ecosystem levels. In the context of ecology, a population is a group of individuals of the same species, and a community is an assemblage of populations of different species that inhabit an area and interact with one another. An ecosystem is the interactive system formed from all living organisms and their abiotic (physical and chemical) environment within a given area (IPCC, 2007). The boundaries of what could be called an ecosystem are somewhat arbitrary, depending on the focus of interest or study. Thus, the extent of an ecosystem may range from very small spatial scales to, ultimately, the entire Earth (IPCC, 2007).

Effects on an individual organism are generally not considered to be adverse to public welfare. However if effects occur to enough individuals within a population, then communities and ecosystems may be disrupted. Changes to populations, communities, and ecosystems can in turn result in an alteration of ecosystem processes. Ecosystem processes are defined as the metabolic functions of ecosystems including energy flow, elemental cycling, and the production, consumption, and decomposition of organic matter (U.S. EPA, 2002). Growth, reproduction, and mortality are species-level endpoints that may be clearly linked to community and ecosystem effects and are considered to be adverse when negatively affected. Other endpoints such as changes in behavior and physiological stress can decrease ecological fitness of an organism, but are harder to link unequivocally to effects at the population, community, and ecosystem level. Support for consideration of adversity beyond the species level by making explicit the linkages

between stress-related effects at the species and effects at the ecosystem level is found in *A Framework for Assessing and Reporting on Ecological Condition: an SAB report* (U.S. EPA, 2002). Additionally, the National Acid Precipitation Assessment Program (NAPAP, 1991) uses the following working definition of "adverse ecological effects" in the preparation of reports to Congress mandated by the Clean Air Act: "any injury (i.e., loss of chemical or physical quality or viability) to any ecological or ecosystem component, up to and including the regional level, over both long and short terms."

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

A consideration in evaluating adversity of climate-related effects is that criteria air pollutants have both direct and indirect effects on radiative forcing. For example, CO has a relatively small direct forcing effect, but it influences the concentrations of other atmospheric species, such as O_3 and methane (CH_4), which are important contributors to climate forcing. PM has both direct and indirect effects. For example, black carbon and sulfate contribute directly to warming and cooling, while aerosols are involved in cloud formation. Thus, it is crucial to consider the role of multiple pollutants together in evaluating the climate impact of criteria pollutants. Although climate effects of criteria air pollutants impact terrestrial and aquatic environments in diverse ways over multiple time scales, their effect on temperature is the main metric of adversity, with some consideration of proximate effects such as precipitation and relatively rapid feedbacks impacting the composition of the troposphere. Downstream effects such as land use changes are more difficult to link back to changes in concentrations that would be produced by the NAAQS. The relative adversity of U.S. versus global emissions and concentrations is informed by regional climate modeling studies, including consideration of uncertainty and spatial and temporal variability.

The adversity of visibility impacts may be expressed in terms of psychological stress, such as impairment of aesthetic quality or enjoyment of the environment, or in monetary

terms, such as willingness to pay to improve air quality. Understanding the relationship between pollutant concentration and perception of visibility, including distinguishing between concerns about health risks due to air pollution and perceived visibility impairment, are crucial in evaluating the level of protection provided by a welfare-based secondary NAAQS.

Adversity of materials damage is evaluated considering the impact to human and economic well being. Physical damage and soiling impair aesthetic qualities and function of materials. Additionally, damage to property and cultural heritage sites due to pollutant deposition may be considered adverse.

b. Quantitative Relationships: Effects on Welfare

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

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PREFACE

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 her "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, 1990a)]. 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, 1990b)] 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]

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 research has not yet identified.[3] Both

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

[2] Welfare effects as defined in Section 302(h) [42 U.S.C. 7602(h)] include, but are not limited to, "effects on soils, water, crops, vegetation, man-made materials, animals, wildlife, weather, visibility and climate, damage to and deterioration of property, and hazards to transportation, as well as effects on economic values and on personal comfort and well-being"(CAA, 2005).

[3] 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*

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 pollution 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.[1] In so doing, protection is provided for both the population as a whole and those groups 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 (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.[2]

In setting standards that are "requisite" to protect public health and welfare as provided in Section 109(b), EPA's task is to establish standards that are neither more nor less stringent than necessary for these purposes. In so doing, EPA may not consider the costs of implementing the standards.[3] Likewise, "[a]ttainability and technological feasibility are not relevant considerations in the promulgation of national ambient air quality standards."[4]

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

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).

[1] See *Lead Industries v. EPA*, 647 F.2d at 1156 n.51; *Mississippi v. EPA*, 723 F. 3d 246, 255, 262−63 (D.C. Cir. 2013).

[2] See *Lead Industries Association v. EPA*, 647 F.2d at 1161−62; *Mississippi v. EPA*, 723 F. 3d at 265.

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

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

appropriate….” Since the early 1980s, this independent review function has been performed by the Clean Air Scientific Advisory Committee (CASAC).[1]

Introduction to the Primary National Ambient Air Quality Standard for Nitrogen Dioxide

Nitrogen dioxide (NO_2) is the indicator for gaseous oxides of nitrogen [e.g., NO_2, nitric oxide (NO)]. Consistent with Section 108(c) of the CAA (42 U.S.C.21 7408), EPA considers the term oxides of nitrogen to refer to all forms of oxidized nitrogen including multiple gaseous species (e.g., NO_2, NO) and particulate species (e.g., nitrates). The review of the primary NO_2 NAAQS focuses on evaluating the health effects associated with exposure to the gaseous oxides of nitrogen. The atmospheric chemistry, exposure, and health effects associated with nitrogen compounds present in particulate matter (PM) were most recently considered in the EPA's review of the NAAQS for PM. The welfare effects associated with oxides of nitrogen are being considered in a separate assessment as part of the review of the secondary NO_2 NAAQS and sulfur dioxide [SO_2; (U.S. EPA, 2013)].

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, considering evidence of effects associated with various time periods of exposure. The level of a standard defines the air quality concentration used (i.e., an ambient concentration of the indicator pollutant) in determining whether the standard is achieved. The form of the standard 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 1-hour NO_2 standard is the 3-year average of the 98th percentile of the annual distribution of 1-hour daily maximum NO_2 concentrations. The Administrator considers these four elements collectively in evaluating the protection to public health provided by the primary NAAQS.

History of the Review of Air Quality Criteria for the Oxides of Nitrogen and the Primary National Ambient Air Quality Standards for Nitrogen Dioxide

In 1971, the EPA added nitrogen oxides to the list of criteria pollutants under Section 108(a)(1) of the CAA and issued the initial air quality criteria [36 Federal

[1] Lists of CASAC members and of members of the CASAC Oxides of Nitrogen Primary NAAQS Review Panel are available at: http://yosemite.epa.gov/sab/sabproduct.nsf/WebCASAC/CommitteesandMembership?OpenDocument.

Register (FR) 1515, January 30, 1971]. Based on these air quality criteria, the EPA promulgated NAAQS for nitrogen oxides using NO_2 as the indicator (36 FR 8186, April 30, 1971). Both primary and secondary standards were set at 100 μg/m^3 [equal to 0.053 parts per million (ppm)], annual average. The standards were based on scientific information contained in the 1971 Air Quality Criteria Document for Nitrogen Oxides (U.S. EPA, 1971). Since then, the Agency has completed multiple reviews of the air quality criteria upon which the NAAQS are set and the primary standards themselves. Table I provides a brief summary of these reviews.

Table I History of the primary National Ambient Air Quality Standards for nitrogen dioxide (NO_2) since 1971.

Final Rule/Decisions	Indicator	Averaging Time	Level	Form
1971 36 FR 8186 Apr 30, 1971	NO_2	1 year	53 ppb[a]	Annual arithmetic average
1985 50 FR 25532 Jun 19, 1985	Primary NO_2 standard retained, without revision.			
1996 61 FR 52852 Oct 8, 1996	Primary NO_2 standard retained, without revision.			
2010 75 FR 6474 Feb 9, 2010	NO_2	1 hour	100 ppb	3-year average of the 98th percentile of the annual distribution of daily maximum 1-hour concentrations
	Primary annual NO_2 standard retained, without revision.			

[a]The initial standard level of the annual NO_2 standard was 100 μg/m^3 which is equal to 0.053 ppm or 53 parts per billion (ppb). The units for the standard level were officially changed to ppb in the final rule issued in 2010 (75 FR 6531, February 9, 2010).

The EPA retained the primary and secondary NO_2 standards, without revision, in reviews completed in 1985 and 1996 (50 FR 25532, June 19, 1985; 61 FR 52852, October 8, 1996). These decisions were informed, respectively, by scientific information contained in the 1982 Air Quality Criteria Document for Oxides of Nitrogen [(U.S. EPA, 1982) which updated the scientific criteria upon which the initial NO_2 standards were based] and the 1993 Air Quality Criteria Document for the Oxides of Nitrogen (U.S. EPA, 1993). In the latter of the two decisions, the EPA concluded that "the existing annual primary standard appears to be both adequate and necessary to protect human health against both long- and short-term NO_2 exposures" and that retaining the existing annual standard is consistent with the scientific data assessed in the 1993 Air Quality

Criteria Document (U.S. EPA, 1993) and the Staff Paper (U.S. EPA, 1995) and with the advice and recommendations of CASAC" (61 FR 52854, October 8, 1996).[1]

The last review of the air quality criteria for oxides of nitrogen (health criteria) and the primary NO_2 standard was initiated in December 2005 (70 FR 73236, December 9, 2005).[2,3] The Agency's plans for conducting the review were presented in the Integrated Review Plan (IRP) for the Primary National Ambient Air Quality Standard for NO_2 (U.S. EPA, 2007a), which included consideration of comments received during a CASAC consultation as well as public comment on a draft IRP. The science assessment for the review was described in the 2008 Integrated Science Assessment for Oxides of Nitrogen—Health Criteria (U.S. EPA, 2008a), multiple drafts of which received review by CASAC and the public. The EPA also conducted quantitative human risk and exposure assessments, after consultation with CASAC and receiving public comment on a draft analysis plan (U.S. EPA, 2007b). These technical analyses were presented in the Risk and Exposure Assessment (REA) to Support the Review of the NO_2 Primary National Ambient Air Quality Standard (U.S. EPA, 2008b), multiple drafts of which received CASAC and public review.

Over the course of the last review, the EPA made several changes to the NAAQS review process. An important change was the discontinuation of the Staff Paper, which traditionally contained staff evaluations to bridge the gap between the Agency's science assessments and the judgments required of the EPA Administrator in determining whether it was appropriate to retain or revise the NAAQS.[4] In the course of reviewing the second draft REA, however, CASAC expressed the view that the document would be incomplete without the addition of a policy assessment chapter presenting an integration of evidence-based considerations and risk and exposure assessment results. CASAC stated that such a chapter would be "critical for considering options for the NAAQS for NO_2" (Samet, 2008). In addition, within the period of CASAC's review of the second draft REA, the EPA's Deputy Administrator indicated in a letter to the CASAC chair, addressing earlier CASAC comments on the NAAQS review process, that the risk and

[1] In presenting rationale for the final decision, the EPA noted that "a 0.053 ppm annual standard would keep annual NO_2 concentrations considerably below the long-term levels for which serious chronic effects have been observed in animals" and that "[r]etaining the existing standard would also provide protection against short-term peak NO_2 concentrations at the levels associated with mild changes in pulmonary function and airway responsiveness observed in controlled human [exposure] studies" (61 FR 52854, October 8, 1996; 60 FR 52874, 52880, October 11, 1995).
[2] Documents related to reviews completed in 2010 and 1996 are available at: http://www.epa.gov/ttn/naaqs/standards/nox/s_nox_index.html.
[3] The EPA conducted a separate review of the secondary NO_2 NAAQS jointly with a review of the secondary SO_2 NAAQS. The Agency retained those secondary standards, without revision, to address the direct effects on vegetation of exposure to oxides of nitrogen and sulfur (77 FR 20218, April 3, 2012).
[4] Initial changes to the NAAQS review process included a policy assessment document reflecting Agency (rather than staff) views published as an advanced notice of public rulemaking (ANPR). Under this process, the ANPR would have been reviewed by CASAC (Peacock, 2006).

exposure assessment will include "a broader discussion of the science and how uncertainties may effect decisions on the standard" and "all analyses and approaches for considering the level of the standard under review, including risk assessment and weight of evidence methodologies" (Peacock, 2008). Accordingly, the final 2008 REA included a policy assessment chapter that considered the scientific evidence in the 2008 ISA and the exposure and risk results presented in other chapters of the 2008 REA as they related to the adequacy of the then current primary NO_2 standard and potential alternative standards for consideration (U.S. EPA, 2008b).[1] CASAC discussed the final version of the 2008 REA, with an emphasis on the policy assessment chapter during a public teleconference on December 5, 2008 (73 FR 66895, November 12, 2008). Following that teleconference, CASAC offered comments and advice on the primary NO_2 standard in a letter to the Administrator (Samet, 2008).

After considering an integrative synthesis of the body of evidence on human health effects associated with the presence of NO_2 in the air and the exposure and risk information, the Administrator determined that the then-existing primary NO_2 NAAQS, based on an annual arithmetic average, was not sufficient to protect the public health from the array of effects that could occur following short-term exposures to ambient NO_2. In so doing, the Administrator particularly noted the potential for adverse health effects to occur following exposures to elevated NO_2 concentrations that can occur around major roads (75 FR 6482). In a notice published in the Federal Register on July 15, 2009, the EPA proposed to supplement the existing primary annual NO_2 standard by establishing a new short-term standard (74 FR 34404). In a notice published in the Federal Register on February 9, 2010, the EPA finalized a new short-term standard with a level of 100 ppb, based on the 3-year average of the 98th percentile of the annual distribution of daily maximum 1-hour concentrations. The EPA also retained the existing primary annual NO_2 standard with a level of 53 ppb, annual average (75 FR 6474). The EPA's final decision included consideration of CASAC (2009) and public comments on the proposed rule. The EPA's final rule was upheld against challenges in a decision issued by the U.S. Court of Appeals for the District of Columbia Circuit on July 17, 2012.[2]

Revisions to the NAAQS were accompanied by revisions to the data handling procedures, the ambient air monitoring and reporting requirements, and the Air Quality

[1] Subsequent to the completion of the 2008 REA, EPA Administrator Jackson called for additional key changes to the NAAQS review process including reinstating a policy assessment document that contains staff analysis of the scientific bases for alternative policy options for consideration by senior Agency management prior to rulemaking (Jackson, 2009). A Policy Assessment will be developed for the current review as discussed in Chapter 7 of the 2014 Integrated Review Plan for the Primary National Ambient Air Quality Standards for Nitrogen Dioxide (U.S. EPA, 2014).

[2] See *American Petroleum Institute v. EPA*, 684 F. 3d 1342 (D.C. Cir. 2012).

Index (AQI).[1] One aspect of the new monitoring network requirements included requirements for states to locate monitors near heavily trafficked roadways in large urban areas and in other locations where maximum NO_2 concentrations can occur. Subsequent to the 2010 rulemaking, the EPA revised the deadlines by which the near-road monitors are to be operational in order to implement a phased deployment approach (78 FR 16184, March 14, 2013). The near-road NO_2 monitors will become operational between January 1, 2014 and January 1, 2017.

[1] The current federal regulatory measurement methods for NO_2 are specified in 40 Code of Federal Regulations (CFR) part 50, Appendix F and 40 CFR part 53. Consideration of ambient air measurements with regard to judging attainment of the standards is specified in 40 CFR part 50, Appendix S. The NO_2 monitoring network requirements are specified in 40 CFR part 58, Appendix D, Section 4.3. The EPA revised the AQI for NO_2 to be consistent with the revised primary NO_2 NAAQS as specified in 40 CFR part 58, Appendix G. Guidance on the approach for implementation of the new standards was described in the Federal Register notices for the proposed and final rules (74 FR 34404; 75 FR 6474).

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EXECUTIVE SUMMARY

Purpose and Scope of the Integrated Science Assessment

This Integrated Science Assessment (ISA) is a comprehensive evaluation and synthesis of policy-relevant science aimed at characterizing exposures to ambient oxides of nitrogen and their relationships with health effects. Thus, this ISA serves as the scientific foundation for the review of the primary (health-based) National Ambient Air Quality Standards (NAAQS) for nitrogen dioxide (NO_2).[1] NO_2 is the indicator for gaseous oxides of nitrogen (i.e., oxidized nitrogen compounds), which also include nitric oxide and gases produced from reactions involving NO_2 and nitric oxide (Figure 2-1, Section 2.2).[2,3] In 2010, the U.S. Environmental Protection Agency (EPA) retained the NAAQS of 53 parts per billion (ppb) annual average concentration to protect against health effects potentially related to long-term NO_2 exposures. In addition, EPA set a new 1-hour NAAQS at a level of 100 ppb, based on the 3-year average of each year's 98th percentile of the highest daily 1-hour concentration. The 1-hour NAAQS was set to protect against respiratory effects related to short-term NO_2 exposures in populations potentially at increased risk, such as people with asthma or people who spend time on or near high-traffic roads. EPA also set requirements for a network of monitors to measure NO_2 near high-traffic roads, one of the places where the highest concentrations are expected to occur.

This ISA updates the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) with studies and reports published from January 2008 through August 2014. EPA conducted searches to identify peer-reviewed literature on relevant topics such as health effects, atmospheric chemistry, ambient concentrations, and exposure. The Clean Air Scientific Advisory Committee (a formal independent panel of scientific experts) and the public also recommended studies and reports. To fully describe the state of available science, EPA also identified relevant studies from previous assessments to include in this ISA.

As in the 2008 ISA, this ISA determines the causality of relationships with health effects only for NO_2 (Chapter 5 and Chapter 6). Key to interpreting the health effects evidence is understanding the sources, chemistry, and distribution of NO_2 in the ambient air (Chapter 2) that influence exposure (Chapter 3), the uptake of inhaled NO_2 in the respiratory tract, and subsequent biological mechanisms that may be affected (Chapter 4).

[1] The ecological effects of oxides of nitrogen are being considered in a separate assessment as part of the review of the secondary (welfare-based) NAAQS for NO_2 and sulfur dioxide (U.S. EPA, 2013).
[2] Total oxides of nitrogen also include several particulate species such as nitrites. Section 108(c) of the Clean Air Act, 42 U.S.C. § 7408(c) specifies that criteria for oxides of nitrogen include consideration of nitric and nitrous acids, nitrites, nitrates, nitrosamines, and other derivatives of oxides of nitrogen. Health effects associated with the particulate species are addressed in the review of the NAAQS for particulate matter (U.S. EPA, 2009).
[3] The blue electronic links can be used to navigate to other parts of this ISA and to information on cited references .

Further, the ISA aims to characterize the independent effect of NO_2 exposure on health rather than its role as just a marker for other air pollutants. The ISA also informs policy-relevant issues (Section 1.6), such as (1) exposure durations and patterns associated with health effects; (2) concentration-response relationship(s), including evidence of potential thresholds for effects; and (3) populations or lifestages at increased risk for health effects related to NO_2 exposure (Chapter 7).

Sources and Human Exposure to Nitrogen Dioxide

A main objective of the ISA is to characterize health effects related to ambient NO_2 exposure. This requires understanding what factors affect exposure to ambient NO_2 and the ability to estimate exposure accurately and accounting for the influence of factors that are related to NO_2 exposure, such as other pollutants and demographic characteristics. For the U.S. as a whole and for major cities, motor vehicle emissions are the largest single source of NO_2 in the ambient air (Section 2.3.1, Figure 2-3). Electric power plants, industrial facilities, other forms of transportation, soil, and wildfires also can contribute considerably to ambient NO_2 concentrations on a national scale and to differences in concentrations and population exposures among locations.

Because many sources of NO_2 are ubiquitous, there is widespread potential for exposure to NO_2. However, given that motor vehicles are a major source, air concentrations of NO_2 can be highly variable across neighborhoods (Section 2.5.2), depending on distance to roads. NO_2 concentrations tend to decrease over a distance of 200−500 meters from the road and can be 30 to 100% higher within 10−20 meters of a road than at locations farther away (Section 2.5.3). The first year of data from a new near-road monitoring network for a small group of U.S. cities show that 1-hour NO_2 concentrations tend to be higher near roads than at most other sites within a city (Table 2-8, Section 2.5.3.2). But, peak NO_2 concentrations are not always higher near roads, indicating that in addition to distance from road, factors such as other local sources, season, wind direction, chemical reactions with ozone in the air (Figure 2-1), and physical features of the environment (Sections 2.2 and 2.5.3) also affect the distribution of ambient NO_2 concentrations.

Because ambient NO_2 concentrations show variability among geographic regions, within communities, and over time, there is potential for large variation in ambient NO_2 exposure among people. Also contributing to variation in ambient NO_2 exposure are the differences in the outdoor and indoor locations where people spend time and the amount of time spent in those locations (Sections 3.4.1 and 3.4.3; Figure 3-3). NO_2 concentrations vary by the type of location, including inside vehicles and buildings (Figure 3-1), and the ventilation of buildings can affect the amount of ambient NO_2 that penetrates indoors (Section 3.4.3.3). Therefore, understanding the extent to which the

methods used to estimate exposure adequately account for variation in ambient concentrations across locations and people's activity patterns is essential to characterize relationships between ambient NO_2 exposure and health effects.

In this ISA, health effects are examined largely in relation to ambient NO_2 concentrations measured at community monitoring sites. These monitors do not cover all locations where people live or spend their time and are not sited to capture the variability in NO_2 concentrations observed within cities, including near roads. Thus, NO_2 measured at these sites have some error in representing people's actual exposures. This error may be reflected in the wide range of relationships observed between total personal NO_2 exposure and ambient concentrations averaged over periods up to 1 week (Section 3.4.2). Such relationships are not well characterized for exposure periods of months to years. These uncertainties do not necessarily mean that ambient NO_2 concentrations are poor measures of personal exposure because variation among people in indoor or in-vehicle exposures and activity patterns may obscure relationships between ambient exposure and concentrations. Compared with NO_2 concentrations at community monitors, there may be more confidence in exposure metrics that account for local variability in NO_2 concentrations and people's activity patterns. These metrics can include short-term personal, home, and school NO_2 measurements and long-term NO_2 exposure estimated at people's homes with models and are examined in some recent health effect studies.

Error in estimating exposure can impact associations observed between ambient NO_2 concentrations and health effects in various ways. In studies of short-term exposure that examine changes in NO_2 over time (e.g., day to day), NO_2 from community monitors has shown lower magnitude associations with health effects (Section 3.4.5) compared with NO_2 measured at people's locations and/or more uncertainty in the association. In studies of long-term exposure that compare people in locations that vary in ambient NO_2 concentrations, NO_2 from community monitors has shown both smaller and larger associations with health effects compared with NO_2 concentrations estimated for people's locations. The impact on health effect associations of using NO_2 concentrations at community sites to represent near-road exposures is not clear. Given the impact of exposure error, this ISA draws conclusions about health effects related to NO_2 exposure by considering the availability of results for NO_2 measured at community monitoring sites versus other locations where people live or spend time and considering how well the various methods represent differences in exposure over time or across locations.

The important contribution of motor vehicles to ambient NO_2 concentrations not only has implications for estimating NO_2 exposure but also indicates the need to consider other traffic-related pollutants. NO_2 concentrations often are moderately to highly correlated

with pollutants also emitted by motor vehicles, such as $PM_{2.5}$,[1] UFP (see footnote 1), elemental or black carbon (EC/BC), and carbon monoxide (Figure 3-6, Section 3.4.4.1). Traffic-related pollutants show effects on many of the same biological processes and health outcomes (Table 5-1). Thus, in characterizing relationships of NO_2 with health effects, the ISA evaluates the extent to which an effect of NO_2 can be distinguished from that of other traffic-related pollutants. Important lines of evidence include epidemiologic studies that statistically adjust the NO_2 association for another pollutant and experimental studies that inform the direct effect of NO_2 exposure on health outcomes and biological mechanisms.

Health Effects of Nitrogen Dioxide Exposure

In this ISA, information on NO_2 exposure, the potential influence of other traffic-related pollutants, and health effects from epidemiologic, controlled human exposure, and toxicological studies is integrated to form conclusions about the causal nature of relationships between NO_2 exposure and health effects. Health effects examined in relation to the full range of NO_2 concentrations relevant to ambient conditions are considered. Based on peak concentrations (Section 2.5) and the ISA definition that ambient-relevant exposures be within one to two orders of magnitude of current conditions (Preamble, Section 5.c), NO_2 concentrations up to 5,000 ppb[2] are defined to be ambient relevant. A consistent and transparent framework (Preamble, Table II) is applied to classify the health effects evidence 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
5) Not likely to be a causal relationship

The conclusions presented in Table ES-1 are informed by recent findings and whether recent findings integrated with information from the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) support a change in conclusion. Important considerations include judgments of error and uncertainty in the collective body of available studies; the consistency of findings integrated across epidemiologic, controlled human exposure, and toxicological studies to inform an independent effect of NO_2 exposure and potential

[1] $PM_{2.5}$: In general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 2.5 µm, a measure of fine particles. UFP: Definitions vary but often refer to particles with an aerodynamic diameter less or equal to a nominal 0.1 µm, a measure of ultrafine particles.
[2] The 5,000-ppb upper limit applies mostly to animal toxicological studies and also a few controlled human exposure studies. Experimental studies examining NO_2 exposures greater than 5,000 ppb were included if they provided information on the uptake of NO_2 in the respiratory tract or on potential biological mechanisms.

underlying biological mechanisms; consistency in epidemiologic evidence across various methods used to estimate NO_2 exposure; and examination in epidemiologic studies of the potential influence of other traffic-related pollutants and other factors that could bias associations observed with NO_2 exposure (Section 5.1.2).

Table ES-1 Causal determinations for relationships between nitrogen dioxide (NO_2) exposure and health effects from the 2008 and current Integrated Science Assessment (ISA) for Oxides of Nitrogen.

Exposure Duration and Health Effect Category[a]	Causal Determination[b]	
	2008 ISA	Current Draft ISA
Short-term NO_2 Exposure (minutes up to 1 month)		
Respiratory effects Section 5.2, Table 5-45	Sufficient to infer a likely causal relationship	Causal relationship
Cardiovascular and related metabolic effects[c] Section 5.3, Table 5-58	Inadequate to infer the presence or absence of a causal relationship	Suggestive, but not sufficient, to infer a causal relationship
Total mortality Section 5.4, Table 5-63	Suggestive, but not sufficient, to infer a causal relationship	Suggestive, but not sufficient, to infer a causal relationship
Long-term NO_2 Exposure (more than 1 month to years)		
Respiratory effects Section 6.2, Table 6-5	Suggestive, but not sufficient, to infer a causal relationship	Likely to be a causal relationship
Cardiovascular and related metabolic effects[c] Section 6.3, Table 6-11	Inadequate to infer the presence or absence of a causal relationship	Suggestive, but not sufficient, to infer a causal relationship
Reproductive and developmental effects[c] Sections 6.4.2, 6.4.3, and 6.4.4, Table 6-14	Inadequate to infer the presence or absence of a causal relationship	Fertility, Reproduction, and Pregnancy: Inadequate to infer a causal relationship
		Birth Outcomes: Suggestive, but not sufficient, to infer a causal relationship
		Postnatal Development: Inadequate to infer a causal relationship
Total mortality Section 6.5, Table 6-18	Inadequate to infer the presence or absence of a causal relationship	Suggestive, but not sufficient, to infer a causal relationship
Cancer Section 6.6, Table 6-20	Inadequate to infer the presence or absence of a causal relationship	Suggestive, but not sufficient, to infer a causal relationship

[a]An array of outcomes is evaluated as part of a broad health effect category: physiological measures (e.g., airway responsiveness), clinical outcomes (e.g., hospital admissions), and cause-specific mortality. Total mortality includes all nonaccidental causes of mortality and is informed by findings for the spectrum of morbidity effects (e.g., respiratory, cardiovascular) that can lead to mortality. The sections and tables referenced include a detailed discussion of the evidence that supports the causal determinations and the NO_2 concentrations with which health effects have been associated.

[b]Since the 2008 ISA for Oxides of Nitrogen, the phrasing of causal determinations has changed slightly, and the weight of evidence that describes each level in the hierarchy of the causal framework has been more explicitly characterized.

[c]In the current ISA, the cardiovascular effects category is expanded to include related metabolic effects. Reproductive and developmental effects are separated into smaller subcategories of outcomes based on varied underlying biological processes and exposure patterns over different lifestages.

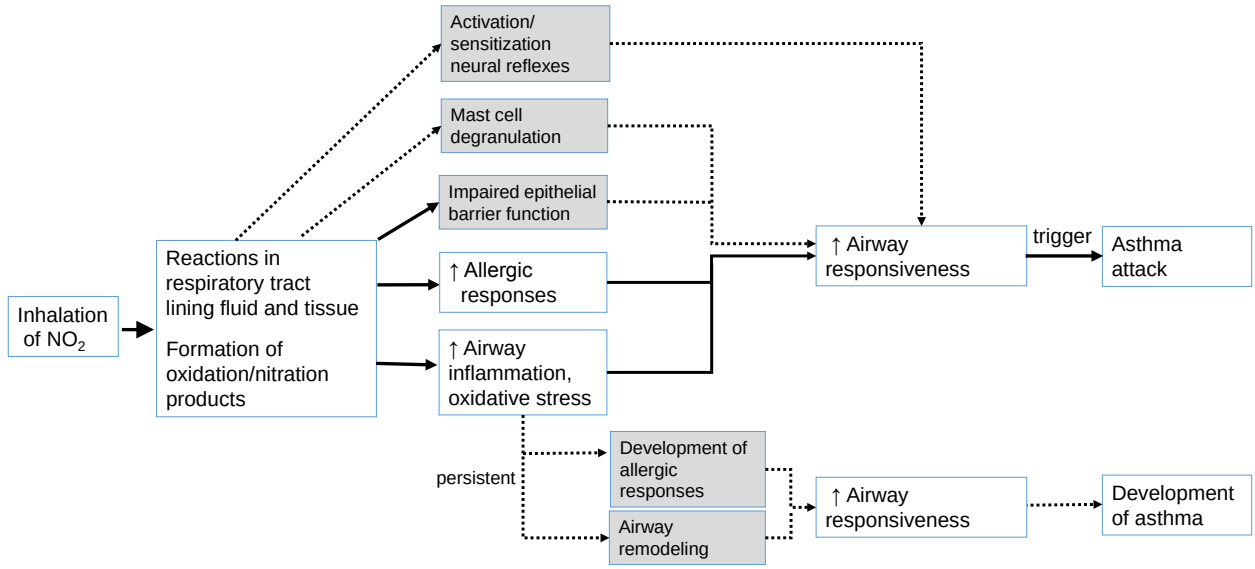
Short-term Nitrogen Dioxide Exposure and Respiratory Effects

A causal relationship is determined for short-term NO_2 exposure and respiratory effects. The conclusion is strengthened from the 2008 ISA for Oxides of Nitrogen from likely to be a causal relationship (Table ES-1) based on the evidence demonstrating that NO_2 exposure can trigger asthma attacks. There is some evidence relating short-term NO_2 exposure to chronic obstructive pulmonary disease, respiratory infection, respiratory effects in healthy populations, and respiratory mortality but uncertainty as to whether the effects of NO_2 exposure are independent of other traffic-related pollutants (Table 5-45).

Supporting a relationship with asthma attacks, epidemiologic studies in diverse locations consistently show that short-term increases in ambient NO_2 concentration are associated with increases in hospital admissions and emergency department visits for asthma, increases in respiratory symptoms and airway inflammation in people with asthma, and decreases in lung function in children with asthma (Section 5.2.9). These associations are found not only with community-average ambient NO_2 concentrations but also with personal NO_2 and NO_2 measured outside children's schools and inside their homes (Sections 5.2.9.3 and 5.2.9.6). Correlations between NO_2 and other traffic-related pollutants are weaker for total personal exposures than for ambient concentrations, and the same may be true for indoor exposures. So, associations with personal and indoor NO_2 may be less influenced by pollutants that are related to outdoor NO_2. Further, studies that measured pollutants at peoples' locations tend to show that NO_2 remains associated with respiratory effects after accounting for the effect of another traffic-related pollutant such as $PM_{2.5}$ or (examined in fewer studies) EC/BC, metals, or UFP (Figure 5-16 and Figure 5-17). The key evidence for an independent effect of NO_2 are the controlled human exposure findings for NO_2-induced increases in airway responsiveness and allergic inflammation, which are hallmarks of asthma attacks (Figure ES-1). An effect of short-term NO_2 exposure on asthma attacks also is plausible given that inhaled NO_2 reacts with substances such as antioxidants in the fluid lining the lung (Section 4.2.2) to form reactive species. The production of such reactive species is an early event involved in increasing airway responsiveness and allergic responses (Figure ES-1).

The 2008 ISA described much of the same evidence and determined a likely to be causal relationship, citing uncertainty as to whether results for NO_2 in epidemiologic studies reflected the effects of other traffic-related pollutants. The 2008 ISA did not explicitly evaluate the extent to which various lines of evidence supported effects on asthma attacks. In this ISA, the determination of a causal relationship is not just based on new evidence but on an evaluation and integration of findings related to asthma attacks. The epidemiologic evidence for asthma attacks and controlled human exposure study findings for increased airway responsiveness and allergic inflammation together are sufficient to

rule out the influence of other traffic-related pollutants with reasonable confidence and demonstrate an independent effect of short-term NO_2 exposure on respiratory effects.



Note: Adapted from Figure 4-1 and Figure 4-2 (Section 4.3.5). White boxes and solid arrows describe pathways well supported by available evidence. Gray boxes and dotted arrows describe potential pathways for which evidence is limited or inconsistent.

Figure ES-1. Biological pathways for relationships of short-term and long-term nitrogen dioxide (NO_2) exposure with asthma.

Long-term Nitrogen Dioxide Exposure and Respiratory Effects

There is likely to be a causal relationship between long-term NO_2 exposure and respiratory effects (Section 6.2.9) based on the evidence for development of asthma. The conclusion is strengthened from the 2008 ISA (Table ES-1) because where previous findings were inconsistent, recent epidemiologic studies consistently observe NO_2-related increases in asthma development in children who are followed over time. There is more uncertainty about relationships with respiratory effects such as development of allergy and respiratory infection (Table 6-5). Asthma development is associated not only with community-level ambient NO_2 concentrations but also with ambient NO_2 exposure estimated at children's homes with models that well capture the spatial variability in communities. Associations between NO_2 and asthma development are independent of factors such as socioeconomic status and exposure to smoking, but the potential influence of other traffic-related pollutants is not well studied. This uncertainty also applies to the findings for NO_2-related decreases in lung function and lung development in children.

There is some support for an independent effect of NO_2 on asthma development provided by findings of increased airway responsiveness in rodents (Figure ES-1). Also, evidence relating short-term NO_2 exposure to airway inflammation in epidemiologic studies of healthy people and allergic responses in experimental studies of rodents and healthy people indicates that repeated short-term NO_2 exposure could lead to the development of asthma. Together, the epidemiologic and experimental evidence for asthma development supports a relationship between long-term NO_2 exposure and respiratory effects, but because experimental evidence is limited, there remains some uncertainty about the potential influence of other traffic-related pollutants in the epidemiologic evidence.

Nitrogen Dioxide Exposure and Other Health Effects

There is more uncertainty about relationships of NO_2 exposure with health effects outside of the respiratory system. NO_2 itself is unlikely to enter the bloodstream, and reactions caused by ambient-relevant concentrations of NO_2 in the airways do not clearly affect concentrations of reactive compounds, such as nitrite, in the blood. Only a few results suggest that compounds that can cause inflammation or oxidative stress may enter the blood from the respiratory tract in response to NO_2 exposure (Section 4.3.2.9). This uncertainty about the effects of NO_2 exposure on underlying biological mechanisms is common to nonrespiratory health effects.

For short-term and long-term NO_2 exposure, evidence is suggestive, but not sufficient, to infer a causal relationship with cardiovascular and related metabolic effects, total mortality, birth outcomes, and cancer (Table ES-1). For short-term NO_2 exposure, recent epidemiologic studies continue to show associations with total mortality and add support for cardiovascular and related metabolic effects by indicating a possible effect on triggering heart attacks. Where there was little previous support, increases in recent epidemiologic evidence result in strengthening conclusions for total mortality and cancer related to long-term NO_2 exposure. New epidemiologic findings for heart disease and diabetes and reduced fetal growth point to possible relationships of long-term NO_2 exposure with health effect categories new to this ISA: cardiovascular and related metabolic effects and birth outcomes. For fertility, reproduction, and pregnancy, as well as postnatal development, evidence is inadequate to infer a causal relationship with long-term NO_2 exposure (Table ES-1) because neither epidemiologic nor toxicological studies consistently show effects. For all nonrespiratory effects, epidemiologic studies do not account for the potential influence of other traffic-related pollutants, which combined with the few or inconclusive results from controlled human exposure or toxicological studies, produces large uncertainty as to whether short-term or long-term NO_2 exposure has independent relationships with health effects outside of the respiratory system.

Policy-Relevant Considerations for Health Effects Associated with Nitrogen Dioxide Exposure

Multiple durations of short-term and long-term NO_2 exposure are observed to be associated with health effects (Section 1.6.1). For short-term exposure, asthma-related effects are associated with total personal NO_2 exposure and NO_2 measured at children's schools or community monitors averaged over 1 to 5 days. These associations are observed with both daily average and the daily highest 1-hour NO_2 concentration. No particular duration of exposure shows a stronger effect. Controlled human studies demonstrate increased airway inflammation and airway responsiveness in adults with asthma following NO_2 exposures of 15 to 60 minutes. These results support the epidemiologic evidence indicating that NO_2 exposures of 2 or 5 hours near high-traffic roads can lead to respiratory effects in adults with and without asthma.

For long-term exposure, asthma development in children is associated with estimates of residential ambient NO_2 exposure averaged over 1 to 10 years, representing various developmental periods, such as infancy, childhood, and lifetime. The pattern of NO_2 exposure underlying these associations is not well characterized, but some evidence from experimental studies in humans and rodents suggests that repeated exposure over many days or weeks can induce allergic responses, which are involved in asthma development.

Information on the shape of the NO_2 concentration-health effect relationship is limited mostly to epidemiologic studies. A few results show that asthma emergency department visits and diagnosis of asthma, respectively, increase with increasing short-term and long-term average ambient NO_2 concentrations (Section 1.6.3). As analyzed for asthma emergency department visits, the association with the daily highest 1-hour NO_2 concentrations is present at low concentrations. But, uncertainty in the relationship is noted at concentrations well below the level of the current 1-hour NAAQS.

Health effects related to NO_2 exposure potentially have a large public health impact. Many people in the U.S. live, work, or spend time near roads and may have high exposures to NO_2. Higher NO_2 exposure also is indicated for urban, low socioeconomic status, and nonwhite populations. Further, people with asthma, children (especially ages 0−14 years), and older adults (especially ages 65 years and older) are identified as being at increased risk of NO_2-related health effects (Chapter 7). Evidence does not clearly identify other at-risk populations in terms of other diseases or behavioral, genetic, or sociodemographic factors. Short-term and long-term NO_2 exposure is linked to clinically relevant increases in airway responsiveness, emergency department visits and hospital admissions for asthma, and development of asthma, which can have a large impact on public health. Given that asthma is the leading chronic illness and the leading cause of

missed school days and hospital admissions among U.S. children, NO_2-related asthma attacks and asthma development have the potential to affect children's overall well-being.

Summary of Major Findings

Expanding on findings from the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008), recent epidemiologic studies show associations of short-term and long-term NO_2 exposure with an array of health effects. However, except for respiratory effects, there remains large uncertainty about whether NO_2 exposure has an effect that is independent of other traffic-related pollutants. As in the 2008 ISA, recent information shows that motor vehicle emissions are the largest single source of NO_2 in the air and that NO_2 concentrations tend to be variable across locations, decreasing with increasing distance from roads. Information to assess whether NO_2 exposure estimates adequately represent the variability in ambient NO_2 concentrations and people's activity patterns varies among the health effects evaluated in this ISA. The major findings from this ISA about NO_2 exposure and health effects and related uncertainties are summarized below.

- Evidence for asthma attacks supports a causal relationship between short-term NO_2 exposure and respiratory effects, and evidence for development of asthma supports a likely to be causal relationship between long-term NO_2 exposure and respiratory effects. These are stronger conclusions than those determined in the 2008 ISA for Oxides of Nitrogen.
- Evidence is suggestive, but not sufficient, to infer a causal relationship for short-term or long-term NO_2 exposure with cardiovascular and related metabolic effects, birth outcomes, total mortality, and cancer. These also are stronger conclusions than those determined in the 2008 ISA for Oxides of Nitrogen.
- Recent and previous findings combined indicate that people with asthma, children, and older adults are at increased risk for NO_2-related health effects.
- There is continued evidence for increased NO_2 exposure among people living or spending time near or on roads, low socioeconomic status populations, and nonwhite populations.
- Data from the U.S. near-road network are starting to become available and may help address gaps in understanding of the:
  - variability in NO_2 concentrations within cities and NO_2 exposure in the population,
  - health effects associated with NO_2 exposures occurring near roads, and
  - potential for other traffic-related pollutants to influence associations observed between NO_2 exposure and health effects.
- Epidemiologic studies continue to examine ambient NO_2 concentrations at community monitoring sites, which represent exposure with error.

- Error in representing changes in short-term exposure over time (e.g., day-to-day) tend to decrease the magnitude of association with health effects and/or increase the uncertainty of the estimates.
- Error in representing differences among people in long-term NO_2 exposure can decrease health effect associations and/or increase the uncertainty of the estimates but sometimes can overestimate associations.
- NO_2 concentrations measured at people's locations may better represent exposure and improve understanding of relationships with health effects.

- An independent effect of short-term NO_2 exposure on respiratory effects is supported by additional epidemiologic evidence for ambient NO_2 measured at people's locations and personal NO_2 exposure and associations that are independent of another traffic-related pollutant integrated with results from previous controlled human exposure studies describing biological pathways.
- For long-term NO_2 exposure and respiratory effects, there is new supporting epidemiologic evidence for NO_2 exposure estimated at or near children's homes. Epidemiologic studies did not examine the influence of other traffic-related pollutants, but limited findings from previous experimental studies provide some support for an independent effect of NO_2 exposure.
- For nonrespiratory effects, there is continued or new epidemiologic evidence, but epidemiologic, controlled human exposure, and toxicological studies have not sufficiently addressed the uncertainty as to whether NO_2 exposure has independent effects.
- In limited investigation, the relationship between the daily highest 1-hour NO_2 concentration and emergency department visits for asthma is present at concentrations well below the current 1-hour NAAQS.

References for Executive Summary

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CHAPTER 1 INTEGRATED SUMMARY

1.1 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" (CAA, 1990). This ISA communicates critical science judgments of the health criteria for a broad category of gaseous oxides of nitrogen (i.e., oxidized nitrogen compounds) for which nitrogen dioxide (NO_2) is the indicator. As such, this ISA serves as the scientific foundation for the review of the current primary (health-based) National Ambient Air Quality Standards (NAAQS) for NO_2. Gaseous oxides of nitrogen include NO_2, nitric oxide (NO), and their various reaction products (Figure 1-1, Section 2.2)[1]. There also are particulate oxides of nitrogen (e.g., nitrates, nitro-polycyclic aromatic hydrocarbons)[2], which were considered in the most recent review of the NAAQS for particulate matter (PM) and evaluated in the 2009 ISA for PM (U.S. EPA, 2009). The welfare effects of oxides of nitrogen are being evaluated in a separate assessment conducted as part of the review of the secondary (welfare-based) NAAQS for NO_2 and sulfur dioxide [SO_2; (U.S. EPA, 2013c)].

This ISA evaluates relevant scientific literature published since the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008), integrating key information and judgments contained in the 2008 ISA and the 1993 Air Quality Criteria Document for Oxides of Nitrogen (U.S. EPA, 1993). Thus, this ISA updates the state of the science that was available for the 2008 ISA, which informed decisions on the primary NO_2 NAAQS in the review completed in 2010. In 2010, the U.S. Environmental Protection Agency (EPA) retained the existing annual average (avg) NO_2 NAAQS with a level of 53 parts per billion (ppb) to protect against health effects potentially associated with long-term exposure. EPA established a new 1-hour (h) NAAQS at a level of 100 ppb NO_2 based on the 3-year (yr) avg of each year's 98th percentile of 1-h daily maximum (max) concentrations.[3] The 1-h standard was established to protect against a broad range of respiratory effects associated with short-term exposures in potential at-risk populations such as people with asthma and people who spend time on or near high-traffic roads. In 2010, EPA also set requirements for a monitoring network in urban areas that includes monitors near (within

[1] The blue electronic links can be used to navigate to other parts of this ISA and to information on cited references.
[2] Section 108(c) of the Clean Air Act, 42 U.S.C. § 7408(c) specifies that criteria for oxides of nitrogen include consideration of nitric and nitrous acids, nitrites, nitrates, nitrosamines, and other derivatives of oxides of nitrogen, including multiple gaseous and particulate species.
[3] The legislative requirements and history of the NO_2 NAAQS are described in detail in the Preface to this ISA.

50 meters [m]) of high-traffic roads, one of the locations where the highest NO_2 concentrations are expected to occur (U.S. EPA, 2010).

This review of the primary NO_2 NAAQS is guided by several policy-relevant questions that are identified in *The Integrated Review Plan for the Primary National Ambient Air Quality Standard for Nitrogen Dioxide* (U.S. EPA, 2014). To address these questions and update the scientific judgments in the 2008 ISA, this ISA aims to:

- Characterize the evidence for health effects associated with short-term (minutes up to 1 month) and long-term (more than 1 month to years) exposure to oxides of nitrogen by integrating findings across scientific disciplines and across related health outcomes and by considering important uncertainties identified in the interpretation of the scientific evidence, including the role of NO_2 within the broader ambient mixture of pollutants.
- Inform policy-relevant issues related to quantifying health risks, such as exposure concentrations, durations, and patterns associated with health effects; concentration-response relationships and evidence of thresholds below which effects do not occur; and populations and lifestages potentially with increased risk of health effects related to exposure to oxides of nitrogen.

Although the scope of the ISA includes all gaseous oxides of nitrogen, much of the information on the distribution of oxides of nitrogen in the air, human exposure and dose, impact of errors associated with exposure assessment methods, and health effects is for NO_2. There is limited information for NO and the sum of NO and NO_2 (NO_X) as well as large uncertainty in relating health effects to NO or NO_X exposure. In the body, NO is produced from nitrates and nitrites derived from diet and enzymatic pathways that are enhanced during inflammation. Ambient NO concentrations generally are in the range of endogenous NO concentrations exhaled from the respiratory tract. It is not clear whether ambient-relevant NO exposures substantially alter endogenous NO production in the respiratory tract or pathways affected by endogenous NO (Section 4.2.3). Thus, the potential for detrimental health effects occurring from ambient-relevant NO exposure is unclear. This lack of evidence leaves NO_2 as the component of NO_X to consider in evaluating health effects in relation to NO_X exposure. Because of the varying ratio of NO_2 to NO_X across locations, time of day, and season (Section 2.5), NO_X may not represent NO_2 exposure consistently. The lack of support for ambient-relevant NO exposure to result in potentially detrimental health effects and the exposure measurement error related to using NO_X to represent NO_2 exposure are the rationale for determining the causal nature of health effects only for NO_2 exposure.

In addressing policy-relevant questions, this ISA aims to characterize the independent health effects of NO_2 exposure, not the role of NO_2 as just a marker for a broader mixture of pollutants in the ambient air. The potential influence of other traffic-related pollutants was the main uncertainty in the conclusions drawn in the 2008 ISA Oxides of Nitrogen

(U.S. EPA, 2008). As described in this ISA, recent epidemiologic findings together with evidence from previous controlled human exposure studies sufficiently describe a coherent, biologically plausible relationship between short-term NO_2 exposure and respiratory effects ranging from increased airway responsiveness to increased symptoms, emergency department (ED) visits, and hospital admissions related to asthma exacerbation. Recent epidemiologic studies provide new evidence supporting a relationship of long-term NO_2 exposure with respiratory effects, specifically, the development of asthma in children, and a small body of previous experimental studies provide some indication that NO_2 exposure may have an independent effect. Recent epidemiologic studies continue to suggest that short-term NO_2 exposure may be associated with cardiovascular and related metabolic effects and mortality, and new findings potentially link long-term NO_2 exposure to cardiovascular and related metabolic effects, poorer birth outcomes, mortality, and cancer. However, for nonrespiratory effects, epidemiologic studies have not adequately accounted for effects of other traffic-related pollutants, and findings from experimental studies continue to be limited. The information in the ISA contributing to these findings will serve as the scientific foundation for the review of the current primary 1-hour and annual NO_2 NAAQS.

1.2 Process for Developing Integrated Science Assessments

EPA uses a structured and transparent process for evaluating scientific information and determining the causality of relationships between air pollution exposures and health effects (see Preamble). 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 causal determinations. As part of this process, the ISA is reviewed by the Clean Air Scientific Advisory Committee (CASAC), a formal independent panel of scientific experts, and the public. As this ISA informs the review of the primary NO_2 NAAQS, it assesses information relevant to characterizing exposure to gaseous oxides of nitrogen and potential relationships with health effects. Relevant studies include those examining atmospheric chemistry, spatial and temporal trends, and exposure assessment, as well as EPA analyses of air quality and emissions data. Also relevant are epidemiologic, controlled human exposure, and toxicological studies on health effects as well as studies on dosimetry and modes of action.

EPA initiated the current review of the primary NAAQS for NO_2 in February 2012 with a call for information from the public (U.S. EPA, 2012). Thereafter, EPA routinely conducted literature searches to identify relevant peer-reviewed studies published since the previous ISA (i.e., from January 2008 through August 2014). Multiple search methods were used (Preamble, Section 2) including searches in databases such as

PubMed and Web of Science. Also, recommendations were made by CASAC and the public. EPA identified additional studies considered to be the definitive work on particular topics from previous assessments to include in this ISA. Some studies were judged to be irrelevant (i.e., did not address a topic described in the preceding paragraph) based on title and were excluded. Studies that were judged to be potentially relevant based on review of the abstract or full text and "considered" for inclusion in the ISA are documented and can be found at the Health and Environmental Research Online (HERO) website. The HERO project page for this ISA (http://hero.epa.gov/oxides-of-nitrogen) contains the references that are cited in the ISA, the references that were "considered" for inclusion but not cited, and electronic links to bibliographic information and abstracts.

Health effects were considered for evaluation in this ISA if they were examined in previous EPA assessments for oxides of nitrogen or multiple recent studies (e.g., neurodevelopment). Literature searches identified one or two recent epidemiologic studies each on outcomes such as gastrointestinal effects, bone density, headache, and depression [Supplemental Table S1-1; (U.S. EPA, 2013d)]. A review of these studies indicated they are similar in design and conducted in areas and populations for which associations between ambient NO_2 concentrations and other health effects have been documented. These few studies were excluded from this ISA because they do not provide new information on particular geographic locations, potential at-risk populations or lifestages, or range of ambient NO_2 concentrations and because these studies more likely are subject to publication bias.

The Preamble describes the general framework for evaluating scientific information, including criteria for assessing study quality and developing scientific conclusions. Aspects specific to evaluating studies of oxides of nitrogen are described in Table 5-1. For epidemiologic studies, emphasis is placed on studies that characterize quantitative relationships between oxides of nitrogen and health effects, examine exposure metrics that well represent the variability in concentrations in the study area, consider the potential influence of other air pollutants and factors correlated with oxides of nitrogen, examine potential at-risk populations and lifestages, or combine information across multiple cities. With respect to the evaluation of controlled human exposure and toxicological studies, emphasis is placed on studies that examine effects relevant to humans and NO_2 concentrations that are defined in this ISA to be relevant to ambient exposures. Based on peak ambient concentrations (Section 2.5) and the ISA definition that ambient-relevant exposures be within one to two orders of magnitude of current levels, NO_2 concentrations 5,000 ppb[1] or less are defined to be ambient relevant. Experimental studies with higher exposure concentrations were included if they informed

[1] The 5,000-ppb upper limit applies largely to animal toxicological studies but also a few controlled human exposure studies.

dosimetry or potential modes of action. For the evaluation of human exposure to ambient NO_2, emphasis is placed on studies that examine the quality of data sources used to assess exposures, such as central site monitors, land use regression (LUR) models, and personal exposure monitors. The ISA also emphasizes studies that examine factors that influence exposure such as time-activity patterns and building ventilation characteristics.

Integrating information across scientific disciplines and related health outcomes and synthesizing evidence from previous and recent studies, the ISA draws conclusions about relationships between NO_2 exposure and health effects. Determinations are made about causation not just association and are based on judgments of aspects such as the consistency, coherence, and biological plausibility of observed effects (i.e., evidence for effects on key events in the mode of action) as well as related uncertainties. The ISA uses a formal causal framework (Table II of the Preamble) to classify the weight of evidence according to the five-level hierarchy summarized below.

- **Causal relationship:** the consistency and coherence of evidence integrated across scientific disciplines and related health outcomes are sufficient to rule out chance, confounding, and other biases with reasonable confidence.
- **Likely to be a causal relationship:** there are studies where results are not explained by chance, confounding, or other biases, but uncertainties remain in the evidence overall. For example, the influence of other pollutants are difficult to address, or evidence among scientific disciplines may be limited or inconsistent.
- **Suggestive, but not sufficient, to infer a causal relationship:** evidence is generally supportive but not entirely consistent or overall is limited. Chance, confounding, and other biases cannot be ruled out.
- **Inadequate to infer a causal relationship:** there is insufficient quantity, quality, consistency, or statistical power of results from studies.
- **Not likely to be a causal relationship:** several adequate studies, examining the full range of human exposure concentrations and potential at-risk populations and lifestages, consistently show no effect.

1.3 Content of the Integrated Science Assessment

The ISA consists of the Preamble, Preface (legislative requirements and history of the primary NO_2 NAAQS), Executive Summary, and seven chapters. Chapter 1 synthesizes the scientific evidence that best informs policy-relevant questions that frame this review of the primary NO_2 NAAQS. Chapter 2 characterizes the sources, atmospheric processes of oxides of nitrogen, and trends in ambient concentrations. Chapter 3 describes methods to estimate human exposure to oxides of nitrogen and the impact of error in estimating exposure on associations with health effects. Chapter 4 describes the dosimetry and

modes of action for NO_2 and NO. Chapters 5 and 6 evaluate and integrate epidemiologic, controlled human exposure, and toxicological evidence for health effects related to short-term and long-term exposure to oxides of nitrogen, respectively. Chapter 7 evaluates information on potential at-risk populations and lifestages.

The purpose of this chapter is not to summarize each of the aforementioned chapters but to synthesize the key findings for each topic that informs the characterization of NO_2 exposure and relationships with health effects. This chapter also integrates information across the ISA to inform policy-relevant issues such as NO_2 exposure durations and patterns associated with health effects, concentration-response relationships, and the public health impact of NO_2-related health effects (Section 1.6). A key consideration in the health effects assessment is the extent to which evidence indicates that NO_2 exposure independently causes health effects versus indicating that NO_2 may be serving as a marker for a broader mixture of air pollutants, especially those related to traffic. To that end, this chapter draws upon information about the sources, distribution, and exposure to ambient NO_2 (Section 3.4.5) and identifies pollutants and other factors related to the distribution of or exposure to ambient NO_2 that can potentially influence epidemiologic associations observed between health effects and NO_2 exposure (Section 1.4.3). The discussions of the health effects evidence and causal determinations (Section 1.5) describe the extent to which epidemiologic studies accounted for these factors and the extent to which findings from controlled human exposure and animal toxicological studies support independent relationships between NO_2 exposure and health effects.

1.4 From Emissions Sources to Exposure to Nitrogen Dioxide

Characterizing human exposure is key to understanding the relationships between ambient NO_2 exposure and health effects. The sources of oxides of nitrogen and the transformations that occur in ambient air influence the spatial and temporal pattern of NO_2 concentrations in the air. These patterns have implications for variation in exposure in the population, the adequacy of methods used to estimate exposure and, in turn, the strength of inferences that can be drawn from associations observed between NO_2 exposure and health effects.

1.4.1 Emission Sources and Distribution of Ambient Concentrations

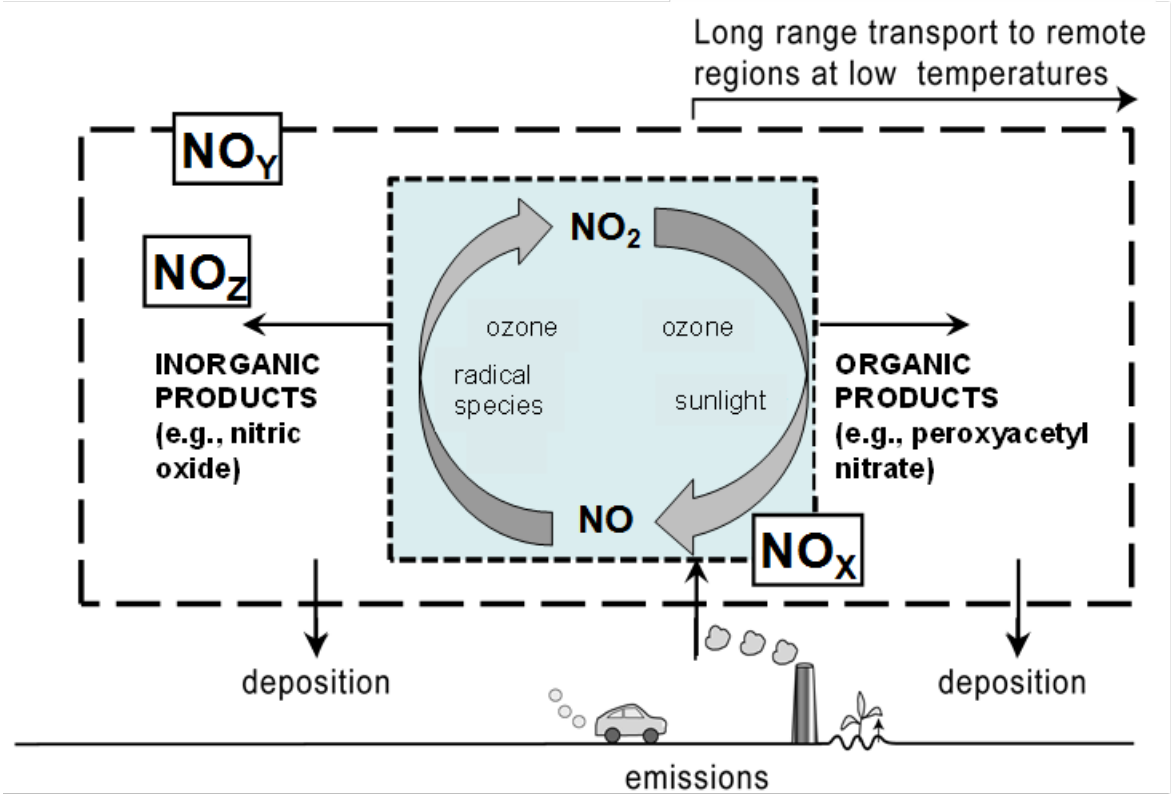
The strength and distribution of emissions sources are important determinants of the distribution of NO_2 in the ambient air and, in turn, human exposure. Information on emissions is available for NO_X, which is emitted primarily as NO. NO rapidly reacts with

radicals and ozone (O_3) to form NO_2 in the air. Based on the 2011 National Emissions Inventory, the largest single source of NO_X emissions in the U.S. overall and in major population centers (city and surrounding communities) is highway vehicles (40−67%; Section 2.3, Table 2-1). Sources such as electric utilities, commercial and residential boilers, and industrial facilities are more variable across locations but can be important contributors to ambient NO_2 concentrations for the U.S. as a whole and in certain populated areas. Some of these sources can affect local air quality with large, transient emissions of NO_X. Natural sources such as microbial processes in soil and wildfires make small (2% of the inventory) contributions to emissions in U.S. population centers, and emissions from natural and anthropogenic sources from continents other than North America (i.e., North American Background) account for less than 1% (typically 0.3 ppb) of ambient concentrations (Section 2.5.6). Although highway vehicles are a large, ubiquitous source of NO_X, the varying presence and mix of specific emissions sources across locations can contribute to heterogeneity in ambient NO_2 concentrations regionally and locally, which has implications for variation in exposure to ambient NO_2 within the population.

In addition to emissions sources, factors that influence NO_2 ambient concentrations include chemical transformations, transport to other locations, meteorology, and deposition to surfaces (Figure 1-1 and in more detail, Figure 2-1). NO and NO_2 react with gas phase radicals and O_3 to form other oxides of nitrogen such as peroxyacetyl nitrate (PAN) and nitric acid (HNO_3; Section 2.2). NO and NO_2 also are involved in reaction cycles with radicals produced from volatile organic compounds (VOCs) to form O_3. The reactions of NO and NO_2 into other oxides of nitrogen typically occur more slowly than the interconversion between NO_2 and NO does, and NO and NO_2 are the most prevalent oxides of nitrogen in populated areas. Compounds such as HNO_3 and PAN can make up a large fraction of ambient oxides of nitrogen downwind of major emission sources.

Sources, atmospheric transformations, and meteorology contribute to the temporal trends observed in ambient NO_2 concentrations. As a result of pollution control technologies on vehicles and electric utilities (Section 2.3.2), NO_X emissions from highway vehicles and fuel combustion decreased by 49% in the U.S. from 1990 to 2013 (Figure 2-2). During that time (1990−2012), U.S.-wide annual average NO_2 concentrations decreased by 48% (Figure 2-20). In addition to long-term trends, ambient NO_2 concentrations show seasonal trends, with higher concentrations measured in the winter than summer. Reflecting trends in traffic, ambient concentrations at most urban sites are higher on weekdays than weekends, and within a day, concentrations peak in early mornings, decrease until late afternoon, then increase again in early evening corresponding with morning and evening commutes. Diurnal trends in ambient NO_2 also are affected by meteorology, with

concentrations rising during the night when atmospheric mixing is reduced because of low wind speeds and low mixing layer heights.



Note: The inner shaded box contains NO_X (sum of nitric oxide [NO] and nitrogen dioxide [NO_2]). The outer box contains oxides of nitrogen (NO_Z) formed from reactions of NO_X.(NO_Z). Oxides of nitrogen in the outer and inner boxes (NO_X + NO_Z) are collectively referred to as NO_Y by the atmospheric sciences community.

Source: National Center for Environmental Assessment. For more details on the various reactions, see Figure 2-1.

Figure 1-1 Reactions of oxides of nitrogen species in the ambient air.

The spatial variation in emissions sources and chemical transformation of oxides of nitrogen likely contribute to the variability in ambient NO_2 concentrations observed at regional, urban, neighborhood, and near-road scales (Section 2.5). Measurements from U.S. air monitoring networks[1] of several hundred sites (Section 2.5.1) show wide variation in ambient NO_2 concentrations across the U.S. Across sites, the mean 1-h daily maximum ambient NO_2 concentration for 2011−2013 was 19 ppb, and the 5th to 99th percentile range was 2−55 ppb (Table 2-3). The mean annual average NO_2 concentration was 8.6 ppb, and the 5th to 99th percentile range was 1.4−22.5 ppb (Table 2-4). Ambient

[1] The air monitoring networks serve many objectives: determining compliance with the NAAQS, providing the public with air pollution data in a timely manner, and providing estimates of ambient exposure for research studies.

NO_2 concentrations are higher in large cities than in less populated locations (Figures 2-11 and 2-12). Ambient NO_2 concentrations also can vary widely across sites within cities where vehicle emissions are the major source (Figure 2-14, Table 2-5). Some city sites agree well in terms of temporal correlations or magnitude of concentration; however, the siting of most monitors away from sources likely does not capture the extent of variability in ambient NO_2 in a city. Preliminary data from the near-road network for 1 year for a small group of U.S. cities tend to show higher NO_2 concentrations near roads than at many other sites in the same city (Table 2-8). Across near-road sites in five cities, mean 1-hour NO_2 concentrations in winter were 21−45 ppb, and maximums were 52−97 ppb. Studies have measured 1-h to 2-week avg NO_2 concentrations of 29 to 65 ppb within 20 m of a road, which are about 20 ppb higher than concentrations 80 to 500 m from the same road (Section 2.5.3, Table 2-6). The wide variation in ambient NO_2 concentrations across spatial and temporal scales, largely influenced by motor vehicle emissions, can contribute to variation in NO_2 exposure within the population and has important implications for accurately characterizing exposure.

1.4.2 Assessment of Human Exposure

Characterizing the adequacy of various exposure assessment methods to represent the variability in ambient concentrations in a location is key in drawing inferences from epidemiologic associations with health effects. Exposure is determined by concentrations in specific ambient, indoor, and in-vehicle locations and time spent in those locations (Section 3.4.1). People vary in the locations where they spend time and time spent in those locations (Section 3.4.3.1), and NO_2 concentrations can vary widely across outdoor, indoor, and in-vehicle locations (Figure 3-1). Measures of NO_2 exposure that do not fully account for the variability in ambient concentrations and people's activity patterns have some amount of error, and this error can alter associations observed with health effects. The extent and impact of error can differ by exposure assessment method and between epidemiologic studies of short-term exposure, which tend to examine temporal (e.g., day to day) changes in NO_2, and studies of long-term exposure, which tend to compare people living in locations that differ in ambient NO_2 concentrations (Section 3.4.5).

Ambient NO_2 concentrations at central site monitors represent both short-term and long-term exposure with some amount of error. Central site monitors do not cover all locations where people live or spend their time and also are not likely to capture the temporal or spatial variability in ambient NO_2 concentrations in a given area. Long-term personal NO_2 exposures and their relationships with ambient NO_2 concentrations are not well characterized. A wide range of correlations is observed between short-term total

personal and ambient NO_2 concentrations (0.12 to 0.43; Table 3-6) and in ambient NO_2 concentrations across sites within some cities (Section 2.5.2). On one hand, poor correlations may not necessarily mean that concentrations at central sites are inadequate exposure metrics because the data may not reflect relationships between ambient NO_2 concentrations and the ambient component of personal exposure (Section 3.4.2). On the other hand, the correlations could indicate heterogeneity among individuals in how well short-term temporal changes in NO_2 concentrations at central site monitors represent temporal changes in ambient exposure. The adequacy of long-term average ambient NO_2 concentrations from central site monitors (from one monitor or combined across multiple monitors) to represent variability in ambient exposures will depend on how close the monitors are to the population or how well local emission sources are dispersed (Section 3.4.5.2). In locations with few or well-dispersed sources and similar ambient NO_2 concentrations across sites, concentrations at central site monitors may adequately capture the spatial variation in long-term ambient NO_2 exposures of the population.

Proximity to roads may contribute substantially to short-term and long-term ambient NO_2 exposure among people living or working near roads or commuting on roads, and the 2008 ISA for Oxides of Nitrogen cited the potential for in-vehicle exposures to dominate short-term personal exposure (U.S. EPA, 2008). Data from the U.S. near-road monitoring network are too preliminary to allow for meaningful comparisons of the temporal or spatial patterns in NO_2 near and away from roads. Data from London, U.K. show that 24-h avg NO_2 concentrations often are higher (26−170%; Table 2-9) at roadside than at background sites but tend to be moderately to highly correlated between sites (correlation coefficient [r] = 0.63−0.86; Table 2-9). These data indicate that central site monitors may capture the short-term temporal variability near roads but may not represent the magnitude of long-term average NO_2 concentrations near roads. Another consideration in estimating exposure from central site monitors is that the chemiluminescence measurement method tends to overestimate ambient NO_2 concentrations because of interference from other oxides of nitrogen. However, interference generally is less than 10% in urban locations (Section 2.4.1) and may not vary widely day to day (Section 3.4.3.4) to produce substantial error in characterizing daily changes in NO_2 concentration. It is not clear how interference compares among locations and what impact interference may have on comparisons of long-term average NO_2 concentrations among locations.

In addition to concentrations at central site monitors, epidemiologic studies examined short-term NO_2 exposures at people's locations, including personal ambient and total NO_2 exposure as well as measurements taken outdoors at schools and indoors at homes and schools. A time-weighted average of NO_2 concentrations in people's locations correlated well with total personal short-term NO_2 exposures (Section 3.4.3.1), indicating

that NO_2 concentrations in locations where a person spends time can represent a component of personal exposure and aid in inference about NO_2-related health effects. Epidemiologic studies examining total personal or indoor NO_2 concentrations also have important roles in characterizing health effects of NO_2 exposure because they can help distinguish NO_2-related health effects from the potential influence of other traffic-related pollutants. Correlations between NO_2 and some copollutants are lower for total personal or indoor metrics than ambient metrics (Section 3.4.4.3, Table 3-10). Results for total personal and indoor NO_2 concentrations also can inform health effects related to ambient exposure for populations with high total personal-ambient NO_2 correlations and populations for whom indoor concentrations are affected by the penetration of ambient NO_2 from open windows or other factors that increase building air exchange rate (Section 3.4.3.3). There is an increase among recent studies in the use of LUR and dispersion models to estimate long-term NO_2 exposures at the neighborhood scale or at an individual's residence. Dispersion models have uncertainties related to systematic bias in representing ambient NO_2 concentrations (Section 3.5). LUR models may account for differences among individuals in residential distance to sources and have been demonstrated to represent well the variability in long-term average ambient NO_2 concentrations in many locations (Section 3.5).

Errors in representing the temporal and spatial variability in short-term and long-term averages, respectively, of ambient NO_2 concentrations in a given area and exposures of the population can impact the characterization of relationships between NO_2 exposure and health effects. For short-term exposure, if ambient NO_2 concentrations and people's ambient exposures are temporally correlated, there may be little error in health effect estimates. NO_2 measurements at people's locations may include error because the metrics may not represent potentially important exposures across the range of locations where people spend time. However, larger magnitude health effects have been estimated for more spatially resolved measures of short-term NO_2 exposure than for NO_2 measured at a single central site monitor or averaged over multiple monitors in a city (Section 3.4.5.1). Such findings indicate that not accounting for the varying population distribution around a central site monitor or varying correlations in NO_2 across monitors can decrease associations with NO_2 (Section 3.4.5.1). Compared with NO_2 estimated by LUR, long-term average NO_2 concentrations at central site monitors often show smaller associations with health effect but larger associations in some cases (Section 3.4.5.2). Thus, spatial misalignment of long-term NO_2 exposure metrics can alter health effect estimates in either direction. Exposure error also can impact the precision [i.e., 95% confidence interval (CI)] of health effect estimates due to variable relationships between personal and ambient NO_2 across people and time and differences in nonambient exposures. It is unclear how error produced from using ambient NO_2 concentrations at central site monitors to represent near-road exposures impacts health effect associations

because differences in temporal or spatial patterns for near-road NO_2 concentrations or correlations with personal NO_2 exposure are not widely characterized. For short-term and long-term exposure, evaluating how well personal, central site, location-specific, or modeled estimates of NO_2 exposure capture the variability in ambient concentrations or exposure and the potential impact of exposure error is a key consideration in drawing inferences from epidemiologic studies about NO_2-related health effects.

1.4.3 Factors Potentially Correlated with Nitrogen Dioxide Exposure to Consider in Evaluating Relationships with Health Effects

The large influence of motor vehicle emissions on the distribution of ambient NO_2 concentrations not only affects the assessment of NO_2 exposure but also has implications for co-exposure to other traffic-related pollutants. NO_2 concentrations are higher near roads as are concentrations of elemental or black carbon (EC/BC), ultrafine particles (UFP), carbon monoxide (CO), and VOCs (Section 3.3.1). The exact nature of gradients varies among pollutants, but concentrations of traffic-related pollutants, including NO_2, decrease with increasing distance from the road. $PM_{2.5}$[1] and organic carbon (OC) do not show clear gradients; however, a portion of $PM_{2.5}$ and OC comes from vehicle emissions. These correlations and evidence that the copollutants show relationships with many of the same health effects as NO_2 and have similar modes of action (Table 5-1, Section 5.1.2.1) point to the importance of evaluating the potential for NO_2-related health effects to be confounded (i.e., biased) by other traffic-related pollutants or for NO_2 to represent a mixture of traffic-related pollution. Common sources, atmospheric reactions, or similar trends due to meteorologic conditions extend the potential for co-exposures to pollutants beyond those emitted from vehicles. Factors such as socioeconomic status (SES), season, and temperature also show correlations with NO_2 concentrations and relationships with similar health effects. The potential for a particular factor to confound NO_2-health effect associations varies depending on the extent of correlation with NO_2 concentrations, the nature of the relationship with the health effect, and study design (i.e., whether temporal variation in short-term exposure or spatial variation in long-term exposure is examined).

Short-term average NO_2 concentrations show a range of correlations with traffic-related copollutants, but high correlations often are observed (Figure 3-6, Table 3-8). For example, for averaging times of 1 to 24 hours, the 25th to 75th percentile ranges of correlation coefficients are 0.59−0.96 for CO, 0.41−0.61 for $PM_{2.5}$, and 0.58−0.67 for EC. Limited data indicate similar correlations with short-term averages of VOCs, and lower correlations with OC. Long-term average ambient NO_2 concentrations show

[1] In general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 2.5 µm, a measure of fine particles.

similar correlations with $PM_{2.5}$ and CO as short-term averages, but the distribution of correlations is shifted to higher values. Correlations of long-term averages of NO_2 with EC/BC, VOCs, OC, and UFP are not well characterized (Figure 3-6). Information on seasonal correlations between ambient concentrations of NO_2 and traffic-related copollutants is sparse, but there is some indication of lower NO_2−$PM_{2.5}$ correlations for short-term averages in the warm season (Section 3.4.4.1). These data point to potentially lower confounding by $PM_{2.5}$ in the warm season. Although traffic-related copollutants have been associated with many of the same health effects as NO_2 (Table 5-1), the wide range of correlations with short-term and long-term average NO_2 concentrations indicates variation among locations in confounding potential.

Much of the data characterizing correlations of NO_2 with traffic-related copollutants are based on measurements at central site monitors. The varying spatial patterns among pollutants may obscure true correlations across study areas or correlations in personal exposure. Except for UFP, the few available data do not indicate systematically higher correlations near roads (Figure 3-6). However, correlations of short-term averages of NO_2 with $PM_{2.5}$ (r = 0.06 to 0.38), EC (r = 0.22 to 0.49), and VOCs (r = −0.42 to 0.14) can be weaker for personal exposures than ambient concentrations (Table 3-11, Section 3.4.4.3). Correlations of short-term averages of NO_2 with $PM_{2.5}$ and BC sometimes can be lower indoors than outdoors (Table 3-8 and Table 3-12). These limited data indicate that associations of short-term personal or indoor NO_2 exposures with health effects may be less subject to confounding by certain traffic-related copollutants. In some locations, short-term average ambient NO_2 concentrations are related more strongly to personal PM than personal NO_2 exposure. However, recent data show negative to moderate correlations between ambient NO_2 concentrations and personal $PM_{2.5}$ or EC (r = −0.19 to 0.44; Table 3-9), suggesting that ambient NO_2 concentrations are not necessarily just a surrogate for personal PM exposure. The varying correlations between short-term average concentrations of NO_2 and other traffic-related pollutants across various microenvironments indicate that the potential for confounding by traffic-related copollutants varies by the exposure assessment method. Similar information to compare copollutant correlations among microenvironments is not available for long-term average NO_2 concentrations.

Other potential confounding factors to consider for long-term NO_2 exposure are measures of traffic proximity or intensity, which could represent exposure to other pollutants that display gradients with distance to road. Although NO_2 is not unique to vehicle emissions and can indicate sources such as off-highway vehicles and electric utilities (Section 2.3), distance to roads, the length of nearby roads, and vehicle counts are predictors of ambient NO_2 concentrations in LUR models (Section 3.2.2.1). Given recent findings linking residential proximity to roads with respiratory effects and possibly with cardiovascular

effects and mortality (HEI, 2010), roadway proximity could confound NO_2-health effect associations by indicating exposure to traffic pollution. Studies considering the potential influence of exposure to traffic, including residential proximity to roads, are another line of evidence to inform the independent effects of long-term NO_2 exposure on health.

Short-term and long-term averages of NO_2 also show a range of correlations with the copollutants PM_{10},[1] SO_2, and O_3. Short-term and long-term average NO_2 concentrations tend to be moderately correlated with PM_{10} (r for 25th−75th percentiles = 0.40−0.66 for short-term averages, 0.44−0.75 for long-term averages) and SO_2 (Figure 3-6, Table 3-8). Short-term averages of O_3 often are inversely correlated with NO_2, and peak correlations are moderate (r for 25th−75th percentile = −0.51 to 0.32) even in the summer, when O_3 concentrations are higher (Table 3-8). Higher correlations are observed between long-term averages of NO_2 and O_3 (r for 25th−75th percentiles = 0.26−0.63). The wide range of correlations observed for short-term and long-term average concentrations of NO_2 with PM_{10}, SO_2, and O_3 indicates the variable potential for these pollutants to confound health effect associations for NO_2. For short-term average NO_2 concentrations, the distributions of correlations with PM_{10} and SO_2 are shifted to lower values compared to correlations with most traffic-related pollutants, indicating the lower potential for confounding. Specific to long-term exposure, relationships of long-term SO_2 and O_3 exposure with many of the health effects evaluated in this ISA are uncertain (Table 5-1) as is their potential to confound NO_2-health effect associations.

Residence near traffic has been linked to higher noise or stress levels, but information on whether noise or stress confounds health effect associations with short-term or long-term NO_2 exposure is limited. Weak to moderate correlations tend to be reported between noise and short-term (r = 0.14−0.62) and long-term (r = 0.22−0.46) average ambient NO_2 concentrations, but high correlations have been observed for short-term NO_2 averages (r = 0.83; Section 3.4.4.4). The impact of short-term changes in noise or stress on health effects is not well characterized, but some data link long-term noise exposure and stress to cardiovascular effects (Section 6.3.2) and decreases in cognitive function (Section 6.4.4). Thus, the potential for stress or noise to confound NO_2-health effect associations is uncertain for short-term exposure but may exist for long-term exposure.

Other potential confounding factors to consider include temperature and humidity for associations of health effects with short-term NO_2 exposure because of similar time-varying patterns as ambient NO_2 concentrations and health effects. Also, similar to many health effects, short-term averages of ambient NO_2 concentration vary by day of the week and season and exhibit long-term time trends. For studies of long-term NO_2

[1] In general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 10 μm, a measure of thoracic particles.

exposure that compare individuals living in different locations, it is important to evaluate confounding by factors such as SES, race (Sections 7.5.2 and 7.5.3), and age, all of which can covary with long-term NO_2 exposures among individuals and spatially with long-term ambient NO_2 concentrations among communities.

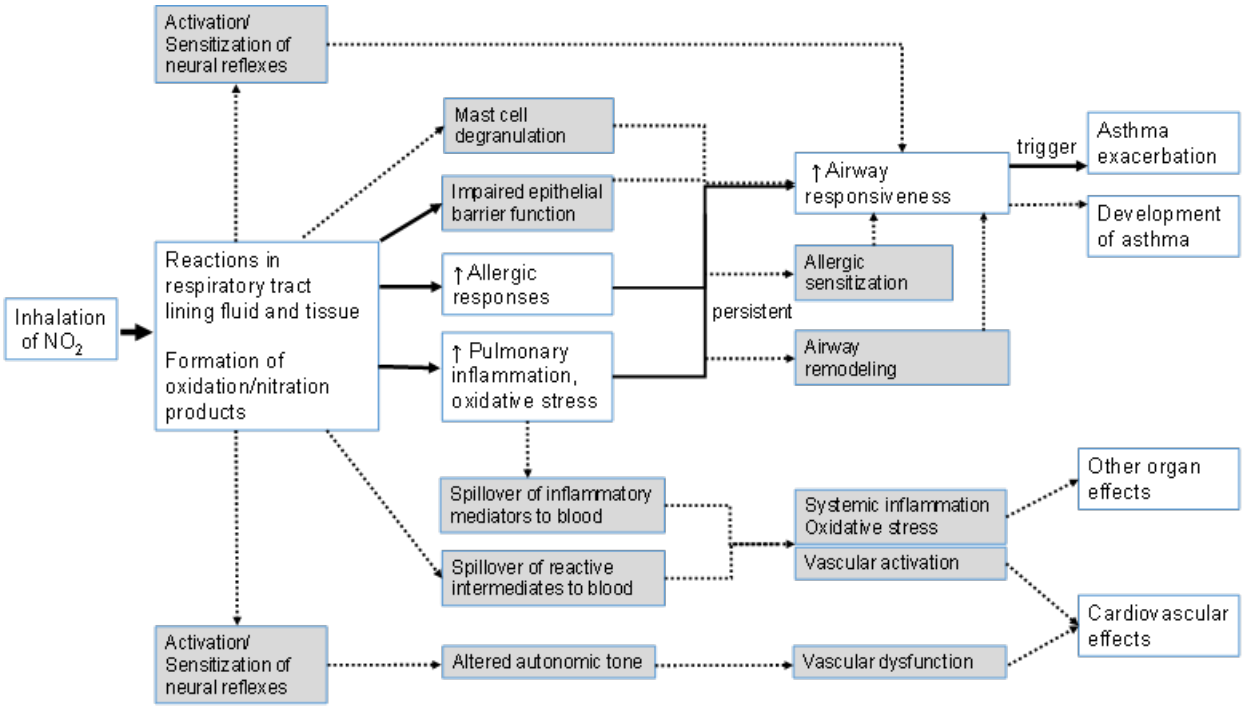
For studies reviewed in this ISA, the main method to account for potential confounding is multivariable models that include NO_2 concentrations and the putative confounder. The NO_2 effect estimate represents the effect of NO_2, keeping the level of the covariate constant. Confounding is assessed by examining the change in the magnitude of the effect estimate and width of the 95% CI, not a change in statistical significance. There are limitations to multivariable models, and correlations between variables and the exposure assessment method are important considerations in drawing inferences about confounding (Section 5.1.2.1). High correlations between NO_2 concentrations and the potential confounder can misleadingly decrease or increase the magnitude or precision of the effect estimate for NO_2 or the covariate and particularly are a concern for models that include a traffic-related copollutant or include three or more pollutants in the same model. Potential differences in exposure measurement error between NO_2 and the copollutant also limit inferences about an independent NO_2 association from copollutant models. Inference from copollutant models may be stronger for pollutants measured at people's locations and for personal exposure than for pollutants measured at central site monitors. As in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008), a key issue in this ISA is the extent to which epidemiologic studies examined potential confounding by traffic-related copollutants and the extent to which other lines of evidence support independent relationships between NO_2 exposure and health effects.

1.5 Health Effects of Nitrogen Dioxide Exposure

This ISA evaluates relationships between an array of health effects and short-term (Chapter 5) and long-term (Chapter 6) exposures to NO_2 as examined in epidemiologic, controlled human exposure, and animal toxicological studies. Short-term exposures are defined as those with durations of minutes up to 1 month, with most studies examining effects related to exposures in the range of 1 hour to 1 week. Long-term exposures are defined as those with durations of more than 1 month to years. Drawing from the health effects evidence described in detail in Chapter 5 and Chapter 6, information on dosimetry and modes of action presented in Chapter 4, as well as issues regarding exposure assessment and potential confounding described in Chapter 3 and Section 1.4, the subsequent sections and Table 1-1 present the key evidence that informs the causal determinations for relationships between NO_2 exposure and health effects.

1.5.1 Respiratory Effects

Relationships of short-term and long-term NO_2 exposure with respiratory effects are supported by the dosimetry and modes of action characterized for inhaled NO_2. Although it is not clear how ambient-relevant NO_2 exposures compare with NO_2 produced endogenously in the lung during inflammation and other immune responses (Section 4.2.2.4), ambient-relevant concentrations of inhaled NO_2 are absorbed throughout the respiratory tract. Dosimetry models predict that total NO_2 dose is relatively constant across the tracheobronchial region and rapidly decreases in the gas exchange (i.e., alveolar) region (Section 4.2.2.3). NO_2 is a reactive gas that reacts with antioxidants and other constituents of the epithelial lining fluid of the respiratory tract to form secondary oxidation products (Section 4.2.2.1). Although these reactions are rapid, antioxidant levels vary across regions of the lung, and untransformed NO_2 that reaches the alveolar region of the airways may penetrate the thin layer of the epithelial lining fluid to reach underlying tissue. Thus, the variable physical and chemical nature of the respiratory tract may influence the site in the respiratory tract of NO_2 uptake and NO_2-induced respiratory effects. The formation of secondary oxidation products likely is the initiating event in the sequence of events comprising the mode of action for NO_2 (Section 4.3.2.1). These products can induce oxidative stress, inflammation, allergic responses, and altered immune function, which are part of the mode of action for respiratory effects (Figures 1-2 and 4-1) as described in the sections that follow.



Note: Adapted from Figures 4-1, 4-2, and 4-3 in Section 4.3.5. Solid arrows and white boxes represent pathways for which there is consistent evidence. Dotted lines and gray boxes represent potential pathways for which the evidence is limited or inconsistent.

Figure 1-2 Characterization of potential modes of action for health effects related to exposure to nitrogen dioxide (NO_2).

Respiratory Effects Associated with Short-term Exposure to Nitrogen Dioxide

A causal relationship exists between short-term NO_2 exposure and respiratory effects based on evidence for asthma exacerbation. The conclusion is strengthened from the likely to be a causal relationship determined in the 2008 ISA for Oxides of Nitrogen because epidemiologic, controlled human exposure, and animal toxicological evidence together describe a coherent and biologically plausible pathway by which NO_2 exposure can trigger an asthma exacerbation (Table 1-1). There is some evidence indicating that short-term NO_2 exposure may be related to other respiratory effects, such as exacerbation of allergy or chronic obstructive pulmonary disease (COPD), respiratory infection, respiratory mortality, and respiratory effects in healthy people. However, because of inconsistency across disciplines and/or limited information to support biological plausibility, there is uncertainty whether short-term NO_2 exposure has independent relationships with nonasthma respiratory effects (Section 5.2.9, Table 5-45).

Supporting a causal relationship, recent epidemiologic studies continue to provide consistent and coherent evidence for NO_2-related exacerbation of asthma. Across diverse

geographic locations, short-term increases in ambient NO_2 concentration are associated with increases in hospital admissions and ED visits for asthma. As uncontrolled symptoms are the major reason for seeking medical treatment, coherence is demonstrated by observations of NO_2-related increases in respiratory symptoms and decreases in lung function in children with asthma. Associations are observed in studies with maximum concentrations of 48 to 106 ppb for 24-h avg NO_2 and 59 to 306 ppb for daily 1-h max NO_2. Epidemiologic evidence is consistent across the various methods used to estimate NO_2 exposure: personal ambient and total NO_2 measurements, NO_2 measured outside children's schools, NO_2 measured inside children's schools and homes, and ambient NO_2 concentrations averaged across central site monitors in a city. NO_2 measured at people's locations may represent exposure better than NO_2 measured at central site monitors, and along with findings for personal and indoor exposure, provide a strong basis for inferring a causal relationship between short-term NO_2 exposure and asthma exacerbation.

NO_2 associations with asthma-related effects persist with adjustment for temperature, humidity, season, long-term time trends, as well as a copollutant such as PM_{10}, SO_2, or O_3. In a few studies, NO_2 associations are attenuated with adjustment for EC/BC, UFP, or a VOC. However, for the most part, recent studies add findings for NO_2 associations that persist with adjustment for a traffic-related copollutant such as $PM_{2.5}$, or as examined in fewer studies, EC/BC, UFP, or CO. Potential confounding by OC, PM metal species, or VOCs is poorly studied, but NO_2 associations with asthma exacerbation tend to persist in the few available copollutant models. In some cases, single-pollutant models indicate asthma-related effects in association with NO_2 but not $PM_{2.5}$ or EC/BC, which were moderately correlated with NO_2 (r = 0.22−0.57). Recent epidemiologic results also suggest asthma exacerbation in relation to exposure indices that combine NO_2 with EC, $PM_{2.5}$, O_3, and/or SO_2 concentrations, but neither epidemiologic nor experimental studies strongly indicate synergistic effects between NO_2 and copollutants. Inference about associations for NO_2 that are independent of $PM_{2.5}$, EC/BC, OC, or UFP is strong particularly in studies that measure pollutants at people's locations and for personal exposure because of comparable measurement error among pollutants. Associations with personal total and indoor NO_2 measurements also support an independent effect of NO_2 exposure because the lower (e.g., r = −0.37 to 0.31) correlations observed with many traffic-related copollutants compared to ambient NO_2 concentrations indicate that the findings for personal and indoor NO_2 may be less prone to confounding by the same traffic-related copollutants than findings for ambient NO_2 concentrations (Section 1.4.3). In the indoor studies, the relative contribution of indoor and outdoor sources to indoor NO_2 concentrations are unknown. And, while associations of outdoor school NO_2 with asthma-related effects persist with adjustment for indoor NO_2 in one group of children, it is unclear whether indoor exposure alters responses of people to outdoor NO_2 exposure.

Because copollutant models have limitations (Section 1.4.3) and are not analyzed for every correlated copollutant or study, evidence from experimental studies is critical. The key evidence for an independent effect of NO_2 exposure in exacerbating asthma are the findings from previous controlled human exposure studies for increases in airway responsiveness in adults with asthma following NO_2 exposures of 200 to 300 ppb for 30 minutes and 100 ppb for 1 hour. Airway hyperresponsiveness is a key feature of asthma and can lead to poorer control of symptoms. A recent meta-analysis shows that NO_2 exposure cut in half the dose of the challenge agent required to increase airway responsiveness, which is a measure of a clinically relevant change. Such evidence for clinically relevant increases in airway responsiveness induced by NO_2 exposures that are not much higher than peak ambient concentrations (Section 2.5) provide plausibility for short-term ambient NO_2 exposures inducing asthma exacerbation. Biological plausibility also is supported by experimental studies of adults with asthma showing that NO_2 exposures of 260 ppb for 15−30 minutes enhanced allergic inflammation, which is a key event in the mode of action for asthma exacerbation via its role in increasing airway responsiveness (Figure 1-2). These results support the NO_2-related respiratory effects observed in populations with asthma that also had high prevalence of allergy. The results for increases in airway responsiveness and allergic inflammation occurring after NO_2 exposures of 100−300 ppb for up to 1 hour also support the few findings of increased respiratory effects in adults with asthma and healthy adults associated with NO_2 exposure (range: 5.7−154 ppb) occurring over 2 or 5 hours at locations near roads.

Not all evidence supports NO_2-related respiratory effects. NO_2 exposure has variable effects on oxidative stress in experimental studies. NO_2-related decreases in lung function are observed in epidemiologic but not controlled human exposure studies. Neural reflexes do not appear to be involved (Figure 1-2, Section 4.3.2.2), but NO_2-induced (500 ppb) mast cell degranulation in rats suggests airway obstruction, which could lead to decreases in lung function.

Much of the evidence from epidemiologic and experimental studies was available in the 2008 ISA. However, compared to the 2008 ISA, this ISA more explicitly evaluates the coherence and biological plausibility for specific respiratory outcome groups. Rather than new evidence, the integration of epidemiologic and experimental evidence for asthma exacerbation—uptake of NO_2 in the respiratory tract and reactions to form reactive oxidation products, allergic inflammation, airway responsiveness, asthma symptoms, and hospital admissions and ED visits for asthma, associations with NO_2 measured in people's locations (which may better represent exposure), associations with adjustment for another traffic-related pollutant—describes a coherent, biologically plausible pathway to support a causal relationship between short-term NO_2 exposure and respiratory effects.

Respiratory Effects Associated with Long-Term Exposure to Nitrogen Dioxide

There is likely to be a causal relationship between long-term NO_2 exposure and respiratory effects based on evidence for the development of asthma. The conclusion is strengthened from that determined in the 2008 ISA for Oxides of Nitrogen because whereas previous epidemiologic evidence was limited and inconsistent, recent evidence consistently indicates associations between ambient NO_2 concentrations and asthma incidence in children (Table 1-1). As with findings for short-term NO_2 exposure, the evidence base varies across respiratory outcome groups, and there is more uncertainty as to whether long-term ambient NO_2 exposure is related to decreased lung function or development or to increases in COPD, respiratory infection, or respiratory mortality.

Epidemiologic studies are noteworthy for isolating the development of asthma from the exacerbation of pre-existing asthma. Studies followed children over time, in several cases from birth, and examined NO_2 exposure for periods preceding asthma diagnosis. Recent epidemiologic studies also associate long-term NO_2 exposure with asthma in adults, but asthma diagnosis or symptoms ascertained only during follow-up could represent recurrence of childhood asthma. Asthma incidence is associated with NO_2 exposures estimated at children's homes based on outdoor measurements or LUR models that often well predicted measured concentrations in study locations (R^2 = 0.42 to 0.69; Section 6.2.9, Table 6-5). Such findings strengthen inference about NO_2-related asthma development. Results also are consistent for less spatially resolved ambient NO_2 concentrations at central site monitors. Asthma incidence is associated with the average NO_2 concentration for the first year of life and NO_2 averaged over multiple years (study means: 14 to 28 ppb), and no single critical exposure period is identified. Associations with asthma are found with adjustment for SES, smoking exposure, gas stove use, community of residence, and in one study, psychosocial stress. However, potential confounding by traffic-related pollutants or proximity to roads is not examined.

A small body of previous experimental studies supports the biological plausibility for a relationship between long-term NO_2 exposure and asthma development by demonstrating effects on key events in the underlying mode of action. As illustrated in Figure 1-2, the information on mode of action is coherent between studies of short-term and long-term NO_2 exposure. NO_2 exposure (1,000 to 4,000 ppb) for 6−12 weeks increased airway responsiveness in a study of rodents. A simultaneous NO_2-induced increase in airway resistance suggests airway obstruction and airway remodeling, which also are linked to asthma development. Findings across disciplines indicate increased oxidative stress and inflammation in relation to long-term NO_2 exposure but not consistently across studies. The temporal pattern of NO_2 exposure underlying the epidemiologic associations with asthma is not well delineated. However, a few experimental studies show that repeated

short-term NO_2 exposures over 4 to 14 days led to the development of allergic responses in healthy adults and healthy rodent models (2,000−4,000 ppb) and to increased airway responsiveness in rodents (4,000 ppb). This evidence for short-term NO_2 exposure supports a relationship between long-term NO_2 exposure and asthma development because it demonstrates the development of asthma-related effects in healthy humans and animal models and indicates that repeated increases in exposure may be important. NO_2 exposures that induce effects related to asthma development are higher than those that induce effects related to asthma exacerbation as described in the preceding section but are within the range of exposures considered to be ambient relevant (Section 1.2).

Epidemiologic studies continue to support associations of long-term NO_2 exposure with decreases in lung function and development and increased respiratory disease severity in children. These outcomes are associated with similar NO_2 concentrations and durations as asthma development and similar exposure assessment methods (Table 6-5) Some studies of lung function observed associations with long-term NO_2 after adjustment for short-term NO_2 exposure, but associations for asthma symptoms do not exclude the potential influence of short-term NO_2 exposure. Compared with asthma development, there is more uncertainty whether long-term NO_2 exposure has an independent effect on decreasing lung function or development or increasing respiratory disease severity. NO_2 exposure does not alter lung function in experimental animals, and the hyperproliferation of lung epithelial cells and fibrosis in adult animals are not directly related to the lung function changes described in children. Associations of long-term NO_2 exposure with bronchitic symptoms or lung function persisted when adjusted for $PM_{2.5}$, EC, OC, or distance to freeway, but such findings are limited in number and not entirely consistent.

Together, evidence from recent epidemiologic studies and previous experimental studies supporting effects on the development of asthma indicates there is likely to be a causal relationship between long-term NO_2 exposure and respiratory effects. Epidemiologic studies observe associations with NO_2 exposure estimated at children's homes with LUR models, which may better represent differences in ambient NO_2 exposure among subjects compared with less spatially resolved NO_2 measurements from central site monitors. Potential confounding by traffic-related copollutants largely is unexamined for asthma development. However, findings from experimental studies for increased airway responsiveness and allergic responses, which are part of the mode of action for asthma development, are considered to provide some support for an independent effect of long-term NO_2 exposure. Because such evidence is limited, some uncertainty remains in attributing epidemiologic associations between long-term NO_2 exposure and asthma development specifically to NO_2 among the array of traffic-related pollutants.

1.5.2 Health Effects beyond the Respiratory System

Epidemiologic studies show associations between NO_2 exposure and health effects in various organ systems, and associations are observed with a similar range of short-term and long-term NO_2 concentrations as respiratory effects (Table 1-1). However, compared to respiratory effects, there is more uncertainty in relationships with NO_2 exposure, largely in identifying an independent effect from other traffic-related pollutants. For some health effects, epidemiologic findings also are inconsistent. A common source of uncertainty across nonrespiratory health effects is the limited availability of controlled human exposure and toxicological studies to inform understanding of how ambient-relevant exposures to NO_2 may affect biological processes that underlie the health effects observed beyond the respiratory system. NO_2 itself is not likely to enter the blood (Section 4.2.2). Among the various products of NO_2 reactions that occur in the epithelial lining fluid of the respiratory tract, nitrite has been identified in the blood. However, nitrite produced from inhaled NO_2 may not appreciably alter levels derived from diet or induce potentially detrimental health effects (Section 4.2.3). Nitrite can react with red blood cell hemoglobin to form methemoglobin. Methemoglobin has been linked with health effects but has not been found with ambient-relevant NO_2 exposure concentrations (Section 4.3.4.1). A recent controlled human exposure study suggests that mediators from the respiratory tract may spillover into the blood. This spillover could lead to systemic inflammation and oxidative stress (Figure 1-2, Section 4.3.5), providing a potential mechanism by which NO_2 exposure could lead to health effects beyond the respiratory system.

Cardiovascular and Related Metabolic Effects

Although it is not clear how inhaled NO_2 affects underlying biological pathways, epidemiologic evidence indicates associations of short-term and long-term NO_2 exposure with cardiovascular and related metabolic effects. For both short-term and long-term NO_2 exposure, the 2008 ISA for Oxides of Nitrogen concluded that evidence was inadequate to infer a causal relationship with cardiovascular effects. There was a lack of supporting evidence for long-term NO_2 exposure. There was supporting evidence for short-term NO_2 exposure but uncertainty about potential confounding by traffic-related copollutants. In this ISA, the health effect category is expanded to include recent studies of metabolic effects, most of which are for long-term NO_2 exposure. New findings relating long-term NO_2 exposure to the development of diabetes and heart disease and additional findings relating short-term NO_2 exposure to the triggering of myocardial infarction support a suggestive, but not sufficient, to infer a causal relationship with cardiovascular and related metabolic effects (Table 1-1). Evidence is inconsistent for the effects of

short-term and long-term NO_2 exposure on cardiovascular effects, such as arrhythmia, cerebrovascular diseases, and hypertension. There still is uncertainty whether NO_2 exposure has effects that are independent of other traffic-related pollutants.

Recent epidemiologic studies continue to indicate that short-term NO_2 exposure may trigger a myocardial infarction. There are consistent findings for associations between short-term increases in ambient NO_2 concentration and hospital admissions or ED visits for myocardial infarction, angina, and their underlying cause, ischemic heart disease (Section 5.3.12, Table 5-58). Coherence is found with epidemiologic evidence for NO_2-related ST segment changes, a nonspecific marker of myocardial ischemia, and increases in cardiovascular mortality, of which ischemic heart disease is the leading cause (Finegold et al., 2013). The robustness of epidemiologic findings is demonstrated as associations consistently observed in studies conducted over several years, in diverse geographic locations, and with data pooled from multiple cities. Also, as with findings for asthma exacerbation (Section 1.5.1), associations of short-term NO_2 exposure with effects related to myocardial infarction persist with adjustment for meteorology, long-term time trends, and a copollutant such as PM_{10}, SO_2, or O_3 (Section 5.3.12.1). Most of the epidemiologic evidence is based on NO_2 exposures assigned as the average ambient concentration across multiple monitors within a city; however, ST segment changes are associated with outdoor residential NO_2, which may better represent temporal changes in subjects' personal exposures.

New epidemiologic evidence for increases in diabetes and heart disease in relation to long-term NO_2 exposure is suggestive, but not sufficient, to infer a causal relationship (Section 6.3.9, Table 6-11). The study reviewed in the 2008 ISA observed a weak association with cardiovascular events. The most consistent recent findings are for diabetes. Similar to asthma development, diabetes is associated with ambient NO_2 concentrations estimated at subjects' homes using LUR, which may capture differences in ambient NO_2 exposure among subjects. Most studies examine concurrent 1-yr avg NO_2 concentrations, but some aim to represent longer exposures more relevant to disease development by examining people who did not change residence. There is also some support for heart disease and mortality from ischemic heart disease related to long-term NO_2 exposure. Heart disease is associated with 1- or 2-yr avg NO_2 concentrations estimated at a neighborhood scale from central site monitors or dispersion models or at subjects' homes with LUR. Most studies assess heart disease by acute cardiovascular events such as myocardial infarction or hospital admissions without considering the potential influence of short-term NO_2 exposure. In some cases, exposures are assessed for periods after the cardiovascular event, and it is uncertain the extent to which these periods represent exposures during disease development. In addition to assessing residential NO_2 exposures, many studies of heart disease and diabetes are noteworthy for

their large sample sizes, prospective follow-up of subjects (up to 20 years), and adjustment for potential confounding by age, sex, SES, and comorbid conditions.

Despite the epidemiologic evidence relating short-term and long-term NO_2 exposure to cardiovascular and related metabolic effects, studies do not adequately account for potential confounding by other traffic-related pollutants, as was the case in the 2008 ISA (U.S. EPA, 2008). In limited examination of copollutant models with $PM_{2.5}$, UFP, or CO, associations of short-term NO_2 exposure with effects related to myocardial infarction are not consistently observed. Confounding by other traffic-related pollutants has not been examined. Also in contrast with findings for asthma exacerbation (Section 1.5.1), copollutant model results are based on NO_2 and copollutant concentrations measured at central site monitors. Differential exposure measurement error may limit the reliability of copollutant model results. Studies of long-term NO_2 exposure and heart disease and diabetes do not examine potential confounding by stress or traffic-related copollutants. Evidence for NO_2 associations that are independent of noise also is limited.

New findings from experimental studies point to the potential for NO_2 exposure to induce cardiovascular effects and diabetes but are not sufficient to address the uncertainties in the epidemiologic evidence. Consistent with findings that reactive products of inhaled NO_2 or mediators of inflammation may spill over from the respiratory tract to the blood (Figure 1-2), some recent experimental studies find increases in mediators of inflammation and oxidative stress in the blood or heart tissue of healthy humans and rodent models in response to short-term NO_2 exposure (Section 5.3.12.1). Evidence does not strongly support the involvement of neural reflexes as examined by changes in respiratory rate or decreases in heart rate variability (Figure 1-2, Sections 4.3.2.2 and 5.3.12.2). Findings for increases in inflammation and oxidative stress describe early, nonspecific changes induced by NO_2 exposure that have the potential to lead to myocardial infarction. Although the findings are mostly for single-day exposures, they also may describe a possible way for recurrent NO_2 exposures to lead to the development of heart disease or diabetes. Limited findings of dyslipidemia in rats and epidemiologic findings of vascular damage in adults in relation to long-term NO_2 exposure also describe potential pathways for NO_2 exposure to lead to heart disease. The limited extent and consistency of findings from experimental studies and nonspecific nature of most of the evidence is not sufficient to demonstrate an independent effect of NO_2 exposure.

In conclusion, evidence is suggestive but not sufficient to infer causal relationships for cardiovascular and related metabolic effects with both short-term and long-term NO_2 exposure. Conclusions were changed from the 2008 ISA based on more epidemiologic evidence linking myocardial infarction to short-term exposure and new evidence linking heart disease and diabetes to long-term exposure. However, an independent effect of NO_2

exposure is not clearly demonstrated. Examination of confounding by other traffic-related pollutants is absent for long-term NO_2 exposure and gives inconsistent results for short-term NO_2 exposure. Some but not all recent experimental studies show that short-term NO_2 exposure increases inflammation and oxidative stress in the blood or heart tissue. Increases in inflammation and oxidative stress describe a potential way for short-term or long-term NO_2 exposure to lead to cardiovascular and related metabolic effects, but because the findings are not linked to any specific health effect, unlike the mode of action evidence for asthma exacerbation or development (Section 1.5.1), they do not rule out chance, confounding, and other biases in the epidemiologic evidence.

Total Mortality

Similar to the evidence described above for cardiovascular and related metabolic effects, epidemiologic evidence supports associations of both short-term and long-term NO_2 exposure with total mortality from all nonaccidental causes. However, potential confounding by other traffic-related pollutants remains largely unresolved, and it is not clear what biological processes NO_2 exposure may affect to lead to mortality. This uncertainty weighed with the supporting epidemiologic evidence is the basis for concluding that evidence is suggestive, but not sufficient, to infer a causal relationship for both short-term and long-term NO_2 exposure with total mortality (Table 1-1). For short-term exposure, the nature of the evidence has not changed substantively, resulting in the same conclusion as the 2008 ISA. For long-term NO_2 exposure, whereas evidence in the 2008 ISA was limited, inconsistent, and inadequate to infer a causal relationship, several recent epidemiologic studies report associations with total mortality, supporting a stronger causal determination.

Evidence is suggestive, but not sufficient, to infer a causal relationship between short-term NO_2 exposure and total mortality based on consistent epidemiologic findings across geographic locations, including several studies pooling data across cities (Section 5.4.8, Table 5-63). Ambient NO_2 exposures were assessed as the average concentration across central site monitors within a city, which has uncertainty in adequately representing the temporal pattern in personal NO_2 exposures. Similar to findings for asthma exacerbation (Section 1.5.1), associations with mortality persist with adjustment for meteorological factors, long-term time trends, and a copollutant among PM_{10}, SO_2, or O_3. A multicontinent study suggests interaction between NO_2 and PM_{10}, with higher PM_{10}-mortality associations observed for periods of higher ambient NO_2 concentrations. However, in contrast with asthma exacerbation, potential confounding of associations between short-term NO_2 exposure and total mortality by traffic-related copollutants remains unexamined.

The generally supportive evidence from the large number of recent epidemiologic studies is suggestive, but not sufficient, to infer a causal relationship between long-term NO_2 exposure and total mortality (Section 6.5.3, Table 6-18). Epidemiologic associations are observed in large cohorts in diverse locations followed for long durations up to 26 years. Increases in total mortality are found in association with NO_2 concentrations averaged over 1 to 16 years and assessed for the year of death and for periods up to 20 years before death. Not all studies observe associations, but the inconsistency does not appear to be due to differences among studies in long-term average ambient NO_2 concentrations or the exposure period examined. Total mortality is associated with long-term NO_2 exposure assigned from central site monitors and exposures estimated at people's homes by LUR models that well represented the spatial variability in ambient NO_2 concentrations in the study areas (R^2 = 0.61 and 0.71). NO_2 associations persist with adjustment for potential confounding by age, sex, smoking, education, and comorbid conditions. In a few studies, associations between long-term NO_2 exposure and mortality persist with adjustment for traffic density or proximity, but confounding by traffic-related copollutants remains a concern because NO_2 associations are inconsistently observed with adjustment for $PM_{2.5}$ or BC exposures estimated from central site monitors or LUR models.

Evidence relating NO_2 exposure to cardiovascular and respiratory effects can inform the uncertainty as to whether NO_2 exposure has an independent effect on mortality by indicating whether NO_2 exposure affects the underlying causes of mortality. In the U.S., cardiovascular disease, namely ischemic heart disease, is the leading cause of death [35% as cited in (Hoyert and Xu, 2012)]. Respiratory causes comprise a smaller fraction of mortality (9% in the U.S.), but COPD and respiratory infections are among the leading causes of all mortality in the world. As described in the preceding sections, independent effects of short-term and long-term ambient NO_2 exposure on myocardial infarction, heart disease, diabetes, COPD, and respiratory infection are uncertain. Strong evidence demonstrates NO_2-related asthma exacerbation, but asthma is not a leading cause of mortality. Thus, it is not clear what spectrum of cardiovascular and respiratory effects NO_2 exposure may induce to lead to mortality and by what biological processes short-term or long-term NO_2 exposure may lead to mortality.

In conclusion, evidence is suggestive, but not sufficient, to infer a causal relationship for total mortality with both short-term and long-term NO_2 exposure based on supporting epidemiologic evidence. The evidence bases for total mortality related to short-term and long-term NO_2 exposure share many characteristics. Although there is supporting epidemiologic evidence, studies do not adequately account for potential confounding by other traffic-related pollutants. Thus, it is uncertain the extent to which epidemiologic findings for total mortality can be attributed specifically to short-term or long-term NO_2 exposure. Also uncertain are the independent effects of NO_2 exposure on the

cardiovascular and respiratory morbidity conditions that make up the leading causes of mortality. Because potential confounding by traffic-related copollutants is largely unaddressed and the biological processes underlying the effects of NO_2 exposure on mortality are unclear, the epidemiologic associations of short-term and long-term NO_2 exposure with total mortality do not rule out chance, confounding, and other biases.

Reproductive and Developmental Effects

The 2008 ISA for Oxides of Nitrogen concluded that evidence was inadequate to infer a causal relationship between NO_2 exposure and a heterogeneous group of reproductive and developmental effects based on limited and inconsistent epidemiologic and animal toxicological evidence for effects on birth outcomes. This ISA presents separate conclusions for more defined categories of outcomes that are likely to occur by different biological processes and exposure patterns over different stages of development: (1) fertility, reproduction, and pregnancy (Section 6.4.2); (2) birth outcomes (Section 6.4.3); and (3) postnatal development (Section 6.4.4). For all three categories, there is a large increase in recent epidemiologic studies. However, only for birth outcomes is there reasonable consistency in the findings to support strengthening the causal determination to suggestive, but not sufficient, to infer a causal relationship with long-term NO_2 exposure (Table 1-1). For all three categories of reproductive and developmental effects, there is large uncertainty in identifying an independent effect of NO_2 exposure. In particular, animal toxicological evidence to support biological plausibility remains limited and inconclusive.

Fertility, Reproduction, and Pregnancy

Evidence is inadequate to infer a causal relationship between long-term NO_2 exposure and effects on fertility, reproduction, and pregnancy (Section 6.4.5, Table 6-14). This conclusion is based heavily on findings from the epidemiologic studies of pre-eclampsia, a pregnancy complication related to hypertension and protein in the urine (Table 1-1). Associations are inconsistently observed with for ambient NO_2 exposures estimated at homes by LUR models that well predicted ambient NO_2 concentrations in the study areas (R^2 = 0.59 to 0.86). Studies that observe associations considered confounding by maternal age, smoking, SES, diabetes, and parity, but few examine other traffic-related pollutants to assess the potential for confounding. Other lines of evidence to inform biological plausibility are not available. Toxicological studies have not examined effects related to pre-eclampsia, and there is a lack of coherence with epidemiologic findings for conditions that contribute to pre-eclampsia, such as gestational hypertension and placental function. Inconsistent and limited findings from animal toxicological and/or epidemiologic studies for detrimental effects on sperm quantity and quality, fertility,

maternal weight gain in pregnancy, and litter size add to the uncertainty regarding a relationship of NO_2 exposure with fertility, reproduction, and pregnancy.

Birth Outcomes

Evidence is suggestive, but not sufficient, to infer a causal relationship between NO_2 exposure and effects on birth outcomes based primarily on recent epidemiologic associations with fetal growth restriction (Section 6.4.5, Table 6-14). The combined epidemiologic and toxicological findings for effects on birth weight and infant mortality are inconsistent as are epidemiologic findings for preterm birth and birth defects. Evidence for NO_2-related decreases in fetal growth is not entirely consistent, but many studies observe associations with ambient NO_2 concentrations at homes estimated by LUR models that well predict NO_2 concentrations in the study areas (R^2 = 0.68 to 0.91; Table 1-1). A few studies observe stronger associations for children whose mothers spent more time at home and less time outdoors in locations other than home, which may be the result of improved relationships of residential ambient NO_2 with personal exposures. Other strengths of recent studies include fetal or neonatal physical measurements and analysis of confounding by season of conception, maternal age, smoking, SES, and in one study, noise. However, epidemiologic studies do not examine potential confounding by traffic-related copollutants. Further, toxicological studies have not examined fetal growth, and a mode of action for NO_2 is not clearly described (Figure 1-2). Prenatal ambient NO_2 exposure is associated with a marker of inflammation in fetal cord blood but not maternal blood. The role of inflammation in affecting birth outcomes is not clearly established, and epidemiologic findings do not rule out effects of other pollutants. Thus, despite the supporting evidence for fetal growth restriction, there is considerable uncertainty in attributing epidemiologic findings specifically to NO_2 exposure.

Postnatal Development

Evidence is inadequate to infer a causal relationship between NO_2 exposure and effects on postnatal development based largely on the inconclusive findings across several recent epidemiologic studies of cognitive function in children (Section 6.4.5, Table 6-14). Associations are inconsistently found for concurrent, infancy, or prenatal NO_2 exposure estimated at children's homes or schools with LUR models that well represent the variability in ambient NO_2 concentrations in the study areas (R^2 = 0.64 to 0.85; Table 1-1). Further, potential confounding by traffic-related copollutants or stress is not examined, although one study shows an association with decreases in memory, adjusting for noise. The recent study indicating that short-term NO_2 exposure of adult rats induced oxidative stress and neuronal degeneration, which potentially could lead to impaired cognitive function, is not sufficient to address the uncertainties in epidemiologic findings. Findings for other effects on postnatal development are both limited and inconsistent.

Specifically, evidence integrated from epidemiologic and toxicological studies is inconclusive for motor function and psychological or emotional distress. Evidence is inconsistent for decrements in attention and limited for autism as examined in epidemiologic studies and for physical development as examined in toxicological studies.

Cancer

The largest evidence base in support of a relationship between NO_2 exposure and cancer is that for lung cancer (Table 1-1). A few recent epidemiologic studies indicate associations between NO_2 exposure and leukemia, bladder cancer, prostate cancer, and breast cancer, but findings for NO_2 exposure inducing carcinogenicity or mutagenicity in bone marrow, spermatocytes, and lymphocytes is inconsistent and based on higher than ambient-relevant NO_2 exposures. The findings from some recent epidemiologic studies for associations of NO_2 exposure with lung cancer and mortality combined with previous findings in rodents that NO_2 exposure may be involved in lung tumor promotion is the basis for strengthening the causal determination from inadequate to infer a causal relationship in the 2008 ISA for Oxides of Nitrogen to suggestive, but not sufficient, to infer a causal relationship (Section 6.6.9, Table 6-20).

Among the many recent epidemiologic studies, some report associations for NO_2 with lung cancer incidence or mortality; others do not. Findings are inconsistent for NO_2 exposure assessed from central site monitors and estimated at subjects' homes with well-validated LUR models. In studies observing associations, NO_2 concentrations were averaged over 1 year at the beginning of the study up to 30 years before the outcome. Thus, in many cases, the exposure period is considered to be relevant for cancer. However, it is not clear whether exposures assessed by LUR or dispersion models for periods of 1 or 5 years before cancer or mortality adequately account for decreases in ambient NO_2 concentration over years or represent longer duration exposures. Studies not finding associations do not differ in mean NO_2 concentrations or exposure duration examined. Many studies examined large numbers of cancer cases, followed adults for 7−30 years, and adjusted for potential confounding by SES, smoking, diet, and occupational exposures. One study observes an association of residential NO_2 exposure with lung cancer mortality that persists with adjustment for $PM_{2.5}$, but examination of confounding by traffic-related copollutants is largely absent.

NO_2 exposure does not independently induce lung tumor formation in various animal models or transform other chemicals in the body into carcinogens at ambient-relevant concentrations. However, a potential role for high-concentration exposures in tumor promotion is indicated by findings of NO_2 exposures of 4,000 to 10,000 ppb increasing lung tumors in mice with spontaneously high tumor rates or with co-exposure to diesel

exhaust particles or known carcinogens. The formation of secondary oxidation products in the respiratory tract (Section 1.5.1) and limited evidence for NO_2-induced increases in hyperplasia of the lung epithelium of rodents are early events that have the potential to mediate NO_2-related lung cancer. While NO_2 exposure impairs host defense in animal models (Section 5.2.9), parameters more directly linked to antitumor immunity such as cytotoxic or regulatory T cells and interferon-gamma have not been studied.

In conclusion, evidence is suggestive, but not sufficient, to infer a causal relationship between long-term NO_2 exposure and cancer based primarily on lung cancer. Associations between ambient NO_2 concentrations and lung cancer incidence and mortality are found in some but not all epidemiologic studies. NO_2 exposures in rodents, some at higher than ambient-relevant concentrations, show an effect on lung tumor promotion, but experimental studies do not support direct effects of NO_2 exposure on carcinogenesis. Because potential confounding by traffic-related copollutants is largely unaddressed and information to support biological plausibility is limited, the associations for long-term NO_2 exposure with lung cancer incidence and mortality observed in some epidemiologic studies do not rule out chance, confounding, and other biases.

Table 1-1 Key evidence contributing to causal determinations for nitrogen dioxide (NO_2) exposure and health effects evaluated in the current draft Integrated Science Assessment (ISA) for Oxides of Nitrogen.

Health Effect Category[a] and Causal Determination[b]		NO_2 Concentrations Associated with Effects
Respiratory Effects and Short-term Exposure (Section 5.2) Current Draft ISA—Causal relationship. 2008 ISA—Sufficient to infer a likely causal relationship.		
Key evidence (Table 5-45)	Strongest evidence is for effects on asthma exacerbation. Consistent epidemiologic evidence for decreases in lung function and increases in respiratory symptoms in children with asthma and increases in asthma hospital admissions and ED visits. Associations observed with NO_2 measured at central site monitors and at subjects' locations (i.e., personal ambient, outdoor school). Copollutant models show NO_2 associations that are independent of $PM_{2.5}$ or as examined in fewer studies, EC/BC, OC, UFP, VOCs, PM metals with pollutants measured at subjects' locations, or CO measured at central site monitors. NO_2 associations persist with adjustment for meteorology, medication use, PM_{10}, SO_2, or O_3. Coherent findings available for total personal and indoor NO_2 with lower potential for copollutant confounding. Independent effect of NO_2 demonstrated in controlled human exposure studies. In adults with asthma, NO_2 exposures not much higher than peak ambient concentrations induce clinically-relevant increases in airway responsiveness and increases in allergic responses, which are part of the mode of action for asthma exacerbation. Inconsistent experimental results for effects on lung function and respiratory symptoms in absence of challenge agent. Uncertainty in independent effect of NO_2 on other respiratory effects (i.e, allergy exacerbation, COPD exacerbation, respiratory infection, respiratory effects in healthy populations) due to limited coherence among findings from epidemiologic and experimental studies.	Overall study ambient maximums Central site monitors: 24-h avg: 55 to 80 ppb 1-h max: 59 to 306 ppb Outdoor school: 24-h avg: 7.5, 16.2 ppb Personal ambient: 2-h avg: 77.7, 154 ppb Total personal: 24-h avg: 48, 106 ppb Airway responsiveness: 200 to 300 ppb for 30 min, 100 ppb for 1 h Allergic inflammation: 260 for 15 min and 581 ppb for 30 min
Reason for change in causal determination	Epidemiologic evidence for NO_2 exposures assessed for subject's locations and in copollutant models with a traffic-related copollutant plus evidence from experimental studies describing mode of action demonstrate consistency, coherence, and biological plausibility for effect of NO_2 exposure on asthma exacerbation to rule out chance, confounding, and other biases with reasonable confidence	
Uncertainty remaining	Strength of inference from copollutant models about independent associations of NO_2, especially with pollutants measured at central site monitors. Potential for NO_2-copollutant mixture effects.	

Table 1-1 (Continued): Key evidence contributing to causal determinations for nitrogen dioxide (NO_2) exposure and health effects evaluated in the current draft Integrated Science Assessment (ISA) for Oxides of Nitrogen.

Health Effect Category[a] and Causal Determination[b]		NO_2 Concentrations Associated with Effects
Respiratory Effects and Long-term Exposure (Section 6.2) Current draft ISA—Likely to be a causal relationship. 2008 ISA—Suggestive but not sufficient to infer a causal relationship.		
Key evidence (Table 6-5)	Strongest evidence is for effects on asthma development. Consistent epidemiologic evidence from recent cohort studies for associations of ambient NO_2 averaged over 1−10 years (early childhood, lifetime) with asthma incidence in children. Associations found with NO_2 estimated at homes and measured at central site monitors. NO_2 associations persist with adjustment for SES and smoking exposure. Potential confounding by traffic-related copollutants or proximity to roads not examined. Small body of experimental studies show effects on hallmarks of asthma. Long-term NO_2 exposure increases allergic responses and airway responsiveness in rodents. Short-term NO_2 exposure induces development of allergic responses in humans and rodents. Inconsistent epidemiologic associations between long-term NO_2 exposure and development of allergic responses in children. More uncertainty in relationships with other respiratory effects because of limited coherence among disciplines. Epidemiologic evidence for increased severity of respiratory disease and decreased lung function and lung development in children. Animal toxicological evidence for respiratory infection.	Overall study ambient means: 14 to 28 ppb for residential annual avg estimates Individual city ambient means: 9.6−51.3 ppb for annual avg; 7.3−31.4 ppb for 10-yr avg Allergic responses: 2,000 ppb for 4 days in humans; 3,000 ppb for 2 weeks and 4,000 ppb for 12 weeks in rodents Airway responsiveness: 1,000 to 4,000 ppb in rodents for 6 or 12 weeks
Reason for change in causal determination	New epidemiologic evidence for associations between estimates of residential ambient NO_2 exposure and asthma development and biological plausibility from a small body of experimental studies.	
Uncertainty remaining	Some uncertainty remains in identifying an independent effect of NO_2 exposure from traffic-related copollutants because evidence from experimental studies for effects related to asthma development is limited, and epidemiologic analysis of confounding is lacking.	
Cardiovascular and Related Metabolic Effects and Short-term Exposure (Section 5.3) Current Draft ISA – Suggestive, but not sufficient, to infer a causal relationship. 2008 ISA – Inadequate to infer a causal relationship.		
Key evidence (Table 5-58)	Strongest evidence is for effects related to triggering myocardial infarction. Consistent epidemiologic evidence for ST segment changes, increases in hospital admissions and ED visits for myocardial infarction and ischemic heart disease, and cardiovascular mortality. Most evidence is based on NO_2 averaged across central site monitors in a city. Associations persist with adjustment for meteorology, PM_{10}, SO_2, or O_3. NO_2 associations inconsistent in copollutant models with $PM_{2.5}$ or CO. Some, but not entirely consistent findings, from experimental studies for early, nonspecific effects with the potential to lead to myocardial infarction: increases in markers of inflammation and oxidative stress in plasma of humans and heart tissue of rats. Inconsistent epidemiologic findings for inflammation. Inconsistent evidence for effects on cerebrovascular effects, arrhythmia, and hypertension.	Individual city ambient 24-h avg: 90th: 22 to 53 ppb, Maximums: 58 to 135 ppb Overall study ambient 1-h max: 90th: 68 ppb Oxidative stress in rats: 5,320 ppb, for 6 h inflammation in rats: 2,660 and 5,320 ppb for 6 h Inflammation in human cells exposed to human plasma, oxidative stress in human plasma: 500 ppb for 2 h
Reason for change in causal determination	Additional epidemiologic evidence for array of effects related to the triggering of myocardial infarction.	
Uncertainty remaining	Effect of NO_2 independent from traffic-related copollutants is uncertain because experimental evidence is limited and not specific to myocardial infarction, and epidemiologic analysis of confounding is limited. Potential exposure error associated with NO_2 measured at central site monitors not well characterized.	

Table 1-1 (Continued): Key evidence contributing to causal determinations for nitrogen dioxide (NO_2) exposure and health effects evaluated in the current draft Integrated Science Assessment (ISA) for Oxides of Nitrogen.

Health Effect Category[a] and Causal Determination[b]		NO_2 Concentrations Associated with Effects
Cardiovascular and Related Metabolic Effects and Long-term Exposure (Section 6.3) Current Draft ISA—Suggestive, but not sufficient, to infer a causal relationship. 2008 ISA—Inadequate to infer a causal relationship.		
Key evidence: (Table 6-11)	Strongest evidence is for development of diabetes and heart disease. Generally supportive but not entirely consistent epidemiologic evidence from recent cohort studies for associations of diabetes, myocardial infarction, and heart failure with ambient NO_2 averaged over 1−2 year periods around time of outcome assessment. Coherence with evidence for cardiovascular mortality. Associations found with NO_2 estimated at homes and measured at central site monitors. NO_2 associations persist with adjustment for age, sex, SES, comorbid conditions, and in a few cases, noise. Potential confounding by traffic-related copollutants, proximity to roads, or stress not examined. Some but not entirely consistent findings from experimental studies for early, nonspecific effects with the potential to lead to heart disease or diabetes: dyslipidemia in rats with long-term NO_2 exposure, increases in markers of inflammation and oxidative stress in plasma of humans and heart tissue of rats with short-term NO_2 exposure. Inconsistent epidemiologic associations between long-term NO_2 exposure and inflammation.	Overall study ambient means: 4.2 to 31.9 ppb for residential annual avg estimates; 34 ppb for 9.5-yr avg at central site monitors Dyslipidemia in rats: 160 ppb for 32 weeks Oxidative stress in rats: 5,320 ppb for 6 h, inflammation in rats: 2,660 and 5,320 ppb for 6 h Inflammation in human cells exposed to human plasma, oxidative stress in human plasma: 500 ppb for 2 h
Reason for change in causal determination	Large increase in recent epidemiologic studies of heart disease and diabetes, with generally supportive but not entirely consistent evidence. New evidence for estimates of residential NO_2 exposure.	
Uncertainty remaining	Effect of NO_2 independent from traffic-related copollutants is uncertain because experimental evidence is limited and not specific to heart disease or diabetes, and epidemiologic analysis of confounding is lacking.	
Total Mortality and Short-term Exposure (Section 5.4) Current Draft ISA and 2008 ISA—Suggestive, but not sufficient, to infer a causal relationship.		
Key evidence: (Table 5-63)	Consistent epidemiologic evidence for increases in total mortality in association with NO_2 averaged across central site monitors in a city. Associations persist with adjustment for meteorology, long-term time trends, PM_{10}, SO_2, or O_3. Potential confounding by traffic-related copollutants not examined. Evidence does not clearly describe independent NO_2 effects on biological processes leading to mortality. Large percentage of mortality is due to cardiovascular causes, for which independent effect of NO_2 is uncertain. The strongest evidence for respiratory morbidity is for asthma and is more limited or inconsistent for COPD and respiratory infection, which are larger causes of mortality in adults.	Individual city ambient 24-h avg maximums: 55 to 135 ppb Individual city ambient 1-h max: 90th: 33 to 133 ppb Maximums: 96 to 147 ppb
Reason for no change in causal determination	Effect of NO_2 independent from traffic-related copollutants is uncertain because epidemiologic analysis of confounding is lacking, and independent effect of NO_2 on biological processes (i.e., effects on morbidity) that lead to mortality not clearly demonstrated. Potential exposure error associated with NO_2 measured at central site monitors not well characterized.	
Uncertainty remaining		

Table 1-1 (Continued): Key evidence contributing to causal determinations for nitrogen dioxide (NO_2) exposure and health effects evaluated in the current draft Integrated Science Assessment (ISA) for Oxides of Nitrogen.

Health Effect Category[a] and Causal Determination[b]		NO_2 Concentrations Associated with Effects
Total Mortality and Long-term Exposure (Section 6.5) Current Draft ISA—Suggestive, but not sufficient, to infer a causal relationship. 2008 ISA—Inadequate to infer a causal relationship		
Key evidence: (Table 6-18)	Generally supportive but not entirely consistent epidemiologic evidence from recent cohort studies, including those with extended follow-up (up to 26 years) of existing cohorts. Associations found with NO_2 averaged over 1 to 16 years for periods 0 to 20 years before death. Most evidence is based on NO_2 measured at central site monitors, but associations also observed with NO_2 estimated at homes. Associations found with adjustment for age, sex, smoking, education, comorbid conditions and in some cases, neighborhood-level SES. In limited analysis, NO_2 associations persist with adjustment for traffic proximity or density but mostly are attenuated in copollutant models with $PM_{2.5}$ or BC. Evidence does not clearly describe independent NO_2 effects on biological processes leading to mortality. Large percentage of mortality is due to cardiovascular causes, for which independent effect of NO_2 is uncertain. The strongest evidence for respiratory morbidity is for asthma and is more limited or inconsistent for COPD and respiratory infection, which are larger causes of mortality in adults.	Overall study ambient means: 12.1 to 21.7 ppb for residential annual avg estimates 13.9 to 33.6 ppb for 1-yr to 15-yr avg at central site monitors
Reason for change in causal determination	Large increase in recent epidemiologic studies, with generally supportive but not entirely consistent evidence. New evidence for estimates of residential NO_2 exposure in some but not all recent studies.	
Uncertainty remaining	Effect of NO_2 independent from traffic-related copollutants is uncertain because epidemiologic analysis of confounding is limited and inconclusive, and independent effect of NO_2 on biological processes (i.e., effects on morbidity) that lead to mortality not clearly demonstrated. Potential exposure error associated with NO_2 measured at central site monitors not well characterized.	
Reproductive and Developmental Effects Long-term Exposure[c] 2008 ISA—Inadequate to infer a causal relationship for broad category.		
Fertility, Reproduction, and Pregnancy (Section 6.4.2) Current Draft ISA—Inadequate to infer a causal relationship.		
Key evidence (Table 6-14)	Heterogeneous group of outcomes related to a successful pregnancy with little support for relationship with NO_2 exposure. Inconsistent epidemiologic evidence among several recent studies for associations of pre-eclampsia, increases in blood pressure, and systemic inflammation in pregnancy with NO_2 estimated at homes with LUR or measured at central site monitors. Studies adjust for maternal age, smoking, SES, diabetes, and parity. Lack of toxicological studies to inform a potential effect of NO_2. More limited and inconsistent epidemiologic evidence for effects on fertility. No effect on fertility in rodents, but change in estrous cyclicity found. No epidemiologic or toxicological evidence for effects on sperm count or quality. Limited, inconclusive evidence in rodents for changes in pregnancy weight.	Overall study ambient mean for pre-eclampsia: 31 ppb for residential 3rd trimester avg estimate
Reason for no change in causal determination	Increase in recent epidemiologic studies, but results lack sufficient consistency, including those for residential estimates of NO_2 exposure. Limited and inconclusive toxicological evidence does not adequately inform a potential effect of NO_2	
Uncertainty remaining		

Table 1-1 (Continued): Key evidence contributing to causal determinations for nitrogen dioxide (NO_2) exposure and health effects evaluated in the current draft Integrated Science Assessment (ISA) for Oxides of Nitrogen.

Health Effect Category[a] and Causal Determination[b]		NO_2 Concentrations Associated with Effects
Birth outcomes (Section 6.4.3) Current Draft ISA—Suggestive, but not sufficient, to infer a causal relationship.		
Key evidence (Table 6-14)	Strongest evidence is for fetal growth restriction. Generally supportive but not entirely consistent recent epidemiologic evidence for decreased head circumference and fetal or birth length, particularly as assessed with fetal or neonatal physical measurements. Associations found with NO_2 estimated at homes and measured at central site monitors. NO_2 associations persist with adjustment for maternal age, SES, smoking, alcohol use, and season of conception. Potential confounding by traffic-related copollutants not examined, and no available toxicological studies to inform a potential effect of NO_2. Evidence for decreased birth weight in a study of rats, but large epidemiologic evidence base is inconsistent. Inconsistent epidemiologic evidence for associations with preterm birth, birth defects, early life mortality, and no or inconclusive toxicological evidence to inform a potential effect of NO_2.	Overall study ambient means: Entire pregnancy: 15.5 to 20 ppb Specific trimesters: 7.8 to 36 ppb Decreased birth weight in rats: 1,300 ppb for 3 mo
Reason for change in causal determination	Large increase in epidemiologic studies, with generally supportive but not entirely consistent evidence for associations between residential ambient NO_2 exposure and fetal growth restriction.	
Uncertainty remaining	Effect of NO_2 independent from traffic-related copollutants is uncertain because evidence from experimental studies and epidemiologic analysis of confounding are lacking.	
Postnatal development (Section 6.4.4) Current Draft ISA—Inadequate to infer a causal relationship		
Key evidence (Table 6-14)	Inconsistent epidemiologic evidence from recent studies for associations with neurodevelopmental effects such as cognitive function, attention, motor function, and emotional responses. Association found with indoor NO_2, but not consistently with ambient NO_2 exposure estimated at home or school by LUR. Associations found with adjustment for SES and in one study, noise. Potential confounding inconsistently examined for smoking and not examined for stress or traffic-related copollutants. Limited and inconclusive toxicological evidence for effects on motor function and emotional responses. In a study of adult rats, short-term NO_2 exposure induced neurodegeneration and oxidative stress, which have the potential to lead to neurodevelopmental effects. Limited and inconclusive toxicological evidence for impaired physical development in rats, and no analogous epidemiologic investigation.	Overall study ambient means for cognitive function: 16.5 ppb for concurrent school annual avg estimate 15.4 ppb for prenatal home annual avg estimate Neurodegeneration in rat brains: 2,500 ppb for 7 days Oxidative stress in rat brains: 5,320 ppb for 7 days
Reason for no change in causal determination	Large increase in epidemiologic studies of cognitive function, but results lack sufficient consistency, including those for residential or school estimates of NO_2 exposure. Limited and inconclusive toxicological evidence does not adequately inform a potential effect of NO_2	
Uncertainty remaining		

Table 1-1 (Continued): Key evidence contributing to causal determinations for nitrogen dioxide (NO_2) exposure and health effects evaluated in the current draft Integrated Science Assessment (ISA) for Oxides of Nitrogen.

Health Effect Category[a] and Causal Determination[b]		NO_2 Concentrations Associated with Effects
Cancer and Long-term Exposure (Section 6.6)		
Current Draft ISA—Suggestive, but not sufficient, to infer a causal relationship. 2008 ISA—Inadequate to infer a causal relationship.		
Key evidence (Table 6-20)	Strongest evidence is for lung cancer. Inconsistent epidemiologic evidence from several recent cohort studies followed for 7−30 years for associations of lung cancer incidence and mortality with NO_2 exposures averaged over 1 to 30 years. Inconsistency observed for NO_2 estimated at homes and measured at central site monitors. Associations persist with adjustment for smoking, diet, SES, and occupational exposures, but confounding by traffic-related copollutants not widely examined. Lack of toxicological evidence for direct effect of NO_2 in lung tumor induction, but findings for high NO_2 exposures with co-exposure to carcinogens suggest possible role for NO_2 in lung tumor promotion. Evidence for formation of secondary oxidation products in the respiratory tract and limited evidence for hyperplasia of lung epithelium, which have the potential to lead to carcinogenicity. Limited epidemiologic evidence for associations with cancers of other sites, but inconsistent findings for mutagenic and genotoxic effects in experimental animals to support independent effect of NO_2.	Overall study ambient means: 12.1 to 23.2 ppb for residential annual avg estimates Individual city ambient means: 1.2 to 33.7 ppb for 10-yr avg at central site monitors 6.4 to 32.4 ppb for 3-yr avg at central site monitors Lung tumor promotion in rodents: 4,000 to 10,000 ppb for 6 to 17 months
Reason for change in causal determination	Evidence in some but not all epidemiologic studies for lung cancer incidence and mortality, including associations with residential estimates of NO_2 exposure. Some toxicological evidence for role of NO_2 in lung tumor promotion	
Uncertainty remaining	Effect of NO_2 independent from traffic-related copollutants is uncertain because epidemiologic analysis of confounding and results from experimental studies that NO_2 acts as a direct carcinogen are lacking.	

BC = black carbon; CO = carbon monoxide; COPD = chronic obstructive pulmonary disease; EC = elemental carbon; ED = emergency department; ISA = Integrated Science Assessment; NO_2 = nitrogen dioxide; O_3 = ozone; OC = organic carbon; $PM_{2.5}$ = particulate matter with an aerodynamic diameter less than or equal to a nominal 2.5 µm; PM_{10} = particulate matter with an aerodynamic diameter less than or equal to a nominal 10 µm; SES = socioeconomic status; SO_2 = sulfur dioxide; UFP = ultrafine particles; VOC = volatile organic compound.

[a]A large spectrum of outcomes is evaluated as part of a broad health effect category including physiological measures (e.g., airway responsiveness, lung function), clinical outcomes (e.g., respiratory symptoms, hospital admissions), and cause-specific mortality. Total mortality includes all nonaccidental causes of mortality and is informed by the nature of the evidence for the spectrum of morbidity effects (e.g., respiratory, cardiovascular) that can lead to mortality. The sections and tables referenced include a detailed discussion of the available evidence that informed the causal determinations.

[b]Since the completion of the 2008 ISA for Oxides of Nitrogen, the phrasing of causal determinations has changed slightly, and the weight of evidence that describes each level in the hierarchy of the causal framework has been more explicitly characterized.

[c]In the 2008 ISA, a single causal determination was made for the broad category of reproductive and developmental effects. In the current draft ISA, separate causal determinations are made for smaller subcategories of reproductive and developmental effects based on varying underlying biological processes and exposure patterns over different lifestages.

1.6 Policy-Relevant Considerations

As described in the Preamble and Section 1.1, this ISA informs policy-relevant issues that are aimed at characterizing quantitative aspects of relationships between ambient NO_2 exposure and health effects and the impact of these relationships on public health. To that end, this section integrates information from the ISA to describe NO_2 exposure durations and patterns related to health effects, the shape of the concentration-response relationship, regional heterogeneity in relationships, the adverse nature of health effects, and at-risk populations and lifestages. In addressing these policy-relevant issues, this section focuses on respiratory effects, for which the evidence indicates there is a causal and likely to be a causal relationship, respectively, with short-term and long-term NO_2 exposure. Because of uncertainty in the independent effects of NO_2 exposure, other health effects are discussed if they potentially provide new insight on a particular issue.

1.6.1 Durations of Nitrogen Dioxide Exposure Associated with Health Effects

The primary NO_2 NAAQS are based on 1-h daily max concentrations (3-yr avg of each year's 98th percentile) and annual average concentrations and were set to protect against a broad range of respiratory effects associated with short-term NO_2 exposures and various health effects potentially associated with long-term exposure, respectively (Section 1.1). Thus, an important consideration in the review of the primary NO_2 NAAQS is whether the nature of the health effects evidence varies among NO_2 exposure durations.

For short-term exposure, the majority of previous and recent evidence associates health effects with 24-h avg ambient NO_2, but a smaller body of evidence is equally consistent for subdaily averages such as 1-h or 8-h max NO_2 and NO_2 averaged over periods of 2 or 5 hours. The 24-h avg and 1-h max ambient NO_2 metrics, assessed primarily from concentrations averaged across multiple monitors within a city, are associated with a spectrum of effects related to asthma exacerbation. In the few within-study comparisons and based on typical increases in 24-h avg and 1-h max ambient NO_2 concentrations (20 and 30 ppb, respectively; Section 5.1.2.3), the magnitude of association with respiratory effects did not clearly differ between 24-h avg and 1-h max NO_2 (Sections 5.2.2 and 5.2.7). A study of asthma-related ED visits in Atlanta, GA observed similar associations for 1-h max and 24-h avg NO_2 with a 1-day lag, and a slightly larger association for 6-h nighttime avg NO_2 (12:00 a.m.−6:00 a.m.; Section 5.2.2.4). Based on measurements from central site monitors, the distribution of concentrations and spatial heterogeneity varied among the array of NO_2 averaging times, which may account for differences in associations with asthma ED visits. For example, nighttime avg NO_2 had a wider range of

concentrations than 24-h avg NO_2. Nighttime avg NO_2 was similar to 1-h max NO_2 in spatial heterogeneity but lower in concentration. The spatial heterogeneity in ambient NO_2 concentrations within urban areas and with distance to roads (Sections 2.5.2 and 2.5.3) and diurnal trends with higher concentrations measured during morning commute hours (Section 2.5.4) are not unique to Atlanta, GA. This heterogeneity in ambient NO_2 concentrations along with diurnal variation in people's time-activity patterns suggest that the array of NO_2 averaging times vary in the extent to which they represent people's exposures, which could obscure true differences in association with health effects

NO_2 measurements aligned with subjects' locations including total and ambient personal, outdoor and indoor school, and indoor home NO_2 are associated with asthma-related effects (Sections 5.2.2.2 and 5.2.2.5) and mostly are integrated over 1 or multiple days. From these integrated exposure metrics, any diurnal pattern of NO_2 exposure that may underlie associations with asthma-related effects cannot be discerned. The relative importance of daily average exposures or acute peaks in exposure occurring as a result of diurnal variation in ambient concentrations is not clear. Any contribution of acute peaks in indoor NO_2 exposures (Table 3-4) to associations observed between 3-day or 4-week avg indoor NO_2 and asthma-related effects also is not known. However, NO_2 exposures of 2 or 5 hours during time spent in outdoor traffic and nontraffic locations are related to pulmonary inflammation and lung function decrements in adults (Section 5.2.9.3). Inference from these results is strong because they are based on personal ambient NO_2 measurements or NO_2 measured at the locations of outdoor exposures. Controlled human exposure studies showing clinically relevant increases in airway responsiveness (Section 5.2.2.2) and allergic inflammation (Section 5.2.2.5) in adults with asthma in response to 100−400 ppb NO_2 exposures in the range of 30 minutes to 6 hours provides biological plausibility for subdaily ambient NO_2 exposures inducing asthma exacerbation.

With respect to long-term exposure, asthma development in children is associated with 1-yr avg ambient NO_2 concentrations estimated at children's homes and 3-yr or 10-yr avg NO_2 concentrations at central site monitors (Section 6.2.2.1). The NO_2 concentrations averaged over 1 year during prenatal or infancy periods could represent critical time windows of exposure for asthma development or represent longer durations of NO_2 exposure for subjects who remain in the same home or neighborhood. Experimental studies do not provide direct insight into what the epidemiologic findings may be indicating are important periods of long-term NO_2 exposure for asthma development because experimental studies examined NO_2 exposures of less than one year in adulthood. However, findings for increased allergic responses and airway responsiveness in humans or rodents indicate that repeated increases in NO_2 exposure over multiple days or exposures over 1 to 3 months may play a role in asthma development (Section 6.2.2.3).

Overall, asthma exacerbation and asthma development are linked to a range of short-term and long-term, respectively, durations of NO_2 exposure. There is no indication of a stronger association for any particular short-term or long-term duration of NO_2 exposure.

1.6.2 Lag Structure of Relationships between Nitrogen Dioxide Exposure and Health Effects

Characterizing the NO_2 exposure lags (i.e., time between exposure and effect) associated with health effects can aid in understanding the nature of relationships between NO_2 exposure and health effects. The lag structure for associations with NO_2 exposure may vary among health effects depending on differences in the time course by which underlying biological processes occur. Identifying important lag structures can depend on whether the lag structure varies within the population according to differences among individuals in time-activity patterns, pre-existing disease, or other factors that influence exposure and responses to exposure. Another consideration in drawing inferences about important lag structures is that differences in associations among exposure lags, particularly single-day and multiday averages NO_2 concentrations, may not only have a biological basis but may be influenced by differences in the extent to which single-day and multiday average ambient NO_2 concentrations represent people's acutal exposures.

Epidemiologic panel studies of children with asthma observed increases in pulmonary inflammation and respiratory symptoms and decreases in lung function in association with increases in NO_2 concentration lagged 0 day (same day as outcome) or 1 day and multiday averages of 2 to 7 days (Section 5.2.2). Consistent with these findings, increases in asthma-related hospital admissions and ED visits were observed in association with NO_2 concentrations lagged 0 or 1 day or averaged over 2 to 5 days. Whereas no particular lag of NO_2 exposure was more strongly associated with decreases in lung function, several studies indicate larger increases in pulmonary inflammation, respiratory symptoms, and asthma-related hospital admissions and ED visits for increases in multiday averages of NO_2 than single-day lags. For measures of personal ambient and total NO_2, outdoor school NO_2, and indoor NO_2, which may better represent exposure compared with measurements from central site monitors, asthma-related effects also were associated with multiday average NO_2 concentrations (i.e., 2 to 4 days).

Studies in which adults with asthma and healthy adults were exposed for 2 or 5 hours in outdoor traffic and nontraffic locations indicate decreases in lung function and increases in pulmonary inflammation immediately or 2 hours after exposures (Sections 5.2.2 and 5.2.7). In both populations, decreases in lung function also were found the day after exposures. In healthy adults, increases in pulmonary inflammation did not persist the day

after outdoor exposure (Section 5.2.7.4). These data based on personal ambient exposure assessment or NO_2 measured at the locations of people's outdoor exposures support other epidemiologic findings showing increases in respiratory effects at lag 0 or 1 day of NO_2 exposure and also indicate a similar lag structure for respiratory effects in people with and without asthma. Experimental studies show that NO_2 exposure affects the biological processes underlying the asthma-related effects observed in epidemiologic studies on a similar time frame. Controlled human exposure studies found airway responsiveness in adults with asthma to increase immediately after or 20 minutes to 4 hours after a single NO_2 exposure and over 4 days of repeated exposure (Section 5.2.2.1). In experimental studies, NO_2 exposure enhanced allergic inflammation 30 minutes up to 19 hours after a single or 2-day exposure in humans and 7 days after exposure in rats (Section 5.2.2.5). Thus, the findings from experimental studies provide biological plausibility for the asthma-related effects observed in epidemiologic studies in association with 2 or 5 hour exposures, same-day NO_2 exposures, as well as exposures averaged over multiple days.

1.6.3 Concentration-Response Relationships and Thresholds

Characterizing the shape of the concentration-response relationship aids in quantifying the public health impact of NO_2 exposure. A key issue is whether the relationship is linear across the full range of ambient concentrations or whether there are deviations from linearity at and below the levels of the current 1-h NAAQS of 100 ppb and annual NAAQS of 53 ppb. Also important for the review of the primary NO_2 NAAQS is understanding at what ambient NO_2 concentrations there is uncertainty in the relationship with health effects. Characterization of the concentration-response relationship in epidemiologic studies is complicated by fewer observations in the low range of ambient concentrations, the influence of other pollutants or risk factors for the health effects, and heterogeneity among individuals in the population in their response to air pollution exposures. The shape of the concentration-response relationship for health effects related to short-term NO_2 exposure is examined in a limited number of epidemiologic studies and is better characterized for respiratory hospital admissions and ED visits and total mortality than for other health effects.

Recent U.S. studies support a linear relationship between short-term NO_2 exposure and asthma ED visits (Section 5.2.2.4). For 1-h max NO_2 concentrations (lag 0−2 day avg) combined across urban monitors by population-weighting, a linear association was observed with pediatric asthma ED visits in Atlanta, GA during 1993−2004. Also, risk estimates increased across quintiles of NO_2 between 28 and 181 ppb (with concentrations less than 28 ppb as the reference). Results from nonparametric models provide evidence of asthma ED visits in the warm season (May−October) increasing with increasing

1-h max NO_2 concentrations between 11 and 37 ppb (5th to 95th percentiles). There is similar confidence in the relationship throughout this range of NO_2 concentrations, with a relatively tight 95% CI even at 11 ppb NO_2. There is uncertainty in the relationship at 1-h max NO_2 concentrations less than 11 ppb, where effect estimates were reported to be unstable. The distribution of 1-h max NO_2 varied across monitors in the Atlanta area, with higher concentrations measured in downtown Atlanta (mean 42 ppb). However, the population-weighted average may better represent concentrations where study subjects live and spend time. For the relationship between 24-h avg NO_2 and pediatric asthma ED visits in Detroit, MI during 2004−2006, evidence does not indicate that associations differ below and above 23 ppb NO_2 (between the 82nd and 85th percentiles), where the investigators tested for a deviation from a linear relationship. The risk was not assumed to be zero below 23 ppb, and the model assuming a deviation from linearity did not fit the data better than the linear model did. NO_2 concentrations were averaged between two Detroit sites, and information on how ambient NO_2 concentrations compared between sites was not reported. These limited findings from Atlanta and Detroit indicate that the association between short-term NO_2 exposure and asthma ED visits in children is present at NO_2 concentrations well below the level of the current 1-h primary NAAQS.

The concentration-response relationship for short-term NO_2 exposure and asthma-related effects is not well examined in controlled human exposure or animal toxicological studies. Combining data across multiple studies, a recent meta-analysis observed that NO_2 exposure cut in half the dose of the challenge agent required to induce an increase in airway responsiveness (i.e., provocative dose) in adults with asthma, but the provocative dose did not change with increasing NO_2 concentration in the range of 100−500 ppb (Figure 5-1). Experimental studies do not strongly inform whether asthma-related effects increase with increasing NO_2 concentration because few studies examined multiple NO_2 exposure concentrations, and the range of these NO_2 concentrations (greater than 100 ppb) exceed those examined in epidemiologic studies of concentration-response.

Linear concentration-response relationships also are indicated for mortality associated with short-term NO_2 exposure in the U.S., Canada, and Asia based on comparisons of linear and various nonlinear models with natural and cubic splines or quadratic and cubic terms for NO_2 (Section 5.4.7). A few previous results point to nonlinear associations but for health effects for which the concentration-response relationship has not been widely examined, including cough in children in the general population or cardiovascular hospital admissions in adults. These studies tend to find NO_2-related increases in effects that are larger in magnitude per increment in NO_2 concentration in the lower range of NO_2 concentrations than in the upper range of concentrations. The implications of results for these nonasthma health effects is less clear given the uncertainty as to whether NO_2 exposure has independent relationships with nonasthma health effects.

For long-term NO_2 exposure, there is evidence for a linear concentration-response relationship with respiratory effects, although from only one or two studies per specific outcome. For asthma development, in analyses of tertiles or quartiles of residential estimates of NO_2 exposure, a linear concentration-response is indicated in one study but not another (Section 6.2.2.2). In the study observing a linear relationship, annual average NO_2 concentrations ranged from 1.8 to 24 ppb, but because tertiles of NO_2 concentration were not reported, the range of NO_2 concentrations where there may be more or less uncertainty in the relationship with asthma development cannot be assessed. Also based on categories of NO_2 concentration or splines, linear relationships are indicated for associations of long-term averages of NO_2 with asthma symptoms in children, chronic bronchitis in adults, and asthma hospital admissions in adults (Section 6.2.3), but some of these findings could reflect associations with short-term NO_2 exposure. Analysis of the concentration-response with categories of long-term average NO_2 concentrations does not provide a strong basis for assessing whether there is a threshold for respiratory effects.

In summary, the shape of the concentration-response relationship is better characterized in epidemiologic studies and for short-term NO_2 exposure than long-term exposure. Previous and recent evidence indicates a linear relationship between short-term NO_2 exposure and hospital admissions or ED visits for asthma and multiple respiratory conditions combined using various methods, including analysis of splines, higher order terms for NO_2 (e.g., quadratic, cubic), and categories of NO_2 concentration. Few controlled human exposure or toxicological studies of asthma-related effects examined multiple NO_2 exposure concentrations; therefore, that evidence lacks strong insight into the concentration-response relationship. Associations with asthma ED visits are present at ambient NO_2 concentrations well below the level of the 1-h NAAQS. In Atlanta, GA, a linear relationship is indicated for 1-h max NO_2 concentrations averaged over 3 days, with similar confidence in the relationship across the range of 11 to 37 ppb but uncertainty in the relationship at concentrations less than 11 ppb. Another source of uncertainty is that 24-h avg or 1-h max NO_2 concentrations were averaged across multiple central site monitors within a city, which may not reflect varying distributions of concentrations within the city or population exposures.

1.6.4 Regional Heterogeneity in Effect Estimates

In addition to examining the shape of the concentration-response relationship for NO_2-related health effects across the distribution of concentrations, studies have examined whether associations vary across geographical regions. In one study, heterogeneity was noted among Asian cities in the shape of the NO_2-mortality relationship. Information on regional heterogeneity is limited, particularly for the U.S.

and for relationships of NO_2 exposure with asthma exacerbation or development. There is no strong indication of heterogeneity in associations of short-term NO_2 exposure with respiratory symptoms in children in the general population among Korean cities (Section 5.2.7.3). A few studies observe regional heterogeneity in associations between short-term NO_2 exposure and total mortality among European and Asian cities (Section 5.4.7). A nonlinear concentration-response relationship observed in one of four Asian cities was hypothesized to be due to differences among cities in mortality from infection, air conditioning use, time spent by the population outdoors, or temperature. On a smaller geographic scale, NO_2-related respiratory effects do not clearly differ between two cities in Ohio with similar ambient NO_2 concentrations (Section 5.2.2.4) or neighboring urban and suburban communities in Europe that differed in ambient NO_2 concentrations (Section 7.5.5). Limited results point to potential within-city differences in asthma exacerbation in relation to short-term NO_2 exposure. NO_2-related asthma ED visits were larger in Bronx than Manhattan, NY (Section 5.2.2.4), and NO_2-related lung function and pulmonary inflammation among children with asthma differed between two El Paso, TX schools (Sections 5.2.2.2 and 5.2.2.5). The reasons for the heterogeneity were not explicitly analyzed. In the El Paso study the schools differed in proximity to road, ambient NO_2 concentrations, racial composition, and asthma medication use.

For long-term NO_2 exposure, differences are observed among Chicago, Houston, San Francisco, New York, and Puerto Rico in the association with asthma prevalence among Latino and African American individuals ages 8−21 years (Section 6.2.2.1). A test for heterogeneity was not statistically significant, but associations are observed only in the San Francisco and New York cohorts. Odds ratios for the average ambient NO_2 concentration for the first year or first 3 years of life are largest in the San Francisco cohort, which comprised only African American individuals. Associations are not observed in Chicago, Puerto Rico, or Houston. The reasons for heterogeneity among the locations were not explicitly analyzed, but the locations differed in the distribution of ambient NO_2, SO_2, and $PM_{2.5}$ concentrations, which may indicate varying air pollution mixtures among locations. San Francisco had lower ambient NO_2 and SO_2 concentrations than New York. $PM_{2.5}$ and SO_2 were associated with asthma prevalence in Houston but not in New York or San Francisco.

In summary, with limited available information, including one U.S. study of asthma prevalence, it is not clear whether there is regional heterogeneity in the relationship between short-term or long-term NO_2 exposure and respiratory effects. There is some evidence of heterogeneity in associations of short-term NO_2 exposure with mortality among cities in Europe and Asia. Given the uncertainty as to whether NO_2 exposure has an independent relationship with mortality, the extent to which the regional heterogeneity in risk is applicable specifically to NO_2 exposure is uncertain.

1.6.5 Public Health Significance

The public health significance of air pollution-related health effects is informed by the adverse nature of the health effects that are observed, the size of the population exposed to the air pollutant or affected by the health outcome, and the presence of populations or lifestages with higher exposure or increased risk of air pollution-related health effects.

Characterizing Adversity of Health Effects

Both the World Health Organization (WHO) and the American Thoracic Society (ATS) have provided guidance in describing what health effects may be considered adverse. WHO defines health as "the state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity" (WHO, 1948). By this definition, changes in health outcomes that are not severe enough to result in a diagnosis of a clinical effect or condition can be considered adverse if they affect the well-being of an individual. ATS also has considered a wide range of health outcomes in defining adverse effects. Distinguishing between individual and population risk, ATS described its view that small air pollution-related changes in an outcome observed in individuals might be considered adverse on a population level. This is because a shift in the distribution of population responses resulting from an increase in air pollution exposure might increase the proportion of the population with clinically important effects or at those at increased risk of a clinically important effect that could be caused by another risk factor (ATS, 2000).

Increases in ambient NO_2 concentrations are associated with a broad spectrum of health effects related to asthma, including those characterized as adverse by ATS such as ED visits and hospital admissions (ATS, 2000). NO_2 exposure also is associated with more subtle effects such as increases in airway responsiveness and pulmonary inflammation and decreases in lung function (Section 1.5.1). Increases in airway responsiveness and pulmonary inflammation are part of the mode of action for both asthma exacerbation and asthma development (Figure 1-2) and show a distribution within populations. NO_2-associated changes in airway responsiveness or pulmonary inflammation may be considered adverse on a population level because they can increase the proportion of the population with clinically important changes that can lead to exacerbation or development of asthma. A meta-analysis of controlled human exposure studies demonstrates that NO_2 exposures of 140−200 ppb for 1−2 hours cuts in half the dose of a challenge agent required to increase airway responsiveness in adults with asthma (Section 5.2.2.1). Such observations that NO_2 concentrations not much higher than peak ambient concentrations can induce clinically relevant effects related to asthma exacerbation further support a role for ambient NO_2 exposures in inducing adverse health effects.

At-Risk Populations and Lifestages for Health Effects Related to Nitrogen Dioxide Exposure

The primary 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 groups potentially at increased risk for health effects from exposure to the air pollutant for which each NAAQS is set (Preface to the ISA). Hence, the public health significance of health effects related to NO_2 exposure also is informed by whether specific lifestages or groups in the population are identified as being at increased risk of NO_2-related health effects. The large proportion of the U.S. population living near roads, where ambient NO_2 concentrations are higher compared to many other locations (Section 2.5.3), indicates the widespread potential for elevated ambient NO_2 exposures. In 2009, 17% of U.S. homes were estimated to be within 91 m of sources of ambient NO_2 such as a four-lane highway, railroad, or airport (Section 7.5.6). The percentage of the population with elevated NO_2 exposures may be greater in cities. For example, 40% of the Los Angeles, CA population was estimated to live within 100 m of a major road (Section 7.5.6). In addition to those living near roads, people spending time near roads and commuting or working on roads have the potential for elevated NO_2 exposure, and in turn, potential for increased risk of NO_2-related health effects.

At-risk populations or lifestages also can be characterized by specific biological, sociodemographic, or behavioral factors, among others. Since the 2008 ISA for Oxides of Nitrogen and as used in the recent ISAs for O_3 (U.S. EPA, 2013b) and lead (U.S. EPA, 2013a), EPA has developed a framework for drawing conclusions about the role of such factors in modifying risk of health effects of air pollution exposure (Table III of the Preamble). Similar to the causal framework, conclusions are based on judgments of the consistency and coherence of evidence within and across disciplines (Chapter 7). Briefly, the evaluation is based on studies that compare exposure or health effect relationships among groups that differ according to a particular factor (e.g., people with and without asthma) and experimental studies conducted in a population or animal model with a particular factor or pathophysiological condition. Where available, information on exposure, dosimetry, and modes of action is evaluated to assess coherence with health effects evidence and inform how a particular factor may increase risk of NO_2-related health effects (e.g., by increasing exposure, increasing biological effect for a given dose).

There is adequate evidence that people with asthma, children, and older adults are at increased risk for NO_2-related health effects (Table 7-26). These conclusions are substantiated by the fact that evidence is for asthma exacerbation, for which an independent effect of short-term NO_2 exposure is demonstrated (Section 1.5.1). Limited, supporting evidence suggests that females, people of low SES, and people with low antioxidant diets have increased risk for NO_2-related health effects. The inconsistent

evidence is inadequate to determine whether genetic variants, COPD, cardiovascular disease, diabetes, obesity, race/ethnicity, smoking, urban residence, or proximity to roads increase NO_2-related health effects. The uncertainty common to many of these factors is that the evidence is for cardiovascular effects, diabetes, or mortality, which are not clearly related to NO_2 exposure.

A causal relationship between short-term NO_2 exposure and respiratory effects is based on the evidence for asthma exacerbation (Section 1.5.1). The increased risk for people with asthma is supported further by controlled human exposure studies demonstrating increased airway responsiveness at lower NO_2 concentrations in adults with asthma than in healthy adults (Section 7.3.1). Differences in NO_2 dosimetry (Section 4.2.2) or exposure among people with asthma are not well described. Epidemiologic evidence does not consistently indicate differences in NO_2-related respiratory effects between children with asthma and without asthma. However, because asthma is a heterogeneous disease and the populations examined varied in prevalence of asthma medication use and atopy, the inconsistent epidemiologic results are not considered to be in conflict with controlled human exposure studies, which examined primarily adults with mild, atopic asthma.

The increased risk of NO_2-related asthma hospital admissions and ED visits for children (Section 7.5.1.1) and older adults (Section 7.5.1.2) suggests that among people with asthma the effects of NO_2 exposure may vary by lifestage. Although not clearly delineated for NO_2, several physiological and behavioral traits may contribute to the increased risk for children. Compared with adults, children have developing respiratory systems and increased oronasal breathing and ventilation rates (Section 4.2.2.3). Limited data do not clearly indicate higher personal NO_2 exposures in children (Table 3-5) but do indicate more time and vigorous activity outdoors (Section 7.5.1.1). Thus, children may have greater NO_2 uptake in the respiratory tract or less exposure measurement error. Many studies reported a higher proportion of asthma ED visits or hospital admissions among children than other lifestages. Thus, higher incidence of asthma exacerbation in children may be a reason for their increased risk.

Because the respiratory system continues to develop throughout childhood, it is possible that critical time windows of exposure exist for NO_2-related respiratory effects. However, the evidence shows that asthma development in children is associated with several different time windows of long-term NO_2 exposure: the prenatal or infancy period, the first year of life, year of diagnosis, or lifetime exposure (Section 7.5.1.1). Studies do not consistently identify a specific time window of long-term NO_2 exposure more strongly associated with the development of asthma as ascertained in children ages 4−18 years.

Children not only comprise a large proportion of the U.S. population (24% in the 2010 U.S. census) but also have a higher rate of asthma health care encounters than adults

(e.g., 10.7 vs. 7.0 per 100 persons with asthma).[1] Further, asthma is the leading chronic illness (9.5% prevalence) and reason for missed school days in children in the U.S. Many U.S. schools are located near high-traffic roads (7% within 250 m; Section 7.5.6). NO_2 concentrations outside schools are associated with asthma-related effects in children (Sections 5.2.2.2 and 5.2.2.5), and school could be an important source of NO_2 exposure. Based on the large number of children in the U.S., the high prevalence of asthma morbidity among children, and potential for high NO_2 exposures, higher risks of asthma exacerbation for children compared with adults can translate into large numbers of people affected, magnifying the potential public health impact of NO_2 exposure.

The public health impact of NO_2-related health effects also is magnified by the growing proportion of older adults in the U.S. As with children, it is not well understood why older adults have increased risk for NO_2-related hospital admissions for asthma. Older adults did not consistently have a higher proportion of asthma hospital admissions compared with younger adults, so higher incidence of asthma exacerbation does not seem to explain their higher NO_2-related risk estimates. Differences in NO_2 dosimetry also are not described for older adults (Section 4.2.2.3). Time-activity patterns have been shown to differ between older and younger adults, but there is not a clear difference in time spent in a particular location that could explain differential exposure to NO_2 in older adults (Section 7.5.1.2). Older adults have higher prevalence of many chronic diseases compared to younger adults (Table 7-2). COPD, cardiovascular diseases, and diabetes did not consistently modify NO_2-related health effects, but studies have not examined whether co-occurring morbidity contributes to the increased risk of NO_2-related asthma exacerbation among older adults or whether age alone influences risk.

Although evidence does not clearly identify increased NO_2-related health effects in populations of low SES or nonwhite race or populations living near roads or in urban areas, there is an indication of higher NO_2 exposure among these groups. In particular, some communities are characterized as having both higher ambient NO_2 concentrations and higher proportions of nonwhite and low SES populations (Section 7.5.2). Further, a few studies characterize schools located near high-traffic roads as having higher nonwhite and low SES populations compared to schools located farther away from roads (Section 7.5.6). Nonwhite and low SES populations also are recognized to have higher risks of health effects, including asthma, though it is not clear whether higher NO_2 exposure and higher risk of health effects interact to influence NO_2-related health effects in these groups. A recent study observed higher risk of NO_2-related asthma hospital admissions among Hispanic children compared with white children only in the low SES group (Section 7.5.2). While these findings suggest that co-occurring risk factors in a

[1]National Center for Health Care Statistics Data Brief. Available: http://www.cdc.gov/nchs/data/databriefs/db94.htm

population could influence the risk of NO_2-related health effects, information at present is too limited to draw firm conclusions.

In summary, the public health significance of NO_2-related health effects is supported by many lines of evidence. A large proportion of the U.S. population lives near roads or spends time near or on roads, resulting in a large number of people potentially with elevated ambient NO_2 exposure. NO_2 exposure is linked to health effects that are clearly adverse such as ED visits and hospital admissions for asthma and development of asthma. NO_2-related effects such as increases in airway responsiveness can be considered adverse on a population level because an increase in NO_2 exposure can lead to an increase in the number of people with clinically important effects. The public health significance of NO_2-related health effects also is supported by the increased risk for people with asthma, children, and older adults. The roles of co-occurring risk factors or combined higher NO_2 exposure and health risk within a population in influencing risk of NO_2-related health effects is not well understood. The large proportions of children and older adults in the U.S. population and the high prevalence of asthma in children can translate into a large number of people affected by NO_2 and thus magnify the public health impact of ambient NO_2 exposure.

1.7 Conclusions

There is a causal relationship between short-term NO_2 exposure and respiratory effects. This conclusion is stronger than that determined in the 2008 ISA for Oxides of Nitrogen and is supported by the evidence integrated from epidemiologic and controlled human exposure studies for asthma exacerbation. Asthma-related effects continue to be associated with NO_2 concentrations at central site monitors. Further, recent epidemiologic studies add evidence for associations with personal ambient and total NO_2 measurements as well as NO_2 concentrations outside schools and inside homes. Epidemiologic evidence continues to support independent associations of NO_2 exposure with asthma-related effects in copollutant models with another traffic-related pollutant such as $PM_{2.5}$, EC/BC, OC, UFP, CO, or a VOC. The potential influence of the full array of traffic-related pollutants or mixtures has not been examined. Thus, the key evidence for an independent effect of NO_2 are the findings from previous controlled human exposure studies that NO_2 exposure not much higher than peak ambient concentrations enhances allergic inflammation and induces clinically relevant increases in airway responsiveness. These are hallmarks of the mode of action for asthma exacerbation. There is likely to be a causal relationship between long-term NO_2 exposure and respiratory effects. The strengthening of the conclusion from the 2008 ISA is based on new epidemiologic evidence for associations of asthma development in children with residential NO_2

exposure estimated by LUR models that well represented the variability in ambient NO_2 concentrations in study areas. Epidemiologic studies did not examine confounding by traffic-related copollutants, but a small body of previous experimental studies showing that long-term and short-term NO_2 exposure increase airway responsiveness and allergic responses in healthy humans and rodent models provide some indication that long-term NO_2 exposure may have an independent effect on asthma development. For both short-term and long-term exposure, results for NO_2 measured in subjects' locations, which may better represent exposure, than concentrations at central site monitors provide a stronger basis for inferring relationships with respiratory effects.

Evidence is suggestive, but not sufficient, to infer a causal relationship for short-term and long-term NO_2 exposure with cardiovascular and related metabolic effects and total mortality and for long-term NO_2 exposure with birth outcomes and cancer. While there is continued or new supporting epidemiologic evidence for these health effects, there still is large uncertainty as to whether NO_2 exposure has an effect independent of traffic-related copollutants. Epidemiologic studies have not adequately accounted for confounding, and there is a paucity of support from experimental studies. Some recent experimental studies show NO_2-induced increases in systemic inflammation or oxidative stress. Such changes are not consistently observed or necessarily linked to any health effect, unlike the mode of action information available for asthma.The insufficient consistency of epidemiologic and toxicological evidence is inadequate to infer a causal relationship for long-term NO_2 exposure with fertility, reproduction, and pregnancy as well as postnatal development.

As described above, key considerations in drawing conclusions about relationships between ambient NO_2 exposure and health effects include evaluating the adequacy of NO_2 exposure estimates to represent the temporal or spatial patterns in ambient NO_2 concentrations in a given area and separating the effect of NO_2 from that of other traffic-related pollutants. In the U.S., although motor vehicle emissions have decreased greatly over the last few decades, vehicles still are the largest single source of ambient NO_2 in U.S. population centers and can contribute to spatial and temporal heterogeneity in ambient NO_2 concentrations. Recent information combined with that in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) shows that ambient NO_2 concentrations can be 30 to 100% higher at locations within 10−20 m of a road compared with locations farther away. Additionally, the first year of data from the U.S. near-road monitoring network generally show higher NO_2 concentrations at near-road monitoring sites than many other sites within a city.

As in the 2008 ISA, many studies assess exposure with ambient NO_2 concentrations measured at monitors whose siting away from sources likely does not capture the variability in ambient NO_2 concentrations within an area. The resulting error in

representing temporal variation in short-term exposure and spatial variation in long-term exposure can produce smaller magnitude or less precise associations with health effects. Such findings are similar to those reported in the 2008 ISA (U.S. EPA, 2008). This ISA additionally indicates that error that results from using NO_2 concentrations at central site monitors to represent long-term exposure in some cases can produce larger health effect estimates compared with residential NO_2 exposure metrics from LUR models. Thus, spatial misalignment of study subjects and ambient NO_2 concentrations potentially can overestimate health effect associations with long-term NO_2 exposure if the difference in exposure between groups that differ in the health effect systematically is underestimated. Given the potential impact of exposure measurement error, the variable relationships observed between short-term personal and ambient NO_2 concentrations, and largely uncharacterized relationships between long-term personal and ambient NO_2 concentrations, the increase in recent epidemiologic findings for exposures assessed for people's locations (e.g., ambient or total personal, outdoor or indoor home or school) may increase confidence in inferences about relationships between ambient NO_2 exposure and health effects. Also, data from the near-road monitoring network may help address gaps in the understanding of the variability in ambient NO_2 concentrations and people's exposures within urban areas and the potential importance of the near-road environment as a source of NO_2 exposure contributing to health effects.

In addition to characterizing causality, characterizing quantitative aspects of NO_2-related health effects is key to the review of the primary NO_2 NAAQS. Limited investigation indicates a linear relationship for short-term ambient NO_2 exposure with asthma ED visits. There is uncertainty about whether the relationship is present at 1-h max NO_2 concentrations far below the level of the current 1-hour NAAQS. Recent evidence continues to indicate that people with asthma, children, and older adults are at increased risk for NO_2-related health effects. While recent evidence points to higher NO_2 exposure among people of low SES or nonwhite race or people living in urban areas or close to roads, it is not clear whether this higher NO_2 exposure leads to increased health effects. Large numbers of people in the U.S. live near (e.g., within 100 m) or travel on major roads and potentially have elevated exposures to ambient NO_2 compared with people away from roads. The large numbers of children and older adults in the U.S. population and the high prevalence of asthma in children can translate into a large number of people potentially affected by NO_2 exposure and thus magnify the public health impact of ambient NO_2 exposure.

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CHAPTER 2 ATMOSPHERIC CHEMISTRY AND AMBIENT CONCENTRATIONS OF OXIDES OF NITROGEN

2.1 Introduction

This chapter presents concepts and findings relating to emissions sources, atmospheric science, and spatial and temporal concentration patterns for oxides of nitrogen. It is intended as a prologue for detailed discussions on the evidence for human exposure to and health effects of oxides of nitrogen that follow in the subsequent chapters, and as a source of information to help interpret those effects in the context of data about atmospheric concentrations.

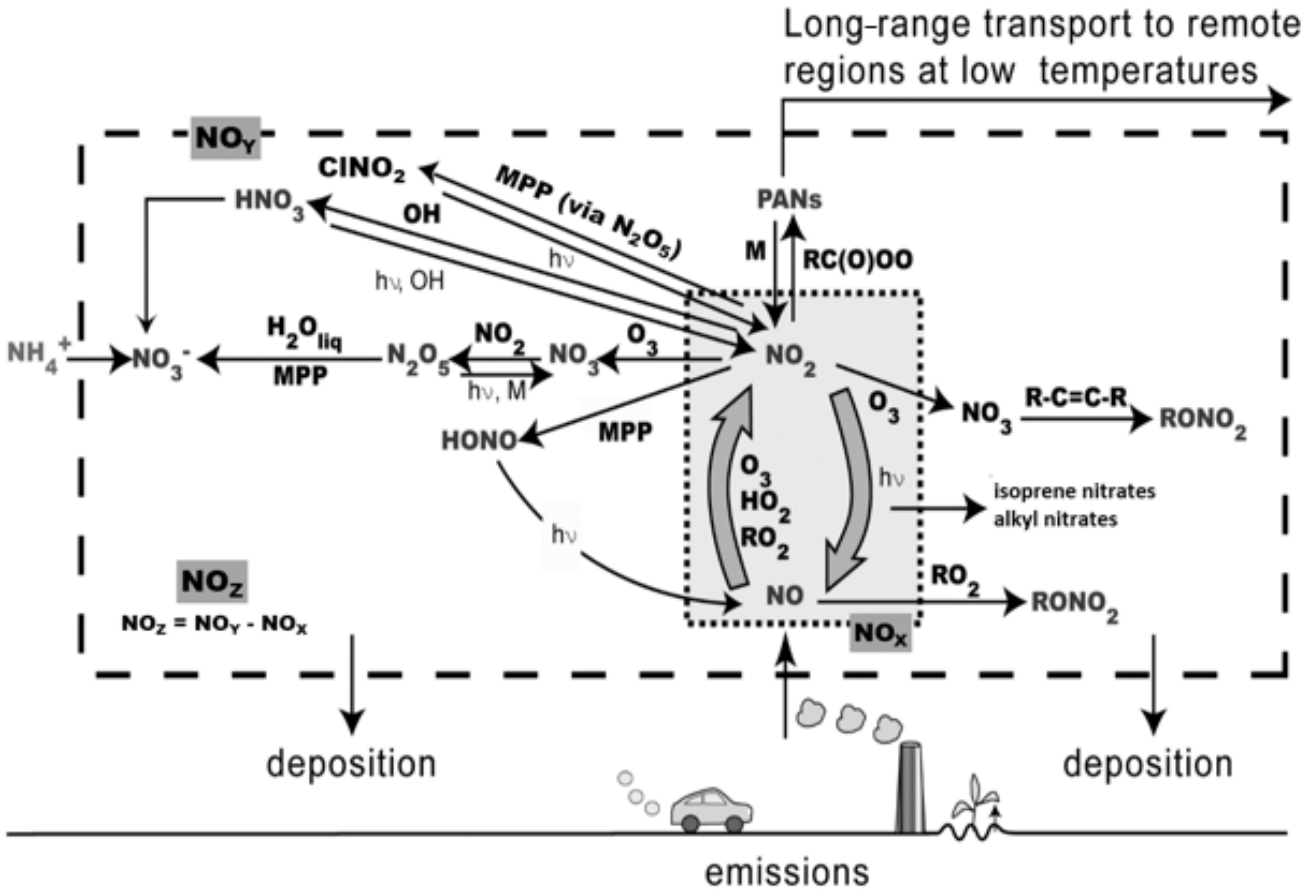
In the Integrated Science Assessment (ISA), the term "oxides of nitrogen" (NO_Y) refers to all forms of oxidized nitrogen (N) compounds, including nitric oxide (NO), nitrogen dioxide (NO_2), and all other oxidized N-containing compounds formed from NO and NO_2. NO and NO_2, along with volatile organic compounds (VOCs), are precursors in the formation of ozone (O_3) and photochemical smog. NO_2 is an oxidant and can react to form other photochemical oxidants such as peroxyacyl nitrates (PANs) and toxic compounds such as nitro-substituted polycyclic aromatic hydrocarbons (nitro-PAHs). NO_2 can also react with a variety of atmospheric species to produce organic and inorganic nitrates, which make substantial contributions to the mass of atmospheric particulate matter (PM) and the acidity of clouds, fog, and rainwater. The abbreviation NO_X refers specifically to the sum of NO and NO_2. This chapter describes the origins, distribution, and fate of gaseous oxides of nitrogen. Aspects of particulate nitrogen species [such as particulate nitrate (pNO_3)] were addressed in the 2009 ISA for Particulate Matter (U.S. EPA, 2009) and will be addressed in the upcoming ISA for Particulate Matter.

2.2 Atmospheric Chemistry and Fate

The chemistry of oxidized nitrogen compounds in the atmosphere was reviewed in the 2008 ISA for Oxides of Nitrogen—Health Criteria (U.S. EPA, 2008a). The role of NO_X in O_3 formation was reviewed in Chapter 3 of the 2013 ISA for Ozone and Related Photochemical Oxidants (U.S. EPA, 2013b) and has been presented in numerous texts (e.g., Jacobson, 2002; Jacob, 1999; Seinfeld and Pandis, 1998). The main points from the

2008 ISA for Oxides of Nitrogen will be presented here along with updates based on recent material.

The overall chemistry of reactive, oxidized nitrogen compounds in the atmosphere is summarized in Figure 2-1. Sources include naturally occurring processes associated with wildfires, lightning, and microbial activity in soils. Anthropogenic sources are dominated by emissions from electricity generating units and motor vehicles. Oxidized nitrogen compounds are emitted to the atmosphere mainly as NO, with only 10% or less emitted as NO_2. Further details about the composition of sources is given in Section 2.3. Freshly emitted NO is converted to NO_2 by reacting with O_3, and NO is recycled during the day by photolysis of NO_2. Thus, NO and NO_2 are often "lumped" together into their own group or family, which the atmospheric sciences community refers to as NO_X (shown in the inner box in Figure 2-1). A large number of oxidized nitrogen species in the atmosphere are formed from the oxidation of NO and NO_2. These include nitrate radicals (NO_3), nitrous acid (HONO), nitric acid (HNO_3), dinitrogen pentoxide (N_2O_5), nitryl chloride ($ClNO_2$), peroxynitric acid (HNO_4), PAN and its homologues (PANs), other organic nitrates, such as alkyl nitrates (including isoprene nitrates), and pNO_3. These reactive oxidation products are referred to collectively as NO_Z. All of the species shown within the dashed lines of Figure 2-1 constitute NO_Y ($NO_Y = NO_X + NO_Z$). The boxes labeled "inorganic" and "organic" in Figure 1-1 (see Chapter 1) contain the species shown in the left and right halves of Figure 2-1.



Note: The inner shaded box contains NO_X (= NO + NO_2). The outer box contains other species (NO_Z) formed from reactions of NO_X. All species shown in the outer and inner boxes are collectively referred to as NO_Y by the atmospheric sciences community. MPP refers to multiphase processes, h*v* to a solar photon, M to a species transferring/removing enough energy to cause a molecule to decompose/stabilize, and R to an organic radical.

Source: National Center for Environmental Assessment.

Figure 2-1 Schematic diagram of the cycle of reactive, oxidized nitrogen species in the atmosphere.

High NO concentrations found near heavy traffic and in power plant plumes are typically associated with O_3 concentrations much lower than in surrounding areas as O_3 can be titrated away, or lost, by reacting with NO. In addition, the reaction of NO with O_3 can produce appreciable amounts of NO_2 rather quickly. For example, 10 ppb NO_2 can be formed in about 20 seconds (for an initial NO concentration of 30 ppb and initial O_3 = 40 ppb at 298° K [25°C])[1]. Higher temperatures and concentrations of reactants

[1] Sample calculation based on solution to an equation for a second order reaction $dx/dt = k([NO]_o - x)([O_3]_o - x)$, where x is the concentration of each species reacted; k is the rate coefficient for the reaction, $= 3 \times 10^{-12}\ e^{(-1500/T)}$ cm^3/sec-molecule (Sander et al., 2011); T = emperature in Kelvin; $[NO]_o$ and $[O_3]_o$ are initial concentrations of NO and O_3.

result in shorter times, while dispersion and depletion of reactants increase this time. A rough estimate of the time for transport away from a broad boulevard is is about a minute (Düring et al., 2011), shorter for more open conditions and ranges up to the order of an hour in Midtown Manhattan street canyons (Richmond-Bryant and Reff, 2012). The time for reaction must be compared to the time for mixing away from the road and for replenishment of O_3, because it is the interplay between these factors that determines how far NO will travel downwind before it is oxidized. These dependencies imply seasonal variability and also geographic variability in the time scale for the reaction. In general, cooler months present the most favorable conditions for NO to travel further before it is oxidized (lower temperature, decreased vertical mixing of O_3 to the surface, generally lower O_3). At any time of the year, if loss of O_3 has been extensive near the surface as happens in many locations at night, then NO could travel a kilometer or more before it is oxidized, resulting in a more uniform downwind distribution of NO_2 than if NO were being oxidized right at its source. The NO_2 that is formed depletes hydroxyl radicals (OH), so that they cannot oxidize hydrocarbons to continue the cycle of new O_3 formation. During the day, NO_2 photolyzes back to NO within a few minutes, setting up the cycle shown in Figure 2-1. Although the assumption of a photostationary state to describe the relations in the $NO/NO_2/O_3$ triad might not be strictly valid, several studies [eg., Düring et al. (2011) and Clapp and Jenkin (2001)] have shown the assumption of a photostationary state can provide a useful approximation of the relationship among these species. Once the sun sets, NO_2 no longer photolyzes to reform NO. If very little or no O_3 is present due to titration in a statically stable, near-surface boundary layer, then NO_2 accumulates through the night solely from direct emissions.

Because of the interplay between dispersion and chemical reaction, the distribution of NO_2 downwind of roads would likely differ from that of a traffic pollutant that is present in ambient air mainly as the result of direct emissions. In addition, day-night differences in both transport and chemistry will also result in day-night differences in the patterns of spatial and temporal variability of NO_2. Examples of the behavior of NO_2 and NO_X downwind of streets and highways are examined in Section 2.5.3. In summary, the major influences on NO_2 concentrations within and downwind of urban centers are the fraction of emissions of NO_X as NO_2, dispersion, and the $NO/NO_2/O_3$ equilibrium, which is established on a time scale of a few minutes during daylight.

All the other species mentioned above in the definition of NO_Y (i.e., NO_Z) are products of reactions of NO or NO_2. Inorganic NO_Z species are shown on the left side of the outer box and organic species are shown on the right side of the outer box in Figure 2-1. Ammonium nitrate and other inorganic particulate species (e.g., sodium [Na^+], calcium [Ca^{2+}] nitrates) are formed from species shown on the left side of the figure; organic nitrates are formed from species shown on the right side of Figure 2-1. The conversion of

NO_X into the inorganic and organic species in the outer box (collectively referred to as NO_Z) typically takes place on much longer time scales than for interconversions between NO and NO_2, e.g., about an hour for conditions in Houston, TX, in April−May of 2009 (Ren et al., 2013) but likely longer in many other areas, especially those at higher latitudes and generally during the cold season. As a result, NO_X emitted during morning rush hour by vehicles can be converted almost completely to products by late afternoon during warm, sunny conditions. However, note the conversion of NO_2 to HNO_3 and hence the atmospheric lifetime of NO_X depends on the concentration of OH radicals, which in turn depends on the concentration of NO_2 [eg., Valin et al. (2013) and Hameed et al. (1979)].

Inorganic NO_Z species shown on the left side of the outer box of Figure 2-1 include HONO, HNO_3, $ClNO_2$, HNO_4, and pNO_3. Pernitric acid (HNO_4) is unlikely to represent an important reservoir for NO_X except perhaps under extremely cold conditions. Mollner et al. (2010) identified pernitrous acid (HOONO), an unstable isomer of nitric acid, as a product of the major gas phase reaction forming HNO_3. However, because HOONO is unstable, it is also not a substantial reservoir for NO_X. Considering the troposphere as a whole, most of the mass of products shown in the outer box of Figure 2-1 is in the form of PAN and HNO_3. The stability of PAN at low temperatures allows its transport to remote regions where it has been shown to exert strong influence on the local production of O_3 [see Fischer et al. (2014) and references therein]. Other organic nitrates (e.g., alkyl nitrates, isoprene nitrates) increase in importance in the planetary boundary layer (PBL), particularly at locations closer to sources (Perring et al., 2013; Horowitz et al., 2007; Singh et al., 2007).

In addition to the above compounds, there is a broad range of gas-phase organic nitrogen compounds that are not shown in Figure 2-1. They are emitted by combustion sources and are also formed in the atmosphere from reactions of NO, NO_2, and NO_3. These compounds include: nitro-aromatics (such as nitrotoluene), nitro-PAHs [such as nitro-naphthalene, e.g., Nishino et al. (2008)], nitrophenols [e.g., Harrison et al. (2005)]; nitriles [such as ethane-nitrile; e.g., de Gouw et al. (2003)]; and isocyanic acid (Roberts et al., 2014).

Sources of NO_X are distributed with height with some occurring at or near ground level and others aloft as indicated in Figure 2-1. NO_X emitted by elevated sources can be oxidized to NO_Z products and/or be transported to the surface, depending on time of day, abundance of oxidants, and strength of vertical mixing. During times of rapid convection, typically in the afternoon on hot sunny days, vertical mixing through the PBL can take place in about1 hour [see e.g., Stull (2000)] and fresh emissions can be brought rapidly to the surface. After sunset, turbulence subsides and emissions entrained into the nocturnal

residual boundary layer, are not mixed downward to the surface. Also, because the prevailing winds aloft are generally stronger than those at the surface, emissions from elevated sources (e.g., the stacks of electrical utilities) can be distributed over a wider area than those emitted at the surface (e.g., motor vehicles). Emissions from elevated sources entrained into the nocturnal residual boundary layer can be transported over long distances, up to a few hundred km overnight depending on location [see e.g., Husar et al. (1978)]. Oxidation of NO_X can occur during the night and in the morning in the residual layer prior to its breakup.Turbulence then mixes NO_X and its oxidation products downward. Emissions directly into the free troposphere are unlikely except in areas such as the Intermountain West where PBL heights can be <200 m during winter, or even <100 m in some locations. Because people live closer to surface sources such as motor vehicles, they are more likely to be exposed to NO and NO_2 from these sources. Thus, atmospheric chemical reactions determine the partitioning of a person's exposure to NO_2 and its reaction products from different sources, and sources of a person's exposure cannot be judged solely by the source strengths given in the National Emissions Inventory (NEI). Issues related to the transport and dispersion of NO_X emitted by traffic are discussed in depth in Section 2.5.3.

Oxidized nitrogen compounds are ultimately lost from the atmosphere by wet and dry deposition to the Earth's surface. Soluble species are taken up by aqueous aerosols and cloud droplets and are removed by wet deposition by rainout (i.e., incorporation into cloud droplets that eventually coagulate into falling rain drops). Both soluble and insoluble species are removed by washout (i.e., impaction with falling rain drops, another component of wet deposition), and by dry deposition (i.e., impaction with the surface and gas exchange with plants). NO and NO_2 are not very soluble, and therefore wet deposition is not a major removal process for them. However, a major NO_X reservoir species, HNO_3, is extremely soluble, and its deposition (both wet and dry) represents a major sink for NO_Y.

Many of the species shown in Figure 2-1, including particulate nitrate (pNO_3) and gas phase HONO, are formed by multiphase processes. Data collected in Houston as part of TexAQS-II summarized by Olaguer et al. (2009) indicate that concentrations of HONO are much higher than can be explained by gas-phase chemistry and by tailpipe emissions.

N_2O_5 is the acid anhydride of HNO_3, and its uptake on aqueous aerosol represents a major sink for NO_X. The uptake of N_2O_5 by atmospheric aerosols or cloud droplets leads to the loss of O_3 and NO_X and the production of aqueous-phase nitric acid, aerosol nitrate, and gaseous halogen nitrites. MacIntyre and Evans (2010) showed that the sensitivity of key tropospheric species, such as O_3, varies from very small to high over the range of uptake coefficients (γ) for N_2O_5 obtained in laboratory studies. For example, global O_3 loss

ranges from 0 to over 10%, with large regional variability over the range of reported N_2O_5 uptake coefficients. However, uptake coefficients for N_2O_5, or $\gamma(N_2O_5)$, on atmospheric particles are not well defined, largely due to uncertainty and variability in aerosol composition. As noted by Brown and Stutz (2012), $\gamma(N_2O_5)$ is largest (≈0.02) for aqueous inorganic aerosols and water droplets, except for nitrate in aerosol, which can reduce $\gamma(N_2O_5)$ by up to an order of magnitude. The uptake of N_2O_5 by mineral particles could also represent an important removal process. For example, values of $\gamma(N_2O_5)$ for calcite and Saharan dust (e.g.) of ≈0.03. However, as noted by Tang et al. (2014) not enough is known to permit a global assessment of the importance of N_2O_5 uptake on mineral surfaces. Organic aerosol and soot can reduce $\gamma(N_2O_5)$ by two orders of magnitude or more, further complicating the task of assessing the importance of uptake of N_2O_5 on aerosol surfaces.

The uptake of N_2O_5 by aqueous aerosols containing chloride (Cl^-) and bromide (Br^-) has been associated with the release of gaseous nitryl chloride ($ClNO_2$) from marine aerosol (sea-spray) (Osthoff et al., 2008). Nitryl chloride has been found not only in coastal and marine environments, but also well inland. For example, Thornton et al. (2010) found production rates of gaseous $ClNO_2$ near Boulder, CO, from reaction of N_2O_5 with particulate Cl^- at levels similar to those found in coastal and marine environments. They also found that substantial quantities of N_2O_5 are recycled through $ClNO_2$ back into NO_X instead of forming HNO_3. Nitryl chloride ($ClNO_2$) readily photolyzes to yield Cl and NO_2 and can represent a significant source of reactive Cl, capable of initiating the oxidation of hydrocarbons (generally with much higher rate coefficients than OH radicals). Riedel et al. (2014) found increases in the production of radicals by 27% and of O_3 by 15% during the 2010 CalNex [California Research at the Nexus of Air Quality and Climate Change in May to June 2010 in Southern California (Ryerson et al., 2013)] field study. However, $ClNO_2$ was found to cause only modest O_3 increases (e.g., ~1 to 1.5 ppb for nominal O_3 concentrations between 60 and 85 ppb) in a model study of the Houston, TX, airshed (Simon et al., 2009). Differences are likely related to differences in the NO_X sensitivity of the two airsheds. Therefore, caution is advised in extrapolating results obtained in one airshed to another.

The lifetimes of PANs are strongly temperature dependent, but these compounds are stable enough at low temperatures to be transported long distances before they decompose to release NO_2. Freshly released NO_2 can then participate in O_3 formation in regions remote from the original NO_X source (cf. Figure 2-1). Nitric acid (HNO_3) acts similarly as a reservoir to some extent, but its high solubility and high deposition rate imply that it is removed from the gas phase faster than PAN; thus HNO_3 would not be as important as a source of NO_X in remote regions. The oxidation of many tropospheric species is initiated by OH radicals during the day. During the night NO_3 radicals, formed

from the reaction of NO_2 and O_3, assume the role of dominant oxidant for many species such as biogenic and anthropogenic alkenes; for some species (e.g., dimethyl sulfide), they are the overall dominant oxidant [see, e.g., Brown and Stutz (2012)]. The reaction of NO_3 with alkenes results in the production of gas phase organic nitrates and secondary organic aerosol formation. Many of the reactions shown in Figure 2-1 occur mainly during the night when NO_3 radicals are most abundant. For example, the formation of N_2O_5, which has a short lifetime with respect to photolysis and thermal decomposition, is favored at low temperatures during the night. Many of the reactions of NO_3, in addition to those of O_3, with alkenes also result in the production of OH and hydroperoxyl (HO_2) radicals during the night.

Isoprene nitrates (INs) and their reaction products could be important for controlling the abundance of NO_X and hence the abundance of O_3 over the eastern U.S. [e.g., Perring et al. (2009)]. Isoprene nitrates (INs) and their reaction products could also be important for exporting reactive nitrogen species to remote areas. Yields for IN formation from isoprene oxidation have been estimated to range from 4% (Horowitz et al., 2007) to 6 to 12% (Xie et al., 2013) based on model simulations of data collected during the International Consortium for Atmospheric Research on Transport and Transformation (ICART) campaign in 2004 and from 7 to 12% in laboratory studies (Lockwood et al., 2010; Paulot et al., 2009; Perring et al., 2009; Horowitz et al., 2007; von Kuhlmann et al., 2004). The initial step in the production of INs involves the reaction of isoprene with OH radicals to produce isoprene peroxy radicals. Under low NO_X conditions, isoprene peroxy radicals will mainly react with HO_2 radicals to produce organic peroxides, with smaller amounts of methacrolein, methyl vinyl ketone, and formaldehyde. Under higher NO_X conditions, isoprene peroxy radicals can also react with NO resulting in the production of many of the same or similar compounds such as methacrolein and methyl vinyl ketone as well as "first generation" INs. Lifetimes on the order of one to a few hours can be estimated for these first generation INs based on their reactions with OH radicals and O_3 (Lockwood et al., 2010; Paulot et al., 2009). The reaction products can further react with NO (after internal rearrangement) to form secondary organic nitrates such as ethanal nitrate, methacrolein nitrate, propanone nitrate, and methylvinylketone nitrate. Lee et al. (2014) found that the rate coefficient for reaction of the first generation INs with O_3 is a factor of ~100 lower than in earlier studies, leading to greater stability and higher levels of first generation INs, particularly at night. Greater stability leads in turn to increased probability of deposition and transport away from source regions. The second generation organic nitrates are more stable than the first generation INs because they lack a double carbon (C=C) bond. Paulot et al. (2009) estimated the yield of NO_X from the destruction of second-generation nitrates to be ~55%. Obviously, the relative importance of pathways forming nitrates or other products depends on the ambient concentrations of NO and other oxides of nitrogen for which many key experimental details are still lacking.

In addition to oxidation initiated by OH radicals, isoprene is also oxidized by NO_3 radicals. Rollins et al. (2009) determined a 70% yield of first generation carbonyl nitrates based on experiments in large reaction chambers. These first generation nitrates can be further oxidized by NO, leading to the production of second generation organic (alkyl) nitrates. Mao et al. (2013) estimated that the global mean lifetime is ~5 days for these second generation organic nitrates. Mao et al. (2013) also suggested that the export of INs and other organic nitrates followed by their decomposition is potentially a larger source of NO_X to the boundary layer of the western North Atlantic Ocean compared to the export of PANs. It should also be noted that some isoprene nitrates (INs) are low enough in volatility that they can partition to the aerosol phase and form PM [e.g., Rollins et al. (2009)].

Describing O_3 formation accurately requires detailed knowledge of the chemistry of INs. Regional or global models that assume a lower yield for forming these nitrates and a higher yield for recycling NO_X tend to over-predict O_3 concentrations in areas with high isoprene emissions, such as the Southeast, compared to those that have a higher yield for the formation of these nitrates and/or a lower yield for their recycling back to NO_X (U.S. EPA, 2013b). The formation rates and the rates that are assumed to recycle INs and other organic nitrates back to NO_X also have implications for calculating the yield of O_3 from isoprene emissions. For example, Fiore et al. (2005) found a negative dependence of O_3 production on isoprene emissions in the eastern U.S. in summer, whereas Mao et al. (2013) found a positive yield for O_3 from isoprene emissions. Xie et al. (2013) determined that the uncertainties in the isoprene nitrates could affect O_3 production by 10% over the U.S. and that uncertainties in the NO_X recycling efficiency had a larger effect than the IN yield. These considerations underlie the importance of further laboratory and field studies to more quantitatively determine the response of O_3 to changes in isoprene emissions at different NO_X levels.

As mentioned earlier, NO and NO_2 are important precursors of O_3 formation. However, because O_3 changes in a nonlinear way with changes in the concentrations of its precursors (NO_X and VOCs), O_3 is unlike many other atmospheric species with rates of formation that vary directly with emissions of their precursors. At the low NO_X concentrations found in environments ranging from remote continental areas to rural and suburban areas downwind of urban centers, the net production of O_3 typically increases with increasing NO_X. In this low-NO_X regime, the overall effect of the oxidation of VOCs is to generate (or at least not consume) radicals, and O_3 production varies directly with NO_X. In the high-NO_X regime, NO_2 reacts with OH radicals to form HNO_3 [e.g., Hameed et al. (1979)]. Otherwise, these OH radicals would oxidize VOCs to produce peroxy radicals, which in turn would oxidize NO to NO_2. In this regime, O_3 production is limited by the availability of radicals (Tonnesen and Jeffries, 1994), and O_3 shows only a

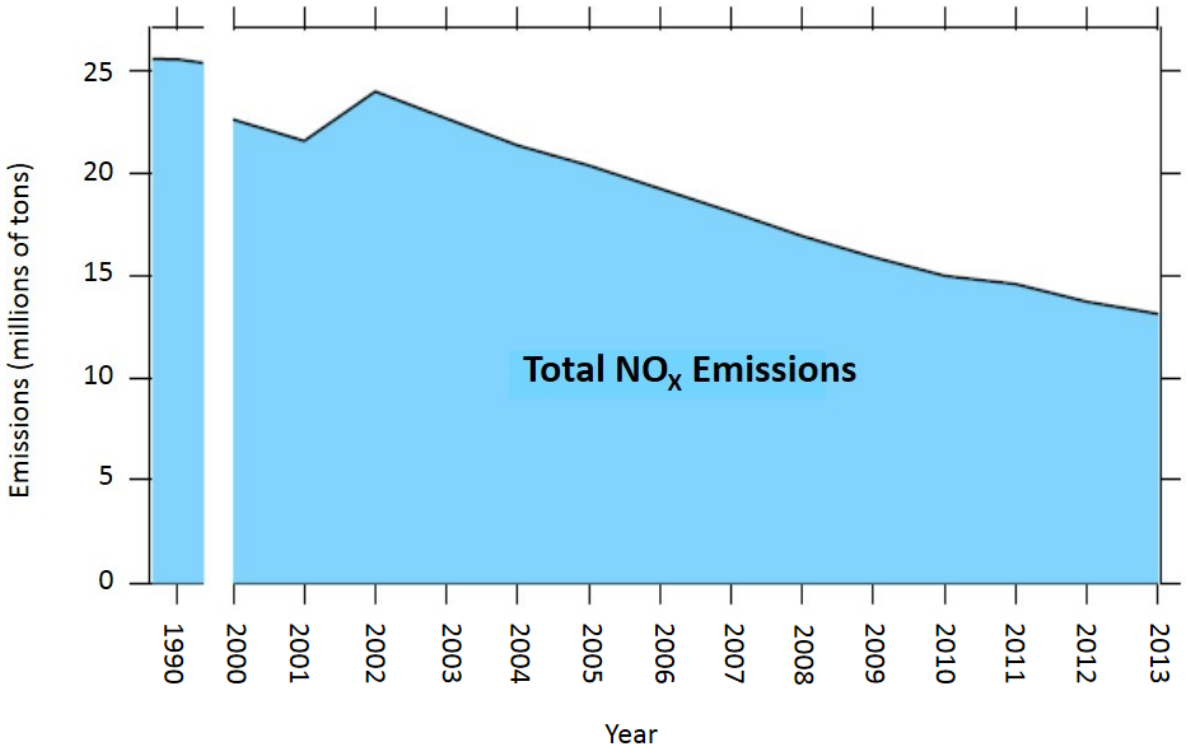
weak dependence on NO_X concentrations. Reaction of O_3 with NO in fresh motor vehicle exhaust depletes O_3 in urban cores, but O_3 can be regenerated during transport downwind of urban source areas, and additional chemical production of O_3 can occur, resulting in higher O_3 concentrations than found upwind of the urban center. Similar depletion of O_3 can occur in power plant plumes with subsequent O_3 regeneration downwind.

Brown et al. (2012) conducted a field study comparing nighttime chemistry in the plumes of two power plants in Texas, one with selective catalytic reduction (SCR) NO_X emissions controls and the other without these controls. They noted that the plume from the power plant with SCR controls did not have enough NO_X to deplete all of the O_3 present in background air. As a result, almost all of the NO_X in the plume was oxidized to NO_Z species, so the NO_X that was oxidized was not available to participate in O_3 production the next day. This situation contrasts with that in the plume from the power plant without controls. In that plume, there was minimal formation of NO_Z species. Instead, NO_X was more nearly conserved and the NO_2 that was formed from the reaction of emitted NO with O_3 photolyzed the following morning, leading to higher O_3 formation rates compared to plumes from the plant with controls.

2.3 Sources

2.3.1 Overview

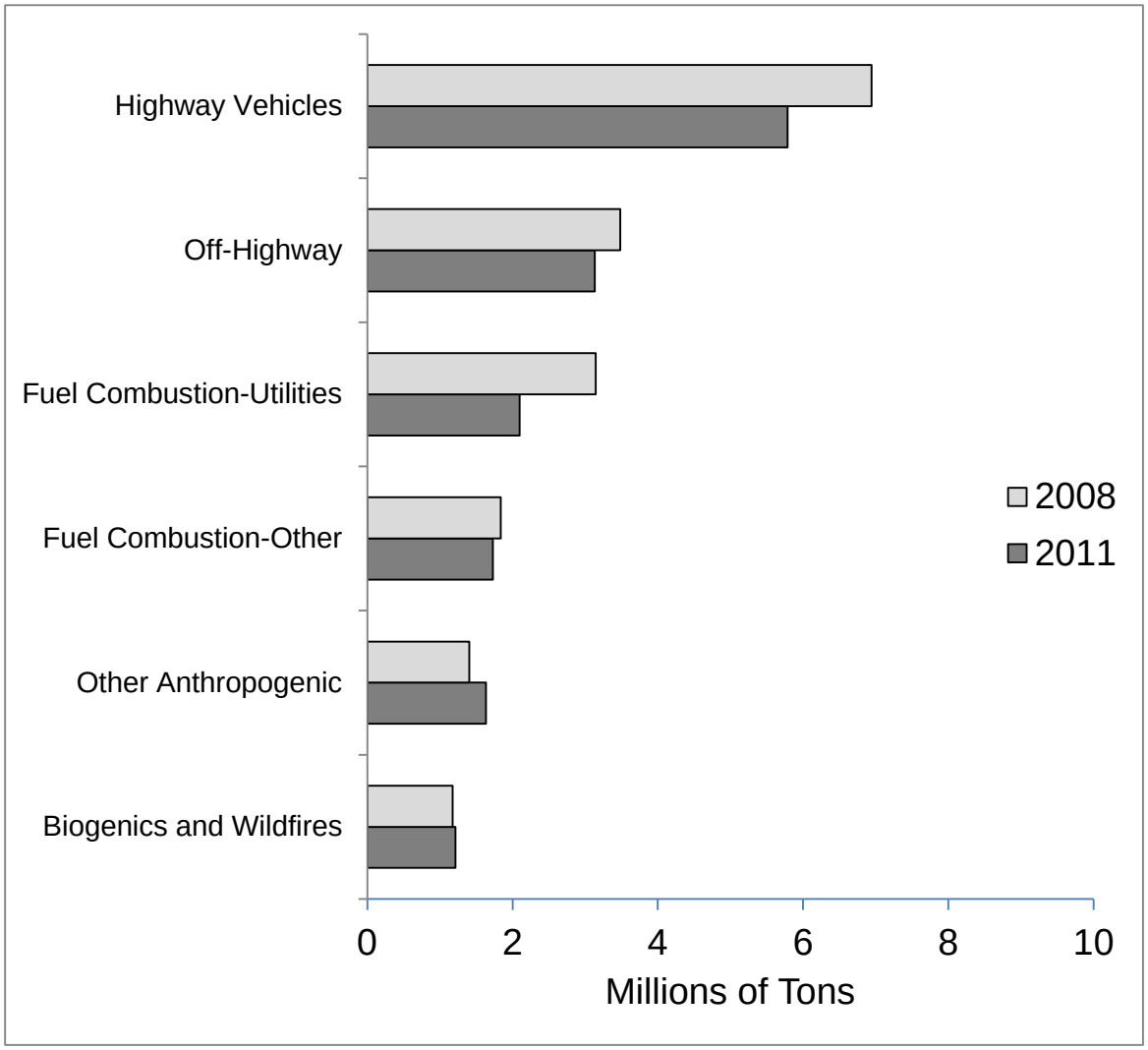
Estimated total NO_X emissions in the U.S. from all sources decreased by 49% over the period from 1990 to 2013, as shown in Figure 2-2. The NEI is a national compilation of emissions sources collected from state, local, and tribal air agencies as well as emission estimates developed by EPA from collected or estimated data by source sector. Emissions after 2011 for mobile sources and electric utilities are regularly added to the 2011 NEI, but emissions for the other sectors are based on 2011 estimates. Through this process some of the major sectors in the 2011 NEI have emission estimates more recent than 2011, while emissions from other source sectors are based on 2011 data. When emissions for later years from these sources are added, the inventory is still referred to as a version of the 2011 NEI.



Source: National Center for Environmental Assessment Analysis of 2011 National Emissions Inventory Data (U.S. EPA, 2013a)

Figure 2-2 U.S. national average NO_X (sum of nitrogen dioxide and nitric oxide) emissions from 1990 to 2013.

The major sources of NO_X in the U.S. identified from the 2008 and 2011 NEI (U.S. EPA, 2013a, 2011) are described in Figure 2-3. The values shown are U.S. nationwide averages and may not reflect the mix of sources relevant to individual exposure in populated areas. For most sources, data are generally available for all 50 states and the District of Columbia (in some cases, such as agricultural burning, data available in the NEI exclude Alaska and Hawaii). Biogenic emissions were estimated using 2011 meteorology and land-use information using the Biogenic Emission Inventory System, version 3.14 (BEIS 3.14) model. Although the BEIS domain includes Canada and Mexico, the NEI uses BEIS estimates from counties that make up the contiguous 48 states.



Source: National Center for Environmental Assessment Analysis of 2011 National Emissions Inventory Data (U.S. EPA, 2013a, 2011).

Figure 2-3 Major sources of NO_X (sum of nitrogen dioxide and nitric oxide) emissions averaged over the U.S. from the 2008 and 2011 National Emissions Inventories.

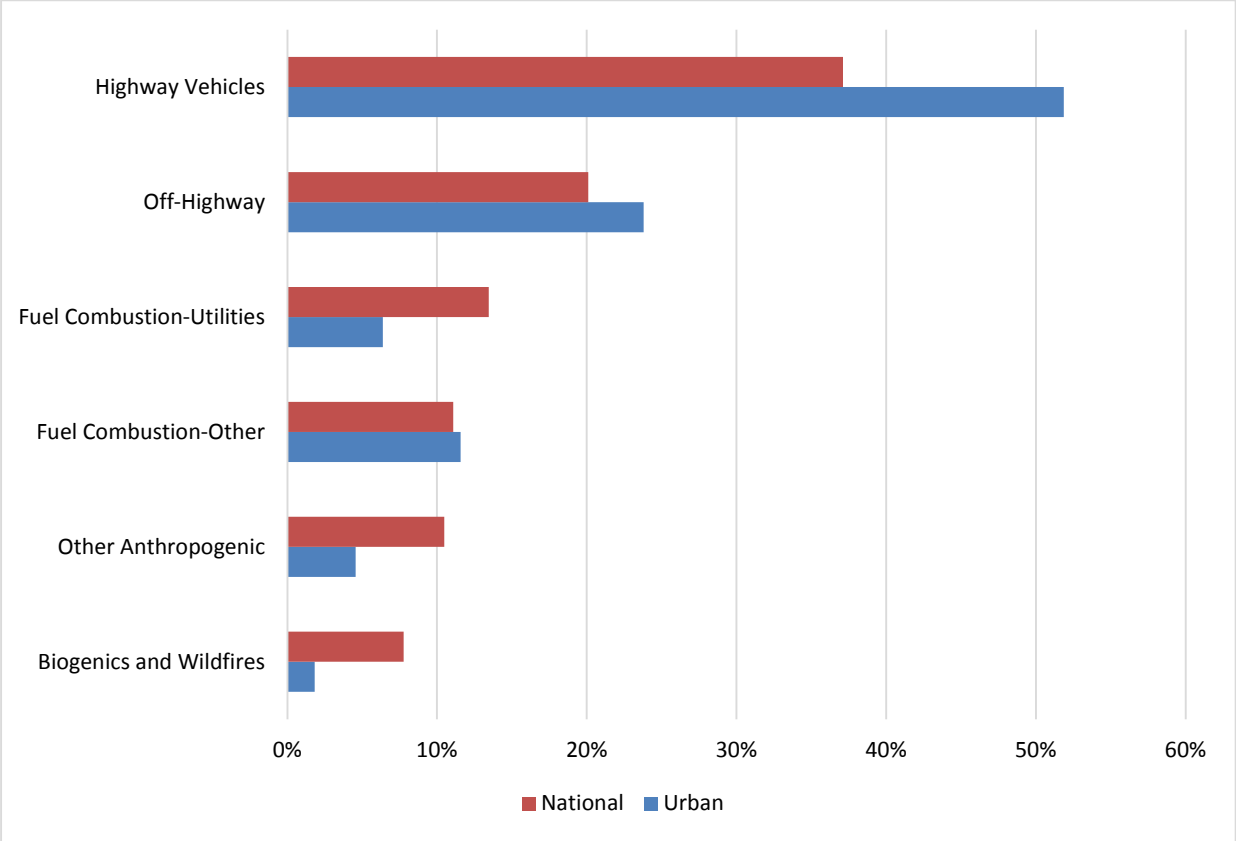
The source categories used in Figure 2-3 represent groups of similar NEI source sectors. Highway Vehicles include all on-road vehicles, including light duty as well as heavy duty vehicles, both gasoline- and diesel-powered. Off-Highway vehicles and engines include aircraft, commercial marine vessels, locomotives, and nonroad equipment. Fuel Combustion-Utilities includes electric power generating units (EGUs), which derive their power generation from all types of fuels, but is dominated by coal combustion, which accounts for 85% of all NO_X emissions from utilities in the 2011 NEI. Fuel

Combustion-Other includes commercial/institutional, industrial, and residential combustion of biomass, coal, natural gas, oil, and other fuels. Other Anthropogenic sources include field burning, prescribed fires, and various industrial processes (e.g., cement manufacturing and oil and gas production). On a national scale, field burning and prescribed fires are the greatest contributors to the Other Anthropogenic sources category. Biogenics and Wildfires include NEI emission estimates for biogenic (plant and soil) emissions and wildfires. For NO_X, biogenic emissions are dominated by soil emissions, which are one to two orders of magnitude greater than vegetation emissions.

Highway Vehicles are the largest source in the 2011 NEI, contributing 37% of the total NO_X emissions. Off-Highway vehicles and engines account for 20% of emissions, Fuel Combustion-Utilities (by EGUs) for 14%, Fuel Combustion-Other for 11%, Other Anthropogenic sources for 10%, and Biogenics and Wildfires for 8% of 2011 NEI national emissions of NO_X. Nationwide estimates of total NO_X emissions in the 2011 NEI are 13% lower than 2008 NEI estimates, decreasing from 18.0 megatons to 15.6 megatons. This decrease reflects lower emission estimates in the 2011 NEI than in the 2008 NEI for the four largest categories in Figure 2-3: 17% lower for Highway Vehicles, 10% lower for Off-Highway vehicles and engines, 33% lower for Fuel Combustion-Utilities, and 6% lower for Fuel Combustion-Other. However, estimated emissions were 17% higher for Other Anthropogenic sources, with the greatest increases observed for oil and gas production, agricultural field burning, prescribed fires, and mining. Although Biogenics and Wildfire emissions have increased as a proportion of total national emissions, anthropogenic sources (i.e., the other categories) still account for more than 90% of emissions in the 2011 NEI.

A somewhat different source mixture than the national average occurs in the most populated areas. Figure 2-4 compares contributions from different groups of sources to the 21 Core-Based Statistical Areas (CBSAs) in the U.S. with populations greater than 2.5 million, where 39% of the U.S. population lives. Relative to the national average, the urban areas have greater contributions to total NO_X emissions from both Highway Vehicle emissions and Off-Highway emissions, and smaller contributions from Fuel Combustion-Utilities (EGUs), Other Anthropogenic emissions, and Biogenics and Wildfires.

Table 2-1 provides details on source distributions from individual CBSAs.



Source: National Center for Environmental Assessment Analysis of 2011 National Emissions Inventory Data (U.S. EPA, 2013a).

Figure 2-4 Percentage contributions from major sources of the annual NO_X (sum of nitrogen dioxide and nitric oxide) emissions averaged over the 21 largest U.S. Core-Based Statistical Areas with populations greater than 2.5 million (blue—urban) compared to the national average (red—national).

Table 2-1 Source distribution of the annual NO_X (sum of nitrogen dioxide and nitric oxide) emissions in the 21 largest U.S. Core-Based Statistical Areas with populations greater than 2.5 million—2011 National Emissions Inventory.

	Highway %	Off-Highway %	Utilities %	Other Fuel %	Other Anth. %	Bio/Wildfire %
New York, NY	44.3	27.6	4.4	21.6	1.8	0.4
Los Angeles, CA	59.6	26.2	0.6	10.4	2.5	0.7
Chicago, IL	40.1	27.1	11.1	14.8	5.4	1.4
Dallas, TX	53.9	21.5	1.2	9.2	9.5	4.7
Houston, TX	46.0	25.6	3.0	9.5	11.4	4.5
Philadelphia, PA	51.2	22.5	4.0	14.7	6.7	0.9
Washington, DC	55.2	23.4	7.8	10.9	1.5	1.3
Miami, FL	50.5	32.2	7.6	4.8	2.7	2.2
Atlanta, GA	57.7	19.6	15.1	5.7	1.1	0.9
Boston, MA	46.4	27.3	2.7	18.7	4.6	0.4
San Francisco, CA	52.8	30.6	0.8	9.7	4.9	1.2
Riverside, CA	53.0	20.8	1.6	8.5	12.4	3.7
Phoenix, AZ	64.0	25.9	1.7	5.2	0.6	2.7
Detroit, MI	52.5	19.4	8.4	14.4	4.1	1.2
Seattle, WA	66.5	25.7	0.1	5.1	2.3	0.4
Minneapolis, MN	50.2	19.4	11.3	13.9	3.3	2.0
San Diego, CA	64.3	25.5	0.6	4.3	0.8	4.6
Tampa, FL	52.7	23.2	13.5	5.6	2.1	2.9
St. Louis, MO	56.9	13.6	15.3	6.7	5.2	2.4
Baltimore, MD	48.5	21.4	11.5	11.0	6.6	1.0
Denver, CO	55.6	22.5	3.2	12.2	2.9	3.6
Urban Average	53.4	23.9	6.0	10.3	4.4	2.0

Source: National Center for Environmental Assessment Analysis of 2011 National Emissions Inventory Data (U.S. EPA, 2013a).

2.3.2 Highway Vehicles

Nationally, Highway Vehicles account for about 37% of NO_X emissions, according to the 2011 NEI. In the 21 largest CBSAs in the U.S. represented in Figure 2-4, more than half of the urban NO_X emissions are from Highway Vehicles, ranging from 40% in Chicago to 67% in Seattle, and together, Highway Vehicles and Off-Highway vehicles and engines account for more than three quarters of total emissions. Other estimates of high contributions from Highway Vehicles have also been reported. For example, on-road vehicles were estimated to account for about 80% of anthropogenic NO_X concentrations in the Los Angeles area (Mcdonald et al., 2012) and 72% in the Atlanta area (Pachon et al., 2012). Highway Vehicle NO_X emissions nationwide are roughly equally split between light duty gasoline engines (48%) and heavy duty diesel engines (46%), according to the 2011 NEI. This is in spite of a national vehicle fleet distribution of more than 230 million mostly gasoline-powered light duty vehicles compared to only 10 million mostly diesel-powered heavy duty vehicles[1]. Mcdonald et al. (2012) estimated that diesel engines were the dominant on-road NO_X sources in the San Joaquin Valley in California, accounting for up to 70% of NO_X emissions. In contrast in Fulton County, GA it was estimated that 60% of on-road NO_X emissions were from gasoline vehicles and 40% from diesel (Pachon et al., 2012). Mcdonald et al. (2012) estimated that in California, gasoline engine-related NO_X emissions steadily decreased by 65% over the period from 1990 to 2010. They also found that the ratio of NO_X emission factors for heavy duty diesel versus light duty gasoline engines grew from ~3 to ~8 between 1990 and 2010 due to improved effectiveness of catalytic converters on gasoline engines.

However, NO_X emissions from on-road diesel engines in the U.S. have also decreased substantially as the result of stricter emission standards, and emissions continue to decline (Mcdonald et al., 2012). Emission standards for heavy duty diesel trucks were first established at 10.7 g/bhp-h in 1988 and the current standard of 0.20 g/bhp-h was gradually phased in for model years 2007 through 2010 (66 FR 5002), so that emission standards from heavy duty diesel trucks were reduced by more than a factor of 50 between 1988 and 2010. The current standard is achieved using a urea-based SCR catalyst in engine exhaust placed downstream of a diesel oxidation catalyst (DOC) and a catalyzed diesel particulate filter (DPF) used for PM emissions control. In extensive testing of diesel engines substantial reductions in NO_X were observed, averaging 61% relative to the 2010 standard requirements and 97% relative to the 2004 standard requirements (Southwest Research Institute, 2013). However, while total diesel NO_X

[1] https://www.fhwa.dot.gov/policyinformation/statistics/2010/vm1.cfm.

emissions have substantially decreased because of urea-based SCR control, the NO_2/NO_X ratio has increased. But these reductions for diesel emissions together with the recent final Tier 3 rule for gasoline engine emissions and lower S 41 gasoline (79 FR 23414) are likely to result in a substantial decline in NO_X emissions as newer vehicles penetrate into the on-road fleet over the next several years.

2.3.3 Off-Highway

Off-Highway engines constitute the next largest group of NO_X emission sources after Highway Vehicles, both on a nationwide basis and in large urban CBSAs as shown in Figure 2-4 and Table 2-1. Emissions from the nonroad source sector can also significantly contribute to local and national air quality. The 2011 NEI estimated that approximately 20% of nationwide NO_X was from Off-Highway engines. Zhu et al. (2011) estimated that nonroad diesel engines contribute 12% of total nationwide NO_X emissions from mobile sources. Off-Highway sources include aviation, marine, and railroad engines, as well as nonroad agricultural and industrial equipment, all of which emit NO_X through combustion processes.

Examples of nonroad equipment include farm tractors, excavators, bulldozers, and wheel loaders. Nationally, agricultural and industrial equipment accounts for more than 60% of Off-Highway NO_X emissions, mostly from diesel-powered equipment (U.S. EPA, 2013a). EPA has set a series of standards to reduce NO_X emissions from nonroad diesel, referred to as Tier 1-4 standards. The most recent standard, Tier 4, was introduced in May 2004, and the phase-in is currently underway, covering a time period between 2008 and 2015. In most cases, advanced diesel engine design, exhaust gas recirculation (EGR), and/or SCR have been used to comply with these standards, with DOC/DPFs used in several engine categories.

Although Fuel Combustion-Utilities is generally a smaller contributor to total NO_X in urban areas than it is nationally, emergency generators are an emerging concern. In urban areas emissions of NO_X have been observed to increase substantially on days of near peak electricity demand because of small natural gas and petroleum powered steam turbines used to generate additional electrical power to meet demand. These generators are classified in the NEI as nonroad equipment that fall into the category of Off-Highway engines. They are typically operated in densely populated areas. They are usually older units with higher emissions and lower stack heights than larger generators, and they are often located close to residential neighborhoods. Because of these factors, emergency generators can have substantial impacts on local air quality. For example, Gilbraith and

Powers (2013) estimated that reducing emissions from emergency generators could decrease NO_X emissions in New York City alone by 70 tons per year.

Aircraft, commercial marine transport, and locomotive emissions account for the remaining 40% of Off-Highway emissions, nationally. Aircraft includes all aircraft types used for public, private, and military purposes, classified into four types: commercial, air taxis, general aviation, and military. Airport-related NO_X emissions can significantly impact local and regional air quality. In the U.K., within a 2−3-km radius of London Heathrow Airport, Carslaw et al. (2006) reported that airport emissions can comprise up to 15% of total ambient NO_X. In Atlanta, GA, Unal et al. (2005) showed that roughly 2.6% of regional NO_X concentrations can be attributed to emissions from activities at Hartfield-Jackson International Airport. Compared to airport-related emissions of other gaseous pollutants (e.g., ammonia [NH_3], carbon monoxide [CO], sulfur dioxide [SO_2], VOC), airport NO_X emissions had the largest contribution to decreased regional air quality in Atlanta, GA.

Commercial marine vessels include boats and ships used either directly or indirectly in the conduct of commerce or military activity. Globally, marine transport is a significant source of NO_X emissions, accounting for more than 14% of all global nitrogen emissions from fossil fuel combustion (mostly NO_X) (Corbett et al., 1999). On a regional scale, the contribution of shipping emissions to total NO_X emissions is variable and can be a substantial fraction near port cities (Kim et al., 2011; Williams et al., 2009; Vutukuru and Dabdub, 2008). In Los Angeles, CA, Vutukuru and Dabdub (2008) estimated that commercial shipping contributed 4.2% to total NO_X emissions in 2002. Using the NEI-05, Kim et al. (2011) estimated that roughly 50% of NO_X concentration near the Houston Ship Channel is associated with commercial shipping emissions. However, this estimation is much higher than observed in satellite and aircraft measurements.

Locomotives powered by diesel engines are a source of NO_X emissions. Using a fuel-based approach to quantify emissions, Dallmann and Harley (2010) estimated that diesel locomotives emitted on average 50% of total NO_X from all nonroad mobile sources and roughly 10% of total NO_X from all mobile sources in the U.S. from 1996−2006 (Dallmann and Harley, 2010). Locomotives can comprise a much larger fraction of NO_X emissions for areas in or near large rail yard facilities (>90% of emissions), including NO_2 nonattainment areas (U.S. EPA, 2010). In a year-long study at the Rougemere Rail Yard facility near Dearborn, MI, 98% of NO_X emissions was attributed to locomotive operation, with only minimal impacts from other sources such as on-road mobile sources and stationary sources (U.S. EPA, 2009). Cahill et al. (2011) measured gaseous and PM pollutants during a 2-week period near the Roseville Rail Yard in Placer County, CA. They observed several transient NO_X emission events, where NO levels of 100s of ppb

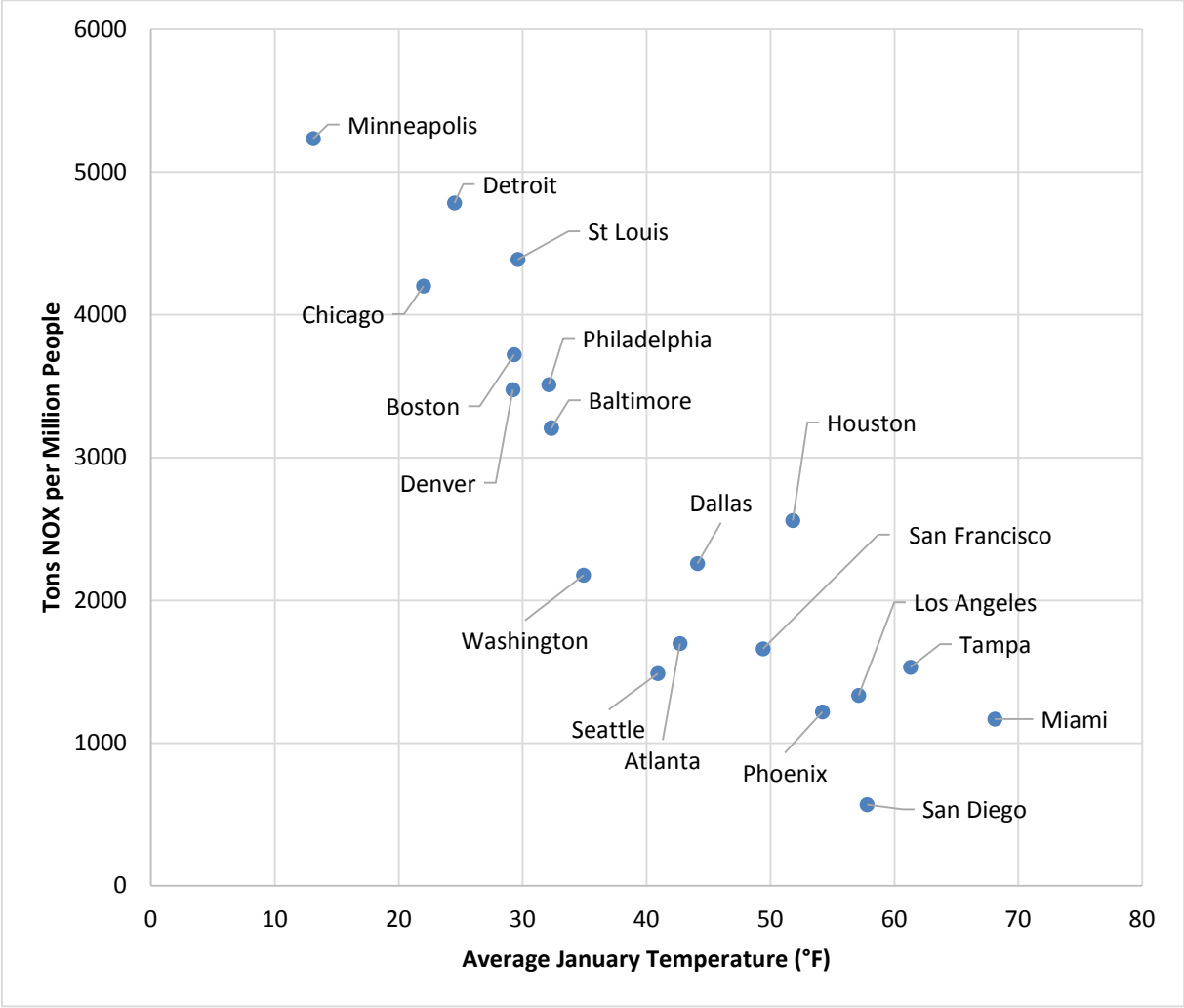
were observed downwind of the Roseville Rail Yard, which was roughly 7 times larger than the observed urban background NO.

2.3.4 Fuel Combustion—Utilities and Other

Fuel combustion for electric power generation and for industrial, residential, commercial, and institutional purposes (excluding motor vehicles and nonroad equipment) accounts for about 25% of NO_X emissions nationwide. As indicated in Figure 2-3, Fuel Combustion-Utilities accounts for the majority of fuel combustion NO_X emissions in the U. S., and about 14% of total NO_X emissions. About 85% of the fuel used for power generation is coal. However, in urban areas, fuel combustion for purposes other than electric power generation appears to be a greater source of emissions (as shown in Figure 2-4).

In contrast to Fuel Combustion-Utilties, coal accounts for only about 1% of Fuel Combustion-Other emissions. However, Fuel Combustion-Other is still dominated by fossil fuels, with natural gas contributing about 68% and oil combustion contributing about 14% of other fuel combustion emissions. Thus, even though biofuels are an important NO_X source globally (Jaegle et al., 2005), only about 10% of Fuel Combustion-Other emissions in the U.S. are due to biomass burning. For Fuel Combustion-Utilities and Fuel Combustion-Other combined, fossil fuels account for more than 90% of U.S. stationary source fuel combustion, and biomass only 4%. Combustion of biofuels accounts for only about 1% of total NO_X emissions nationwide.

Fuel Combustion-Other accounts for an additional 12% of urban NO_X emissions, but ranges as high as 22% in New York and 19% in Boston, as shown in Table 2-1. Figure 2-5 shows that the contribution Fuel Combustion-Other to overall urban NO_X emissions varies with average January temperatures. This trend suggests that winter heating is the driving factor for Fuel Combustion-Other emissions, and that in winter the Fuel Combustion-Other contribution is likely to be considerably greater than the contribution presented on an annual basis in Table 2-1, possibly rivaling Highway Vehicle emissions in winter.



Source: National Center for Environmental Assessment Analysis of 2011 National Emissions Inventory Data (U.S. EPA, 2013a).

Figure 2-5 Fuel Combustion-Other emissions vs. average ambient January temperature for the 21 largest U.S. Core-Based Statistical Areas >2.5 million population.

2.3.5 Other Anthropogenic Sources

Other Anthropogenic sources include prescribed and agricultural fires as well as industrial operations such as oil and gas production and mining. As emissions estimates from other major source categories have decreased between 2008 and 2011, emissions from these sources have increased by 17%, from about 1.4 megatons in 2008 to more than 1.6 megatons in 2011. On a national scale, agricultural burning and prescribed fires

are responsible for a large fraction of the Other Anthropogenic sources category and its the increase in national emissions for Other Anthropogenic sources between 2008 and 2011. However, in urban areas, fires are less of a contributor, and Other Anthropogenic sources are mainly industrial. Other Anthropogenic sources vary considerably among the 21 largest CBSAs with populations greater than 2.5 million. In three CBSAs, NO_X emissions from Other Anthropogenic sources exceed 10,000 tons per year. Emissions in these three CBSAs are separated by industrial sector in Table 2-2.

In Chicago, emissions from several different sources make substantial contributions to Other Anthropogenic emissions. In contrast, Other Anthropogenic emissions in Dallas are dominated by oil and gas production, which is not an important source in Chicago. The oil and gas production sector is an increasing source of NO_X, with a 2011 emission estimate of more than 600,000 tons, compared to slightly more than 400,000 tons in 2008. Pacsi et al. (2013) estimated that routine operating activities from the Barnett Shale production facility near Dallas, TX can emit roughly 30 to 46 tons NO_X/day, depending on the demand for natural gas electricity generation. Nonroutine gas flares can also result in episodic peaks of large NO_X emissions, affecting local air quality (Olaguer, 2012). Houston, TX, presents yet another variation, with anthropogenic emissions mainly from petroleum refining and chemical manufacturing. These data demonstrate that sources with relatively small nationwide or annual emissions may contribute substantially to emissions on a local scale. For example, cement manufacturing, which is listed in Table 2-2 as an important source in the local Dallas, TX, airshed, accounts for less than 1% of annual national emissions, but has been characterized by variable emissions with high peaks (Walters et al., 1999).

Table 2-2 Relative contributions to Other Anthropogenic NO_X (sum of nitrogen dioxide and nitric oxide) sources in selected cities[a].

	Chicago %	Dallas %	Houston %
Bulk gasoline terminals	nr[b]	nr	nr
Fires—agricultural field burning	nr	1	3
Fires—prescribed fires	1	4	1
Gas stations	nr	nr	nr
Industrial processes—cement manufacturing	nr	19	nr
Industrial processes—chemical manufacturing	8	nr	43
Industrial processes—ferrous metals	9	2	nr
Industrial processes—not elsewhere classified	35	6	3
Industrial processes—nonferrous metals	21	nr	nr
Industrial processes—oil & gas production	0	66	9
Industrial processes—petroleum refineries	13	nr	37
Industrial processes—pulp & paper	nr	nr	nr
Industrial processes—storage and transfer	nr	nr	nr
Miscellaneous nonindustrial not elsewhere classified	nr	nr	nr
Solvent—degreasing	nr	nr	nr
Solvent—graphic arts	nr	nr	nr
Industrial surface coating & solvent use	1	nr	1
Waste disposal	11	1	4
Total	100	100	100

[a]NO_X emissions as percent of "Other Anthropogenic sources" emissions in the Core-Based Statistical Area.
[b]nr indicates that no emissions were reported for this sector, i.e., there were no sources with emissions above the reporting threshold.
Source: National Center for Environmental Assessment Analysis of 2011 National Emissions Inventory Data (U.S. EPA, 2013a).

2.3.6 Biogenics and Wildfires

The NEI's Biogenics sector includes emissions from plants and soil. In the case of NO_X, biogenic emissions are dominated by emissions from soil. Biogenic emissions account for about 6% of total NO_X emissions in the 2011 NEI. However, spatial and temporal variability in NO_X emissions from soil leads to considerable variability in emission estimates. For example, satellite-based estimates that 15−40% of the total NO_2 column in various locations over the Great Plains region can be attributed to soil emissions in spring and summer months Hudman et al. (2010). This is consistent with geographic differences in soil contributions described in the 2011 NEI, in which soil contributions accounted for 13−34% of NO_X emissions in Iowa, Kansas, Nebraska, North Dakota, and South Dakota. About 60% of the total NO_X emitted from soils is estimated to occur in the central corn belt of the U.S. Because of low population density and the wide area over which emissions are distributed, soil emissions are a less important concern for exposure than more concentrated sources in more highly populated areas.

Biogenic emissions for the 2011 NEI were computed based on the BEIS model. The BEIS modeling domain includes the contiguous 48 states in the U.S., parts of Mexico, and Canada. The NEI uses the biogenic emissions from counties from the contiguous 48 states and DC. Both nitrifying and denitrifying organisms in the soil can produce NO_X, mainly in the form of NO. Emission rates depend mainly on the amount of applied fertilizer, soil temperature, and soil moisture. As a result, a high degree of uncertainty is associated with soil emissions, and estimates obtained from satellite observations can be greater than source-based estimates (Jaegle et al., 2005).

Emissions from wildfires can produce enough NO_X to cause local and regional degradation of air quality in some regions (Pfister et al., 2008). Roughly 15% of global NO_X emissions are from biomass burning (Denman et al., 2007). Burling et al. (2010) reported that NO_X emissions from Southwest U.S. vegetation ranged from 2.3 to 5.1 g/kg, with the majority of the NO_X present as NO. Emissions vary considerably among different species of biota, making it difficult to estimate emissions for key ecosystems, such as extratropical forests (Mcmeeking et al., 2009). Emissions from forest wildfires can be more than double per amount of energy released than for shrubs (Mebust et al., 2011).

2.3.7 Emissions Summary

Major categories of NO_X emissions in the U.S. are Highway Vehicles, Off-Highway vehicles and engines, Fuel Combustion-Utilities, Fuel Combustion-Other, Other

Anthropogenic emissions, and Biogenics and Wildfire emissions. Of these, Fuel-Combustion-Utilities and Biogenics and Wildfire emissions are less important in populated urban areas with the highest NO_2 concentrations and thus, potentially have less impact on human exposure to NO_2. Instead, in urban areas, emissions are generally dominated by Highway Vehicles and Off-Highway vehicles and engines, which make up more than three-quarters of emissions in the 21 largest CBSAs with populations greater than 2.5 million. Other sources can make important contributions. For example, in cities with average January temperatures below freezing, NO_X emissions from Fuel Combustion-Other can also be important, and episodic emissions from Other Anthropogenic sources can be important locally. However, Highway Vehicles is generally the greatest source of NO_X emissions in urban areas.

2.4 Measurement Methods

2.4.1 Federal Reference and Equivalent Methods

This discussion focuses on current methods and on promising new technologies, but no attempt is made here to cover in detail the development of these methods, or of methods such as wet chemical techniques, which are no longer in use. More detailed discussions of the histories of these methods can be found elsewhere (U.S. EPA, 1996, 1993).

NO is routinely measured using the chemiluminescence induced by its reaction with O_3 at low pressure. The Federal Reference Method (FRM) for NO_2 makes use of this technique of NO detection with a prerequisite step that is meant to reduce NO_2 to NO on the surface of a molybdenum oxide (MoO_X) substrate heated to between 300 and 400°C. On June 1, 2012, an automated Federal Equivalent Method (FEM) for measuring NO_2 using a photolytic convertor to reduce NO_2 to NO met the equivalency specifications outlined in 40 CFR Part 53 and was approved by the U.S. EPA (77FR 32632). Although photolytic convertors have lower conversion efficiencies than FRM-based analyzers, they have been found to be stable over a period of at least two months (Pollack et al., 2011). Also, two monitors using cavity attenuated phase shift (CAPS) spectroscopy have been approved more recently as FEMs (78 FR 67360, November 12, 2013; 79 FR 34734, June 18, 2014). These techniques are described below.

Because the FRM monitor cannot detect NO_2 specifically, the concentration of NO_2 is determined as the difference between the NO in the air stream passed over the heated MoO_X substrate and the NO in the air stream that has not passed over the substrate.

However, the reduction of NO_2 to NO on the MoO_X catalyst substrate also reduces other oxidized nitrogen compounds (i.e., NO_Z compounds shown in the outer box of Figure 2-1) to NO. This interference by NO_Z compounds has long been recognized following Winer et al. (1974) who found over 90% conversion of PAN, ethyl nitrate, ethyl nitrite, and n-propyl nitrate; and 6−7% conversion of nitroethane to NO with a MoO_X converter. HNO_3 produced a response but its form could not be determined. As a result of their experiments, Winer et al. (1974) concluded that "the NO_X mode of commercial chemiluminescent analyzers must be viewed to a good approximation as measuring *total* gas phase 'oxides of nitrogen,' not simply the sum of NO and NO_2." Numerous later studies have confirmed these results (Dunlea et al., 2007; Steinbacher et al., 2007; U.S. EPA, 2006; McClenny et al., 2002; Parrish and Fehsenfeld, 2000; Nunnermacker et al., 1998; Crosley, 1996; U.S. EPA, 1993; Rodgers and Davis, 1989; Fehsenfeld et al., 1987). The sensitivity of the FRM to potential interference by individual NO_Z compounds was found to be variable, depending on characteristics of individual monitors, such as the design of the instrument inlet, the temperature and composition of the reducing substrate, and on the interactions of atmospheric species with the reducing substrate.

Only recently have attempts been made to systematically quantify the magnitude and variability of the interference by NO_Z species in ambient measurements of NO_2. Dunlea et al. (2007) found an average of about 22% of ambient NO_2 (~9 to 50 ppb), measured in Mexico City over a 5-week period during the spring of 2004, was due to interference from NO_Z compounds. However, similar comparisons have not been carried out under conditions typical for state and local air monitoring stations (SLAMS) monitoring sites in the U.S. Dunlea et al. (2007) compared NO_2 measured using the conventional chemiluminescent instrument with other (optical) techniques. The main sources of interference were HNO_3 and various organic nitrates. Efficiency of conversion was estimated to be ~38% for HNO_3 and ~95% for PAN and other organic nitrates. Peak interference of up to 50% was found during afternoon hours and was associated with O_3 and NO_Z compounds, such as HNO_3 and the alkyl and multifunctional alkyl nitrates.

Lamsal et al. (2008) used data for the efficiency of reduction of NO_Z species on the MoO_X catalytic converters to estimate seasonal correction factors for NO_2 measurements across the U.S. These factors range from <10% in winter to >80% with the highest values found during summer in relatively unpopulated areas. In general, interference by NO_Z species in the measurement of NO_2 is expected to be larger downwind of urban source areas and in relatively remote areas because of the conversion of NO_2 to NO_Z during transport downwind of source areas.

In a study in rural Switzerland, Steinbacher et al. (2007) compared continuous measurements of NO_2 from a chemiluminescence analyzer with a MoO_X catalytic converter (CL/MC) with measurements from a photolytic converter (CL/PC) that reduces NO_2 to NO. They found the conventional technique using catalytic reduction (as in the FRM) overestimated the measured NO_2 compared to the photolytic technique, on average by 10% during winter and 50% during summer.

Villena et al. (2012) and Kleffmann et al. (2013) suggested that negative interference in the chemiluminescent method using the photolytic converter could occur by the production of HO_2 and RO_2 radicals by the photolysis of VOCs (e.g., glyoxal) in the photolytic converter. Subsequent to photolysis and prior to detection, these radicals react with NO that is either produced by the photolytic converter or already in the sampling stream. Because the chemiluminescent techniques rely on detection of NO, a negative artifact results. The most direct evidence for this artifact was found at high concentrations in a smog chamber containing 1 ppm glyoxal, a concentration more than a thousand times higher than typically found in ambient air. Similar indications were also found by Kleffmann et al. (2013) in a street canyon (at the University of Wuppertal, Germany) and in an urban background environment (University of Santiago, Chile). However, Kleffmann et al. (2013) also found that the magnitude of the negative artifact is smaller when a light source with a smaller spectral range is used and that this artifact is expected to be most apparent under high VOC conditions, such as in street canyons.

Within the urban core of metropolitan areas, where many of the ambient monitors are sited close to strong NO_X sources such as motor vehicles on busy streets and highways (i.e., where NO_2 concentrations are highest), the positive artifacts due to the NO_2 oxidation products are much smaller on a relative basis. Conversely, the positive artifacts are larger on a relative basis away from NO_X sources. Data for PAN and HNO_3 were collected in Houston, TX in April and May of 2009 during the Study of Houston Atmospheric Radical Precursors (SHARP) campaign (Olaguer et al., 2014). Median concentrations of PAN and HNO_3 during the afternoon were 181 (interquartile range [IQR] 94) ppt and 164 (IQR 158) ppt for NO_2 <1 ppb measured by CL/PC during SHARP; and 157 (IQR 54) ppt and 146 (IQR 402) ppt for NO_2 >10 ppb. These results suggest that potential interference in CL/MC caused by HNO_3 and PAN is estimated to be <1 ppb using the conversion efficiencies obtained by Dunlea et al. (2007) and concentrations of HNO_3 and PAN obtained during SHARP. However, the extent of interference could be expected to be most problematic for NO_2 <~1 ppb.

In summary, the current FRM for determining ambient NO_X concentrations and then reporting NO_2 concentrations by subtraction of NO is subject to a consistently positive interference by NO_X oxidation products, including HNO_3, PAN, and its analogues, and

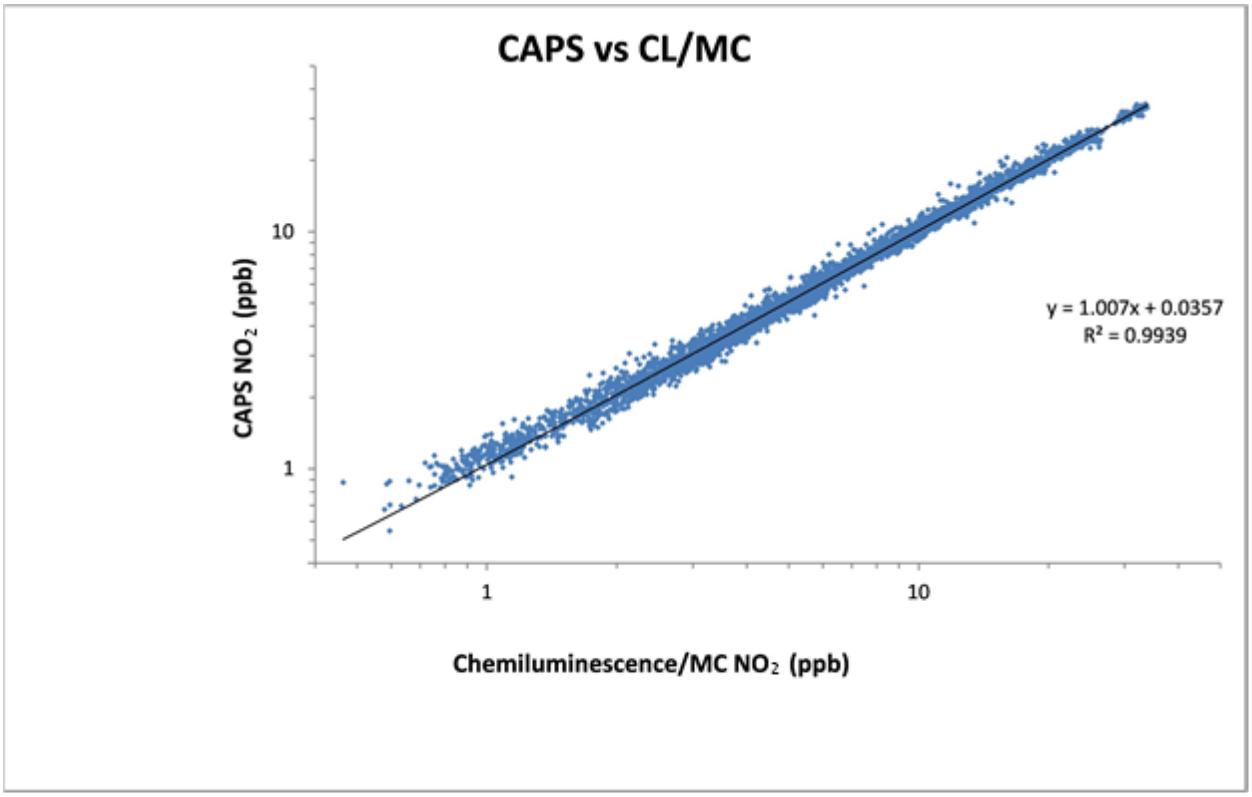
total organic nitrates ($RONO_2$). The magnitude of this positive bias is largely unknown as measurements of these oxidation products in urban areas are sparse.

2.4.2 Other Methods for Measuring Nitrogen Dioxide

Optical methods such as those using differential optical absorption spectroscopy (DOAS) or laser-induced fluorescence (LIF) are also available. However, these particular methods, even those that have been commercialized (e.g., DOAS), can be more expensive than either the FRM monitors or photolytic reduction technique and also require specialized expertise to operate; moreover, the DOAS obtains a path-integrated rather than a point measurement. Cavity attenuated phase shift (CAPS) monitors are an alternative optical approach requiring much less user intervention and expense than either DOAS or LIF (Kebabian et al., 2008). At first glance, it might appear that this technique is not highly specific to NO_2, as it is subject to interference by species that absorb at 440 nm such as 1,2-dicarbonyl compounds. However, this source of interference is expected to be small (~1%), and if necessary, the extent of this interference can be limited by shifting the detection to longer wavelengths and adjusting the lower edge of the detection band to 455 nm. In principle, detection limits could be <30 ppt for a 60 second time scale.

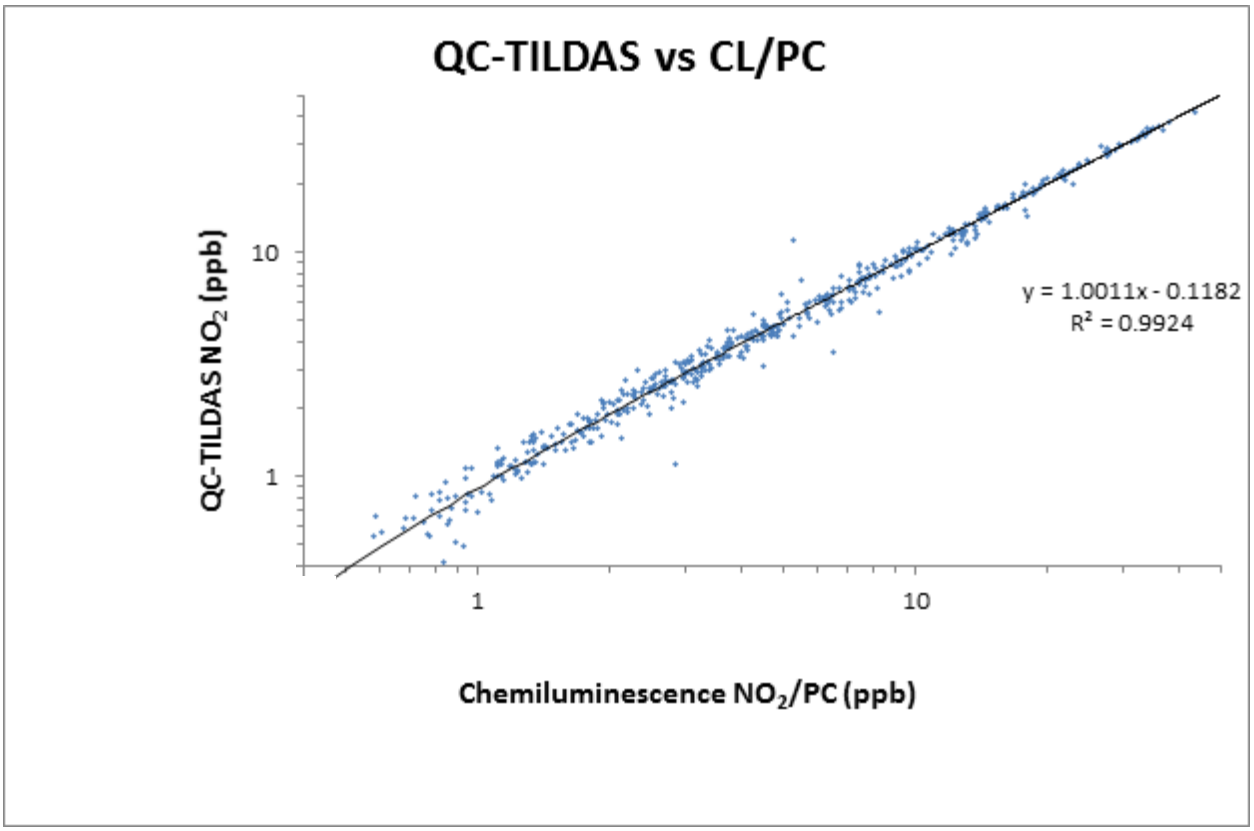
Lee et al. (2011) describe the development of a dual continuous wave mode quantum cascade – tunable infrared laser differential absorption spectrometer or QC-TILDAS to measure NO_2 and HONO simultaneously. The one-second detection limit (signal-to-noise ratio [S/N] = 3) is 30 ppt. A field comparison of measurements of NO_2 by CAPS and by chemiluminescence monitors with MoO_X converters (CL/MC) is shown in Figure 2-6. The CAPS—CL/MC (Thermo Electron 42I) data were obtained over 4 days in a parking lot located ~200 m from a major arterial highway (Route 3 in Billerica, MA) in October 2007. Figure 2-7 shows the results of a comparison of NO_2 measured by QC-TILDAS to NO_2 measured by chemiluminescence with a photolytic converter (CL/PC). The QC-TILDAS-CL/PC data were collected in Houston, TX in April and May of 2009 during the SHARP campaign (Olaguer et al., 2014). Both comparisons show very high R^2 (>0.99) and close agreement over concentrations ranging from <1 ppb to >30 ppb, and both comparisons are characterized by small nonzero intercepts. For the CAPS instrument (see Figure 2-6), slightly higher values than those reported by the CL/MC monitor are seen at concentrations <~2 ppb. Figure 2-7 shows that the QC-TILDAS obtains slightly lower concentrations than reported by CL/PC for NO_2 concentrations <~1 ppb. Although CAPS presents a practical alternative to chemiluminescence for NO_2 measurements, an important consideration in routine network deployment of CAPS or any other method that only measures NO_2 (e.g., does not measure NO) is the potential

loss of NO_X data, which has been used as an indicator for traffic- or other combustion-related pollution.



Source: National Center for Environmental Assessment, using data from Kebabian et al. (2008).

Figure 2-6 Comparison of nitrogen dioxide (NO_2) measured by cavity attenuated phase shift (CAPS) spectroscopy to NO_2 measured by chemiluminescence/MoO_X catalytic converter (MC) for 4 days in October 2007 in Billerica, MA.



Source: National Center for Environmental Assessment, using data from Lee et al. (2013)

Figure 2-7 Comparison of nitrogen dioxide (NO_2) measured by quantum cascade−tunable infrared differential absorption spectroscopy (QC-TILDAS) to NO_2 measured by chemiluminescence with photolytic converter during April and May 2009 in Houston, TX.

Villena et al. (2011) describe the development of a long path absorption photometer (LOPAP) to measure NO_2. In this technique, NO_2 is sampled in a stripping coil using a modified Griess-Saltzman reagent with the production of an azodye whose visible absorption is measured by long-path photometry. This reaction was the basis for a much earlier manual method for measuring NO_2 (Saltzman, 1954). Interference, which can be minimized by additional stripping coils, could be caused by HONO, O_3, and PAN. In an intercomparison with a CL/PC carried out over 4 days in March 2007 on the 5th floor balcony of a building at the University of Wuppertal in Germany, very good agreement (mean deviation of 2%) was obtained. Interestingly, in the entire range of measurements (~0.5 ppb to ~40 ppb) the relation between LOPAP and CL/PC can be characterized by LOPAP (ppb) = 0.984 × CL/PC − 0.42 (ppb); but if the range <6 ppb only is considered, the relation becomes LOPAP (ppb) = 0.998 × CL/PC + 0.19 (ppb).

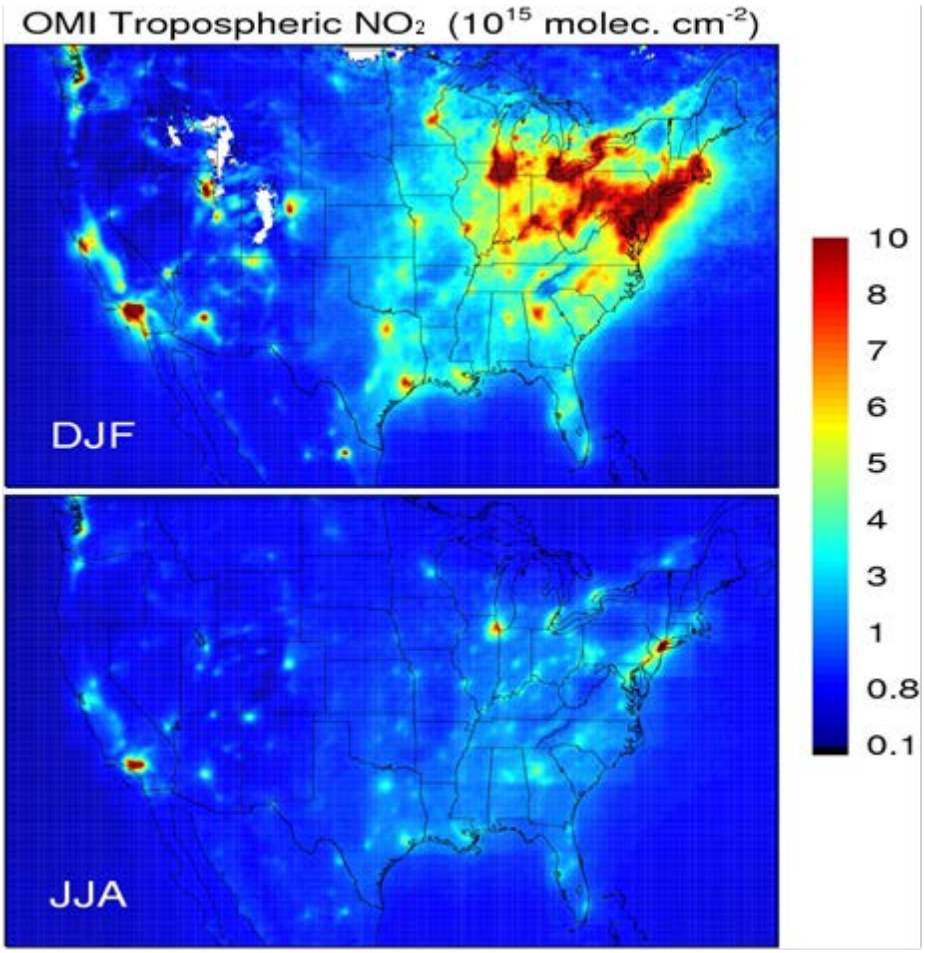
Diode laser-based cavity ring-down spectroscopy (CRDS) has also been used to detect NO_2. Fuchs et al. (2009) developed a portable instrument that relies on NO_2 absorption at 404 nm, with 22 ppt detection limit at 1 second (S/N = 2). As opposed to chemiluminescence monitors that measure NO_2 indirectly based on direct measurement of NO, NO_2 (formed by reaction of NO with excess O_3) is directly measured in CRDS. NO is then determined by subtracting NO_2 measured in the first cavity from the sum of NO_2 and NO (i.e., NO_X) measured in the second cavity. The O_3 is generated by photolysis of O_2 in the Schumann-Runge bands at 185 nm. This conversion should be much more complete than relying on the reduction of NO_2 and NO_Z species with variable efficiency on a Molybdenum oxide converter. Note that the optical methods relying on NO_2 absorption at ~400 nm described above (i.e., CAPS, CRDS) might be subject to positive interference from absorption by trace components (e.g., glyoxal and methyl glyoxal). However, absorption cross sections for these dicarbonyls are much lower than for NO_2 at this wavelength, and concentrations for these potentially interfering species are generally lower than for NO_2. Furthermore, it is possible that thermal decomposition of NO_Z species, such as PAN, in inlets or their reduction on inlet surfaces or in optical cavities can be a source of NO_2 in these or other instruments requiring an inlet.

2.4.3 Satellite Measurements of Nitrogen Dioxide

Remote sensing by satellites is an approach that could be especially useful in areas where surface monitors are sparse. Retrieving NO_2 column abundances from satellite data involves three steps: (1) determining the total NO_2 integrated line-of-sight (slant) abundance by spectral fitting of solar backscatter measurements, (2) removing the stratospheric contribution by using data from remote regions where the tropospheric column abundance[1] is small, and (3) applying an air mass factor to convert tropospheric slant columns into vertical columns. The retrieval uncertainty is largely determined by Steps 1 and 2 over remote regions where there is little tropospheric NO_2, and by Step 3, over regions of elevated tropospheric NO_2 (Boersma et al., 2004; Martin et al., 2002). Satellite retrievals are largely limited to cloud fractions <20%. The algorithm used here to derive the tropospheric column of NO_2 is given in Bucsela et al. (2013). This algorithm was used to generate the maps in Figure 2-8 for 2005 to 2007 and in Figure 2-9 for 2010 to 2012 showing seasonal average NO_2 columns obtained by the Ozone Monitoring Instrument (OMI) on the AURA satellite. Other algorithms, for example the Berkeley High-Resolution product (Russell et al., 2011), which is based on higher resolution input

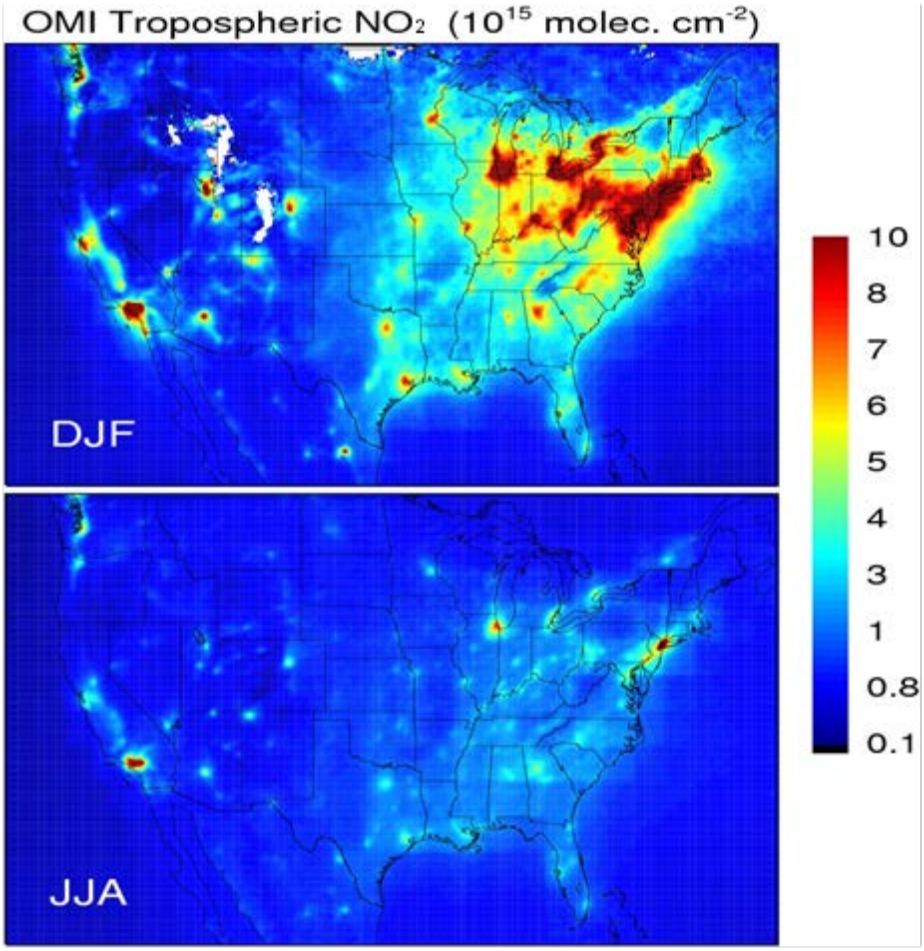
[1] Column refers to the integrated line-of-sight abundance in a unit cross section, such that its units are molecules/cm^2.

fields (topography, albedo, and NO_2 vertical profile shape) in the retrievals, can reduce the uncertainty in the measurements.



Note: Images shown were constructed by Dr. Lok Lamsal of Universities Space Research Association from data obtained by the OMI instrument on the AURA satellite (http://aura.gsfc.nasa.gov/scinst/omi.html) using the algorithm described in Bucsela et al. (2013). Top panel (winter; DJF: December, January, February). Lower panel (summer; JJA: June, July, August).

Figure 2-8 Seasonal average tropospheric column abundances for nitrogen dioxide (NO_2: 10^{15} molecules/cm^2) derived by ozone monitoring instrument (OMI) for winter (upper panel) and summer (lower panel) for 2005 to 2007.



Note: Images shown were constructed by Dr. Lok Lamsal of Universities Space Research Association from data obtained by the OMI instrument on the AURA satellite (http://aura.gsfc.nasa.gov/scinst/omi.html) using the algorithm described in Bucsela et al. (2013). Top panel (winter; DJF: December, January, February). Lower panel (summer; JJA: June, July, August).

Figure 2-9 Seasonal average tropospheric column abundances for nitrogen dioxide (NO_2: 10^{15} molecules/cm^2) derived by ozone monitoring instrument (OMI) for winter (upper panel) and summer (lower panel) for 2010 to 2012.

Areas of high column NO_2 abundance are found over major source areas during both 2-year periods shown in Figures 2-8 and 2-9. High column abundances are found over many major urban areas, such as Los Angeles, CA; Houston, TX; Chicago, IL; and New York City, NY; and over major power plant complexes such as the Four Corners (Colorado, New Mexico, Arizona, and Utah) and the Ohio River Valley. A diffuse area with column abundances above background is found over the Bakken Shale fields in northwestern North Dakota in winter. However, in general, the area of very high column abundance of NO_2 (shown in red) is smaller in the 2010 to 2012 composite than from

2005 to 2007. The photochemical lifetime of NO_2 is longer in winter than in summer resulting in lower column abundances of NO_2 in summer than in winter during the 2-year periods shown in Figures 2-8 and 2-9.

Because satellite instruments do not return surface concentrations directly, information on NO_2 surface concentrations must be inferred from the column measurements. Lamsal et al. (2008) and Lamsal et al. (2010) combined satellite data for column NO_2 from OMI with results from the GEOS-Chem global scale chemistry-transport model to derive surface concentrations of NO_2 (see Figure 2-13 for an example of seasonally averaged surface NO_2 concentrations derived by this method). This method accounts for the feedback from the abundance of NO_2 on the lifetime of NO_2. Note however that data are collected only during the daily satellite overpass in early afternoon and this method has only been applied for the time of satellite overpass. Some other means must be used to extend the time period of applicability, for example by scaling the afternoon value by the diel variation in a model, provided the model bias in simulating NO_2 has been characterized over the times of interest in a 24-hour cycle (Stavrakou et al., 2008; Kim et al., 2006).

2.4.4 Measurements of Total Oxides of Nitrogen in the Atmosphere

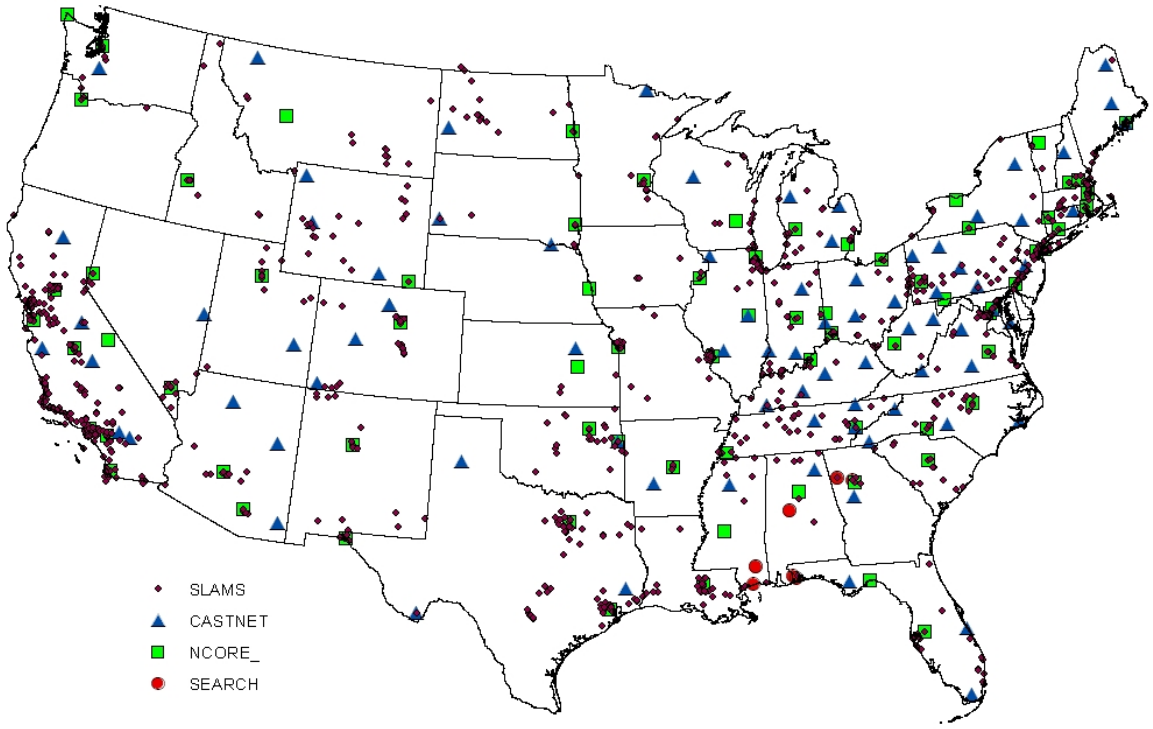
Commercially available NO_X monitors have been converted to NO_Y monitors by moving the MoO_X convertor to interface directly with the sample inlet. Because of losses on inlet surfaces and differences in the efficiency of reduction of NO_Z compounds on the heated MoO_X substrate, NO_X concentrations cannot be considered as a universal surrogate for NO_Y. However, most of the NO_Y is present as NO_X close to sources of fresh combustion emissions, such as highways during rush hour. To the extent that all the major oxidized nitrogen species can be reduced quantitatively to NO, measurements of NO_Y concentrations should be more reliable than those for NO_X concentrations, particularly at typical ambient levels of NO_2. Exceptions might apply in locations near NO_X sources, where NO_X measurements are likely to be less biased and confidence in measurement accuracy increases.

Alternatively, multiple methods for observing components of NO_Y have been developed and evaluated in some detail. As a result of these methods, as applied in the field and the laboratory, knowledge of the chemistry of odd-N species has evolved rapidly. Recent evaluations of methods can be found in Arnold et al. (2007) for HNO_3, (Wooldridge et al., 2010) for speciated PANs, and Pinto et al. (2014) for HONO. However, it is worth reiterating that the direct measurements of NO are still the most reliable method. Reliable measurements of NO_Y and NO_2 concentrations, especially at the low concentrations

observed in many areas remote from sources, are also crucial for evaluating the performance of three-dimensional, chemical transport models of oxidant and acid production in the atmosphere.

2.4.5 Ambient Sampling Network Design

Figure 2-10 shows routinely operating monitoring sites for approximately 500 ambient air oxidized nitrogen across the U.S. Four networks are highlighted: (1) regulatory-based SLAMS designed to determine National Ambient Air Quality Standards (NAAQS) compliance, (2) Clean Air Status and Trends Network (CASTNET) which provides weekly averaged values of total nitrate (HNO_3 and pNO_3) in rural locations, (3) National Core (NCORE) network of approximately 70 stations designed to capture area-representative multiple-pollutant concentrations that provides routinely measured NO_Y, and (4) the Southeast Aerosol Research Characterization (SEARCH), a privately funded network of 6−10 sites that provides direct measurements of true NO_2 as well as NO_Y and other nitrogen species (oxidized and reduced forms).



Note: SLAMS = State and Local Air Monitoring Stations, CASTNET = Clean Air Status and Trends Network, NCORE = National Core Network, SEARCH = Southeast Aerosol Research Characterization.

Source: U.S. Environmental Protection Agency.

Figure 2-10 Map of monitoring sites for oxides of nitrogen in the U.S. from four networks.

With the exception of 4−6 sites in the SEARCH network, direct or true NO_2 is not measured routinely (Hansen et al., 2003). The regulatory networks rely on chemiluminescence difference techniques that provide NO concentration directly and report a calculated NO_2 concentration as the difference between NO_X concentration and NO concentration as discussed above. Criteria for siting ambient monitors are given in the State and Local Air Monitoring Stations/National Air Monitoring Stations/Photochemical Monitoring Stations (SLAMS/NAMS/PAMS) Network Review Guidance (U.S. EPA, 1998). NO_2 monitors are meant to be representative of several scales: microscale (in close proximity, up to 100 m from the source), middle (several city

blocks, 100 to 500 m), neighborhood (0.5 to 4 km), and urban (4 to 50 km) (40 CFR Part 58, Appendix D). Microscale to neighborhood-scale monitors are used to determine the highest concentrations and source impacts, while neighborhood- and urban-scale monitors are used for monitoring population exposures.

EPA promulgated new minimum monitoring requirements in February of 2010, mandating that state and local air monitoring agencies install near-road NO_2 monitoring stations within the near-road environment in larger urban areas. Under these new requirements, state and local air agencies will operate one near-road NO_2 monitor in any CBSA with a population of 500,000 or more, and two near-road NO_2 monitors in CBSAs with 2,500,000 or more persons or roadway segments carrying traffic volumes of 250,000 or more vehicles. These monitoring data are intended to represent the highest population exposures that may be occurring in the near-road environment throughout an urban area over the averaging times of interest. The near-road NO_2 network is intended to focus monitoring resources on near-road locations where peak ambient NO_2 concentrations are expected to occur as a result of on-road mobile source emissions and to provide a clear means to determine whether the NAAQS is being met within the near-road environment throughout a particular urban area. The network is now being phased in, and the first phase became operational in January of 2014.

2.5 Ambient Concentrations of Oxides of Nitrogen

This section provides a brief overview of ambient concentrations of NO_2 and associated oxidized N compounds in the U.S.; it also provides estimates of background concentrations used to inform risk and policy assessments for the review of the NAAQS.

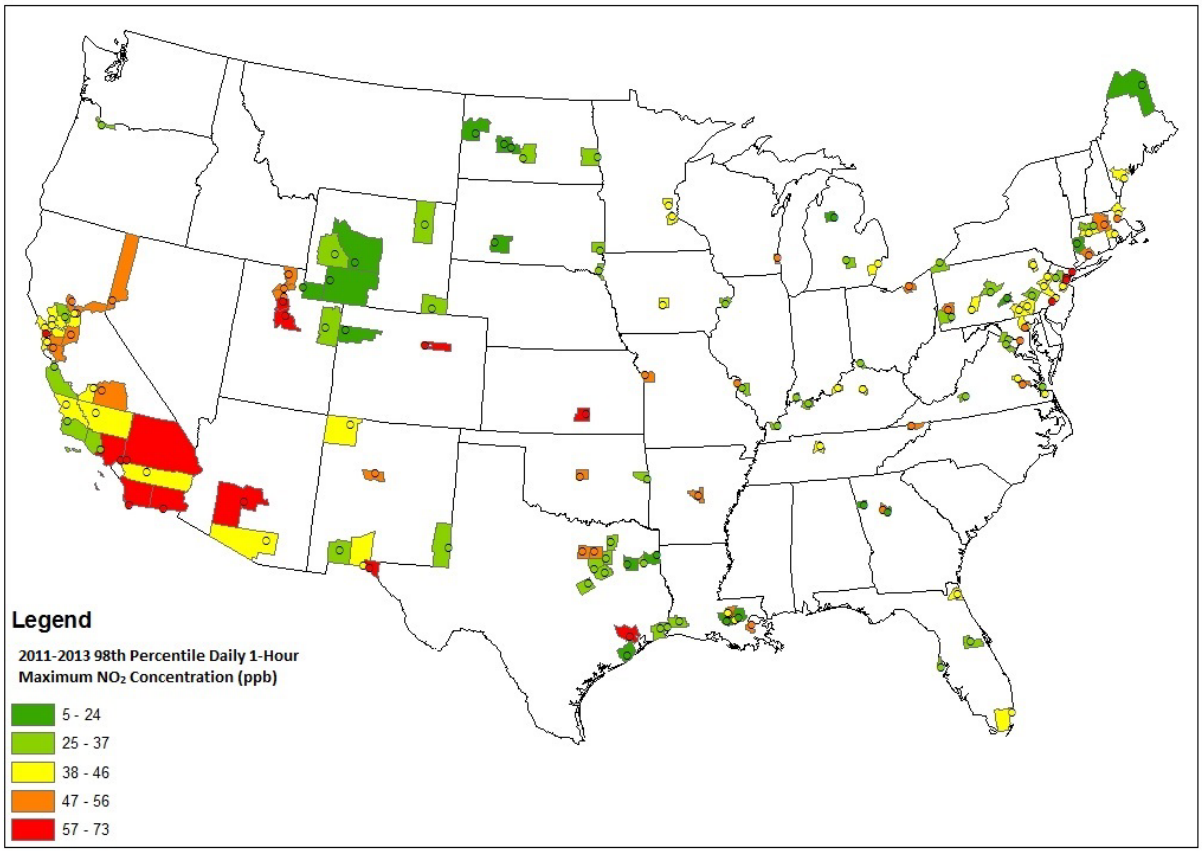
In the 2008 ISA for Oxides of Nitrogen, NO_2 concentrations were summarized with an explanation that the annual average NO_2 concentrations of ~15 ppb reported by the regulatory monitoring networks were well below the level of the NAAQS (53 ppb), but that the 1-hour daily maximum concentrations can be much greater in some locations, especially in areas with heavy traffic (U.S. EPA, 2008a).

2.5.1 National Scale Spatial Variability

In the 2008 ISA for Oxides of Nitrogen, data were analyzed for NO_2 measured at monitoring sites located within urbanized areas in the U.S. (U.S. EPA, 2008a). NO_2 concentrations were ~15 ppb for averaging periods ranging from a day to a year, and the 1-hour daily maximum NO_2 concentration was ~30 ppb, about twice as high as the

24-h avg. Data on NO_Z concentrations were very limited but indicated that HNO_3 and HONO concentrations were considerably lower than NO_2 concentrations. HNO_3 concentrations ranged from <1 to >10 ppb and HONO concentrations were reported as <1 ppb even under heavily polluted conditions. HNO_3 concentrations were highest downwind of an urban center. HONO concentrations were present in areas with traffic, at concentrations several percent of NO_2 concentrations (U.S. EPA, 2008a). Field study results indicating much higher NO_Z concentrations than NO_X concentrations in relatively unpolluted rural air were also described (U.S. EPA, 2008a).

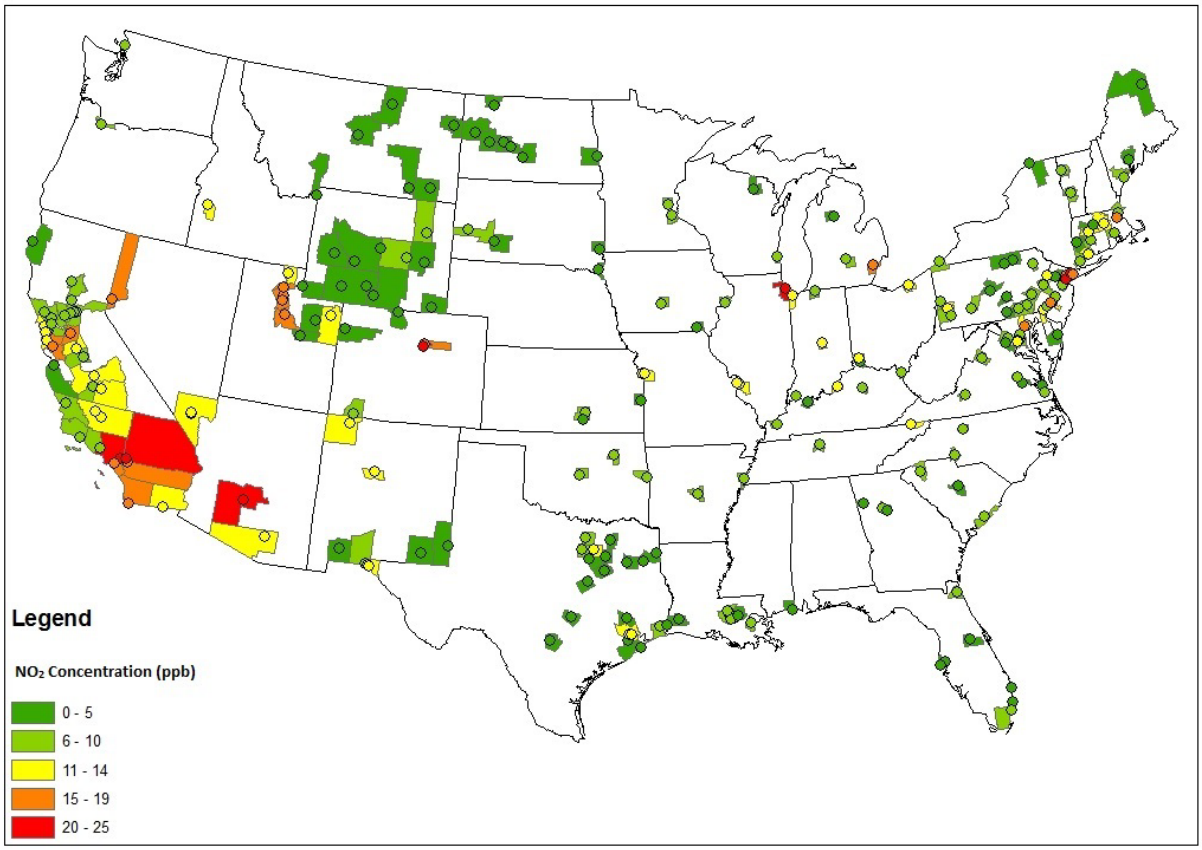
Figure 2-11 presents a national map of the 98th percentile of 1-hour daily maximum concentrations based on 2011−2013 data, and Figure 2-12 presents annual average NO_2 concentrations based on 2013 calendar year data. In both figures data are included only for monitors with 75% of days reported for each calendar quarter over the 3-year period and only for days with 75% of all hours reported. Because of the completeness requirements, there are cases where sites have valid annual average data but not valid 1-hour daily maximum concentrations. The highest concentrations are in the Northeast Corridor, California, and other urbanized regions, and the lowest concentrations are in sparsely populated regions, most notably in the West. These observations are consistent with those described in the 2008 ISA (U.S. EPA, 2008a). Tables 2-3 and 2-4 present summary data on 1-hour daily maximum and annual average concentrations for the period 2011−2013. Table 2-3 also includes summary data by individual years and by quarters averaged over the 3 years, as well as summary data for selected urban areas that are examined in recent U.S. epidemiologic studies on the health effects of NO_2 (Chapters 5 and 6).



Note: Concentrations indicated are the highest concentration in the county, and not intended to represent county-wide concentrations.

Source: U.S. Environmental Protection Agency Analysis of data from state and local air monitoring stations in 2014.

Figure 2-11 98th percentiles of U.S. 1-hour daily maximum nitrogen dioxide (NO_2) concentrations (ppb) for 2011−2013.



Note: Concentrations indicated are the highest concentration in the county, and not intended to represent county-wide concentrations.

Source: U.S. Environmental Protection Agency 2014 analysis of data from state and local air monitoring stations.

Figure 2-12 U.S. annual average nitrogen dioxide (NO_2) concentrations (ppb) for 2013.

Table 2-3 Summary statistics for 1-hour daily maximum nitrogen dioxide (NO_2) concentrations based on state and local air monitoring stations (ppb).

				Percentiles								
	Year	*n*	Mean	1	5	10	25	50	75	90	95	99
NO_2	2011−2013	390,713	19	1	2	3	8	16	27	38	44	55
NO_2	2011	127,610	19	1	2	4	8	16	28	39	45	57
NO_2	2012	130,170	18	1	2	3	8	16	27	37	43	55
NO_2	2013	132,933	18	1	2	3	7	15	26	37	43	54
NO_2	1st Quarter	94,612	22	1	2	4	10	20	32	41	47	58
NO_2	2nd Quarter	96,962	16	1	2	3	6	12	22	33	40	52
NO_2	3rd Quarter	99,125	16	1	2	3	7	13	22	32	38	50
NO_2	4th Quarter	100,101	21	1	2	4	10	20	31	40	46	58
Atlanta[a]	2011−2013	3,215	13	2	2	3	4	8	18	34	41	52
Atlanta—all[b]	2011−2013	3,215	13	2	2	3	4	8	18	34	41	52
Boston[a]	2011−2013	6,246	25	5	8	11	16	24	32	39	44	52
Boston—all[b]	2011−2013	10,986	19	1	3	4	9	17	28	36	41	49
Denver[a]	2011−2013	966	38	6	14	22	30	39	46	53	58	68
Denver—all[b]	2011−2013	2,184	41	9	21	26	33	41	48	55	61	73
Houston[a]	2011−2013	9,525	21	1	3	5	10	18	29	45	45	56
Houston—all[b]	2011−2013	16,610	18	1	3	4	8	15	26	36	43	54
Los Angeles[a]	2011−2013	8,328	27	4	7	10	16	26	36	44	49	60
Los Angeles—all[b]	2011−2013	30,612	28	4	7	10	17	28	38	47	52	63
New York[a]	2011−2013	9,469	27	1	3	5	13	27	38	47	52	64
New York—all[b]	2011−2013	11,803	27	1	3	5	15	27	38	47	52	63
Seattle[a]	2011−2013	none										
Seattle—all[b]	2011−2013	1,649	13	3	4	5	7	11	17	24	31	46

[a]City name only rows contain hourly data that meet 75% completeness criteria.
[b]City—all rows report data regardless of whether completeness criteria are met.
Source: Office of Air Quality Planning and Standards and National Center for Environmental Assessment Analysis of Air Quality System Network Data 2011−2013.

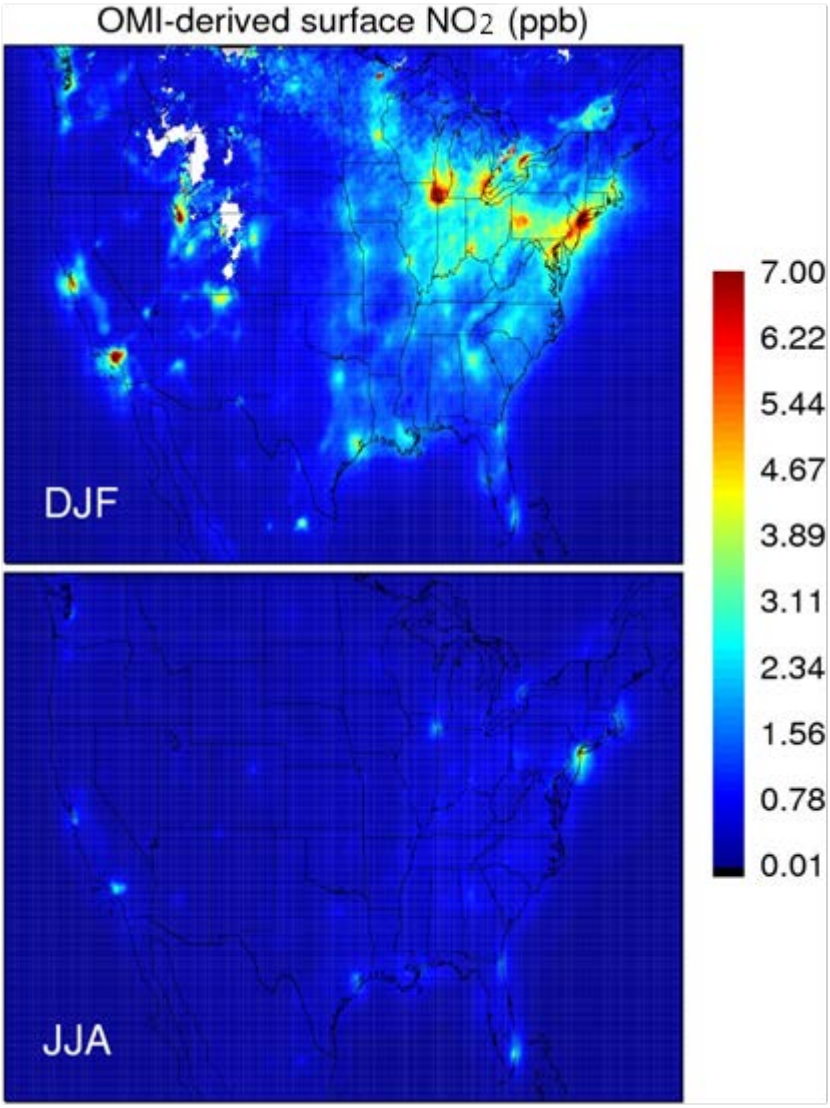
Table 2-4 Summary statistics for nitrogen dioxide (NO_2), nitric oxide (NO), and NO_X (sum of NO_2 and NO) annual average concentrations based on state and local air monitoring stations (ppb).

Pollutant	Year	n	Mean	Min	Percentiles									
					1	5	10	25	50	75	90	95	99	Max
NO_2														
NO_2	2011−2013	1,041	8.6	0.1	0.6	1.4	2.2	4.3	8.1	11.8	16.2	18.6	22.5	26.0
NO_2	2011	338	9.0	0.2	0.6	1.5	2.5	4.7	8.4	12.3	16.8	19.6	23.9	25.3
NO_2	2012	347	8.5	0.1	0.7	1.4	2.2	4.2	8.1	11.6	15.9	18.6	22.1	26.0
NO_2	2013	356	8.3	0.3	0.6	1.3	2.1	4.2	7.7	11.6	15.8	18.1	21.8	24.6
NO														
NO	2011−2013	1,127	4.8	0.01	0.02	0.1	0.3	1.0	2.9	6.8	11.3	15.3	25.3	48.8
NO	2011	363	5.0	0.01	0.03	0.1	0.3	1.1	3.1	7.4	12.7	15.1	23.9	46.9
NO	2012	377	4.8	0.01	0.03	0.1	0.2	1.0	2.9	6.6	10.9	15.0	27.7	48.8
NO	2013	387	4.6	0.01	0.03	0.1	0.2	1.0	2.6	6.5	11.0	15.7	21.5	36.2
NO_X														
NO_X	2011−2013	1,011	13.4	0.1	0.6	1.5	2.6	5.4	11.3	18.6	28.1	31.8	45.4	68.4
NO_X	2011	320	13.7	0.1	0.6	1.5	2.6	5.8	11.8	19.3	28.9	31.7	44.8	68.4
NO_X	2012	342	13.2	0.1	0.7	1.3	2.6	5.2	11.2	18.5	26.8	31.3	48.9	61.0
NO_X	2013	349	13.3	0.3	0.7	1.7	2.6	5.4	10.9	18.3	28.0	32.7	44.1	61.7

Source: Office of Air Quality Planning and Standards and National Center for Environmental Assessment analysis of Air Quality System network data 2011−2013.

The relatively short lifetime of NO_2 with respect to conversion to NO_Z species results in gradients and low concentrations away from major sources that are not adequately captured by the existing monitoring networks (see Figure 2-10 for location of monitoring sites). Satellite data coupled with model simulations might be more useful for showing large-scale features in the distribution of NO_2. Winter and summer seasonal average NO_2 concentrations for 2009−2011 derived from the OMI instrument on the AURA satellite and the GEOS-Chem global, three-dimensional chemistry-transport model are shown in Figure 2-13. In this method, integrated vertical column abundances of NO_2 derived from the OMI instrument are scaled to surface mixing ratios using scaling factors derived from GEOS-Chem [see (Lamsal et al., 2010; Lamsal et al., 2008); also see Section 2.4 for more complete descriptions of the method]. A nested version of GEOS-Chem at 50 km × 50 km horizontal resolution is used in this method. A description of the capabilities of GEOS-Chem and other three-dimensional chemistry transport models is given in the ISA for Ozone and Related Photochemical Oxidants (U.S. EPA, 2013b).

Large variability in NO_2 concentrations is apparent in Figure 2-13. As expected, the highest NO_2 concentrations are seen in large urban regions, such as in the Northeast Corridor, and lowest values are found in sparsely populated regions located mainly in the West. Minimum hourly values can be less than ~10 ppt, leading to a large range between maximum and minimum concentrations. Although overall patterns of spatial variability are consistent with the current understanding of the behavior of NO_2, not much confidence should be placed on values <~100 ppt due to limitations in the satellite retrievals. NO_2 concentrations tend to be higher in January than in July, largely reflecting lower planetary boundary layer heights in winter. Such seasonal variability is also evident on a local scale, as measured by surface monitors. For example, in Atlanta, GA, NO_X measurements also exhibited higher concentrations in winter and lower concentrations in summer, when NO_X is more rapidly removed by photochemical reactions (Pachon et al., 2012).



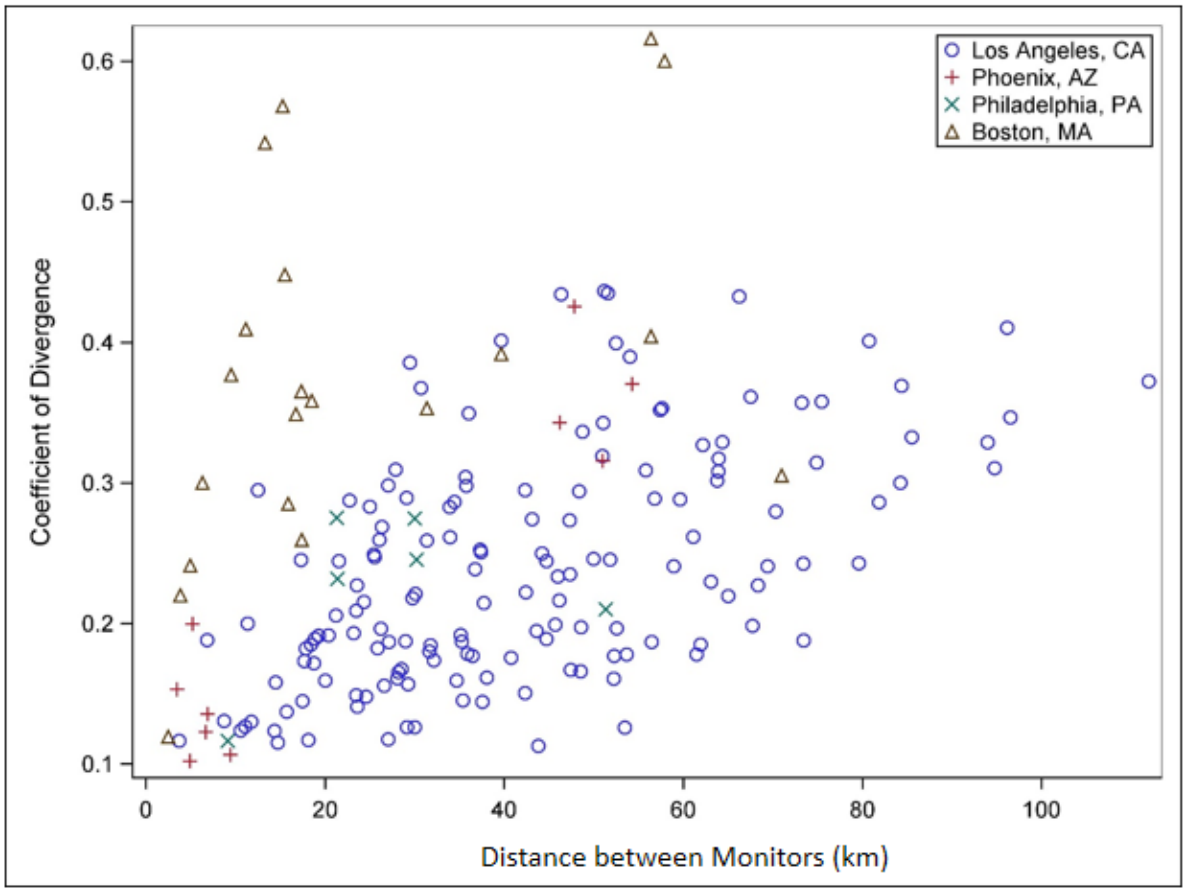
Note: Images shown were constructed by Dr. Lok Lamsal of Universities Space Research Association from data obtained by the OMI instrument on the AURA satellite (http://aura.gsfc.nasa.gov/scinst/omi.html) using the algorithm described in Bucsela et al. (2013). Output from the GEOS-Chem, global-scale, three-dimensional, chemistry-transport model to derive surface concentration fields from the satellite data as described in Lamsal et al. (2008) and Lamsal et al. (2010).

Top panel (winter; DJF: December, January, February). Lower panel (summer; JJA: June, July, August).

Figure 2-13 Seasonal average surface nitrogen dioxide (NO_2) concentrations in ppb for winter (upper panel) and summer (lower panel) derived by ozone monitoring instrument (OMI)/Goddard Earth Observing System (GEOS)-Chem for 2009−2011.

2.5.2 Urban-Scale Spatial Variability

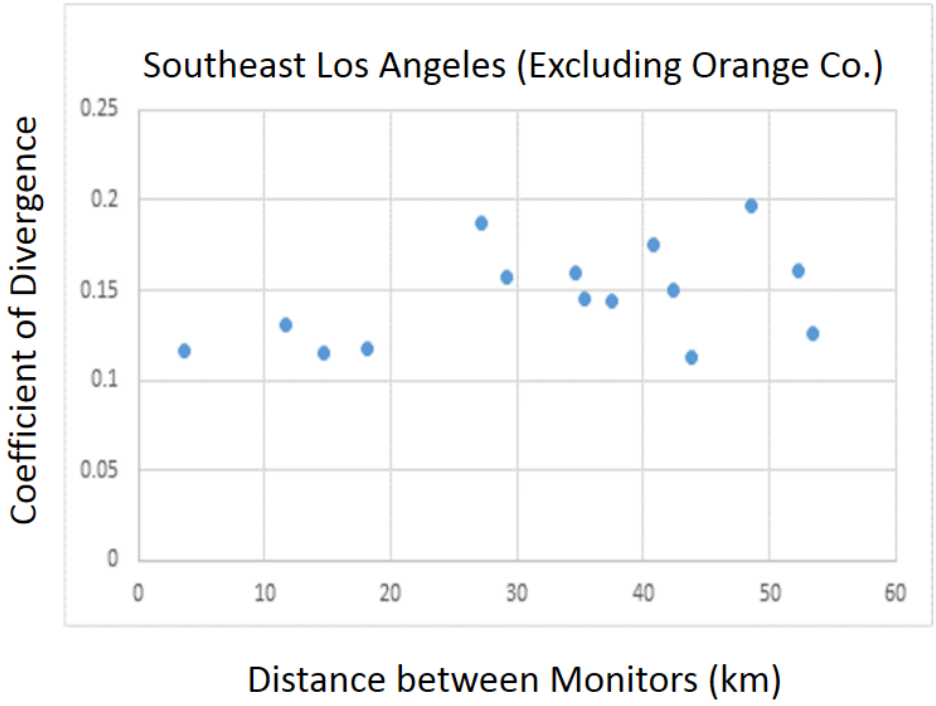
Figure 2-14 describes 1-hour daily maximum concentration agreement between pairs of SLAM monitors from 2011−2013 for selected U.S. CBSAs with more than one monitor. Agreement is expressed as coefficient of divergence (COD), which has been widely used to assess spatial variability of air pollutant concentrations (U.S. EPA, 2008a; Wilson et al., 2005; Pinto et al., 2004). In practical terms, a COD = 0 indicates perfect agreement and COD values increase as spatial variability increases. COD values in Figure 2-14 generally range from about 0.1 to 0.4, with a few higher values. This indicates a range of variability across CBSAs from fairly uniform to a moderate degree of variability (Wilson et al., 2005). At first glance, distance between sites does not appear to be an important factor for explaining variability between site pairs on an urban scale. However, for extremely short distances, a trend with distance is observed, especially for data within the same city. For example, for Boston, MA, the six observations with the shortest distances between them exhibit a trend of increasing COD with distance, from about 3 km to about 10 km. These data are for the four sites closest to the city center. As indicated by the COD values, there is a substantial degree of variability for all but the closest sites, with CODs ranging above 0.4 even for comparison between site pairs near the city center.



Source: National Center for Environmental Assessment 2014 analysis of Air Quality System network data.

Figure 2-14 Coefficient of divergence between monitor pairs in four U.S. cities.

A similar trend is observed for Los Angeles, CA but over a broader scale. The highest COD increases somewhat regularly with distance, up to about 40 km. Also, for all sites within 15 km of each other, a fairly high degree of agreement is observed. Another major difference between Boston and Los Angeles is that in Los Angeles good agreement (COD ~0.1) is often observed between sites up to 50 km or more apart, suggesting that other factors besides distance are important. Five of the Los Angeles monitors (Main Street Los Angeles, Burbank, Pasadena, Pomona, and Long Beach North) form a subset of monitors with distinctly lower variability than the area as a whole, with low CODs for each possible combination of monitors within this group, as shown in Figure 2-15. Other sites near the ocean or mountains exhibit poorer agreement with these monitors, even if the distances between them are shorter.



Source: National Center for Environmental Assessment 2014 analysis of Air Quality System network data.

Figure 2-15 Coefficient of divergence among a subset of five Los Angeles, CA monitors.

Yet another pattern is observed for Phoenix, AZ and Philadelphia, PA. For these cities, low COD values are observed for all sites except rural locations outside of the urbanized area. The Phoenix data in Figure 2-14 fall into two clusters, with urban site pairs ranging up to 10 km distance from each other and urban-rural site pairs 40 to 60 km from each other. All of the comparisons between urban sites exhibit a COD <0.2, but poorer agreement is observed between urban and rural site pairs. Similarly, good agreement (COD ~0.1) is observed between two monitoring sites operating within the city of Philadelphia about 10 km apart, but poorer agreement is observed for more distant suburban sites. This result is consistent with observations of Sarnat et al. (2010), who observed that using monitors in rural areas of counties considered part of the Atlanta, GA metropolitan area affected relative risk estimates for associations with health effects, but that using different urban monitors within approximately 15 to 30 km of a study subject did not.

To summarize urban scale spatial variability for NO_2, good agreement between nearby sites in city cores is not unusual, and was observed for 2011 to 2013 data for all sites in

Philadelphia and Phoenix. In Los Angeles, good agreement was also usually observed over similar distances to those compared in Philadelphia and Phoenix (i.e., between sites separated by less than 15 km). In contrast, agreement between monitors in Boston became poorer over a shorter distance, but followed a trend of increasing variability with distance between sites over 3 to 10 km, a smaller spatial scale than the other cities.

Similar results are observed for annual averages. Tables 2-5A and 2-5B present the difference in annual average NO_2 concentrations between pairs of sites divided by the average between the two sites for that year to get a percent difference in concentration for Boston and Los Angeles. The CODs of annual average NO_2 concentrations show wide ranges in agreement similar to those reported for 1-hour daily maximum NO_2 concentrations for the NO_2 concentrations measured in both Boston and Los Angeles. The nearest site pairings in Boston agree within 3 to 20%, while the other two site pairings exhibit poorer agreement ranging from 38 to 65% and 31 to 90%.

For Los Angeles, the 14 sites in Los Angeles County that reported data for 2011 are shown in Table 2-5B. A number of site pairings agree within 10 to 15%. For example, concentrations at the Pico Rivera, Pomona, and Long Beach Hudson sites all agree within 10% of the concentrations reported at the Los Angeles Main Street site.

Table 2-5A Percent difference in annual average nitrogen dioxide concentration between monitors in Boston.

	0002 vs. 0040 %	0002 vs. 0042 %	0040 vs. 0042 %
2011	41	10	31
2012	65	20	47
2013	38	3	90

Source: National Center for Environmental Assessment Analysis of Air Quality System Network Data from 2011−2013.

Table 2-5B Percent difference in annual average nitrogen dioxide concentration between monitors in Los Angeles 2011.

Site	0002 %	0016 %	0113 %	1103 %	1201 %	1302 %	1602 %	1701 %	2005 %	4002 %	4006 %	5005 %	6012 %
0016	7												
0113	17	20											
1103	21	57	38										
1201	16	22	2	36									
1302	3	35	15	23	13								
1602	22	58	39	1	37	25							
1701	26	62	43	5	41	28	4						
2005	7	44	24	14	23	10	15	19					
4002	7	31	10	28	9	4	29	33	14				
4006	12	49	29	9	28	15	10	14	5	19			
5005	32	5	15	52	17	30	53	57	39	25	44		
6012	35*	3	18	55	20	32	56	60	42	28	47	3	
9033	47	10	30	66	32	45	67	71	54	40	58	4	13

Source: National Center for Environmental Assessment Analysis of Air Quality System Network Data from 2011.

While these results indicate that relatively good agreement in 1-hour daily maximum and annual average NO_2 concentrations between pairs of nearby urban monitors in the same metropolitan area occurs in some cases, it does not rule out the possibility of greater variability on a smaller spatial scale. Vardoulakis et al. (2011) described a distinction between "intra-urban" and "street scale" variability, explaining that long-term monitoring sites tend to be situated away from sources and hot spots that can strongly influence variability. They compared results from long-term monitoring sites to short-term networks of passive samplers placed in areas between the long-term monitors at varying distances from key roads and intersections, and found that "street level" variability of passive sampler measurements exhibited greater variability than "intra-urban" variability based on long-term monitors. Spatial variability near roads is described in detail in the following section.

2.5.3 Microscale- to Neighborhood-Scale Spatial Variability, Including near Roads

2.5.3.1 Near-Road Gradient Observations

The spatial trends described in this section provide a background for understanding traffic related NO_2 exposure on and near roads, described in Section 3.3.1.1. Numerous observations have been summarized in several recent reviews, each of which concluded that a zone of elevated NO_2 concentration typically extends from 200 to 500 m from roads with heavy traffic (HEI, 2010; Karner et al., 2010; Zhou and Levy, 2007). Table 2-6 describes observations from studies that were included in these reviews and/or in the 2008 Risk and Exposure Assessment for Oxides of Nitrogen (U.S. EPA, 2008b) to estimate on-road concentrations, as well as more recent observations. A direct comparison of the observations included in Table 2-6 is not appropriate because different experimental designs, measurement methods, averaging times, distances from the road, time of year, and other important factors vary among studies. However, Table 2-6 provides a broad overview of the magnitudes of concentration differences observed and the spatial extent over which differences in concentration have been observed.

Table 2-6 Summary of near-road nitrogen dioxide concentration gradients from different studies.

Author	Location	Method	Time of Year	Averaging Time	Nearest Conc. C_{near}	Farthest Conc. C_{far}	Spatial Extent	Difference $(C_{near} - C_{far})/C_{far}$ %	Difference $C_{near} - C_{far}$ ppb
Gilbert et al. (2003)	Montreal	Passive	September	1 week	29 ppb (0 m)	18 ppb (200 m)	200 m	60	11 ppb
Monn (2001)	Zurich	Passive	Summer	1 week	~20 ppb (15 m)	~12 ppb (80 m)	>80 m	>30	~8 ppb
			Winter		~25 ppb (15 m)	~25 ppb (80 m)	None	~0	~0 ppb
Pleijel et al. (2004)	Rural Sweden	Passive		1 month	8−18 ppb (10 m)	4−10 ppb (100 m)	500 m	80−100	2−8 ppb
Roorda-Knape et al. (1998)	Netherlands	Passive		2 weeks	24−25 ppb	16−17 ppb (~300 m)	>300 m	10−60	~7 ppb
Singer et al. (2004)	Oakland	Passive	Fall/Spring	1 week	30 ppb (60 m)	20 ppb (>350 m)	350 m	60	11 ppb
Smargiassi et al. (2005)	Montreal	Passive	May−June	1 day	33 ppb (<10 m)	20 ppb (>1 km)		70	13 ppb
Beckerman et al. (2008)	Toronto	Passive	August	1 week	(4−28 m)		300 m	30−100	
Zou et al. (2006)	Shanghai	Passive	All year	2 weeks	~50−65 ppb (0 m)	39−48 ppb (350 m)	>350 m	~30−40	~12−18 ppb
Gonzales et al. (2005)	El Paso	Passive	Winter	1 week	~25 ppb (0.25 m)	~15 ppb	>3.75 km	~70	~10 ppb
Cape et al. (2004)	Scotland	Passive	April−May	1 week	3−50 ppb (1 m)	3−40 ppb (10 m)	>10 m	<0−70	0−11 ppb
Bell and Ashenden (1997)	Rural Wales	Passive	All year	1 week	8−28 ppb (<1 m)	2−14 ppb (200−350 m)		20−660	3−20 ppb
Bignal et al. (2007)	Rural England	Passive		11−17 days	~25 ppb	5−15 ppb (250 m)	~100 m	70−400	~10−20 ppb
Uemura et al. (2008)	Tokyo	Passive	All year	48 h	35−47 ppb (<50 m)	23−37 ppb (>200 m)			<10 ppb
Maruo et al. (2003)	Sapporo	Sensor	July	½ day	~28−43 ppb	~20−23 ppb (150 m)		~40	~10 ppb
Rodes and Holland (1981)	LA—high O_3	Chemilum.	July−August	1 h	~120 ppb (8 m)	~40 ppb (388 m)	400−500 m	~200	80 ppb
	medium O_3				~80 ppb (8 m)	~40 ppb (388 m)	400−500 m	~100	40 ppb
	Low O_3				~70 ppb (8 m)	~40 ppb (388 m)	400−500 m	~80	30 ppb

Table 2-6 (Continued): Summary of near-road nitrogen dioxide (NO_2) concentration gradients from different studies.

Author	Location	Method	Time of Year	Averaging Time	Nearest Conc. C_{near}	Farthest Conc. C_{far}	Spatial Extent	Difference $(C_{near} - C_{far})/C_{far}$ %	Difference $C_{near} - C_{far}$ ppb
Massoli et al. (2012)	New York	Chemilum.	July	4:30−9:30 a.m.	25−40 ppb (10 m)	25−40 ppb (500 m)	None	~0	~0
Polidori and Fine (2012)	Southern Calif	Chemilum.	Summer	1 h	28 ppb (15 m)	18 ppb (80 m)		56	10 ppb
			Winter		37 ppb (15 m)	32 ppb (80 m)		15	5 ppb
Kimbrough et al. (2013)	Raleigh	Chemilum.	All year	5 min	25 ppb (20 m)	20 ppb (300 m)		30	5 ppb
	Downwind only				28 ppb (20 m)	23 ppb (300 m)		20	5 ppb
McAdam et al. (2011)	Ontario	Chemilum.	Summer	1 h	0−30 ppb (10 m)	0−17 ppb (60 m)	None	~0	<1 ppb (avg)
Durant et al. (2010)	Somerville, MA	TILDAS	January	Real time (a.m.)	~15−35 ppb (<50 m)	~10−30 ppb (400 m)	100−250 m	>0	<10 ppb
Nitta et al. (1993)	Tokyo	Colorimetry	All year	Continuous	34−57 ppb (0 m)	24−42 ppb (150 m)		10−50	8−17 ppb
Nakai et al. (1995)	Tokyo	Colorimetry	All year	Continuous	46 ppb (0 m)	35 ppb (150 m)		30	11 ppb

C_{far} = concentrations measured at the farthest distance; C_{near} = concentrations measured at the nearest distance.

If sufficient detail is given on individual experiments, ranges for concentrations measured at the nearest distance (C_{near}), concentrations measured at the farthest distance (C_{far}), and differences between them are provided in Table 2-6. Otherwise averages over the entire study, or over various categories such as season, wind direction, location, or O_3 concentration are given. Studies using passive sampling methods are listed first in Table 2-6. With the exception of Rodes and Holland (1981), earlier studies were mainly limited to passive samplers that required collection for 1 or 2 weeks, making it difficult to explore effects of time of day or wind direction, which typically shifts on shorter time scales. More recently, more studies have used chemiluminescence, QC-TILDAS, and other methods. These methods not only provide greater time resolution, but also result in the collection of larger numbers of samples, both of which are useful for better understanding the factors influencing near-road concentration patterns. There are essentially three types of experimental designs used in the studies listed in Table 2-6: (1) samples are collected simultaneously at varying distance from the same road; (2) samples are collected by a mobile laboratory with high time resolution, with samples collected at different distances from a road, not simultaneously, but with minimal elapsed time between sampling at different distances from the road; or (3) samples are collected over a wider spatial scale at varying distances from a number of heavily trafficked roads and distance parameters are not linked to the same road for all samples.

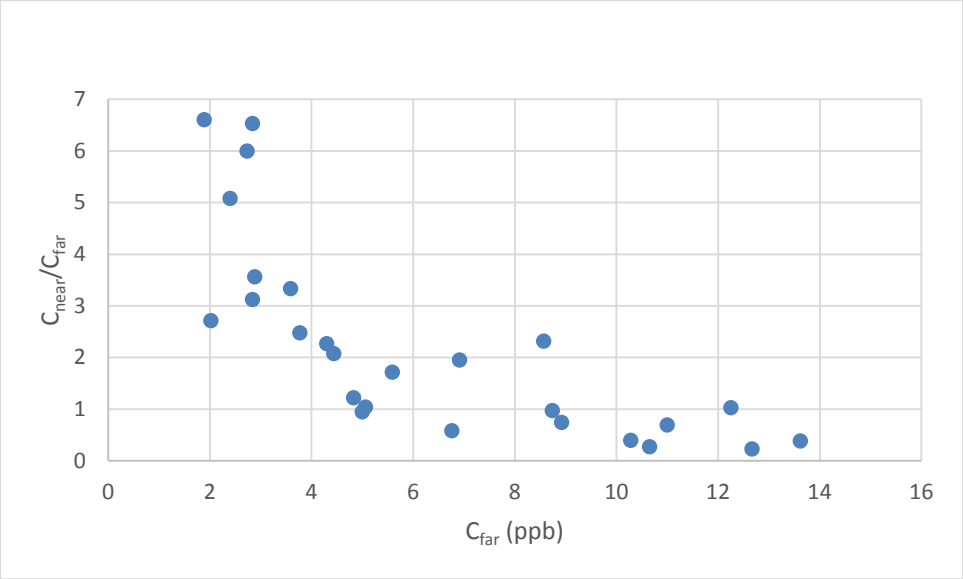
Most of the studies conclude that the spatial extent of elevated NO_2 concentrations is within the range of the 200 to 500 m described by HEI (2010) or Zhou and Levy (2007). However, some recent studies (not necessarily included in Table 2-6) concluded that the influence of the road on NO_2 concentrations can still extend to several kilometers, but with smaller differences in concentration (Gilbert et al., 2007; Gonzales et al., 2005). In other studies, remarkably greater differences in NO_2 concentration with distance were observed within 10 to 20 m of the road than at further distances from the road, suggesting the possibility of an exponential relationship of decreasing concentration with distance from the road with a steeper decrease right next to the road than further than 10 to 20 m from the road (Cape et al., 2004; Bell and Ashenden, 1997). Compared to NO, ultrafine particles (UFP), and other traffic-related pollutants, NO_2 concentrations decrease less rapidly with distance from the road over a range of about 200 to 500 m, and exhibit a somewhat greater spatial extent of elevated concentration (Gordon et al., 2012; Chaney et al., 2011; McAdam et al., 2011; Beckerman et al., 2008; Zhou and Levy, 2007). This has been attributed to chemical production downwind of roads (cf. Section 2.2) and other nontraffic related sources of NO_2 (Chaney et al., 2011; Zhou and Levy, 2007; Rodes and Holland, 1981). Because of the interplay between dispersion and chemical reaction described in Section 2.2, the distribution of NO_2 downwind of roads would likely differ from that of a strictly primary traffic pollutant. For example, Massoli et al. (2012), in a

study examining the behavior of traffic-related pollutants upwind and downwind of the Long Island Expressway (LIE) during morning rush hour(s), found that the concentrations of carbon dioxide [CO_2] and NO_X are highest closest to the highway and decreased by approximately 50% within 150 m downwind of the LIE, whereas the concentration of NO_2 was found to be nearly constant over this distance from the road.

Table 2-6 also compares differences in C_{near} and C_{far} distance reported in each study. The nearest distance ranges from 0 to 60 m and the farthest distance from 80 m to several km. In early studies using passive monitors with usual sampling periods of 1 to 2 weeks, NO_2 C_{near} ranged from 30 to 100% higher than C_{far} in the majority of the observations listed.

A few observations of NO_2 concentrations were more than 100% higher at the location nearest the road than at the location farthest from the road. Most of these greater than usual differences were observed when C_{far} was much lower than usual. This is illustrated in Figure 2-16, which shows that on a major road in a rural area of Great Britain (Bell and Ashenden, 1997) NO_2 C_{near} ranged up to six times higher than C_{far}, but the greatest differences were observed only when C_{far} was lower than usual. Differences were consistently greater than 200% when C_{far} was less than 4 ppb, but less than 100% when C_{far} exceeded 10 ppb. Because C_{far} was so low, even for the greatest differences in concentrations observed by Bell and Ashenden (1997), the absolute difference in concentration between distances of <1 m and 200 m never exceeded 20 ppb. Differences of similar magnitude were observed by Bignal et al. (2007) for a British rural area where C_{far} ranged from 5 to 10 ppb. Because data were collected in a rural area, the differences observed by Bignal et al. (2007) would not necessarily be applicable for absolute differences that might be observed in urban areas where NO_2 concentrations are typically higher. However, Figure 2-16 they clearly demonstrates that when concentration differences are expressed in terms of a ratio of concentrations, the ratio observed at an average concentration could be greater than that observed at higher concentrations.

Although a concentration dependence for $(C_{far} - C_{near})/C_{far}$ was observed for rural areas in Figure 2-16, low C_{far} measurements do not explain all of the high ratios of $(C_{far} - C_{near})/C_{near}$ in Table 2-6. Rodes and Holland (1981), observed concentration ratios for $(C_{far} - C_{near})/C_{far}$ ranging from 100 to 200% along with C_{far} concentrations averaging about 40 ppb, which they attributed to rapid formation of NO_2 between the road and the nearest monitor 8 m from the road because of high O_3 concentrations. Most of the NO_X emitted from vehicles is emitted as NO, which can be rapidly converted into NO_2 in the presence of O_3 as described in Section 2.2. However, the results of Table 2-6 in general indicate that concentrations nearest the road rarely appear to be more than 100% higher at a distance of 10 to 20 m from the road than at 80 to 500 m away from the road.



Source: National Center for Environmental Assessment analysis of data from Bell and Ashenden (1997).

Figure 2-16 Influence of nitrogen dioxide concentration magnitude on the ratio of NO_2 concentrations at <1 m from the road (C_{near}) to concentrations at 200−350 m (C_{far}) in rural Wales.

As studies with better time resolution have been conducted, more observations of the lack of any difference between concentrations nearest the road and farthest from the road ($C_{near} - C_{far} = \sim 0$) have been reported. Monn (2001) observed little difference in winter with passive samplers, and in more recent studies a lack of near-road NO_2 concentration gradient appears to be common in early morning measurements (Massoli et al., 2012; McAdam et al., 2011).

There are now thousands of individual chemiluminescence measurements from two new studies (Kimbrough et al., 2013; Polidori and Fine, 2012) that solidly support observations from earlier passive sampling studies. Kimbrough et al. (2013) reported average concentrations of more than seven thousand 5-minute measurements, and showed that NO_2 concentrations 20 m from the road were an average of 27% higher than at 300 m from the road in Las Vegas, NV. In Southern California, Polidori and Fine (2012) reported that NO_2 concentrations at 15 m were 56% higher in summer and 15% higher in winter than at 80 m. This seasonal difference has been noted in other studies (Monn, 2001) and is consistent with the difference in seasonal ratio distributions estimated in the 2008 Risk and Exposure Assessment for Oxides of Nitrogen (U.S. EPA, 2008b). Averaging over the two seasons gives an NO_2 concentration 36% higher at 15 m than at 80 m, which is remarkably similar to the observation of Kimbrough et al. (2013).

The absolute difference in measured NO_2 concentrations between the nearest and farthest locations ($C_{near} - C_{far}$) is also consistent across most studies, with concentration differences rarely exceeding 20 ppb. The exception is the Rodes and Holland (1981) study from Los Angeles in the early 1980s. Because this is an older study than the others, the vehicle fleet was not strictly regulated for NO_X emissions. As a result, the concentrations observed may not be relevant to current conditions. Excluding this study, the range in $C_{near} - C_{far}$ is somewhat smaller than the range for C_{far} across all of the studies, which further implies that a ratio of concentrations at different distances from the road could be more strongly influenced by the concentration away from the road (C_{far}) than by the concentration nearest the road (C_{near}).

Several investigators have attempted to fit NO_2 concentration data as a function of distance from the road. NO_2 concentrations followed a logarithmic function with distance from a road over a range of 100 m (Pleijel et al., 2004), more than 300 m (Roorda-Knape et al., 1998), and more than 1,000 m (Gilbert et al., 2003):

$$C_x = C_b + C_v - k \log x$$

Equation 2-1

where

x = distance from the road

k = decay constant derived from empirical data

C_x = NO_2 concentration at a distance x from a road

C_b = NO_2 concentration contribution away from the influence of the road

C_v = NO_2 concentration contribution from vehicles on a roadway

Cape et al. (2004) used an exponential decay function to fit measured NO_2 concentrations measured from 1 m to 10 m from the road:

$$C_x = C_b + C_v e^{-kx}$$

Equation 2-2

This approach was also used in the 2008 Risk and Exposure Assessment for Oxides of Nitrogen (U.S. EPA, 2008b) to estimate on-road concentrations from the published studies.

A shifted power law model has also been used (Zou et al., 2006):

$$C_x = C_b(x+1)^{-k}$$

Equation 2-3

Table 2-6 also provides some preliminary insight into factors that affect near-road concentrations. For example, the difference between NO_2 concentrations at different distances from a road is consistently greater in summer than in winter (Kimbrough et al., 2013; Monn, 2001; Bell and Ashenden, 1997), as also described in the 2008 Risk and Exposure Assessment for Oxides of Nitrogen (U.S. EPA, 2008b).

There is less consistency concerning time of day. Near-road NO_2 concentrations have been observed consistently to decrease after sunrise (Gordon et al., 2012; Massoli et al., 2012; Durant et al., 2010). However, both increases (Gordon et al., 2012) and decreases (Durant et al., 2010) in concentration gradient have been observed after sunrise. In the morning, observations of no variation of NO_2 concentration with distance for short time intervals have been observed before sunrise (Gordon et al., 2012), after sunrise (Durant et al., 2010), or both before and after sunrise (Massoli et al., 2012).

A slight effect of wind conditions has been observed. Concentration varies with distance from the road under all wind conditions, but is more pronounced downwind from the road (Kimbrough et al., 2013; McAdam et al., 2011; Beckerman et al., 2008; Roorda-Knape et al., 1998). When air is sampled both upwind and downwind of the road, more gradual gradients are observed on the downwind side of the roadway (Durant et al., 2010; Clements et al., 2009; Hu et al., 2009; Beckerman et al., 2008). Also, higher concentrations are observed at low wind speeds, especially for winds blowing from the road (Kimbrough et al., 2013).

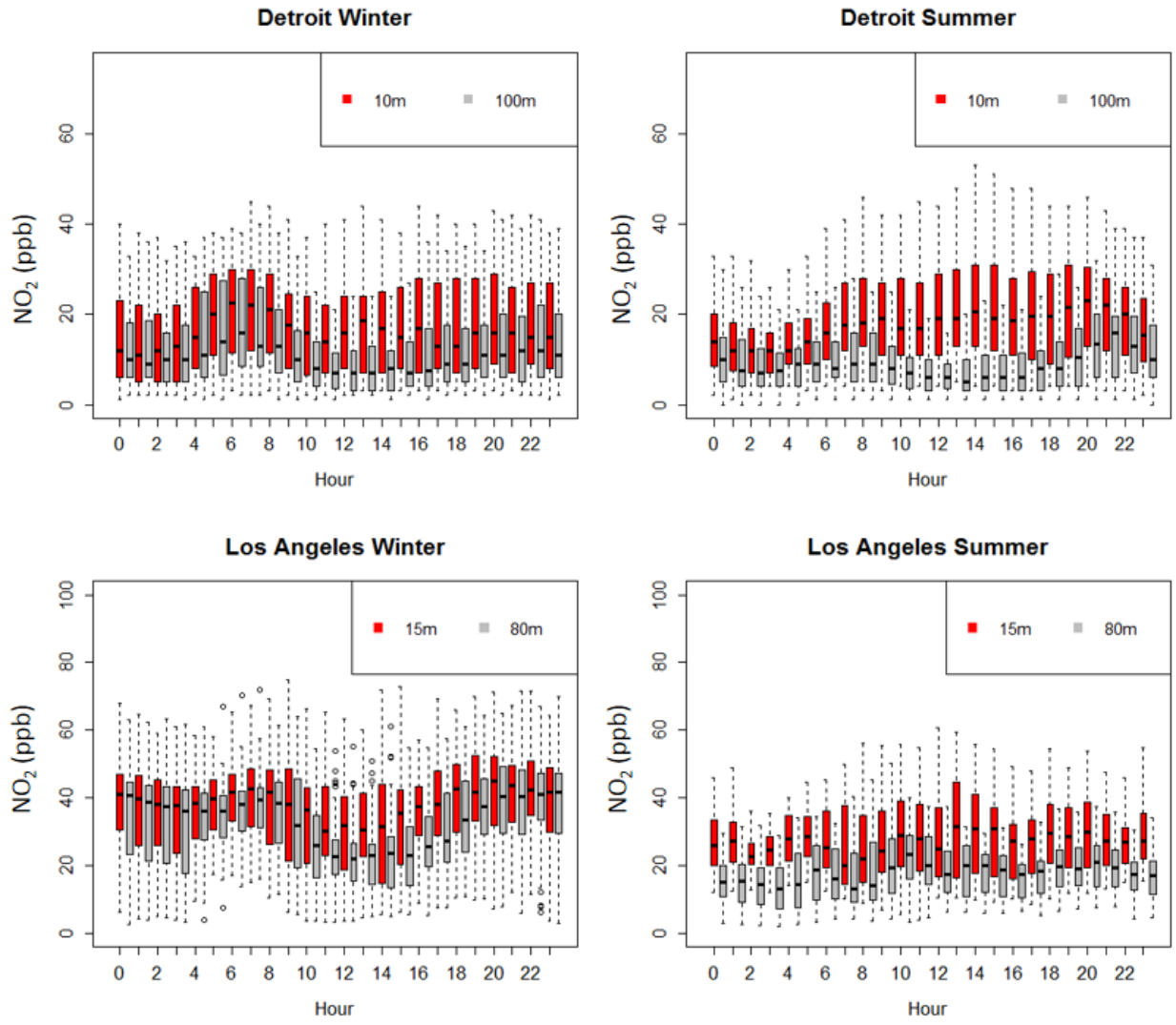
To summarize the results and conclusions of the studies in Table 2-6, a zone of elevated NO_2 concentration typically extends up to a distance of 200 to 500 m from roadways with sufficient traffic. NO_2 concentrations measured from 0 to 20 m from the road range up to 20 ppb higher, or up to 100% higher than concentrations measured between 80 and 500 m from a road. More recent data from intensive studies suggest concentrations at 15 to 20 m average 20−40% higher than concentrations 80 m from the road, with greater differences during daylight hours and in the summer.

Figure 2-17 explores the near-road gradient in more detail for a single study, providing observations of seasonal and diurnal variations based on near-road field measurements in Los Angeles, CA (Polidori and Fine, 2012) and Detroit, MI (Vette et al., 2013). In both cities, hourly NO_2 measurements at near-road (within 15 m from a major interstate) and

downwind sites (within 100 m from major interstate) exhibit the evolving nature of NO_2 concentrations and roadway gradients during different seasons and hours of the day. On a seasonal basis, higher average NO_2 concentrations generally occur during winter months, when atmospheric inversions are more prevalent, although some of the highest concentrations in Detroit are observed during the day in summer. Additionally, because absolute NO_2 concentrations tend to decrease more during the summer when concentrations are lower compared to winter, larger ratios of concentrations at different distances from the road are observed during the summer, as described earlier in this section.

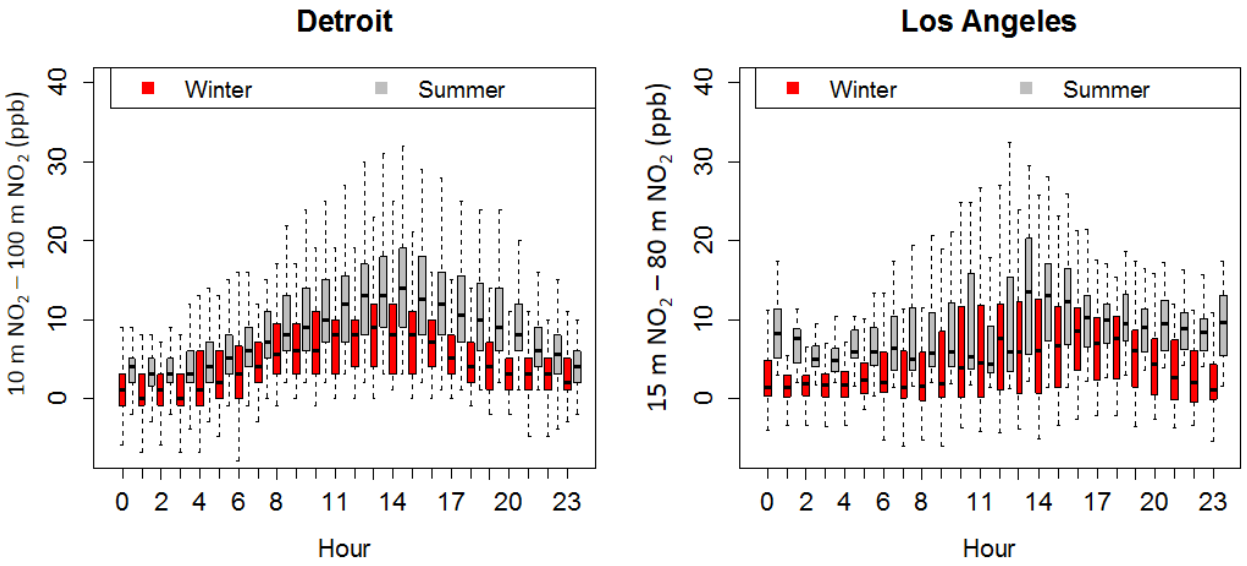
On a diurnal basis, NO_2 roadway concentrations typically increase during morning rush hour (6:00−10:00 a.m.) then gradually decrease from late morning to mid afternoon as the atmospheric mixing layer expands. Roadway NO_2 concentrations begin to increase again during afternoon rush hour and nighttime, and are generally similar to or slightly lower than NO_2 concentrations during morning rush hour. As demonstrated in Figure 2-17, this diurnal profile is more evident in the winter compared to the summer.

Notably, while maximum concentrations tend to occur during morning rush hour and nighttime, especially during the winter, the NO_2 roadway gradient is largest during afternoon hours (10:00 a.m.−5:00 p.m.). This trend is further demonstrated in Figure 2-18, which shows the absolute difference in NO_2 concentrations between near-road and downwind sites during winter and summer. In both cities, the absolute difference between sites is below 15 ppb during morning rush hour and nighttime, whereas a somewhat larger difference is observed during mid-afternoon hours (12:00 p.m.−5:00 p.m.).



Source: National Center for Environmental Assessment analysis of data obtained from Polidori and Fine (2012) and Vette et al. (2013). The box represents the interquartile range of concentrations observed during a given hour, with the 10th and 90th percentiles of concentrations shown by bottom and top whiskers, respectively.

Figure 2-17 Diurnal variation of near-road (red: within 15 m of major interstate) and downwind (gray: within 100 m of major interstate) nitrogen dioxide (NO_2) concentrations observed during year-long field campaigns in Los Angeles, CA and Detroit, MI.



Note: National Center for Environmental Assessment analysis of data obtained from Polidori and Fine (2012) and Vette et al. (2013). The box represents the interquartile range of concentrations observed during a given hour, with the 10th and 90th percentiles of concentrations shown by bottom and top whiskers, respectively.

Figure 2-18 Absolute difference between nitrogen dioxide (NO_2) concentrations at near-road sites during year-long field campaigns in Los Angeles, CA and Detroit, MI.

2.5.3.2 Near-Road Monitoring

The near-road monitoring network described in Section 2.4.5 is scheduled to be implemented in three phases, and monitors in the first phase were scheduled to become operational January 1, 2014. Data presented here from the near-road monitoring network is very limited both in terms of the number of operational monitoring sites and the number of monitoring days reported. Therefore, concentration trends and patterns should be considered very preliminary.

Because some monitoring sites became operational earlier than January 1, 2014, and some of them have already reported second quarter 2014 data, several near-road monitors have already reported a full year of data. Warm season and cold season NO_2 concentrations from these monitors are summarized and compared to other monitors in the same city in Table 2-7. The near-road monitor usually had the highest annual average

of all 1-hour NO_2 measurements from all monitors in the city, but usually not the highest 98th percentile or maximum concentration.

Table 2-7 Comparison of near road and area wide 1-hour daily maximum concentrations for monitors with year round data (ppb).

City	Period	AW Sites	Season	NR Mean	AW Mean	NR 98%	AW 98%	NR Max	AW Max
Boston	6/2013–5/2014	7	Warm	26.2	7–27	43	16–49	51	25–70
			Cold	35.5	13–34	58	33–63	64	40–68
Denver	6/2013–5/2014	2	Warm	39.0	32–37	54	62–77	62	58–63
			Cold	45.0	41–47	71	71–83	97	106–136
Des Moines	2013 (all year)	1	Warm	16.7	15	30	41	34	44
			Cold	21.3	17	35	35	42	39
Detroit	2012–2013 (2 years)	2	Warm	30.4	22–23	50	40–42	78	51–54
			Cold	30.0	25–26	51	42–45	71	48–54
Minneapolis	4/2013–3/2014	3	Warm	22.2	9–15	42	20–38	54	26–42
			Cold	31.1	15–24	51	31–59	52	43–70

AW = area wide; NR = near road

Source: National Center for Environmental Assessment and Office of Air Quality Planning and Standards Analysis of Air Quality System Network Data from 2012–2014.

Comparing near-road NO_2 concentrations to those measured in another part of the city is not the same as comparing them to concentrations a few hundred meters away from the road, as illustrated in Figure 2-14. In many cases, the other monitors have been intentionally sited in areas of high NO_2 concentrations, and considerable variability has been observed on the same spatial scale as the distance between monitors (e.g., Boston in Figure 2-14). A good example of this is the highest 1-hour daily maximum NO_2 concentration of 136 ppb in Denver in Table 2-7 at a monitor located 3 km from the near-road monitor, but one block from high rise buildings that form the edge of the high-density central business district.

Additional monitors have not yet operated for a full year, but were operational in the first quarter of 2014. Results from these monitors are presented in Table 2-8. 1-hour daily maximum NO_2 concentrations ranged from 49 ppb in Houston, TX to 97 ppb in Denver, CO, with 98th percentile concentrations for the quarter ranging from 48 to 71 ppb. More than half of the monitors reported maximum and 98th percentile concentrations of 1-hour daily maximum NO_2 concentrations exceeding 60 ppb. Seasonal average NO_2 concentrations ranged from 23.5 to 46.0 ppb, with the highest average concentrations in Denver, CO and Phoenix, AZ. These are two of only a few cities that fell into the highest concentration classes for both 1-hour daily maximum and annual average concentrations of NO_2 during the previous 3 years, as indicated in Figures 2-11 and 2-12 in Section 2.5.1. This suggests other influences besides roads with heavy traffic can also influence NO_2 concentrations, even at locations very close to the road (i.e., within 50 m).

Table 2-8 Near-road network 1-hour daily maximum nitrogen dioxide concentration summary for first quarter 2014 (ppb).

City	Maximum	98th Percentile	Seasonal Mean
Denver, CO	97	71	44.7
St. Louis, MO	71	66	35.3
Cincinnati, OH	68	67	42.3
Philadelphia, PA	65	60	36.3
Indianapolis, IN	64	64	38.4
Boston, MA	64	60	36.8
Phoenix, AZ	62	62	46.0
Detroit, MI	62	61	34.9
S.F.-Oakland, CA	61	55	30.2
Richmond, VA	59	55	34.6
Birmingham, AL	55	54	23.5
Columbus, OH	53	53	29.3
Minneapolis, MN	52	51	32.9
San Antonio, TX	51	51	28.1
Houston, TX	49	48	29.2

Source: Office of Air Quality Planning and Standards and National Center for Environmental Assessment analysis of Air Quality System Network Data 2014.

These preliminary results from the U.S. near-road network are similar to data from the London, U.K. network with several near-road monitors that have been in operation for a longer period, despite potential differences from the U.S. in fleet mix, traffic mitigation policies, and small geographic scope that may limit generalizability. London data were analyzed because the city has a well-established system of roadside and urban background monitors. Air quality data were obtained from the Airbase database (EIONET, 2014) for 2004 to 2006 and 2010 to 2012 in the form of hourly NO_2 measurements, and monitors of interest were those whose city was listed as London and were within 10 m of the roadway to capture NO_2 primarily derived from mobile sources. Overall, there were large differences in NO_2 concentrations between roadside and urban background monitors, which ranged from 2.4 to 9.8 km apart as shown in Tables 2-9A and 2-9B. The differences in 24-h avg NO_2 concentrations ranged from approximately 24% lower to 170% higher at the roadside than urban background site. The largest relative differences in 24-h avg NO_2 concentrations were observed when the ambient urban background concentrations were less than 20 ppb. All roadside monitors were positively correlated with the overall urban background monitors, and the Pearson correlations were greater than $r = 0.70$ for two out of three. Interquartile ranges were generally similar between roadside monitor-urban background monitor pairs, indicating that while in the majority of cases roadside monitors had higher NO_2 concentrations than urban background monitors, temporal variability was similar between the two monitors. As for the preliminary results from the U.S. near-road network, these results suggest that while concentrations measured at roadside monitors were generally higher than those measured at urban background monitors, there were still large ranges in mean differences.

Table 2-9A Roadside and urban background nitrogen dioxide concentrations in London, U.K. 2010−2012.

Monitor Pairs		Distance Between Monitors km	Mean Concentration ppb	Δ Mean[a] %	98th Percentile of 1-Hour Daily Max[b] ppb	Δ 98th Percentile %	24-H Avg IQR	1-H Max IQR	24-H Avg. Correlation With Urban Background Monitors 95% CI
Roadside	London Marylebone Rd	2.4	52.34	68	140.53	102	25.7	59.58	0.30
Urban Bkg	London Bloomsbury		31.17		69.68		12.01	14.36	(0.25, 0.36)
Roadside	Camden Kerbside	2.8	43.76	124	132.02	108	16.89	32.45	0.74
Urban Bkg	London N.Kensington		19.58		63.56		12.61	15.96	(0.71, 0.77)
Roadside	Haringey Roadside	9.8	23.78	−24	64.22	−7.9	12.03	18.62	0.84
Urban Bkg	London Bloomsbury		31.17		69.68		12.01	14.36	(0.83, 0.86)

CI = confidence interval; IQR = interquartile range.
[a]Difference in mean NO_2 between roadside and urban background monitors.
[b]3-year average.
Source: National Center for Environmental Assessment Analysis of European Air Quality Database Data from 2010−2012.

Table 2-9B Roadside and urban background nitrogen dioxide concentrations in London, U.K. 2004−2006.

	Monitor Pairs	Distance Between Monitors km	Mean Concentration ppb	Δ Mean[a] %	98th Percentile of 1-H Daily Max[b]* ppb	Δ 98th Percentile %	24-H Avg IQR	1-H Max IQR	24-H Avg. Correlation With Urban Background Monitor 95% CI
Roadside	London Marylebone Rd	2.4	58.34	87.84	163.52	131.77	28.62	45.49	0.24
Urban Bkg	London Bloomsbury		31.06		70.55		13.1	15.96	(0.18, 0.29)
Roadside	Southwark Roadside	3.3	32.98	95.9	80.69	50.25	11.13	13.3	0.86
Urban Bkg	London Eltham		16.84		53.7		10.66	16.49	(0.84, 0.88)
Roadside	London Cromwell Rd 2	3.4	42.69	131.15	97.68	51.44	12.1	21.01	0.63
Urban Bkg	London Bexley		18.47		64.5		11.86	15.96	(0.59, 0.66)
Roadside	Camden Kerbside	3.8	37	74.51	116.28	62.01	15.66	26.87	0.78
Urban Bkg	London N.Kensington		21.2		71.77		12.65	17.02	(0.75, 0.80)
Roadside	Tower Hamlets Roadside	4.1	32.34	25.65	80.05	−12.23	15.18	19.15	0.81
Urban Bkg	London Hackney		25.74		91.2		14.29	19.68	(0.79, 0.83)
Roadside	Haringey Roadside	4.5	24.4	−5.21	66.08	−27.54	12.02	16.49	0.80
Urban Bkg	London Hackney		25.74		91.2		14.29	19.68	(0.78, 0.82)
Roadside	London Bromley	5.2	24.73	−4.98	80.36	−8.1	12.36	17.56	0.70
Urban Bkg	London Lewisham		26.03		87.44		12.59	17.02	(0.67, 0.73)
Roadside	London A3 Roadside	6.2	35.33	169.95	93.01	73.94	14.66	19.15	0.64
Urban Bkg	London Teddington		13.09		53.47		11.15	19.21	(0.77, 0.81)

CI = confidence interval; IQR = interquartile range; Bkg = background.
[a]Roadside vs urban background comparison.
[b]3-year average.
Source: National Center for Environmental Assessment Analysis of European Air Quality Database Data from 2004−2006.

While NO_2 measurements are more widely used than NO_X for exposure estimates and epidemiologic studies, NO_2 accounts for only a fraction of NO_X near roads with heavy traffic. For example, Clements et al. (2009) measured concentrations of NO, NO_2, and NO_X, 5 m downwind from a state road in Austin, TX, and observed NO_X concentrations of approximately 40−50 ppb, NO concentrations of approximately 15−40 ppb, and NO_2 concentrations of approximately 5−15 ppb under downwind conditions. NO_2 accounted for 10−38% of the NO_X.

It follows that NO is often a greater contributor to NO_X near roads. Baldauf et al. (2008a) presented time-series of pollutants measured 5 m from I-40 in Raleigh, NC, and reported that NO concentrations reached near 250 ppb between 8:00 a.m. and 9:00 a.m., with minimum NO concentrations around 50 ppb during that time period. The predominance of NO (rather than NO_2) in the near-road environment contrasts with nationwide annual average concentrations in Table 2-4, for which annual average NO_2 accounts for more than 60% of annual average NO_X.

Wind speed and atmospheric stability also impact roadway NO_X concentrations. Peak roadway concentrations are often observed during presunrise hours when winds are weak and atmospheric inversions are present (Gordon et al., 2012; Durant et al., 2010; Hu et al., 2009). During these presunrise hours, the NO_X concentrations exhibit a more gradual decay from the roadway than after sunrise. Hu et al. (2009) observed this effect during a near-road field campaign in Santa Monica, CA. They observed elevated NO concentrations (90−160 ppb) as far as 1,200 m downwind of the roadway during pre-sunrise hours, which is much larger than the expected spatial extent of NO (100−300 m) (Karner et al., 2010; Zhou and Levy, 2007). NO_X concentration gradients continue to change throughout the day as atmospheric stability evolves. After sunrise, near-road NO_X concentrations drop as vertical mixing increases (Gordon et al., 2012; Durant et al., 2010) until concentrations reach a minimum during the late afternoon (Gordon et al., 2012). In some studies, no clear gradient is observed in NO_X concentrations (or other traffic-related species) during mid-morning or early evening hours (Gordon et al., 2012; Durant et al., 2010). However, the exact response of the horizontal concentration gradient to changes in boundary layer height is unresolved to some extent.

Dispersion of NO_X in the near-road environment is influenced by several factors: atmospheric turbulence, vehicle-induced turbulence, and roadway-induced turbulence (Baldauf et al., 2009; Wang and Zhang, 2009). Atmospheric turbulence occurs as a result of meteorological factors within the urban boundary layer. Vehicle-induced turbulence results from the air disturbances caused by the direction and speed of vehicle motion. Roadway-induced turbulence happens when wind-driven air masses undergo separation

following impact with a roadway structure in the built environment. These sources of turbulence interact with each other to create complex, unique dispersion profiles at a given road segment to influence NO_X concentrations. This discussion addresses the physical factors influencing dispersion of NO_X.

Several atmospheric conditions affect regional or urban airflow profiles and potentially can impact the dispersion profile of NO_X even in the absence of adjacent buildings, roadway structures, or traffic-related turbulence. In urban areas, effects of the built environment can be seen at regional-, urban-, neighborhood-, and street-level scales (Fernando, 2010; Britter and Hanna, 2003). Roughness created by upstream buildings contributes to local turbulence levels, even in the absence of adjacent buildings. Land forms such as slopes and valleys can also affect the atmospheric turbulence level because they interact with atmospheric stability conditions to restrict air movement. Finn et al. (2010) observed that tracer gas concentration increased with increasing atmospheric stability. This finding is consistent with results with other studies (Gordon et al., 2012; Durant et al., 2010; Hu et al., 2009) that observed the highest concentrations of NO, NO_2, and NO_X before sunrise when traffic levels and atmospheric stability are high. Hu et al. (2009) also argued that atmospheric stability potentially extends the decay profile of near-roadway pollutants. Additionally, the presence of slopes and valleys can cause spots where airflow converges or diverges (Fernando, 2010). Heat flux can be sizeable in urban areas where the "heat island" effect from roadways and buildings can raise local temperatures by several degrees (Britter and Hanna, 2003); heat flux potentially contributes to convection near roadways and other structures in the built environment. Underscoring the dominant role of local turbulence on dispersion patterns, Venkatram et al. (2007) measured meteorological factors potentially affecting NO concentrations near a road segment in Raleigh, NC and found that among meteorological variables vertical velocity fluctuations had the largest effect on NO concentration.

Vehicle motion creating high levels of turbulence on and near roads can contribute to the dispersion of traffic-related air pollution in the vicinity of a roadway (Baldauf et al., 2008a). An early description of this was provided by Sedefian et al. (1981) for the General Motors experiments, in which groups of vehicles were driven along a test track while towers with mounted anemometers measured mean and fluctuating velocities. It was observed that vehicle-induced turbulence dissipates slowly under low mean wind conditions and vice versa. Vehicle-induced turbulence was found in that study to contribute to vertical dispersion of emitted pollutants. Computational fluid dynamics (CFD) simulations by Wang and Zhang (2009) also found that vehicle-induced turbulence contributed to vertical dispersion. Rao et al. (2002) observed large measurements of turbulence kinetic energy in the wake of a vehicle outfitted with a trailer carrying sonic anemometers driving along a runway. Sedefian et al. (1981) found that

advection of vehicle-induced turbulence away from the roadway was related to the speed and direction of mean winds. di Sabatino et al. (2003) showed that vehicle-induced turbulence is related to traffic levels. In light traffic, the wake behind a vehicle is isolated, but for increasing traffic, the wakes interact and turbulence is a function of the number of vehicles and vehicle length scale. At congested traffic levels, the vehicle-induced turbulence becomes independent of the number of vehicles. For street canyon simulations and measurements, Kastner-Klein et al. (2003) observed that predictions of tracer concentrations were overestimated when vehicle-induced turbulence was not considered; this implies additional dispersion related to vehicle-induced turbulence. Traffic directionality was investigated by He and Dhaniyala (2011) and Kastner-Klein et al. (2001). He and Dhaniyala (2011) observed that turbulent kinetic energy from two-way traffic was roughly 20% higher than for one-way traffic, and they found that the turbulent kinetic energy increased with decreasing distance between the traffic lanes. Kastner-Klein et al. (2001) observed that two-way traffic suppresses the mean flow of vehicle-induced air motion along a street canyon, whereas one-way traffic produces a piston-like effect [note that the Kastner-Klein et al. (2001) study was for the geometrical case of a street canyon]. Substantially higher turbulence levels were produced with two-way traffic compared with one-way traffic for the Kastner-Klein et al. (2001) study as well.

The presence of near-road structures results in recirculating airflow regions that may trap air pollutants on one side and disperse them on another side, depending on wind conditions (Baldauf et al., 2008b). Finn et al. (2010) simulated transport from a roadway using a point source tracer gas with barrier and open terrain conditions. With airflow from the simulated roadway and high atmospheric stability, high concentrations were trapped in the roadway region with a negligible tracer gas in the wake downstream of the barrier with considerable lateral and vertical plume dispersion. For open terrain, transport of the tracer was characterized by a narrow plume. Hagler et al. (2011) used CFD to model airflow and concentrations around barriers of different heights and similarly found reductions in inert tracer concentration downwind of the barrier compared with the open terrain case with trapping of air pollutants upstream of the barrier. With the barrier in place, downwind tracer concentrations were observed at elevations of twice the barrier height. Mean airflow vectors also illustrate a wind disturbance at elevations of twice the barrier height. Even for the open terrain case, vertical dispersion occurs. In additional simulations involving a service road just downstream of the barrier, Hagler et al. (2011) observed entrainment of tracer in the wake downstream of the barrier. Tokairin and Kitada (2005) used CFD to investigate the effect of porous fences on contaminant transport near roads and observed tracer gas retention and airflow recirculation when the fences were designed with less than 40−50% porosity. Heist et al. (2009b) investigated the effect of geometry of road cuts and noise barriers in wind tunnel tracer gas

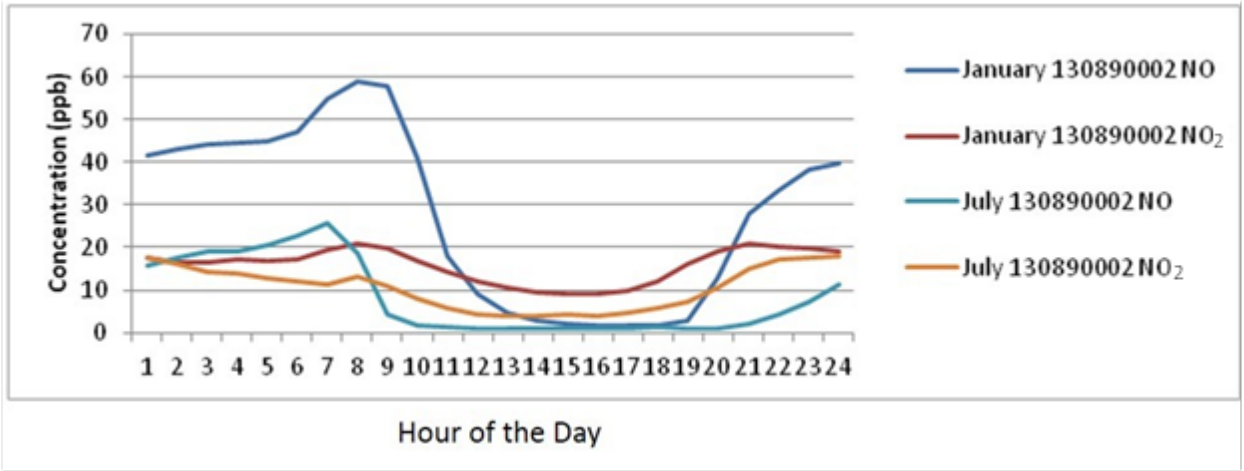
experiments. They observed that elevated roadways, depressed roadways, and noise barriers all resulted in lower downwind concentrations compared with the open terrain case with elevated roadways producing the least reduction in concentration. As in Hagler et al. (2011), Heist et al. (2009b) observed measurable concentrations at elevations that resulted from Gaussian dispersion for all geometries of the road cut or barrier, but vertical dispersion was enhanced or dampened depending on the specific geometry. Similarly, for wind tunnel simulations of a single tower above a matrix of street canyons, the tower was shown to induce both airflow and tracer concentration along the leeward edge of the building to a height exceeding the tower height (Brixey et al., 2009; Heist et al., 2009a).

For the special case of street canyons, retention time for traffic-based pollution increases on the roadway with increasing building height-to-road width ratio because recirculating airflow forms closed streamlines within the canyon (Li et al., 2005; Liu et al., 2005). For wind tunnel simulations of tracer emission at street level with and without traffic, Kastner-Klein et al. (2001) observed measurable tracer concentrations near the top of the street canyon but with some dispersion from maximum tracer levels at the canyon floor. Dilution of NO_X concentrations through these recirculating air structures leads to a steep decrease in concentration with increasing distance from the ground (Lee et al., 2012). For low-aspect-ratio street canyons, secondary recirculating structures can arise; while contaminant retention still occurs in this case, ventilation occurs more readily than for the high-aspect-ratio case (Simoëns and Wallace, 2008; Simoëns et al., 2007). Cheng et al. (2008) used CFD to evaluate factors leading to contaminant retention in street canyons and observed that the exchange rate for air and a tracer gas was driven by the turbulent component of airflow at the roof-level interface of the street canyon. Subsequent simulations showed that exchange rate was also aided by unstable atmospheric conditions (Cheng et al., 2009). CFD simulations by Gu et al. (2010) of transport within a street canyon with and without vegetation suggested that the recirculating flow is dampened by the presence of vegetation.

2.5.4 Seasonal, Weekday/Weekend, and Diurnal Trends

Month-to-month variability in 24-h avg NO_2 concentrations was described in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a). Strong seasonal variability in NO_2 was reported, with higher concentrations in winter and lower concentrations in summer. Monthly maxima varied regionally. Day-to-day variability in NO_2 concentration was generally larger during the winter.

Recent data presented in Table 2-3 continue to show similar seasonal trends for average seasonal NO_2 concentrations across 3 years. Mean and 99th percentile concentrations are highest in the first and fourth quarters. Concentration patterns of NO and NO_2 are affected strongly by emissions and meteorology, as concentrations peak during early morning hours and in winter when PBL heights are lowest (Figure 2-19). NO_2 exhibits flatter profiles relative to NO as secondary formation processes influence concentration patterns.



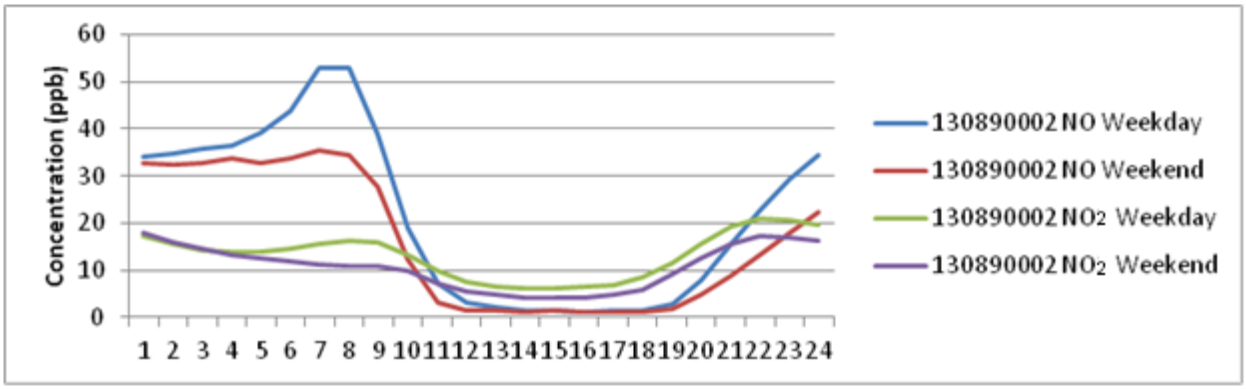
Source: National Center for Environmental Assessment analysis of Air Quality System Network Data.

Figure 2-19 January and July hourly profiles of nitric oxide (NO) and nitrogen dioxide (NO_2) (ppb) for Atlanta, GA (site in Atlanta with maximum 1-hour NO_2 concentrations).

Figure 2-19 shows a typical diurnal cycle for a nonnear-road site for NO and NO_2. As described in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a), the NO_2 concentration typically exhibits a daily maximum during morning rush hour, although the concentration maximum can also occur at other times of day. This pattern is shown for Atlanta, GA, in Figure 2-19, but it is also typical for other urban sites. Although the concentration trends shown in Figure 2-19 are for a nonnear-road monitoring site, they are similar to trends observed for the Las Vegas and Detroit near-road concentration patterns in Figure 2-17. NO levels well above zero at night imply that O_3 has been completely titrated.

Differences between weekdays and weekends are shown for the same monitor in Figure 2-20. Typically, weekday concentrations of NO_x, particularly NO, exceed

weekend concentrations, and diurnal cycles are more compressed on weekends. The weekend effect for NO was first observed by Cleveland et al. (1974) and is a general characteristic of urban NO and NO_X concentrations observed in many locations (Tonse et al., 2008; Pun et al., 2003; Marr and Harley, 2002). Differences between weekdays and weekends were also noted in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a), with more pronounced differences at sites more influenced by traffic. Both empirical observations and modeling simulations of weekly cycles of NO_X based on summer satellite column data converted to concentrations using a chemistry transport model of the vertical NO_2 distribution (see Section 2.4.5) also indicate higher concentrations on weekdays than on weekends regardless of land coverage, for urban, forest, and other regions (Choi et al., 2012). In southern California, NO_X concentrations were an average of 46% lower in ground-based measurements, and 34% lower in airborne measurements (Pollack et al., 2012). In Atlanta, NO_X concentrations were 24% higher on weekdays than on weekends (Pachon et al., 2012).



Source: National Center for Environmental Assessment analysis of Air Quality System Network Data.

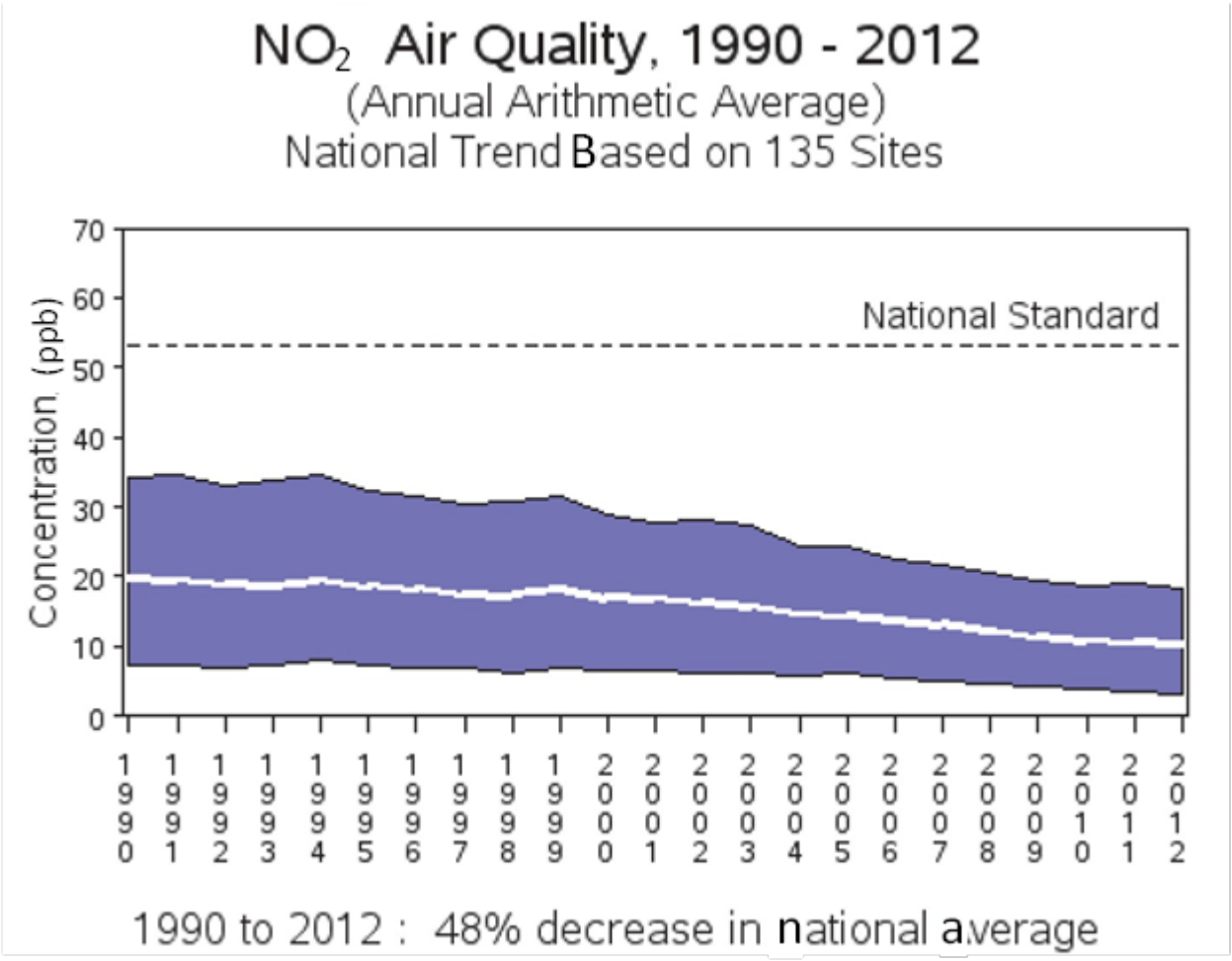
Figure 2-20 Weekend/weekday hourly profiles of nitric oxide (NO) and nitrogen dioxide (NO_2) (ppb) for Atlanta, GA (site in Atlanta with maximum NO_2 concentrations).

2.5.5 Multiyear Trends in Ambient Measurements of Oxides of Nitrogen

The annual average NO_2 concentration across the U.S. based on concentrations from the national air quality monitoring network decreased by 49% from 1990 to 2012, as shown in Figure 2-21. The blue band shows the distribution of air pollution levels among the

trend sites, displaying the middle 80%. The white line represents the average among all the trend sites. Ninety percent of sites have NO_2 concentrations below the top line, while 10% of sites have concentrations below the bottom line.

Information on trends on a regional basis and at individual, local air monitoring sites can be found at http://www.epa.gov/air/airtrends/nitrogen.html; National Trends in Nitrogen Dioxide Levels. The steady decline in NO_2 concentrations over the years can be attributed mainly to reductions in emissions from mobile and stationary sources (see Figure 2-2).



Source: http://www.epa.gov/airtrends/nitrogen.html.

Figure 2-21 U.S. national annual average ambient nitrogen dioxide concentration trends, 1990−2012.

In Atlanta, GA, NO_X concentrations decreased from 1999 to 2001, increased during 2002 and 2003, and decreased again until 2007. The decrease from 1999 to 2001 was attributed to the implementation of EPA's acid rain program, and the decrease from 2002 to 2007 to decreases in on-road NO_X emissions (Pachon et al., 2012).

2.5.6 Background Concentrations

In the context of a review of the NAAQS, EPA generally defines "background concentrations" in a way that distinguishes among concentrations that result from precursor emissions that are relatively less controllable from those that are relatively more controllable through U.S. policies or through international agreements. The most commonly used form in the past and in this document is North American Background (NAB), which refers to simulated NO_2 concentrations that would exist in the absence of anthropogenic emissions from the U.S., Canada, and Mexico. This definition of background includes contributions resulting from emissions from natural sources (e.g., soils, wildfires, lightning) around the world. Other definitions can also be used. For example, in the 2013 ISA for Ozone and Related Photochemical Oxidants (U.S. EPA, 2013b), a U.S. background, which includes emissions from Canada and Mexico in addition to those in the definition of a North American background, and a natural background, which includes only emissions from natural sources globally, were used. Background is used to inform policy considerations regarding the current or potential alternative standards.

As can be seen from Figure 2-13, maximum seasonally averaged concentrations of NO_2 occur along the Northeast Corridor, the Ohio River Valley, and in the Los Angeles basin. While NO_2 concentrations are often above 5 ppb, NAB is less than 300 ppt over most of the continental U.S., and less than 100 ppt in the eastern U.S., as shown in Figure 2-4 through Figure 2-18 in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a). The distribution of background concentrations in the 2008 ISA was shown to largely reflect the distribution of soil NO emissions and lightning, with some local increases due to biomass burning, mainly in the western U.S. In the northeastern U.S., where present-day NO_2 concentrations are highest, NAB contributes <1% to the total.

The only updates to the results given in the 2008 ISA (U.S. EPA, 2008a) are the global-scale model calculations of Lin et al. (2012). In addition to U.S. and other North American sources, various NO_Y species from sources outside North America have long enough residence times in the atmosphere enabling them to be transported to the U.S. (Lin et al., 2012). As noted in the 2013 ISA for Ozone and Related Photochemical Oxidants (U.S. EPA, 2013b), spring is the dominant season for effects of intercontinental

transport of pollution to be detected in the U.S. Lin et al. (2012) calculated that transport of NO_X from other continents contributes less than 10 ppt to the regional background in the western U.S., but concentrations of PAN could range from 50 to 80 ppt.

The annual median NO_2 concentration of ~8 ppb reported by the SLAMS monitoring network is well below the level of the current annual NAAQS (0.053 ppm) and the hourly NAAQS (100 ppb). Background concentrations of NO_2 are much lower than average ambient concentrations and are typically less than 0.1 ppb over most of the U.S., with the highest values found in agricultural areas. All these values indicate that background concentrations of NO_2 are well beneath the level of the current NO_2 NAAQS.

2.6 Conclusions

A large number of oxidized nitrogen species occur in the atmosphere. They are emitted to the atmosphere mainly as NO, which interconverts with NO_2. Thus, NO and NO_2 are often combined into their own group and referred to as NO_X. NO_X plays an important role in the formation of atmospheric O_3 and particulate matter. The conversion of NO_X into other species, such as PAN, HNO_3, or particulate nitrate typically takes place on much longer time scales than does interconversions between NO and NO_2. As a result, near sources, such as in heavily populated areas or busy roads with heavy traffic, oxides of nitrogen are mainly present as NO_X. However, in remote areas downwind of major sources, more oxidized species account for a greater fraction of oxides of nitrogen.

NO_X emissions in the U.S. have been roughly cut in half since 1990. In most of the largest urban areas in the U.S., motor vehicle traffic accounts for 40−67% of emissions and Off-Highway diesel and gasoline engines contribute an additional 20−30%. Off-Highway vehicles and engines, electric power generation, other stationary fuel combustion, industrial and agricultural process, and fires are all important NO_X sources on a national scale, with highway vehicles, Off-Highway vehicles and engines, and stationary fuel combustion especially important in urban areas. Urban stationary fuel combustion emissions account for a greater fraction of NO_X emissions in colder climates. In some cities, specific industrial sources like oil and gas production, petroleum refining, or cement manufacturing account for a greater fraction of NO_X emissions locally than they do nationally. However, traffic emissions are generally responsible for the greatest share of NO_X in the U.S., especially in populated areas.

NO and NO_2 are most commonly measured by a Federal Reference Method based on chemiluminescence of NO induced by its reaction with O_3. NO_2 is measured by first reducing it to NO, and then measuring the chemiluminescence of NO. Recent advancements in NO_2 measurements include improved methods of conversion of NO_2 to

NO, development of optical methods to measure NO_2 directly, and development of satellite measurement methods. NO_2 is measured at hundreds of monitors in several national monitoring networks. In 2014 the first phase of the new near-road monitoring network was initiated in recognition that millions of people live within a few hundred meters of a major roadway, and that concentrations of NO_2 typically decrease with increasing distance from a major road.

If annual average NO_2 concentrations for individual monitoring sites are averaged over all monitoring sites in the U.S., the overall average is about 15 ppb. Similarly, the average daily 1-hour maximum NO_2 concentration over all U.S. monitoring sites is about 30 ppb. Average NO_2 concentrations are usually somewhat higher in winter than in summer. Concentrations are highest in populated urban areas where sources are dominated by vehicle emissions. Within urban areas there can be a high degree of spatial variability, although good intra-urban agreement has also been frequently observed. Concentrations within urban areas are usually highest near major roadways and major stationary sources. Near roadways, an NO_2 concentration gradient is often observed, especially in the summer and during daylight hours. NO_2 concentrations are typically up to 20 ppb higher within 20 m of a major road than at a distance a few hundred meters from the road, and the spatial extent of elevated concentration typically ranges from 200 to 500 m. Preliminary results from EPA's new near-road monitoring network indicate that seasonal average NO_2 concentrations are usually higher near roads with heavy traffic than in other locations in the same city.

Much of the most recent research on atmospheric NO_2 and NO_X has focused on its role as a traffic pollutant and its spatial variability, especially in proximity to major roads. Because traffic is the largest source of NO_X in the U.S., especially in populated areas, this research is highly relevant to human exposure, and the results described in this chapter provide a useful context for NO_2 exposure and health assessment.

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CHAPTER 3 EXPOSURE TO OXIDES OF NITROGEN

3.1 Introduction

Assessment of exposure to ambient oxides of nitrogen builds from the characterization of concentrations and atmospheric chemistry presented in Chapter 2. The primary conclusions from Chapter 2 were that NO_2 concentrations have declined over the past 20 years, but concentrations are still elevated near roads and in urban areas, with vehicular traffic and off-highway vehicles contributing the majority of NO_2 emissions. For this reason, NO_2 exposure assessment focuses predominantly on urban and near-road settings.

True personal exposure to ambient oxides of nitrogen is given by the concentration of oxides of nitrogen emitted from ambient sources and encountered by an individual over a given time. Personal ambient exposure is influenced by a number of 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); and,
- microenvironmental emission sources (e.g., roadways, construction equipment, indoor gas stoves) and concentrations.

Surrogates for personal exposure to ambient oxides of nitrogen include ambient NO_2 measured at a central site monitor or modeled using spatial techniques such as land use regression (LUR), Gaussian dispersion models, or chemical transport models (CTM). All exposure surrogates are subject to measurement errors related to spatial and temporal variability of the ambient concentration field, quality of additional input data, representativeness of predictor variables, and accuracy of the monitoring or modeling methodology. The following sections describe methods to estimate personal exposure, current data used to characterize exposure to ambient oxides of nitrogen, exposure-related factors that influence interpretation of epidemiologic models of the health effects of oxides of nitrogen, and considerations for use of exposure metrics in epidemiologic studies of different design. This chapter focuses on the ambient component of personal exposure to NO_2, because the NAAQS regulates ambient oxides of nitrogen, for which NO_2 is the indicator. However, studies using total personal NO_2 exposure and indoor NO_2 concentrations as exposure metrics can also inform the understanding of exposure and related health effects and so are included as supporting evidence where appropriate. This

chapter focuses on studies of exposure among the general population. Exposure of at-risk groups, based for example on socioeconomic status, race, and proximity to roadways, is addressed in Chapter 7; occupational exposures to ambient NO_2 are discussed in Chapter 7 within the subsections for socioeconomic status and proximity to roadways. Intake of NO_2 based on ventilation rate, and in relation to physical activity, is described in Section 4.2. The information provided in this chapter will be used to help interpret the health effects studies of NO_2 exposure presented in Chapters 5, 6, and 7.

3.2 Methodological Considerations for Use of Exposure Data

The following sections outline various facets of characterizing NO_2 exposure, including research-grade (i.e., central site) and personal NO_2 exposure sampling techniques and NO_2 exposure modeling. The section ends with a discussion of the application of measurement and modeling techniques in epidemiogic studies of different designs.

3.2.1 Measurement

3.2.1.1 Central Site and Near-Road Monitoring

Monitoring of NO_2 concentrations by chemiluminescent sampling is described in detail in Section 2.4.1 along with limitations of the monitoring methodology. In summary, NO_2 concentrations are calculated by FRM as the difference between NO measured in the air stream that has passed over a heated MoO_X substrate (measuring total oxides of nitrogen) and NO in the air stream that was diverted away from the substrate. FRMs are subject to positive bias because oxidized nitrogen compounds other than NO_2 are often detected by the MoO_X substrate. A FEM is also available to measure NO_2 directly using a photolytic converter to reduce NO_2 to NO. Evaluation of the chemiluminescent method is provided in Section 2.4.1 along with a description of the measuring technique. Monitors set up by state agencies as part of the SLAMS network that report to the AQS are typically centrally sited, although the same monitors are used in select cases for near-road monitoring. See Section 2.4.5 for more details.

In addition to judging compliance with the NAAQS, NO_2 concentrations measured by centrally sited or near-road FRMs and FEMs are frequently used by epidemiologic researchers as exposure metrics for studies of the health effects of exposure to oxides of nitrogen, as described further in Section 3.4. Central site monitoring data can be used in epidemiologic studies of short-term exposure to NO_2 when focused on the time series of

exposure or in epidemiologic studies of long-term exposure when comparing average NO_2 concentrations among different geographic areas. Section 3.4.3 explores the factors causing errors associated with siting central site or near-road monitors at a single location, and Section 3.4.5 considers the influence of those errors on health effect estimates, respectively. Briefly, with respect to time-series exposure estimation for epidemiologic studies of short-term exposure, correlation between measured concentration and concentration and some distant point decreases with distance. For epidemiologic studies of long-term exposure to NO_2, difference between the measured concentration and the true exposure would result in exposure misclassification. These issues are potentially exacerbated by the fact that there are a limited number of samplers in the network.

3.2.1.2 Personal and Area Sampling

Personal sampling for NO_2 was described in detail in Annex 3.3 to the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) and is briefly summarized here. Active sampling systems typically involve air pumped past a chemiluminescent device; they enable measurements of NO_2 over short time periods to produce near real-time data. Given the weight of most active sampling systems, they are not used extensively for personal sampling. Passive samplers based on Fick's first law of diffusion are more commonly deployed for personal or area NO_2 sampling in a badge, tube, or radial manifold. These are typically deployed over periods ranging from a few days to several weeks. Passive sampling results are integrated over the time period during which the sorbent material is exposed, which is selected by the user and usually spans days to weeks. The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) reported that, depending on the sorbent material, personal NO_2 samplers may be subject to biases related to interferences from HONO, PAN, HNO_3 (Gair et al., 1991), and high RH (Centro di Ricerche Ambientali, 2006). These biases depend on ambient temperature and atmospheric levels of the copollutants and relative humidity. Personal sampling for NO_2 exposure is most commonly used in epidemiologic panel studies.

Recent work has been performed to evaluate passive sampling device performance. Sather et al. (2007) compared Ogawa passive samplers with a collocated NO_2 FRM monitor over a 4-week field study in El Paso, TX and observed good agreement, with an average absolute difference of 1.2 ppb with $R^2 = 0.95$. For measurements in Umeå, Sweden, Hagenbjork-Gustafsson et al. (2009) observed that, when using the manufacturer's recommended uptake rates to calculate concentration, passive NO_2 measurements were negatively biased by 9.1%, and NO_X concentration measurements were positively biased by 15% compared with an FRM. When uptake rates were derived

in the field based on the chemiluminescent FRM, NO_2 measurements were positively biased by 2%, and NO_X concentration measurements were unbiased compared with the FRM. These results suggest that deviation from temperature conditions under which the samplers were laboratory tested may lead to biased results. Jimenez et al. (2011) used Palmes-type passive diffusion tubes to measure both NO_2 and NO_X concentrations and investigated specific sources of biases in their measurements. They found that, within the passive diffusion tubes, NO and O_3 were reacting to form NO_2, causing NO measurements to be negatively biased while NO_2 measurements were positively biased. Wind was also a source of positive bias in the NO_2 and NO_X concentration measurements because increased airflow effectively reduced the diffusion lengths of the gas collection tubes. In laboratory and field evaluation of NO_2 passive diffusion tubes, Buzica et al. (2008) observed negligible difference between the diffusion tubes and FRM measurements; however, uncertainty increased with decreasing concentration. When comparing biases among samplers, note that the FRM is subject to positive biases related to sensitivity to PAN, $RONO_2$, and HNO_3 (see Sections 2.4.1 and 3.2.1.1).

Triethanolamine (TEA) is often employed as a sorbent material in denuders used for capturing NO_2 during active sampling and in passive sampling because it can be applied in an even coating. However, sampling efficiency is sensitive to sampler flow rate (Vichi and De Santis, 2012), relative humidity (Poddubny and Yushketova, 2013; Šerevičienė and Paliulis, 2012; Vardoulakis et al., 2009), averaging time (Vardoulakis et al., 2009), and ambient temperature (Poddubny and Yushketova, 2013). Heal (2008) found that NO_2 bias was sensitive to method of application of the TEA to the substrate. Sekine et al. (2008) and Nishikawa et al. (2009) experimented with size and number of filters, respectively, in a passive sampler and found minimal effect on NO_2 or NO_X concentration. However, Ozden and Dogeroglu (2008) observed that TEA-complexed NO_2 was sensitive to photodegradation if not stored in a dark glass tube, resulting in underprediction of NO_2 exposure.

Recent attention has been given to using passive or miniature active monitors for saturation sampling, i.e., siting monitors over a dense grid. This is typically done in urban areas. For example, Ross et al. (2013) sited roughly 150 passive NO_2 monitors across the five boroughs of New York City to create a dense concentration map for exposure estimates and to provide training and validation data for LUR. Similarly, Shmool et al. (2014) deployed Ogawa passive badges for NO_2 sampling, along with $PM_{2.5}$, BC, relative humidity, and barometric pressure, across metropolitan Pittsburgh, PA. The monitoring boxes were sited to capture air pollution gradients along the urban-to-suburban land use gradient and included areas influenced by industrial sources and highways. Skouloudis and Kassomenos (2014) deployed sensors for NO_2, NO_X, CO, O_3, and C_6H_6 to correspond to the population distribution on the island of Malta. Active samplers were used in this

scheme, with a global positioning system (GPS) and data transmission capabilities for near real-time analysis. Skouloudis and Kassomenos (2014) proposed that these dense area samplers could also be assimilated with satellite measurements to improve the accuracy of the exposure estimates.

3.2.2 Modeling

Computational models can be employed to provide estimates of exposure in epidemiologic studies when measurements are not available at locations and/or times needed to estimate spatial and temporal variability in concentration within communities and the epidemiologic study design requires greater spatial variability than attainable through ambient NO_2 measurements. These methods can sometimes account for complex urban morphometry and meteorology, which can interact to cause turbulence that may affect pollutant residence times (Fernando, 2010) or incorporate localized sources that might not otherwise be detected by central site monitoring (Goldman et al., 2012). Such estimates can then be used as inputs to exposure models described in Section 3.4. These modeling approaches produce data at times and/or locations where exposures are uncharacterized, but each method carries its own uncertainty (Fuentes, 2009). Detailed descriptions of computational models used for predicting spatially resolved concentration profiles for exposure assessment have been provided in Section AX 3.6 of the 2008 ISA for Oxides of Nitrogen Annex (U.S. EPA, 2008) and Section 3.8 of the 2009 ISA for PM (U.S. EPA, 2009). Methods include LUR models, spatial interpolation through statistical techniques, CTM, and dispersion models.

3.2.2.1 Statistical Modeling

Land Use Regression Models

LUR modeling has been applied extensively to estimate the spatial distribution of ambient NO_2 or NO for exposure assessment on a neighborhood or urban scale for application in epidemiologic studies of long-term exposure (Clougherty et al., 2013; Hatzopoulou et al., 2013; Cesaroni et al., 2012; Gonzales et al., 2012; Mukerjee et al., 2012a; Mukerjee et al., 2012b; Oiamo et al., 2012; Esplugues et al., 2011; Fernández-Somoano et al., 2011; Hystad et al., 2011; Oiamo et al., 2011; Rose et al., 2011; Smith et al., 2011; Szpiro et al., 2011; Adamkiewicz et al., 2010; Aguilera et al., 2009; Cohen et al., 2009; Hart et al., 2009; Iniguez et al., 2009; Karr et al., 2009; Mukerjee et al., 2009; Su et al., 2009b; Aguilera et al., 2008; Atari et al., 2008; Cesaroni et al., 2008; Rosenlund et al., 2008). As such, LUR was used in many of the long-term epidemiologic

studies described in Chapter 6. LUR fits a multiple linear regression model of concentration based on land use data and then applies that model to locations without monitors as an attempt to increase heterogeneity in the spatial resolution of the concentration field compared with other methods, such as central site monitoring (Marshall et al., 2008). Recently, LUR has been implemented to examine local-scale concentration estimates across the U.S. (Novotny et al., 2011; Hart et al., 2009) and Canada (Hystad et al., 2011). Models are typically calibrated using data from NO_2 or NO from passive sampler measurements and several predictor variables, such as land use, road length, population density, and proximity to areas of high concentrations (city center, major road and/or highway, and point sources). Given that most passive measurement methods are not designed for short-term sampling, LUR models are typically based on several days, weeks, or years of data and hence do not account for short-term temporal variability well. Hence, LUR is commonly used to estimate air pollution exposure in long-term epidemiologic studies. Several methodological issues must be considered when interpreting LUR model results; these issues include number of measurement sites used to fit the statistical model, predictor variable selection, and comparison of LUR performance among LUR model formulations and with other models. These issues affect how well the spatial variability of NO_2 or NO_X concentration in a city is represented by the LUR.

Finer spatial resolution of calibration points can improve goodness of fit of the model for the city in which it was fit. Using 155 monitoring sites throughout New York City, Clougherty et al. (2013) ran an LUR with resolutions down to 50 m (R^2 = 0.67[1]). At this fine scale, roadways and localized sources can be better represented than at coarser scales. Parenteau and Sawada (2012) examined LUR model performance when basing the model on successively finer spatial resolution from 2 km down to 50 m, with the geographic borders of the finely resolved regions tied to population groupings based on population density mapping. The two finer resolution approaches yielded better agreement with measured NO_2 data (R^2 = 0.80−0.81) than the less spatially resolved approach (R^2 = 0.70). Likewise, Dijkema et al. (2011) compared LUR based on spatial resolution and observed better agreement with NO_2 observations for neighborhood-level simulations (R^2 = 0.57) compared with whole-city simulations (R^2 = 0.47). Janssen et al. (2012) proposed using LUR to improve performance of a CTM by downscaling the CTM to the LUR. Downscaling entails a redistribution of the CTM-modeled concentrations through a statistical model to conform to measured concentrations at points in space where measurements are available using the LUR-derived regression parameters. Janssen et al. (2012) found that the spatial representativeness of the CTM for NO_2 improved by roughly 20% when incorporating the LUR downscaler, based on a comparison of the

[1] Unless otherwise noted for the LUR studies, R^2 refers to model fit.

CTM and downscaled CTM with central site monitor measurements. It is worth noting that any errors and uncertainties associated with a particular LUR run would transfer to the downscaled result if LUR were used as a basis for downscaling CTM results.

Studies have evaluated LUR model performance when the LUR was fit with different numbers of NO_2 measurement sites and observed that the LUR model design is sensitive to the number of measurement sites. Basagaña et al. (2012) evaluated LUR models for 24−120 NO_2 measurement sites in Girona, Spain and different numbers of predictor variables, starting with 106 prediction variables related to land use and then reducing the set to 18 components through principal component analysis (PCA). Johnson et al. (2010) evaluated LUR performance in New Haven, CT when the LUR model was fit with NO_2 data from 25−285 measurement sites. Wang et al. (2012) also evaluated LUR performance when fit with 24−120 NO_2 monitors distributed across the Netherlands. These studies (Basagaña et al., 2012; Wang et al., 2012; Johnson et al., 2010) observed that, when a large number of prediction covariates were used, the model performed better (higher adjusted R^2 and R^2 for cross-validation) for a smaller number of NO_2 measurement sites compared with the model using a larger number of NO_2 sites, but when the number of prediction covariates was reduced through PCA, then a larger number of NO_2 measurement sites was needed.

LUR results may not be generalizable between or across cities unless the model fit is randomly distributed in space across the city, includes model training data covering the complete ambient concentration distribution, and the cities are similar with respect to source strength, source distribution, and topography. Allen et al. (2011) developed separate LUR models for two Canadian cities (Winnipeg, Manitoba and Edmonton, Alberta) with 50 calibration points each and then applied the models to the other city to compare performance. As anticipated, locally generated model performance (NO_2: R^2 = 0.81−0.84; NO: R^2 = 0.55−0.56) was superior to performance of the model fit for the other city (NO_2: R^2 = 0.37−0.52; NO: R^2 = 0.24−0.41) and to bivariate local models using only road proximity ($R^2 \leq 0.19$). NO_2 models consistently performed better than NO models. Wang et al. (2014) developed a LUR model for NO_2 based on data from 23 European study areas (containing 20−40 sites within each study area) with NO_2, $PM_{2.5}$, land use, and traffic data. Given the continental design of the study, a regional background concentration variable was also imposed on the model. The LUR model fit was R^2 = 0.56 for all of the urban areas combined. After fitting the LUR model, Wang et al. (2014) tested the LUR model's ability to predict concentrations for different configurations of cities by leaving one out of different analyses and found comparable results (R^2 = 0.59). Generally, the R^2 for NO_2 were either comparable or lower than R^2 for single city studies. This would be expected given the smoothing effect of fitting a model over a large geographic area.

Selection of predictor variables, such as meteorology, traffic, land use, and population density, influences the ability of the LUR model to predict concentrations of oxides of nitrogen and depends on the specific city for which the model is fit. Su et al. (2008a) and Ainslie et al. (2008) developed the Source Area-LUR (SA-LUR) to incorporate the effects of meteorology on the model results. The SA-LUR integrates data for wind speed, wind direction, and cloud cover variables in estimates for NO and NO_2 and was found to perform better when seasonal variability in concentrations was high. Su et al. (2008b) included a street canyon aspect ratio as a LUR predictor variable to account for retention of pollutants in street canyons. They observed that, upon adding the aspect ratio to the LUR model, R^2 increased from 0.56 to 0.67 for NO_2 and from 0.72 to 0.85 for NO. Similarly, when Clougherty et al. (2013) added "built space within 1 km" to their LUR model of NO_2, R^2 increased by 0.41. Franklin et al. (2012) explored bivariate correlations between NO_2, NO, and NO_X concentrations and several predictors reflecting traffic, population, elevation, and land use in twelve southern California communities. For NO_2, Pearson correlations of concentration with distance to road were $R = -0.42$ and -0.35 for freeway and nonfreeway roads, respectively, and produced an −8.2% change in concentration per IQR increase in distance in the LUR model. Correlations with traffic volume within a 300-m buffer were $R = 0.41$, and traffic volume within a 300-m buffer produced a 2.4% change in the LUR prediction per IQR. Correlation with neighborhood elevation was $R = -0.50$, and neighborhood elevation produced a −6.7% change in LUR-modeled concentration per IQR increase in elevation. Su et al. (2009a) developed a method to optimize the SA-LUR variable selection process in which correlations between several land use variables and NO_2 concentrations were computed across a 3-km buffer of the NO_2 measurement (1.5-km buffer for traffic-related variables), and the data for correlation versus distance were fit to a curve describing that relationship. The variable with highest correlation at the optimum buffer distance was added to the model if its addition produced a statistically significant change ($p < 0.1$) in the model R^2. Su et al. (2009a) found the important variables to be distance from monitor, 24-hour traffic levels, expressway casement, open land use, railway, major road, land grade, population density, and distance to coast. It can be anticipated that the important variables might be different depending on city-specific factors.

Several studies of LUR have explored temporal treatments in the model. LUR has been evaluated across seasons and spatial variability in the NO_2 concentration profile has not been found to change substantially with season, despite more temporal variability during mild weather compared with either cold or warm weather (Dons et al., 2014; Crouse et al., 2009). Therefore, the authors concluded that an annual average would be acceptable for LUR simulations. LUR models applied several years after model development have demonstrated moderate-to-good predictive ability in a few studies. Eeftens et al. (2011) compared LUR obtained from NO_2 measurements at 35 locations in

the Netherlands over the years 1999−2000 with LUR developed from NO_2 measurements at 144 locations in the Netherlands during 2007. Both the NO_2 measurements and the LUR models agreed well for the two time periods studied (β = 0.9998; R^2 = 0.89). Wang et al. (2013b) tested stability of an LUR model for Vancouver, Canada between 2003 (based on 116 sites) and 2010 (based on 116 sites, with 73 from the 2003 study). Wang et al. (2013b) evaluated the model by testing how much variability in the measurements was predicted by models from the other year, with moderate results. Linear regression for comparison of the 2003 model with 2010 measurements produced R^2 = 0.58−0.60 for NO and R^2 = 0.52−0.61 for NO_2, while comparison of the 2010 model with 2003 measurements produced R^2 = 0.50−0.55 for NO and R^2 = 0.44−0.49 for NO_2. Wang et al. (2013b) attributed the diminished performance for the 2003 model using 2010 data (compared with using the 2010 model for 2003 data) to reductions in NO and NO_2 concentrations over the 7-year time period. Visual inspection of the NO and NO_2 concentration maps from the Wang et al. (2013b) study suggests that changes in spatial correlation over time may have contributed to reduced model performance in comparison with the Eeftens et al. (2011) study.

LUR evaluation depends on the validation algorithm, model conditions, and basis for validation (i.e., to what the modeling results are compared when computing R^2). In a recent study of LUR application in 20 European study areas, Wang et al. (2013a) found that leave-one-out cross-validation (LOOCV), typically used to validate LUR, produced higher R^2 for NO_2 compared with hold-out evaluation (HEV) (LOOCV: R^2 = 0.83; HEV: R^2 = 0.52). LOOCV involves repeatedly withholding a fraction of the monitoring sites from the fitting process for performance evaluation and then computing an ensemble R^2, whereas HEV entails prediction with the LUR at locations not fit by the model. Therefore, HEV may provide a more conservative estimate of model fit. Mercer et al. (2011) compared 10-fold cross-validated LUR with universal kriging (UK), in which a surface of concentrations was built based on measured values, for three seasons in Los Angeles with roughly 150 measurement sites. UK performance was slightly better than LUR for all seasons with little difference in model performance among the seasons (UK: R^2 = 0.75, 0.72, and 0.74; LUR: R^2 = 0.74, 0.60, 0.67). Li et al. (2012) developed a new formulation for LUR using generalized additive models (GAM) and cokriging to boost the performance of LUR over other LUR model variations of models and evaluated it for Los Angeles, CA. GAM enables incorporation of localized nonlinear effects among the prediction covariates, while cokriging is intended to improve spatial smoothing. The LUR using GAM and cokriging, had the highest cross-validation (R^2 = 0.88−92), compared with universal kriging (R^2 = 0.68−0.75) and multiple linear LUR (R^2 = 0.42−0.64).

LUR comparison with other models has produced variable results, in part because the comparison data does not always have the same spatial resolution or account for the same physical phenomena. Beelen et al. (2010) compared LUR with a dispersion model incorporating a near-road module for modeling NO_2 concentrations in a Rotterdam, Netherlands neighborhood. The dispersion model agreed better (Pearson $R = 0.77$) compared with LUR ($R = 0.47$) with NO_2 measurements from 18 evaluation sites. Dijkema et al. (2011) also compared LUR for the city of Amsterdam and neighborhoods therein with a dispersion model and found better agreement of the dispersion models with observations for the city-wide model than for LUR (dispersion: $R^2 = 0.74$; LUR: $R^2 = 0.47$) although agreement was comparable for the neighborhood specific model ($R^2 = 0.57$ for both models). Marshall et al. (2008) compared LUR with inverse distance-weighted (IDW) spatial interpolation of NO and NO_2 measurements, nearest NO and NO_2 measurements, and a Community Multiscale Air Quality (CMAQ) model run for Vancouver, Canada. The LUR location was matched to each CMAQ grid cell centroid and compared with the grid cell concentration. LUR and CMAQ produced similar average absolute difference in the concentration compared with measured central site concentrations for NO (LUR: 42%, CMAQ: 47%) and NO_2 (LUR: 17%, CMAQ: 17%), while nearest monitor and spatial interpolation methods produced less than 5% difference for both pollutants and methods. However, it is important to recognize that these methods were compared to a central site monitor, which cannot capture the spatial variability of the NO_2 concentration distribution. Specifically, IDW, central site monitoring of NO_2 concentration, and nearest monitor NO_2 concentration estimation approaches cannot account for localized sources unless the sources are close to the monitors. Therefore, agreement among the models does not necessarily signify accurate depiction of the spatial distribution of NO_2 concentration.

Recent studies have explored hybrid application of LUR and dispersion models. For example, Wilton et al. (2010) included concentrations computed with the CALINE3 dispersion model in their LUR to estimate NO_X and NO_2 concentrations in Los Angeles and Seattle. They observed modest improvements in model R^2 (Los Angeles, NO_X: $R^2 = 0.71-0.74$ vs. $R^2 = 0.53-0.55$; Los Angeles, NO_2: $R^2 = 0.79$ vs. $R^2 = 0.74$; Seattle, NO_2: $R^2 = 0.81$ vs. $R^2 = 0.72$) when CALINE3-computed concentration was included as one variable along with land use, roadway length, and traffic density variables. However, Lindström et al. (2013) applied LUR with CALINE3-computed NO_X concentration for Los Angeles participants in the Multi-Ethnic Study of Atherosclerosis and found no appreciable improvement (R^2 within ± 0.04) in model performance for a variety of averaging times (daily "snap shot," 2-week, 10-year). Mölter et al. (2010) also used dispersion modeling data in lieu of measurement data when fitting an LUR for Greater Manchester, U.K. and found reasonable agreement of NO_2 predictions with monitoring data ($R^2 = 0.86$) and with a separate data set where 25% of the data were set

aside for evaluation (R^2 = 0.62). Note that the nature of the monitoring data (i.e., central site or other) was not explicitly stated in the Mölter et al. (2010) study.

Although not used specifically for prediction at alternate locations, multiple linear regression has been used to predict point concentrations based on meteorological and source characteristics, in a manner similar to LUR but not including land use variables. This technique is not employed for exposure assessment in any of the epidemiologic studies cited in Chapter 6, but it is noted as an emerging exposure assessment method. Vlachogianni et al. (2011) used multiple regression to forecast concentrations at two locations each in Helsinki, Finland and Athens, Greece. They noted that the model including only measured meteorological parameters (temperature, relative humidity, wind speed, and wind direction) did not capture the true variability of the NO_2 concentration time series as well as a second model that also included atmospheric turbulence parameters (Monin-Obukhov length and mixing height). This was particularly true during cold weather. Carslaw and Taylor (2009) modeled NO_X concentrations as a function of meteorological and temporal variables. They found that wind speed, temperature, temporal parameters (a trend metric, hour of the day, Julian day), and wind direction were most important in the NO_X model. Carslaw and Taylor (2009) highlighted that the boosted regression tree methodology enabled examination of the influence of interactions among the variables in addition to the direct dependence of NO_X on each individual variable.

Spatiotemporal Interpolation Modeling

Spatiotemporal modeling can also be used to describe NO_2 concentrations for application in exposure assessment in epidemiologic studies of long-term exposure. These methods are not typically used in epidemiologic studies but can be considered emerging methods. Le and Zidek (2006) developed an approach to use autoregressive integrated moving average (ARIMA) models to capture the temporal pattern of the concentration field in conjunction with an empirical Bayes maximum likelihood model to estimate the spatial pattern of the true concentration field. Pollice and Jona lasinio (2010) applied a first-order autoregression model to estimate the NO_2 concentration field in Taranto, Italy and observed reasonable agreement between observations and the predictions. Similarly, ARIMA models can be used on their own at the location of a single model to forecast the concentration time series at a specific location. Kumar and Jain (2009) found that observations generally agreed with an ARIMA model of a point concentration within the model's ±95% confidence interval (CI). Kumar and Jain (2009) tested several orders of autoregression, integration, and moving average and found that different goodness-of-fit measures favored selection of different autoregression and moving average orders. However, Chaudhuri and Dutta (2014) tested different model orders and found that a

model with no autoregression but with second-order integration and moving average best fit the NO_2 data, with $R^2 = 0.83$ and negative bias of 16%.

Artificial neural networks (ANN) provide another technique for predicting NO_2 concentrations in a manner similar to LUR. In ANN, data are input through "nodes" in the system, which are weighted based on different criteria to represent the influence of various predictor variables. Nodes can reflect influential parameters such as meteorology and source presence, and it is flexible to analyze data over space and/or time. They can also be included as latent variables that aggregate the effects of the input variables (Arhami et al., 2013). In generally, ANN has been shown to validate well with observation data (Baawain and Al-Serihi, 2014; Arhami et al., 2013; Vlachogianni et al., 2011; Konovalov et al., 2010; Moustris et al., 2009). Singh et al. (2012) tested variations of ANNs, which vary based on the number of layers of nodes and the interactions between layers in the model. Based on the model output of Singh et al. (2012), it was found that suspended particulate matter concentration was the variable that contributed the most in explaining variability in NO_2 concentrations (with other variables for SO_2 concentration, temperature, relative humidity, and wind speed). The Singh et al. (2012) study illustrates how ANN may be used to gain insight into mechanistic processes influencing NO_2 concentration.

3.2.2.2 Mechanistic Models

Chemical Transport Models

CTMs can be used to develop estimates of human exposure to NO, NO_2, or NO_X. CTMs, such as CMAQ, are deterministic of chemical transport that account for physical processes including advection, dispersion, diffusion, gas-phase reaction, and mixing while following the constraint of mass conservation (Byun and Schere, 2006). These models provide regional concentration estimates and are typically run with surface grid resolutions of 4, 12, or 36 km. Temporal resolution of CTMs can be as fine as 1 hour, although larger temporal aggregation often occurs for the purpose of maintaining reasonable data file size.

CTMs can be applied in epidemiologic studies of either short- or long-term exposure to NO_2 or NO_X but are more commonly used in long-term exposure studies. These models are used to compute interactions among atmospheric pollutants and their transformation products, the production of secondary aerosols, the evolution of particle size distribution, and transport and deposition of pollutants. CTMs are driven by emissions inventories for primary species such as NO_2, SO_2, NH_3, VOCs, and primary PM, and by meteorological

fields produced by other numerical prediction models. Values for meteorological state variables such as winds and temperatures are taken from operational analyses, re-analyses, or weather circulation models. In most cases, these are off-line meteorological analyses, meaning that they are not modified by radiatively active species generated by the air quality model. Work to integrate meteorology and chemistry was done in the mid-1990s by Lu et al. (1997a) and Lu et al. (1997b) and references therein, although limits to computing power prevented their wide-spread application. More recently, new, integrated models of meteorology and chemistry are now available as well; see, for example, Binkowski et al. (2007) and the Weather Research and Forecast model with chemistry (WRF Chem) (http://ruc.noaa.gov/wrf/WG11/). Given observed biases in the CTMs [e.g., U.S. EPA (2008)], much attention has been given to bias correction of these models for application in exposure assessment, as detailed below under the Hybrid Models section below.

Dispersion Models

Dispersion models, or Gaussian plume models, estimate the transport and dispersion of ambient air pollutants emanating from a point or line source through solution of an equation that estimates the spread of the pollutant to follow a Gaussian curve that is a function of distance from the source. Given that dispersion models typically capture average concentrations, they are most commonly used in epidemiologic studies of long-term exposure. Several studies of health effects related to NO_X exposure employ dispersion models to estimate NO_X concentrations [e.g., Gruzieva et al. (2013), McConnell et al. (2010), and Oftedal et al. (2009)] because NO_2 has high local spatial variability (Section 2.5.3). The grid spacing in regional CTMs, usually between 1 and 12 km^2, is too coarse to resolve spatial variations on the neighborhood scale. More finely resolved spatial scales that better represent human exposure scales are provided by local-scale dispersion models. Several dispersion models are available to simulate concentration fields near roads, and each has its own set of strengths and weaknesses.

Several line source Gaussian dispersion models are available to simulate the dispersion of emissions from a roadway. The California Department of Transportation developed the CALINE model (http://www.dot.ca.gov/hq/env/air/software/caline4/calinesw.htm) for this purpose. The CALINE family of models is not supported by the California Department of Transportation for modeling of highway source NO_2 and does not include NO_X transformation chemistry. Benson (1992) validated the CALINE3 and CALINE4 model versions using data from field studies at U.S. Highway 99 in Sacramento, CA and a General Motors test track in Michigan. Benson (1992) found that more than 85% of model predictions fell within a factor of two of measured observations for SF_6 (an inert tracer gas). Among those that fell outside the factor of two envelope, 85% were positively

biased and mostly occurred when wind speeds were below 1 m/s. Additionally, the NO_2 module of CALINE4 was tested by Benson (1992) under a limited set of conditions, and it was recommended that CALINE4 not be used to predict NO_2 dispersion under parallel wind conditions without ample data to calibrate the model predictions.

The University of California, Davis (UCD) 2001 model was designed to improve upon the design of CALINE by using an array of point sources to represent a three-dimensional highway source of emissions and by using power law functions for wind speed and vertical eddy diffusivity (Held et al., 2003). UCD 2001 exhibited improved performance for parallel, low speed winds (<0.5 m/s), with 87% and 83% reduction in error compared with CALINE3 and CALINE4, respectively, for the General Motors SF_6 evaluation data set. Snyder et al. (2013) recently released a Research Line-source (RLINE) dispersion model that incorporates improved formulations of horizontal and vertical dispersion and found that the predictions were within a factor of two of the observations for neutral, convective, and weakly stable atmospheric conditions, but negative bias was observed for stable conditions based on a line source SF_6 experiment in Idaho Falls, ID. During comparison with the U.S. 99 data set, 81% of data were within a factor of two for downwind measurements, but only 19% for upwind measurements when winds were within 30° of perpendicular to the road; 75% of downwind predictions were within a factor of two of observations when winds were less than 1.5 m/s, and 88% were within a factor of two for wind speeds greater than 1.5 m/s. Only 51% were within a factor of two when winds were within 30° of parallel to the road. Additionally, a hybrid optimization model fitting CALINE3 line-dispersion calculations for concentration to observations of NO_2 was developed and applied in the greater Tel Aviv, Israel area (Yuval et al., 2013). Cross-validation was reported to have negligible bias in the model predictions with 36% error; note that the authors did not clearly distinguish bias and error in this manuscript.

The American Meteorological Society/Environmental Protection Agency Regulatory Model (AERMOD; http://www.epa.gov/scram001/dispersion_prefrec.htm) is a steady state point source plume model formulated as a replacement to the Industrial Source Complex (ISC3) dispersion model (Cimorelli et al., 2005). In the stable boundary layer, it assumes the concentration distribution to be Gaussian in both the vertical and horizontal dimensions. In the convective boundary layer, the horizontal distribution is also assumed to be Gaussian, but the vertical distribution is described with a bi-Gaussian probability density function. AERMOD has provisions that can be applied to flat and complex terrain and multiple source types (including point, area, and volume sources) in both urban and rural areas. It incorporates air dispersion based on the structure of turbulence in the planetary boundary layer and scaling concepts and is meant to treat surface and elevated sources, in both simple and complex terrain in rural and urban areas. The dispersion of emissions from line sources like highways in AERMOD is handled as a source with

dimensions set using an area or volume source algorithm in the model; however, actual emissions usually are not in a steady state.

Most simple dispersion models including AERMOD are designed without explicit chemical mechanisms but have nondefault options to estimate conversion of NO to NO_2 based on a NO_X/O_3 titration model. Hendrick et al. (2013) evaluated two modules used with AERMOD to compute NO_2 concentrations: the plume volume molar ratio method (PVMRM) and the ozone limiting method (OLM). Both methods assume ratios of NO_2:NO_X that are based on the concentration of co-occuring O_3. Hendrick et al. (2013) validated the models against more than 12 months of hourly observations taken near a small power plant in Wainwright, AK, and they observed that the PVMRM overpredicted NO_2 at low concentrations and underpredicted at high concentrations, although the average bias was small; the OLM also overpredicted NO_2 concentrations at high observed NO_2.

AERMOD results have been compared with measurements and other models to evaluate relative performance. Gibson et al. (2013) found poor agreement with respect to magnitude of NO_X concentrations and correlations (R^2 = 0.001−0.003) at hourly, monthly, and annual timescales when comparing AERMOD results with observations in Halifax, Canada where several industrial facilities emit NO_X. Cohan et al. (2011) compared AERMOD output with 24-hour central site monitoring observations averaged over August 2005 from San Jose, CA, where there are combined emissions from a port, rail yard, and roadways. They observed that the AERMOD model consistently underpredicted the observations; negative bias was more pronounced for simulations from January compared with August. Misra et al. (2013) compared AERMOD with the Quick Urban and Industrial Complex (QUIC) model. QUIC approximates average airflow around buildings in urban environments then models pollution parcels based on Lagrangian particle dispersion. In this case, AERMOD underpredicted NO_X concentrations in an urban street canyon, while most QUIC predictions were within a factor of two of the observed NO_X concentrations.

There are also nonsteady state models for different types of sources. For example, CALPUFF (http://www.src.com/calpuff/calpuff1.htm), which is EPA's recommended dispersion model for transport in ranges >50 km, is a nonsteady-state puff dispersion model that simulates the effects of time- and space-varying meteorological conditions on pollution transport, transformation, and removal and has provisions for calculating dispersion from surface sources (U.S. EPA, 1995). However, CALPUFF was not designed to treat the dispersion of emissions from roads, and like AERMOD has some limited chemistry options to estimate production of secondary pollutants. The distinction between a steady-state and time-varying model may not be important for studying health

effects where long-exposure timescales are relevant; however, when short-exposure timescales are of interest (e.g., 1-hour), it would be more important to approximate the short-term variability in concentrations. CALPUFF was validated against SF_6 data at two military test sites in Nevada (Chang et al., 2003), where it was shown that 53% of CALPUFF predictions were within a factor of two under SF_6 observations for one site and 29% of predictions were within a factor two under the observations at a second site; the second test site had surrounding mountains acting to increase vertical dispersion for which CALPUFF did not account well. Cui et al. (2011) evaluated CALPUFF by releasing SF_6 from a weather tower at the bank of the Gan Jiang River in China, which has a combination of open field, agricultural land, and forest. CALPUFF was found to be negatively biased with only 25−27% of data within a factor of two of observations. The authors concluded that CALPUFF did not predict hourly dispersion well. Similarly, Ghannam and El-Fadel (2013) compared NO_2 concentrations calculated using CALPUFF with NO_2 measurements and observed that the model severely underpredicted the measurements, sometimes by up to three orders of magnitude, but was stated to have captured the temporal variability, although correlations were not reported. Ghannam and El-Fadel (2013) attributed this underprediction to underestimation of the emissions input to the model. The results of Cui et al. (2011) and Ghannam and El-Fadel (2013) indicating negative bias are consistent with those of Chang et al. (2003) for the site where vertical dispersion may have played a larger role in the airflow characteristics.

An example of where AERMOD has been used in better understanding the relationship between ambient concentrations and health risks is found in Maantay et al. (2009). These researchers coupled AERMOD with geographic information system proximity buffers around a stationary point source in Bronx, NY. They observed that buffers based on the predicted plume shape to set levels of human exposure to NO_X, along with PM_{10}, $PM_{2.5}$, CO, and SO_2, corresponded better with asthma hospitalization rates compared with circular buffers centered around the emissions source.

Hybrid Models

Substantial uncertainties at the subgrid scale remain when using CTM to model concentrations at resolutions of 4−36 km (U.S. EPA, 2008). In densely populated regions of the country, monitor density may be finer than CTM surface grid resolution. Moreover, CMAQ and other CTMs suffer from pollutant-specific concentration biases, such as underestimation of total nitrate that require correction (Fuentes and Raftery, 2005) prior to interpretation for exposure assessment. Bayesian Maximum Entropy models for merging CMAQ and concentration data (Fuentes and Raftery, 2005) and downscaling (Berrocal et al., 2010a, b) have recently been developed to improve spatial resolution and provide bias correction for the modeled concentration used as an exposure

surrogate, but such methods must be used with caution. For instance, Chen et al. (2014) ran a 36-km resolution CMAQ for NO_2, NO_X, and other copollutants, fused the CMAQ results with monitor observations, and compared both the raw and fused model results with monitor observation data. The raw CMAQ simulations overpredicted NO_2 and NO_X concentrations, particularly in the winter. These overpredictions were substantially reduced (and in some cases the model slightly underpredicted concentrations) for the fused model. Isakov et al. (2009) modeled subgrid spatial variability within CMAQ using the AERMOD dispersion model prior to linking the modeled results with stochastic population exposure models to predict annual and seasonal variation in urban population exposure within urban microenvironments. In each case, these papers have referred to other air pollutants, but the methodology is still applicable for NO_2 exposure prediction.

Berrocal et al. (2010b) proposed a downscaling approach to combine monitoring and CMAQ modeling data to improve the accuracy of spatially resolved modeling ozone data. Specifically, a Bayesian model is developed to regress CMAQ model estimates on monitoring data, and then the regression model is used to predict concentrations using the CMAQ model results as an input field. Berrocal et al. (2010a) extended the approach to include two pollutants (ozone and $PM_{2.5}$) in a single modeling framework, and Berrocal et al. (2012) added smoothing processes that incorporate spatial autocorrelation and correction for spatial misalignment between monitoring and modeled data. Although these papers did not specifically use NO_2 data, the methods can be applied for NO_2 as they have been for O_3 and $PM_{2.5}$. Bentayeb et al. (2014) applied a similar data assimilation method in which local measurements and elevation data were combined with CTM output in a geostatistical forecasting model. This algorithm was applied for NO_2 as well as PM_{10}, $PM_{2.5}$, SO_2, C_6H_6, and O_3. Correlations between assimilated values and measurements ranged between Pearson $R = 0.75-0.90$. Debry and Mallet (2014) also employed data assimilation for forecasting but combines three CTMs in an ensemble average to minimize the influence of their errors in conjunction with assimilation of observation data. The method of Debry and Mallet (2014) reduced error in hourly, daily, and peak NO_2 concentrations by 19, 26, and 20%, respectively.

In a slightly different approach, Crooks and Isakov (2013) blended CMAQ, AERMOD, and monitoring data for NO_X, $PM_{2.5}$, and CO using a Bayesian model based on a wavelet basis series. In this method, the true exposure is represented by the B-spline wavelet series, and then the CMAQ grid cell concentrations, AERMOD receptor concentrations, and measurement points are represented by the wavelet field modified by some assumed error. These components each comprise linear contributions to a Gaussian likelihood model. For NO_X, the model was found to favor CMAQ data when modeling background and monitor data in dense urban areas where spatial variability is higher. The blended model results had lower prediction error and bias compared with kriging when smaller

numbers of points were used for the kriging surface, although the blended model did not perform as well as kriging when densely gridded data were available for that purpose. Similarly, Robinson et al. (2013) used geographically weighted regression, which used a combination of dispersion model results and monitoring data as input for a regression model to compute concentrations in local population centers and then used kriging to fill in gaps between those population centers. When compared with other kriging methods, the geographically weighted regression approach produced the smallest residual mean squared errors when modeling average NO_2 concentrations across the U.K. for the year 2004. Beevers et al. (2012) also blended CMAQ with a near-road dispersion model and applied the blended model for estimation of human exposure to NO_X in London, U.K. (Beevers et al., 2013). Predicted peak rush hour (0600−0900) NO_X exposures exceeded observed NO_X concentrations by roughly 25% at a heavily trafficked road but performed better when averaged over multiple sites.

Nondimensional Scale Models

Although nondimensional scale models are not currently used for exposure assessment in epidemiologic studies, they are described briefly here as emerging methods for potential use in exposure assessment. Existing wind tunnel and observational data have been used in nondimensional scale models of wind movement that support NO_X fate and transport modeling in the presence of built structures. For example, the Ausbreitungsmodell gemäß der Technischen Anleitung zur Reinhaltung der Luft (AUSTAL2000) airflow model has been developed using a combination of wind speed and direction, mixing layer height, and stability classifications. Air pollutant transport is then modeled using a Lagrangian dispersion model with a random walk to simulate the influence of turbulence on the air pollution parcels' movement. Langner and Klemm (2011) compared AUSTAL2000 to the AERMOD dispersion model using five test cases with varied topography and building presence for which experimental field data existed. Although these runs simulated SO_2 and SF_6 transport, the model performance is instructive for analysis of NO_X transport as well. In every case, AERMOD performed better than AUSTAL2000 in capturing the observed concentrations.

The Operational Street Pollution Model also uses nondimensional scale modeling but is developed specifically to capture street canyon recirculation. Berkowicz et al. (2008) developed a model that includes a turbulent mixing velocity in the street canyon and free convection. Monthly and 6-mo avg NO_2 concentrations were calculated using a turbulent plume model. Modeled concentrations were compared with NO_2 measurements from a 1995 panel study and found to agree reasonably well (6−12% negative bias; R^2 = 0.75−0.81).

3.2.2.3 Stochastic Exposure Models

Although they are not typically used for exposure assessment in epidemiologic modeling, stochastic exposure models inform the risk assessment performed as part of the national ambient air quality standard review process. The state of the science for stochastic population exposure models has not changed substantially since the 2008 ISA for Oxides of Nitrogen, as described in detail in 2008 Annex 3.6 (U.S. EPA, 2008). Examples of stochastic population exposure models include Air Pollution Exposure (APEX), Stochastic Human Exposure and Dose Simulation (SHEDS), and exposure in polis (or cities) (EXPOLIS), which involve stochastic treatment of the model input factors (Kruize et al., 2003; Burke et al., 2001). Advancement in exposure modeling has come from its integration with chemical transport models of outdoor air quality through a hybrid approach (Isakov et al., 2009) and characterization of the uncertainty in these models (Ozkaynak et al., 2009; Zidek et al., 2007).

Hybrid exposure modeling uses ambient air quality input from grid-based models rather than from central site monitoring data, as is typically done (Isakov et al., 2009). In the hybrid version, the CMAQ model is used to simulate concentrations for a coarse discrete grid, e.g., 12 km × 12 km. Next, local scale concentrations from point and mobile sources are estimated using Gaussian dispersion modeling through AERMOD. In combination, these models produce an ambient air quality estimate at the location of the receptor that is then input into APEX or SHEDS to estimate total human exposure. Isakov et al. (2009) observed that the omission of specific point and traffic sources led to an underestimate in median concentration by up to a factor of two, depending on location; these simulations were for benzene and $PM_{2.5}$; NO_X tends to be comparable in spatial variability compared with benzene and more spatially variable compared with $PM_{2.5}$ (Beckerman et al., 2008).

Recent studies have considered the variability and uncertainty associated with exposure modeling. Ozkaynak et al. (2009) considered uncertainty and variability in simulations involving estimation of concentration, exposure, and dose in separate compartments of a model. They found that uncertainty and variability propagated from one compartment to the next. Zidek et al. (2007) addressed uncertainty and variability in exposure modeling by using distributions of input parameters in the exposure model framework rather than point estimates. These models estimate time-weighted exposure for modeled individuals by summing exposure in each microenvironment visited during the exposure period. Zidek et al. (2007) found that use of distributions of input enabled examination of cases for potential subpopulations with common characteristics. Note that both of these studies model PM, but the findings are applicable to NO_X.

Sarnat et al. (2013) recently compared risks of cardiovascular and respiratory morbidity with 24-hour NO_X and other primary and secondary air pollutants in Atlanta using

various exposure metrics. Epidemiologic results based on the mean, median, and 95th percentile of the exposure distributions from APEX were compared with measures from a central site monitor, regional background, AERMOD, and a hybrid model merging AERMOD output with regional background data. NO_X concentrations modeled with APEX were generally higher than those obtained with the hybrid model, likely because the APEX model incorporates road activity levels in their exposure estimates. Epidemiologic analyses for asthma/wheeze produced statistically significantly higher risk ratios for the APEX mean, median, and 95th percentile compared with the hybrid model and central site and background metrics but a negligible difference among the APEX and hybrid results for respiratory or cardiovascular diseases.

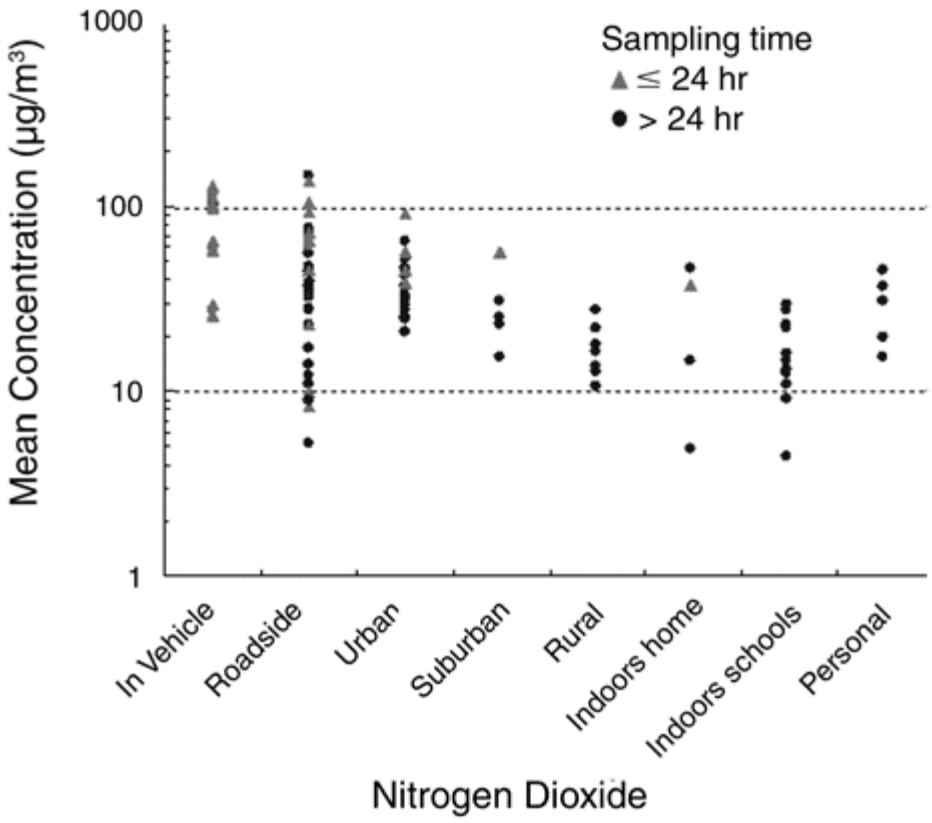
3.2.3 Choice of Exposure Metrics in Epidemiologic Studies

Appropriateness of the exposure metric for a given study depends in part on epidemiologic study design and spatial variability of the pollutant. Table 3-1 summarizes the methods described in Sections 3.2.1 and 3.2.2. Based on epidemiologic studies using various methods for exposure assessment, Figure 3-1 illustrates the range of NO_2 concentrations to which people may be exposed in different locations (HEI, 2010). Because this figure is the result of the HEI (2010) review, the data points included were obtained based on varying temporal scales. The figure illustrates variability in exposures across locations and also the variability measured within a type of location. Given the natural variability of exposures over space and time and nuances of the specific exposure assessment techniques, it is important to be cognizant of the specific applicability and limitations of each approach, as summarized in Table 3-1.

Table 3-1 Summary of sampling methods, their typical use in epidemiologic studies, and related errors and uncertainties.

Method	Epidemiologic Application	Errors and Uncertainties in Exposure Estimates
Central site monitors	Short-term community time-series exposure of a population within a city	Correlation between exposure and measurement decreases with increasing distance from the monitor (Section 3.4.5)
	Long-term exposure for comparison of populations among different cities	Potential for exposure misclassification if the monitor site does not correspond to the exposed population (Section 3.4.5)
		Positive instrument bias (Section 3.2.1.1)
Passive monitors	Short-term panel	Positive instrument bias (Section 3.2.1.2)
	Long-term exposure across a city (or for LUR model fit)	Positive instrument bias (Section 3.2.1)
		Potential for exposure misclassification if the monitors are sited at fixed locations (Section 3.4.5)
LUR	Long-term exposure, usually across a city but sometimes fit among multiple cities	Potential for exposure misclassification if grid is not finely resolved (Section 3.2.2.1)
		Potential for bias if the model is misspecified or applied to a location different from where the model was fit (Section 3.4.5)
IDW	Long-term exposure across a city	Potential for negative bias if sources are not captured or overly smoothed (Section 3.2.2.1)
Spatiotemporal modeling	Not reported	Not yet well understood (Section 3.2.2.1)
CTM	Long-term exposure, sometimes within a city but more typically across a larger region	Potential for exposure misclassification when grid cells are too large to capture spatial variability of exposures (Section 3.2.2.2)
Dispersion modeling	Long-term exposure within a city	Potential for bias where the dispersion model does not capture boundary conditions and resulting fluid dynamics well, e.g., in large cities with urban topography affecting dispersion (Section 3.2.2.2)
Parameterization modeling	Not reported	Not yet well understood (Section 3.2.2.2)

LUR = land use regression; IDW = inverse distance-weighted; CTM = chemical transport model.



Source: HEI (2010)

Figure 3-1 Average nitrogen dioxide concentrations measured in studies using different monitor siting.

Concentrations measured by central site or near-road monitoring are commonly used as a surrogate for human exposure, and they can be used in studies of both short-term and long-term exposure to NO_2 (Section 3.2.1.1). Central site measurements are subject to positive bias from instrument error. For epidemiologic studies of short-term exposure, correlation between measured central site concentration and concentration at some distant point decreases with distance. For epidemiologic studies of long-term exposure to NO_2, the difference between the measured concentration and the true exposure would result in exposure misclassification. Passive sampling techniques such as Palmes tube measurements are subject to positive instrumentation biases. Additionally, passive monitors left in place for sampling durations of days to weeks may produce data having errors and uncertainties that are similar to those associated with using a fixed-site monitor to capture exposures for a population that is dispersed over space and moving in time. The influence of exposure error in passive sampling methods is discussed in more detail

in Sections 3.4.5.2 and 3.4.5.3. Passive sampling can be used for panel studies, or when samples are integrated over a month or averaged over several months, as input for long-term studies (Section 3.2.1.2). The integrated nature of the passive samples limits their application in time-resolved studies. Passively sampled concentrations are also used commonly as input for LUR model fitting (Section 3.2.2.1).

LUR is generally thought to illustrate spatial variability of NO_2 exposures well for use in long-term exposure studies. The quality of the exposure metric provided by the model depends on several factors, including spatial resolution of the model, representativeness of the model fit locations for the city and population under study, and inclusion of the right variables in fitting the model. IDW is also used for exposure estimation between spatially distributed passive NO_2 measurements (Section 3.2.2.1). However, if the monitors are not dense enough to capture the true spatial variability of NO_2 related to localized sources, exposure is likely to be underestimated.

CTMs and dispersion models are based on physics of air flow and contaminant transport (Section 3.2.2.2). Like central site monitors, CTM can be used to compare NO_2 exposures among different cities for long-term exposure studies. However, coarse spatial resolution of CTMs limits its applicability within cities. Dispersion models are frequently used for within-city NO_2 exposure estimation in long-term exposure studies, but the simplifying assumption of Gaussian dispersion can add error to the exposure estimate if meteorology or topography of the built environment are complex. Given this complexity, the direction of exposure error is not predictable. Biases in dispersion model output can occur in either direction, and they depend strongly on the specific environment (i.e., topography, meteorology, source representation) being modeled. Correction methods may sometimes be applied to minimize such error for a given location, but the effectiveness of error minimization must be determined on a case-by-case basis. Subsequent sections will describe characterization of NO_2 exposures, a conceptual model of exposure, relationships among exposure metrics, sources of exposure error, confounding, and implications of exposure error for epidemiologic studies of different designs.

3.3 Characterization of Nitrogen Dioxide Exposures

3.3.1 Nitrogen Dioxide Concentration as an Indicator of Source-based Mixtures

3.3.1.1 Mobile Source Emissions

Seventeen percent of U.S. homes are located within 91 m of a highway with four or more lanes, a railroad, or an airport (U.S. Census Bureau, 2009). Moreover, 7% of U.S. schools serving 3,152,000 school children are located within 100 m of a major roadway, and 15% of U.S. schools serving 6,357,000 school children are located within 250 m of a major roadway (not specifically defined in this study in terms of annual average daily traffic [AADT], number of lanes, or other criteria) based on data from the National Center for Education Statistics (Kingsley et al., 2014). Average one-way commuting times for the U.S. labor force working outside the home are 19.3 minutes for bicyclists, 11.5 minutes for walkers, and 25.9 minutes for all other modes of transportation. Among the populace working outside the home, 15.6% spend 45 minutes or more commuting to work each day (U.S. Census Bureau, 2007). Based on Figure 2-4, the proportion of NO_X emissions from mobile sources in the 21 CBSAs with at least 2.5 million residents is 16% higher than it is among the general population. Hence, a large share of the U.S. population is exposed to the on- and near-road environment on a regular basis, and those exposures are likely to be higher for the 38% of the population living in urban areas (U.S. Census Bureau, 2013). This has implications for potential NO_2 exposure. Section 2.5.3 describes spatial patterns of NO_2 concentrations near roads as a background for understanding traffic-related NO_2 exposure. This section builds on the observations of NO_2 concentration gradients described in Chapter 2 to consider how near-road concentrations influence traffic-related NO_2 and NO_X exposure.

Time spent in traffic can be an important determinant of personal NO_2 exposure. Mölter et al. (2012) calculated associations with time spent in several home, transit, and school microenvironments for a cohort of 12−13 year-old children from Greater Manchester, U.K. based on 2-day sampling periods per season and observed that time spent in transit was positively associated with both NO_2 exposure and mean prediction error of a microenvironmental model of personal NO_2 exposure, where mean prediction error compares the microenvironmental model with NO_2 measurements. Together, these findings suggest that exposures are higher on roads and consequently that time spent in transit may comprise a larger share of daily NO_2 exposure compared with the proportion of time in a day that is spent in transit. Ragettli et al. (2014) estimated exposures among commuters reporting to the 2010 Swiss Mobility and Transport Microcensus in Basel,

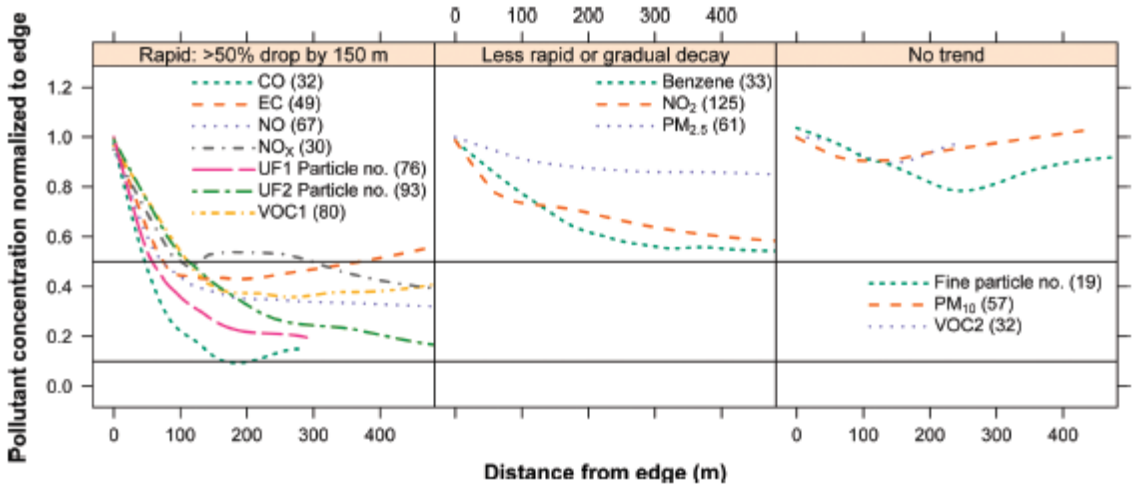
Switzerland based on the times and routes they reported for their commutes and modes of transport and using concentration estimates from a combination of dispersion modeling and LUR. Ragettli et al. (2014) found that travel in motor vehicles produced the highest exposure (reported as the product of concentration and time), followed closely by bicyclists and those taking public transit. Pedestrians had measurably lower exposures.

Health studies often focus on the independent effects of NO_2 or use NO_2 concentration as a surrogate for exposure to traffic pollution mixtures when measurements of other pollutants are not available. NO_2 concentration is routinely measured at sampling sites nationwide, and NO_2 is a prevalent reaction product of NO, which is a component of vehicle exhaust (see Section 2.2). Section 3.4.4 concludes that NO_2 concentration generally correlates spatially with other traffic-related pollutants in urban areas. NO_2 concentration has also been observed in at least one study to correlate with nonconcentration measures of traffic. With respect to exposure, these observations make it hard to distinguish NO_2 from other pollutants when considering the health impacts potentially attributable to each.

As a surrogate for traffic-related exposure, NO_2 concentration may do an adequate job of capturing spatial and temporal trends of traffic pollution. Microscale spatial variability of NO_2 concentrations near roads has been studied extensively, and NO_2 concentration gradients from a number of studies are summarized and compared in Section 2.5.3. Based on 1−2 weeks of passive sampling measurements for NO_2, Wheeler et al. (2008) and Beckerman et al. (2008) reported correlations among NO_2 and several traffic-related air pollutants, including benzene (Pearson $R = 0.85$) and toluene ($R = 0.63$). The near-road air pollutant gradients displayed in the review by Karner et al. (2010) suggested that NO_2 is correlated with traffic-related air pollutants across various distances from a roadway. These studies concluded that gradients in NO_2 concentrations were spatially correlated with gradients in traffic-related pollution.

The size and shape of the near-road gradient for NO_2 determines the spatial zone where near-road exposures are most likely. Observations of the structure of the NO_2 near-road concentration gradient are summarized in Table 2-6 in Section 2.5.3. Although NO_2 tends to correlate with most roadway pollutants in a near-road environment, the NO_2 concentration gradient tends to be shallower than gradients for other primary traffic-related pollutants (e.g., CO, UFP). These gradients influence how exposure and copollutant correlations change spatially across the near-road environment. For example, near road NO_2 concentration is typically 30−100% higher than the urban background concentration, defined here as the lowest concentration measured upwind of the road. In contrast, peak near-road UFP counts are approximately 5−6 times higher than the urban background concentration (Karner et al., 2010). These results suggest that, although NO_2

may capture many aspects of pollutant gradients from the roadway, NO_2 concentration used as a marker for traffic may underestimate the magnitude of the concentration gradient for other near-road pollutants, such as UFP and CO. Figure 3-2 presents the spatial variability of NO_2 and copollutants at various gradients from the roadway reported in Karner et al. (2010) to compare the spatial near-road gradient of NO_2, NO, and NO_X concentrations with those of other traffic-related pollutants (Beckerman et al., 2008). The review of Karner et al. (2010) showed that the NO_2 gradient was much less steep compared with the gradients for NO and NO_X, with decay to background levels within 400−500 m. In a later study of near-road concentrations in Medford, MA, Padró-Martínez et al. (2012) used continuous instrumentation mounted on a mobile sampling unit operated over the course of a year, to illustrate a similar gradient.



Note: NO_2, NO, and NO_X concentration gradients are presented in the center panel. NO_2 = nitrogen dioxide, NO = nitric oxide, NO_X = sum of NO_2 and NO, CO = carbon monoxide, $PM_{2.5}$ = in general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 2.5 µm, a measure of fine particles, PM_{10} = in general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 10 µm, a measure of thoracic particles, EC = elemental carbon, VOC = volatile organic compound, UF = ultrafine.

Data presented from Karner et al. (2010) were synthesized from 41 peer-reviewed references, 11 of which reported data for NO_2, 5 of which reported data for NO, and 6 of which reported data for NO_X. The number in parentheses refers to regression sample size. UF1 and UF2 are measures of ultrafine particle number.

Source: Reprinted with permission of the American Chemical Society, Karner et al. (2010).

Figure 3-2 Spatial variability in concentrations of near-road pollutants, including NO_2, NO, NO_X, CO, $PM_{2.5}$, PM_{10}, EC, benzene, VOCs, and UF Particles. Concentrations are normalized by measurements at the edge of the road.

As also pointed out in Section 2.5.2, near road NO concentrations are typically much higher than near road NO_2 concentrations; Table 2-6 describes the near road concentration gradient for NO_2 only. Table 3-2 expands on these observations to consider

on-road exposure to NO_2 and NO while in transit. Recent on-road and near-road measurements of both NO and NO_2 concentrations indicate that on-road NO exposures can be much higher than on-road NO_2 exposures immediately upon their emission. In particular, the Los Angeles data for NO_X and NO suggest that rush hour NO_2 concentrations are roughly 50−60 ppb, but NO concentrations reach roughly 200−360 ppb in the morning and 95−260 ppb in the afternoon, based on 2-h avg of 1-minute data (Fujita et al., 2011). Beckerman et al. (2008) measured 1-week integrated NO and NO_2 samples next to two highways in Toronto, Canada and also observed that mean NO levels were 3−4 times higher than mean NO_2 levels.

The relationship between NO_2 concentration and traffic metrics informs exposure assessment because it establishes potential for exposure among those commuting or living in the near-road environment. In Minneapolis, MN, Pratt et al. (2014) compared direct traffic metrics, such as traffic volume, with LUR-computed NO_2 concentrations (which were not estimated from traffic volume although road length was included in the model). They observed a correlation (type unstated) of 0.58 between NO_2 concentration and traffic density (AADT per km^2), with a slope of 0.103 on a log-log model of NO_2 versus traffic density. Gauderman et al. (2005) measured the correlation between NO_2 concentrations and various traffic metrics in 12 Southern California communities. On average across the communities, the Spearman correlation between NO_2 concentration and increasing distance to freeway was $r = -0.54$, but the correlation between NO_2 concentration and traffic volume within 150 m of a freeway was $r = 0.24$. The contribution of mobile source emissions to NO_2 concentration varies with strength of additional sources. For example, Ducret-Stich et al. (2013) modeled NO_2 concentration as a function of background NO_2 levels; light duty and heavy duty traffic counts; and meteorological, topographic, and temporal variability in the Swiss Alps with a model $R^2 = 0.91$. They observed that background NO_2 concentration contributed 83% of the variability in the model, while heavy duty and light duty traffic counts contributed 8 and 7%, respectively. Similarly, NO_X has been found to have mixed correlation with traffic density in a nationwide long-term exposure epidemiologic study of the U.S. Veterans Cohort [1976−2001 (Lipfert et al., 2009)]. In areas deemed high traffic density (higher traffic than the average 1985 traffic density), Pearson $R = 0.27$, while for areas of low traffic density (lower traffic than the average 1985 traffic density), $R = 0.56$.

Table 3-2 Near- and on-road measurements of nitrogen dioxide (NO_2), nitric oxide (NO), and the sum of NO and NO_2 (NO_X).

Reference	Location and Date	Distance to Road (m)[a]	Averaging Time	NO (ppb)	NO_2 (ppb)	NO_X (ppb)
Beckerman et al. (2008)	Toronto site 1, August 2004	28, 47, 57, 107, 126, 194, 209, 382, 507, 742, 986	1-week integrated	44.2 (19.9)[b]; 77.6[c]	14.6 (2.8)[b]; 18.6[c]	NR
	Toronto site 2, August 2004	4, 28, 38, 56, 105, 114, 175, 246, 335, 346, 438, 742, 875	1-week integrated	70.5 (62.7)[b]; 239.3[c]	17.5 (4.6)[b]; 28.2[c]	NR
Zhu et al. (2008)	Los Angeles I-710 (mostly diesel trucks), NR	0	2-h avg of 1-min data unfiltered	NR	NR	432 (0.9)[b]
	Los Angeles I-405 (mostly autos), NR	0	2-h avg of 1-min data unfiltered	NR	NR	267 (114)[b]
Fujita et al. (2011)	Los Angeles I-110 (mostly autos), Sep−Dec 2004	0	2-h avg of 1-min data (morning)	347 (235)[b]	NR	411 (250)[b]
	Los Angeles I-405 (mostly autos), Sep−Dec 2004	0	2-h avg of 1-min data (morning)	198 (94)[b]	NR	245 (100)[b]
	Los Angeles SR-60 (mostly autos), Sep−Dec 2004	0	2-h avg of 1-min data (morning)	329 (114)[b]	NR	388 (120)[b]
	Los Angeles truck route, Sep−Dec 2004	0	2-h avg of 1-min data (morning)	361 (143)[b]	NR	426 (154)[b]
	Los Angeles I-110 (mostly autos), Sep−Dec 2004	0	2-h avg of 1-min data (afternoon)	95 (49)[b]	NR	148 (62)[b]
	Los Angeles I-405 (mostly autos), Sep−Dec 2004	0	2-h avg of 1-min data (afternoon)	98 (56)[b]	NR	140 (64)[b]

Table 3-2 (Continued): Near- and on-road measurements of nitrogen dioxide (NO_2), nitric oxide (NO), and the sum of NO and NO_2 (NO_X).

Reference	Location and Date	Distance to Road (m)[a]	Averaging Time	NO (ppb)	NO_2 (ppb)	NO_X (ppb)
	Los Angeles SR-60 (mostly autos), Sep−Dec 2004	0	2-h avg of 1-min data (afternoon)	112 (55)[b]	NR	170 (65)[b]
	Los Angeles truck route, Sep−Dec 2004	0	2-h avg of 1-min data (afternoon)	258 (114)[b]	NR	321 (125)[b]
Fruin et al. (2008)	Los Angeles I-10 (mostly autos), Feb−April 2003	0	2-to-4-h avg of 20-s data	280[d]	NR	NR
	Los Angeles I-710 (mostly diesel trucks), Feb−April 2003	0	2-to-4-h avg of 20-s data	390[d]	NR	NR
MacNaughton et al. (2014)	Boston bike path separate from vehicle traffic, NR	0	Average over 40 3-h sampling periods with 1-min data	NR	14.7 (0.582)[b]	NR
	Boston bike lane adjacent to vehicle traffic, NR	0	Average over 40 3-h sampling periods with 1-min data	NR	19.5 (0.343)[b]	NR
	Boston designated bike lane shared between bikes and buses, NR	0	Average over 40 3-h sampling periods with 1-min data	NR	24.2 (1.72)[b]	NR

avg = average; NR = not reported.

[a]Distance of 0 m indicates on-road measurements.

[b]Average (standard deviation).

[c]Maximum.

[d]Average of medians.

Several recent studies have evaluated the use of central site NO_2 or NO_X concentration as a surrogate for personal exposure to traffic pollution mixtures. In a near-road environment, NO_X concentration can be correlated with pollutants that are also associated with health effects, including UFP and water soluble metals (Sánchez Jiménez et al., 2012); PAHs (Brook et al., 2007); BTEX (Beckerman et al., 2008); and EC (Minguillón et al., 2012) or BC (Clougherty et al., 2013). Correlations generally in the range of 0.6−0.8 of NO_2 with CO, NO_X, and EC (or BC) concentrations forms the basis for a proposed multipollutant mobile source indicator that combines these three species into an Integrated Mobile Source Indicator (IMSI) for traffic-related air pollution. The IMSI is a weighted average of mobile source pollutant concentrations weighted by the ratio of mobile source to total emissions for each pollutant, which Pachon et al. (2012) developed using CO, NO_X, and EC. Although the IMSI is not currently used in any epidemiologic studies of the health effects of NO_2 or NO_X, the IMSI is an informative tool that may shed light on the relationship between traffic-related sources and human exposures, as shown in Equation 3-1.

$$IMSI_{EB} = \frac{\frac{Emission_{EC,mobile}}{Emission_{EC,total}} \times C'_{EC} + \frac{Emission_{NO_{X,mobile}}}{Emission_{NO_{X,total}}} \times C'_{NO_X} + \frac{Emission_{CO,mobile}}{Emission_{CO,total}} \times C'_{CO}}{\frac{Emission_{EC,mobile}}{Emission_{EC,total}} + \frac{Emission_{NO_{X,mobile}}}{Emission_{NO_{X,total}}} + \frac{Emission_{CO,mobile}}{Emission_{CO,total}}}$$

Equation 3-1

Note that *C'* = average concentration normalized by the standard deviation of concentration. Urban street-side (mostly street canyon) NO and NO_2 concentrations have been measured and compared with downwind sites, including those located in parks and reference sites (i.e., sites that are located away from or upwind from traffic-related emissions). A study where criteria pollutant concentrations were sampled using high density siting throughout the five boroughs of New York City with 2-week integrated samples per season (Ross et al., 2013). Consistent with Karner et al. (2010), the street-side sites generally showed higher NO concentrations compared with NO_2 (NO: mean 31.82 ppb, max 151.76 ppb; NO_2: mean 27.60 ppb, max 87.18 ppb) in Ross et al. (2013) (see Table 3-3). The NO on average was lower than the NO_2 away from the road, for example at park sites (NO: mean 18.88 ppb, max 45.15 ppb; NO_2: mean 22.13 ppb max 36.94 ppb). The ranges for overall and truck traffic density, Census population, and building areas were all higher for the street-side sites compared with the park sites. In a mobile van study of street canyons in Helsinki, Finland operating continuous monitors during rush hour (sampling interval: 1-minute), Pirjola et al. (2012) found that the topographical characteristics of the roadway influenced the concentration gradient. They studied concentration profiles on the upwind and downwind sides within a street canyon

and observed downwind-to-upwind ratios of 0.28 and 0.70 for NO and NO_2, respectively, when the street canyon aspect ratio (building height-to-street width) was 0.55. When the aspect ratio increased to 0.70, downwind-to-upwind ratios decreased to 0.18 and 0.65 for NO and NO_2, respectively.

Table 3-3 Summary (mean, range) within 300 m of monitoring sites, by site type, in a spatially dense monitoring campaign in New York City, based on 2-week integrated samples per season.

	Street-Side Sites (*n* = 138)	Park Sites (*n* = 12)	Reference Sites (*n* = 5)	Regulatory Sites (*n* = 5)
NO_2 concentration (ppb)	27.6 (8.32−87.2)[a]	22.1 (8.10−36.9)[a]	20.2 (9.43−38.2)[a]	22.7 (17.1−34.2)[a]
NO concentration (ppb)	31.8 (2.69−152)[a]	18.9 (4.93−45.2)[a]	15.9 (5.42−54.8)[a]	12.1 (3.30−40.0)[a]
Roadway length (km)	4.3−6.0	2.1−3.7	1.9−2.5	
Traffic density (vehicle-km/h)	561−2,800	302−2,560	119−783	
Truck density (vehicle-km/h)	13.4−83.2	0.910−24.4	5.80−13.5	
2000 Census population (number)	1,316−5,819	117−3,455	0−522.7	
Building area (m^2)	90.7−382	0−163	0−38.5	
Residential space area (m^2)	53.79−242	0−124	0−30.7	
Commercial space area (m^2)	15.6−105	0−29.0	0−18.7	
Industrial space area (m^2)	0−7.19	0−4.65	0−0	

NO_2 = nitrogen dioxide; NO = nitric oxide.
Source: Matte et al. (2013).
[a]avg (range).

NO_2 and NO emissions, concentrations, and therefore exposures, are also subject to interventions in the built environment. After a tunnel was built in Sydney, Australia to reduce urban pollution levels, Cowie et al. (2012) observed statistically significant reductions in NO_2 and NO_X concentrations by 1.4 and 4.6 ppb, adjusted for meteorology, based on 2-week passive sampler measurements taken at three periods during Fall 2006−2008. Beevers and Carslaw (2005) studied the impact on annual NO_X emissions of the London congestion pricing zone implemented in 2003 to reduce traffic in central London. Overall, they reported a 12% decrease in NO_X emissions within the congestion pricing zone and a 1.5% increase in NO_X emissions at the surrounding ring road, related

to some individuals re-routing their drives to the surrounding ring road where no payment was required. Similarly, Panteliadis et al. (2014) studied the impact of congestion pricing in Amsterdam, Netherlands and observed a 6.6% reduction in NO_2 concentrations at a roadside measurement, with an 11% reduction in the traffic contribution to ambient NO_2 concentrations. However, Masiol et al. (2014) analyzed the effects of traffic-free Sundays over 13 years on air quality in the Po Valley of Italy and saw no appreciable change in NO_2 levels. Rao et al. (2014) studied the influence of tree canopies on NO_2 levels in Portland, OR using LUR modeling and observed a 38% reduction in NO_2 related to increasing the tree canopy at higher elevations in the city. MacNaughton et al. (2014) measured NO_2 exposures of bicyclists in Boston using real-time monitoring (3-h avg of 1-minute data) equipment and GPS and observed that riding in a shared bicycling/bus lane, traffic density, background NO_2 concentration, and vegetation density were associated with measured NO_2 exposures. The city of Beijing, China restricted traffic during the 2008 Olympics, thus creating a natural experiment in pollution reduction. Zhang et al. (2013) reported that the average of 1-hour NO_2 concentration measurements dropped from 25.6 ± 3.66 ppb to 14.6 ± 3.76 ppb when comparing periods before (June 2−July 20) and during (July 21−September 19) the Olympic games. After the Olympics (September 20−October 30), concentrations increased back up to 41.4 ± 3.81 ppb. Huang et al. (2012) reported reductions of 21.6 and 12.9% for the periods before and during the Olympics compared with the previous year. The reduced NO_2 concentrations that followed these interventions suggest that controls can lead to reduced NO_2 exposures.

3.3.1.2 Other Outdoor Sources

As described in Section 2.3, other sources contributing to ambient NO_X emissions include nonroad mobile sources, electric generating units, industrial sources, and wildfires. Nonroad mobile sources, such as airports, shipping ports, and rail yards, can contribute substantially to local and regional ambient NO_X concentrations (Kim et al., 2011; Williams et al., 2009; Vutukuru and Dabdub, 2008; Carslaw et al., 2006; Unal et al., 2005). Carslaw et al. (2012a) took advantage of the natural experiment of the Icelandic volcano eruption of 2010, when airports across Europe were shut down for 6 days, to evaluate the local effect on airport NO_X. Downwind of the airport, a 38% reduction in average NO_X concentrations (from 42 ppb down to 26 ppb) was observed. At shipping ports and airports, traffic from ground-level support activities can also contribute a large portion to NO_X emissions from these sources (Klapmeyer and Marr, 2012; Kim et al., 2011). Outside of urban centers where traffic is not a dominant source, other sources of NO_X may include wildfires and residential wood-burning. As such, NO_X concentration

may not always be a reliable proxy for traffic pollution. Section 2.3 discusses different sources of NO_X in more detail.

3.3.2 Indoor Dynamics

3.3.2.1 Sources, Sinks, and Penetration

The general understanding of oxide of nitrogen production indoors has not changed since the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008). Indoor sources of oxides of nitrogen are combustion-based, including gas stoves, gas heating, oil furnaces, coal stoves, wood burning stoves, kerosene heaters, smoking, candle burning, and to a lesser extent, electric cooking. The magnitude of indoor oxides of nitrogen depends on ventilation of the indoor space and appliances, source strength, and rate of pollutant reaction. Recent studies show associations between indoor combustion and indoor NO_2 levels (Vrijheid et al., 2012; Kornartit et al., 2010; Park et al., 2008) or indoor NO_X levels (Cattaneo et al., 2014), depending on what was measured during the study. HONO can also be emitted directly during combustion or through surface reactions. Park et al. (2008) measured HONO and NO_2 during combustion and compared their results with older studies in the peer-reviewed literature, as shown in Table 3-4. High peak-to-mean ratios suggest high temporal variability of the exposures that might be differentiated from exposures of outdoor origin through time-series analysis. This review also generally found higher HONO concentrations in the presence of indoor combustion sources. Oxides of nitrogen can be lost through indoor deposition and ventilation (U.S. EPA, 2008). Sarwar et al. (2002) reported deposition velocities of $6-7 \times 10^{-5}$ m/sec for NO_2, HONO, HNO_3, HO_2NO_2, NO_3^-, and N_2O_5. Much lower deposition velocities (not detected -2×10^{-6} m/s) were reported for NO, PAN, and organic NO_3^- species.

Table 3-4 Indoor nitrogen dioxide (NO_2) and nitrous acid (HONO) concentrations in the presence and absence of combustion.

Study	Combustion Source	Measurement Frequency	NO_2 (ppb)		HONO (ppb)	
			Peak	24-h avg	Peak	24-avg
Brauer et al. (1990)[a]	No source (background)	15 min	29	17	8	5
	Gas range[a]	15 min	157	36	35	13
	Convective space heater[a]	15 min	955	209	106	42
Brauer et al. (1990)[b]	No source	15 min	5.0	1.8	3.5	3.4
	Gas range[b]	15 min	37	8	31	9.6
Brauer et al. (1991)[c]	Unknown	15 min	NR	NR	NR	1−12
Spengler et al. (1993)[d]	Gas range, stove, furnace	24-h	NR	60 (24−115)	NR	4.7 (2−8)
Simon and Dasgupta (1995)[e]	Kerosene heater	8 min	NR	NR	5−10	NR
Leaderer et al. (1999)[f]	No source[f]	24-h	NR	NR	NR	0.8 (0.0−2.9)
	Gas stoves[f]	24-h	NR	NR	NR	4.0 (0.0−11.3)
	Kerosene heaters[f]	24-h	NR	NR	NR	6.8 (0.2−35.9)
	No source[f]	24-h	NR	NR	NR	2.4 (0.1−20.1)
	Gas stoves[f]	24-h	NR	NR	NR	5.5 (0.4−20.1)
Khoder (2002)[g]	Gas appliances (summer)	24-h	NR	39 (20−73)	NR	3.7 (1.3−7.3)
	Gas appliances (winter)	24-h	NR	65 (27−120)	NR	6.8 (1.6−12.5)
Lee et al. (2002)[h]	Gas range, etc.	6-day	NR	28 (4.3−52.0)	NR	4.6 (0.1−21.1)
Jarvis et al. (2005)[i]	Gas hob		NR	12.8	NR	4.1
	Gas oven		NR	12.8	NR	5.0
Hong et al. (2007)[j]	Gas range	4 min	81.1	NR	9.3	NR
Park et al. (2008)[k]	Gas range	4 min	189.3	19.4	15.2	2.1

avg = average; NR = not reported.
[a]Location: Chicago, IL research home, unvented combustion condition; gas range operation hours: 1 h (with one burner and 2,320 kcal/h); convective space heater operation hours: 4 h (with one burner and 2,785 kcal/h).
[b]Location: Maryland research home, unvented combustion condition; gas range operation hours: 1 h (with one burner and 2,320 kcal/h).
[c]Location: 11 Boston, MA homes (winter).
[d]Location: 10 homes in Albuquerque, NM (winter).
[e]Location: Four different home environments with a small kerosene heater (2,270 kcal/h).
[f]Location: 58 homes (summer) and 223 homes (winter) in southwest Virginia and Connecticut; 39 inside homes without gas stoves (summer); 19 inside homes with gas stoves (summer); 74 inside kerosene-heater homes (winter); 96 inside homes without kerosene heaters and gas stoves (winter); 52 inside homes without kerosene heaters and with gas stoves (winter).
[g]Location: Four homes in suburban residential areas in Greater Cairo, Egypt.
[h]Location: 119 homes in southern California (spring).
[i]Location: Homes in European community.
[j]Location: Living room of an apartment in Gwangju, Korea (May 2006).
[k]Location: Korean apartment (city and year unspecified, October).
Source: Reprinted with permission of Elsevier, Park et al. (2008).

3.3.2.2 Indoor Chemistry

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) described well-established reactions involving oxides of nitrogen and other indoor air pollutants for gas-phase and surface chemistry that serves as both a source and sink for oxides of nitrogen. Knowledge of indoor chemistry helps identify potential sources of uncertainty in estimates of indoor exposure to ambient oxides of nitrogen. Moreover, epidemiologic studies of indoor exposure may providing supporting evidence to the assessment of health effects from ambient NO_2 exposure. Identification of the uncertainty in those exposure estimates can aid interpretation of those studies.

For gas phase reactions, indoor NO can be oxidized to NO_2 via reaction with O_3 or HO_2 radicals generated by indoor O_3 chemistry or VOCs found in household products. NO_2 can react with O_3 to form NO_3 radicals that may subsequently oxidize organic compounds. NO_2 also reacts with free radicals to produce PAN. NO_2 removed through surface reactions was known to contribute to NO levels indoors either by surface reduction of NO_2 or by reaction of NO_2 with aqueous HONO on indoor surfaces (Spicer et al., 1989). Conversion of NO_2 to HONO occurs through a number of indoor surface reactions, and the reaction increases with increased relative humidity (U.S. EPA, 2008). A recent study has demonstrated the role of irradiance in humidity-driven surface reaction of NO_2 to HONO on paints (Bartolomei et al., 2014). Surface reactions of NO and OH radicals may also produce HONO, but the reaction rate is slower than for NO_2.

Indoor combustion can lead to direct emission of NO and HONO, and conversion of NO to NO_2 can lead to secondary HONO production from heterogeneous reactions involving NO_2 on indoor surfaces. Park et al. (2008) observed HONO to be correlated with both NO (Spearman $r = 0.64$) and NO_2 ($r = 0.68$) during combustion. They noted that HONO concentrations were 4−8% of NO_2 concentrations during gas range operations but rose to ~25% of NO_2 concentrations after combustion ceased, which underscores the role of surface reaction as the major source of HONO production. In a model of combustion products for oxides of nitrogen during candle and incense burning, Loupa and Rapsomanikis (2008) observed simultaneous NO and HONO production, the latter of which were in agreement with older test chamber results of HONO production during combustion (De Santis et al., 1996). These studies on surface reactions of NO_2 provide insight into indoor NO_2 sinks that may reduce NO_2 exposures as well as exposures to HONO, of which health effects are less well understood.

Recent gas-phase indoor chemistry work has shed light on processes involving organic compounds and/or secondary organic aerosols (SOA). Carslaw et al. (2012b) modeled indoor reactions forming SOA and observed that for their base case simulation, organic nitrates constituted 64% of the overall SOA, while PANs constituted an additional 21%.

In sensitivity tests varying ambient concentrations and meteorological conditions, organic nitrates varied from 23−76% of the SOA, and PAN varied from 6−42%. Nøjgaard et al. (2006) investigated the interference of NO_2 in ozonolysis of monoterpenes in a simulation of indoor air chemistry and observed that NO_2 reacted with O_3 and hence reduced SOA formation from ozonolysis of alkenes α-pinene and β-pinene while increasing the mode of the SOA size distribution. Intermediate NO_3 products may play a role in this process, as described above. However, the presence of NO_2 had less effect on ozonolysis of *d*-limonene, and this is thought to occur because the ozonolysis reaction rate is faster. In chamber experiments and computational chemistry models, Cao and Jang (2008) and Cao and Jang (2010) tested toluene SOA formation in the presence of low (≤3 ppb), medium (90−135 ppb), and high (280−315 ppb) NO_X concentrations and found that the organic matter component of the toluene SOA yield generally decreased with increasing NO_X concentrations, especially when high NO levels (~222−242 ppb) were present. Ji et al. (2012) explored rate constants of NO_2 reactions with various low molecular weight aldehydes found indoors and observed that the reaction rates, k, increased in the following order: $k_{formaldehyde} < k_{acetaldehyde} < k_{propanal} < k_{butanal}$. Ji et al. (2012) concluded from this observation that NO_2 reacts more with longer chain, low molecular weight aldehydes compared with shorter chain, low molecular weight aldehydes. $RC(=O)\cdot$ radicals and HONO were both observed to be products of these reactions. These sinks may result in lower NO_2 exposures, but little information is available regarding organic nitrate reaction product exposures.

Reactions involving N_2O_5 (formed by reaction of NO_2 and NO_3 in the presence of another molecule) in an indoor context have been studied in recent years. In an examination of NO_3 and N_2O_5 (measured as the sum of those two species) in an office building, Nøjgaard (2010) observed that alkenes remove more indoor NO_3 and N_2O_5 than either ventilation or surface deposition. Griffiths et al. (2009) studied N_2O_5 uptake by organic aerosols in a reaction cell and large chamber (260 m^3) and observed little N_2O_5 uptake by solid organic aerosols, more efficient uptake by liquid aerosols, and uptake that increased with increasing RH. N_2O_5 uptake by dicarboxylic acids (oxalic acid, malonic acid, succinic acid, and glutaric acid) was 30−90% of that by $(NH_4)_2SO_4$ and $(NH_4)_2SO_4$-mixed dicarboxylic acid aerosols at similar RH. N_2O_5 uptake by malonic or azelaic acid in the presence of higher RH is consistent with findings of Thornton et al. (2003) for experiments conducted in a reaction cell. Raff et al. (2009) suggested that N_2O_5 autoionizes to $NO_2 + NO_3^-$ and then reacts quickly with water to form HNO_3; it is possible that HNO_3 might then participate in the liquid aerosol reactions described by Griffiths et al. (2009) and Thornton et al. (2003). Raff et al. (2009) also proposed autoionization of N_2O_5 as a likely mechanism for reaction with HCl, which would result in ClNO and HNO_3 formation while NO_2 and water vapor experienced an intermediate surface reaction to form HONO, which would react with HCl. Complexity of reactions

involving N_2O_5 in creating NO_2 as an intermediary reaction product also lends uncertainty to NO_2 exposure assessment. This uncertainty may lead to variability in personal or indoor NO_2 exposure measurements.

3.4 Exposure Assessment and Epidemiologic Inference

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) examined several factors influencing exposure to ambient oxides of nitrogen and measurements used to represent exposures. These include high spatial and temporal variability of NO_2 concentrations in urban areas and near roads, location of NO_2 samplers, and ventilation of indoor microenvironments. The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) concluded that errors associated with the use of NO_2 concentrations measured at central site monitors as exposure metrics for epidemiologic studies tended to bias the health effect estimate towards the null for both short-term exposure and long-term exposure epidemiologic studies. The following sections explore new evidence regarding a conceptual exposure model, exposure metrics employed in epidemiologic studies, personal-ambient relationships, factors that introduce exposure error, potential confounding, and how the exposure errors may or may not introduce bias and uncertainty into epidemiologic health effect estimates, depending on the epidemiologic study design.

3.4.1 Conceptual Model of Total Personal Exposure

Total personal exposure (E_T) integrates the product of microenvironmental concentration (C) and fraction of time spent in a microenvironment across an individual's microenvironmental exposures, t:

$$E_T = \sum_{j}^{n} C_j t_j$$

Equation 3-2

where C_j = average NO_2 concentration in the jth microenvironment, t_j = fraction of total time spent in the jth microenvironment, and n = total number of microenvironments which the individual has encountered (U.S. EPA, 2008) (Klepeis et al., 2001). Hence, both the microenvironmental NO_2 concentration and time-activity aspects of total exposure must be considered.

Alternatively, based on the principle of mass balance, an individual's total NO_2 exposure can be expressed as the sum of its ambient NO_2 exposure (E_a) and nonambient NO_2 exposure (E_{na}) components (U.S. EPA, 2008) (Wilson and Brauer, 2006):

$$E_T = E_a + E_{na}$$

Equation 3-3

E_a represents the amount of NO_2 exposure derived from outdoor sources, and E_{na} represents the amount of NO_2 exposure from indoor sources. The microenvironmental formulation presented in Equation 3-2 and the component formulation presented in Equation 3-3 can be rectified by recognizing that E_a and E_{na} can both be expressed in terms of microenvironmental concentrations and time spent in each outdoor and indoor microenvironment. During the fraction of a day spent in each outdoor microenvironment ($y_{o,j}$), ambient exposure to NO_2 having an outdoor concentration of $C_{o,j}$ is:

$$E_{o,j} = y_{o,j} C_{o,j}$$

Equation 3-4

Indoor NO_2 exposures in the jth microenvironment ($E_{i,j}$) are more complicated because some part of indoor exposure may emanate from nonambient sources, and some part of indoor exposure infiltrates from outdoors. Indoor exposures from nonambient sources are given as $E_{na,j}$. Exposures in each indoor microenvironment from ambient sources are also influenced by infiltration of outdoor NO_2 (INF_j), time spent indoors ($y_{i,j}$), and $C_{o,j}$:

$$E_{i,j} = y_{i,j} INF_j \cdot C_{o,j} + E_{na,j}$$

Equation 3-5

Infiltration is a function of the jth microenvironment's air exchange rate (a_j), air pollutant penetration (P_j), and decay rate (k_j):

$$INF_j = P_j\, a_j/(a_j + k_j)$$

Equation 3-6

Hence, indoor NO_2 exposure for microenvironment *j* is the sum of the ambient and a nonambient components:

$$E_{i,j} = y_{i,j}[P_j\, a_j/(a_j + k_j)]C_{o,j} + E_{na,j}$$

Equation 3-7

Finally, E_a can be described as the sum of the outdoor NO_2 exposure and the ambient component of the indoor NO_2 exposure, summed over *j* indoor microenvironments (U.S. EPA, 2008) (Wilson and Brauer, 2006; Wilson et al., 2000):

$$E_a = \sum_{j=1}^{n} y_{o,j}C_{o,j} + \sum_{j=1}^{n} y_{i,j}[P_j\, a_j/(a_j + k_j)]C_{o,j}$$

Equation 3-8

Ambient concentration of NO_2 is often used as a surrogate for human exposure. In concert, a second simplifying assumption is often made that the exposed individual resides in one indoor microenvironment, such that time-activity data are reduced to "time indoors" and "time outdoors." Errors associated with this approach, which may vary depending on the epidemiologic study design in which the exposure surrogate is used, are described in detail in Section 3.4.3. In this case, outdoor microenvironmental NO_2 exposures (E_o) are expressed simply as the product of the fraction of all time spent outdoors (y_o) and ambient NO_2 concentration (C_a): $E_o = y_o C_a$. Furthermore, based on the assumption that the individual occupies only one indoor and one outdoor microenvironment, then the infiltration term can be simplified to $y_i[P{\cdot}a/(a + k)]$, and because $y_o + y_i = 1$:

$$E_a = \{y_o + y_i[Pa/(a + k)]\}C_a$$

Equation 3-9

Then, an exposure factor (α) can be defined to express the influence of time-weighting and infiltration on NO_2 exposure:

$$\alpha = y_o + (1 - y_o)[Pa/(a + k)]$$

Equation 3-10

Last, an approximate expression for total personal exposure is obtained:

$$E_T = \alpha C_a + E_{na}$$

Equation 3-11

Comparison of Equations 3-3, 3-9, and 3-11 reveals that α can also be approximated as the ratio E_a/C_a. Subsequent sections examine how E_a, α, and C_a are modeled or measured, and how errors and uncertainties in the simplifying assumptions behind Equations 3-9, 3-10, and 3-11 may influence health effect estimates computed from epidemiologic studies of varying design.

3.4.2 Personal-Ambient Relationships and Nonambient Exposures

Personal exposure measurements typically capture both ambient and nonambient exposure contributions; for the purpose of this document, these are referred to as "total personal exposure" measurements. The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) concluded that literature relating ambient NO_2 concentrations measured by a central site monitor to personal NO_2 exposures was mixed for studies of both short-term and long-term NO_2 exposure, with some studies finding associations between the personal and central site monitors and other studies finding no association. These inconsistencies reflected various factors that influence exposure in respective studies, including proximity and strength of sources of ambient and nonambient NO_X, spatiotemporal variability of NO_2 concentrations, and time-activity behavior of the exposed sample population. Recent studies have found that personal NO_2 concentration measurements taken for adults and children tend to be more highly correlated with indoor

concentrations compared with personal correlations with outdoor or ambient concentrations, although wide variability in the correlations was observed (see Tables 3-5 and 3-6). Personal-outdoor (i.e., measurements taken outdoors but not at a central site monitor) correlations also tended to be higher for summer compared with winter. This is not surprising because open windows and greater time spent outdoors during summer likely increase exposure to outdoor air (Brown et al., 2009). The study results indicate that, for epidemiologic studies of short-term exposure, indoor sources of NO_2 can add noise to the ambient NO_2 exposure signal. As described further in Section 3.4.5.1, uncertainty in the NO_2 exposure term can lead to negative bias and added uncertainty in the epidemiologic health effect estimate for short-term exposure studies.

Table 3-5 Ambient, outdoor, transport, indoor, and personal nitrogen dioxide measurements (ppb) across studies.

Study	Location	Time Period	Sampling Interval	Ambient (Central Site)	Outdoor (Outside Residence)	Transport	Indoor	Personal	Personal-Ambient Slope[a,b]
Sarnat et al. (2012)	El Paso, TX (large city)	January–May, 2008	96-h	14.0–20.6[c]	4.5–14.2[c]	NR[d]	4.0–8.1[c]	NR	NR
	Ciudad Juarez, Mexico (large city)			NR	18.7–27.2[c]	NR	23.1–120.8	NR	NR
Williams et al. (2012b); Meng et al. (2012a)	Detroit, MI (large city)	Summer, 2004–2007	24-h	Williams: 22.0[e]; Meng: 22.0[e]; 22.7[c]	NR	NR	NR	Total: Williams: 25.5[c]; Meng: 25.4[c] Ambient: 16.0[e]; 21.0[c]	Meng: 0.24; 0.13[f]
		Winter, 2004–2007		24.0[e]; 23.9[c]	NR	NR	NR	Total: 24.0[e]; 35.6[c] Ambient: 18.0[e]; 20.4[c]	Meng: 0.08; 0.07[f]
Suh and Zanobetti (2010)	Metropolitan Atlanta, GA (large city)	Fall, 1999–Spring, 2000	24-h	17.96[e]; 17.13[c]	NR	NR	NR	8.08[e]; 11.60[c]	NR

Table 3-5 (Continued): Ambient, outdoor, transport, indoor, and personal nitrogen dioxide measurements (ppb) across studies.

Study	Location	Time Period	Sampling Interval	Ambient (Central Site)	Outdoor (Outside Residence)	Transport	Indoor	Personal	Personal-Ambient Slope[a,b]
Brown et al. (2009)	Metropolitan Boston, MA (large city)	Nov 1999−Jan 2000	24-h	25.8[g]; 26.8[c]	NR	NR	NR	10.4[g]; 12.9[c]	All: 0.19 Windows closed: 0.09 Windows open: 0.31 Low AER: 0.21 High AER: 0.15
		June−July 2000		22.0[g]; 22.8[c]	NR	NR	NR	13.9[g]; 17.4[c]	All: 0.23 Windows closed: 0.64 Windows open: 0.10 Low AER: 0.34 High AER: 0.19
Delfino et al. (2008)	Riverside, CA; Whittier, CA (SoCAB) (large city)	July−Dec 2003 (Riverside); July−Dec 2004 (Whittier)	24-h	25.3[e]; 25.0[c]	NR	NR	NR	26.7[e]; 28.6[c]	NR
Delgado-Saborit (2012)	Birmingham, U.K. (large city)	July−Oct 2011	5-min	47[c]	64[c]	Car: 40[c] Bus: 71[c] Bike: 125[c] Train: 58[c]	Office: 14[c] Home: 17[c]	All: 23[c] Gas oven: 31[c] Electric oven: 19[c]	1-h avg: 0.044 Sampling event: 0.14

Table 3-5 (Continued): Ambient, outdoor, transport, indoor, and personal nitrogen dioxide measurements (ppb) across studies.

Study	Location	Time Period	Sampling Interval	Ambient (Central Site)	Outdoor (Outside Residence)	Transport	Indoor	Personal	Personal-Ambient Slope[a,b]
Kornartit et al. (2010)	Hertfordshire, U.K. (Greater London Area) (large city)	Winter 2000	7-day	NR	NR	NR	Electric oven: Bedroom: 7.8[c] Living room: 7.9[c] Kitchen: 7.1[c] Gas oven: Bedroom: 10.8[c] Living room: 13.7[c] Kitchen: 20.6[c]	Electric oven: 8.1[c] Gas oven: 11.2[c]	NR
		Summer 2001		NR	NR	NR	Electric oven: Bedroom: 12.7[c] Living room: 13.1[c] Kitchen: 11.0[c] Gas oven: Bedroom: 14.3[c] Living room: 14.7[c] Kitchen: 14.2[c]	Electric oven: 13.3[c] Gas oven: 14.6[c]	NR

Table 3-5 (Continued): Ambient, outdoor, transport, indoor, and personal nitrogen dioxide measurements (ppb) across studies.

Study	Location	Time Period	Sampling Interval	Ambient (Central Site)	Outdoor (Outside Residence)	Transport	Indoor	Personal	Personal-Ambient Slope[a,b]
Lee et al. (2013)	Seoul, Korea (large city)	July 2008	NR	29.5[f]; 30.7[c]	NR	NR	Home: 24.4[g]; 25.7[c] Work: 19.2[g]; 21.5[c]	25.3[g]; 27[c]	NR
		Jan 2009	NR	29.5[g]; 31.1[c]	NR	NR	Home: 20.9[g]; 24.9[c] Work: 27.9[g]; 29.9[c]	22.5[g]; 24.2[c]	NR
	Daegu, Korea (mid-sized city)	July 2008	NR	19.9[g]; 21.1[c]	NR	NR	Home: 19.3[g]; 20.3[c] Work: 21.3[g]; 22.8[c]	21.4[g]; 22.6[c]	NR
		Jan 2009	NR	23.0[g]; 24.3[c]	NR	NR	Home: 23.3[g]; 25.1[c] Work: 20.3[g]; 22.9[c]	20.3[g]; 21.7[c]	NR
	Asan, Korea (small city)	July 2008	NR	26.0[g]; 27.9[c]	NR	NR	Home: 23.8[g]; 24.9[c] Work: 21.1[g]; 25.6[c]	22.6[g]; 24.3[c]	NR
		Jan 2009	NR	21.6[g]; 23.9[c]	NR	NR	Home: 20.3[g]; 22.9[c] Work: 13.0[g]; 18.6[c]	19.9[g]; 22.3[c]	NR

Table 3-5 (Continued): Ambient, outdoor, transport, indoor, and personal nitrogen dioxide measurements (ppb) across studies.

Study	Location	Time Period	Sampling Interval	Ambient (Central Site)	Outdoor (Outside Residence)	Transport	Indoor	Personal	Personal-Ambient Slope[a,b]
Lee et al. (2013) (Continued)	Suncheon, Korea (rural)	July 2008	NR	15.0[g]; 15.9[c]	NR	NR	Home: 13.0[g]; 14.3[c] Work: 12.0[g]; 14.5[c]	14.0[g]; 16.3[c]	NR
		Jan 2009	NR	12.5[g]; 15.2[c]	NR	NR	Home: 15.9[g]; 20.4[c] Work: 9.3[g]; 12.9[c]	12.9[g]; 15.7[c]	NR
	Total	July 2008	NR	21.7[g]; 23.7[c]	NR	NR	Home: 19.5[g]; 21.2[c] Work: 18.4[g]; 21.4[c]	20.5[g]; 22.6[c]	NR
		Jan 2009	NR	20.6[g]; 23.6[c]	NR	NR	Home: 19.9[g]; 23.3[c] Work: 16.4[g]; 21.1[c]	18.6[g]; 21.0[c]	NR
Du et al. (2011)	Beijing, China	Oct 2006	Varied with transit times	NR	NR	Subway: 20[c]; Nonsubway: 22[c]; Taxi drivers: 25[c]	NR	NR	NR

Table 3-5 (Continued): Ambient, outdoor, transport, indoor, and personal nitrogen dioxide measurements (ppb) across studies.

Study	Location	Time Period	Sampling Interval	Ambient (Central Site)	Outdoor (Outside Residence)	Transport	Indoor	Personal	Personal-Ambient Slope[a,b]
Physick et al. (2011)	Melbourne, Australia (large city)	May 2006; June 2006; April 2007; May 2007	Ambient: 1 h; Personal: Participants wore two sets of passive samplers. One was worn for 48 h. One was worn only during the hours spent at home, at work, in transit, or while performing other activities.	6:00 p.m.to 8:00 a.m.: 19.8[e]; 18.7[c] 8:00 a.m. to 6:00 p.m.: 20.3[e]; 21.2[c]	NR	NR	Home: 17.2[e]; 16.8[c] Work: 21.6[e]; 21.7[c]	Total: 12.2[h] Home: 8.2[h] Work: 14.7[h] Transit: 23.4[h] Other: 17.4[h]	NR
Sahsuvaroglu et al. (2009)	Hamilton, Canada (mid-sized city)	Oct 2003	72-h	NR	All: 32.0[c] Non-ETS: 31.7[c]	NR	All: 22.4[c] Non-ETS: 21.9[c]	All: 23.3[c] Non-ETS: 22.4[c]	All: 0.06 Nonsmoking: 0.13
		May 2004		NR	All: 17.6[c] Non-ETS: 16.8[c]	NR	All: 13.5[c] Non-ETS: 12.3[c]	All: 14.4[c] Non-ETS: 14.0[c]	All: 0.31 Nonsmoking: 0.28
		Aug 2004		NR	All: 9.7[c] Non-ETS: 9.6[c]	NR	All: 8.2[c] Non-ETS: 7.4[c]	All: 8.8[c] Non-ETS: 8.2[c]	All: 0.50 Nonsmoking: 0.15
		Total		NR	All: 19.3[c] Non-ETS: 18.9[c]	NR	All: 14.4[c] Non-ETS: 13.6[c]	All: 15.2[c] Non-ETS: 14.6[c]	All: 0.62 Nonsmoking: 0.62

Table 3-5 (Continued): Ambient, outdoor, transport, indoor, and personal nitrogen dioxide measurements (ppb) across studies.

Study	Location	Time Period	Sampling Interval	Ambient (Central Site)	Outdoor (Outside Residence)	Transport	Indoor	Personal	Personal-Ambient Slope[a,b]
Schembari et al. (2013)	Barcelona, Spain (large city)	Nov 2008 and Oct 2009	7-day	NR	18.7[g,j]; 19.4[c,i]	NR	19.2[g,i]; 20.6[c,i]	17.7[g,i]; 18.6[c,i]	1.01[k]
Molloy et al. (2012)	Melbourne, Australia (large city)	Aug 2008−Dec 2008; Jan 2009−April 2009	7-day	NR	9.5[e]; 10.0[c]	NR	7.9[e]; 8.4[c]	NR	0.9[k]
Pegas et al. (2012)	Aveiro, Portugal (small city center, suburb)	April−June 2010	7-day	NR	City center: 10.5[c,i]; Suburb: 10.1[c,i]	NR	City center: 7.4[c,i,j]; Suburb: 6.9[c,i,j]	NR	NR
Chatzidiakou et al. (2014)	Suburban London, England	Nov 2011	5-day	NR	7.4	NR	3.7[l]	NR	NR
				NR	5.1	NR	2.9[l]	NR	NR
				NR	5.1	NR	2.7[l]	NR	NR
	London, England			NR	19	NR	13[l]	NR	NR
				NR	20	NR	16[l]	NR	NR
				NR	22	NR	18[l]	NR	NR
Rivas et al. (2014)	Barcelona and Sant Cugat, Spain	Jan−June 2012	4-day	22[c]; 20[d]	25[c]; 24[d]	NR	16[c]; 16[d]	NR	NR

AER = air exchange rate; avg = average; ETS = environmental tobacco smoke; NR = not reported; SoCAB = South Coast Air Basin.
[a]Unadjusted models only.
[b]Total personal NO_2 exposure vs. ambient concentration unless noted otherwise.
[c]Average.
[d]Median.
[e]Personal exposure to ambient NO_2 vs. ambient concentration.
[f]Geometric mean.
[g]Data provided by the authors for Figure 1 of Physick et al. (2011).
[h]Reported in $\mu g/m^3$ and converted to ppb assuming 25°C and 760 mmHg.
[i]Averaged over 4 classrooms and 2 weeks.
[j]Indoor-outdoor ratio, rather than slope, is reported for Schembari et al. (2013).
[k]Integrated measurement over 2 weeks.
[l]Estimated from reported indoor-outdoor ratio and outdoor NO_2 concentration.

Table 3-6 Correlations between measured nitrogen dioxide (NO_2) concentrations from personal, outdoor, indoor, and ambient monitors.

Study	Location	Personal-Ambient	Personal-Outdoor	Personal-Indoor	Outdoor-Indoor
Sarnat et al. (2012)[a]	Ciudad Juarez, Mexico; El Paso, TX	NR	NR	NR	CJ-A: 0.36; CJ-B: 0.92; EP-A: 0.66; EP-B: 0.01
Williams et al. (2012a)[a]	Wayne County, MI	All Subjects: 0.11; Vest-compliant (>60%)[c]: 0.14	NR	NR	NR
Suh and Zanobetti (2010)[a]	Atlanta, GA	0.12	NR	NR	NR
Brown et al. (2009)	Boston, MA	Winter: 0.00; Summer: 0.03	NR	NR	NR
Delfino et al. (2008)	2 southern California cities	0.43	NR	NR	NR
Kousa et al. (2001)	Helsinki, Finland; Basel, Switzerland; Prague, Czech Republic	NR	0.61	0.73	0.66
Delgado-Saborit (2012)	Birmingham, U.K.	1-h NO_2: 0.024; Sampling event NO_2: 0.15	NR	NR	NR
Lee et al. (2013)	Seoul, South Korea	NR	Summer: 0.39; Winter: 0.47	Summer: 0.50; Winter: 0.55	Summer: 0.71; Winter: 0.22
	Daegu, South Korea	NR	Summer: 0.43; Winter: 0.47	Summer: 0.32; Winter: 0.59	Summer: 0.65; Winter: 0.57
	Asan, South Korea	NR	Summer: 0.62; Winter: 0.11	Summer: 0.63; Winter: 0.37	Summer: 0.67; Winter: 0.37
	Suncheon, South Korea	NR	Summer: 0.46; Winter: 0.56	Summer: 0.46; Winter: 0.60	Summer: 0.77; Winter: 0.80
	All 4 cities	NR	Summer: 0.58; Winter: 0.53	Summer: 0.60; Winter: 0.55	Summer: 0.78; Winter: 0.55

Table 3-6 (Continued): Correlations between measured nitrogen dioxide (NO_2) concentrations from personal, outdoor, indoor, and ambient monitors.

Study	Location	Personal-Ambient	Personal-Outdoor	Personal-Indoor	Outdoor-Indoor
Sahsuvaroglu et al. (2009)[b]	Lake Ontario, Canada (winter)	NR	All Subjects: 0.002; Non-ETS: 0.020	All Subjects: 0.430; Non-ETS: 0.283	NR
	Lake Ontario, Canada (spring)	NR	All Subjects: 0.233; Non-ETS: 0.187	All Subjects: 0.589; Non-ETS: 0.599	NR
	Lake Ontario, Canada (summer)	NR	All Subjects: 0.067; Non-ETS: 0.011	All Subjects: 0.822; Non-ETS: 0.783	NR
	Lake Ontario, Canada (all seasons)	NR	All Subjects: 0.517; Non-ETS: 0.540	All Subjects: 0.729; Non-ETS: 0.693	NR
Schembari et al. (2013)[b]	Barcelona, Spain	NR	0.58	0.78	0.53
Vieira et al. (2012)[a]	São Paolo, Brazil	NR	<0.35	NR	All subjects: 0.13; Non-ETS: 0.42
Van Roosbroeck et al. (2008)[a]	Netherlands (3 schools)	NR	0.35	NR	NR

CJ-A = Ciudad Juarez Site A; CJ-B = Ciudad Juarez Site B; EP-A = El Paso Site A; EP-B = El Paso Site B; ETS = Environmental Tobacco Smoke; NR = not reported.
[a]Spearman coefficient.
[b]Pearson coefficient.
[c]Subjects wore the sampling vests at least 60% of the sampling period.

Several studies have investigated factors that influence the relationship between short-term personal exposure measurements and ambient concentrations. It was observed that, even when the median or average total personal NO_2 exposures and ambient concentrations were comparable, the total personal exposure measurements and central site monitor concentrations might not have always been correlated. For example, Williams et al. (2012a) measured total personal NO_2 exposures for the Detroit Exposure and Aerosol Research Study (DEARS) population of nonsmoking adults in 24-hour intervals and found a low association (Spearman $r = 0.14$ for participants complying with study protocols; $r = 0.11$ for all participants) between total personal NO_2 exposure with NO_2 measured at central site monitors. This result indicated the influence of nonambient sources on the DEARS participants' total personal NO_2 exposures, suggesting that total personal and ambient NO_2 exposures are not always well correlated. Likewise, Suh and Zanobetti (2010) measured correlation of Spearman $r = 0.12$ between 24-hour total

personal exposure and central site NO_2 measurements among an Atlanta panel of 30 adults. Vieira et al. (2012) calculated Spearman correlations between 12-hour outdoor, indoor, and personal NO_2 measurements. All correlations between personal and outdoor NO_2 measurements were below $r = 0.35$. Indoor and outdoor NO_2 concentrations were more correlated ($r = 0.42$), although when smokers were included, correlation between indoor and outdoor NO_2 dropped ($r = 0.13$). Van Roosbroeck et al. (2008) compared personal NO_2 measurements for children obtained over 1 to 4 weeks in a panel study with measurements taken outside the children's schools, and they observed correlation of Pearson $R = 0.35$. Outdoor school NO_2 underestimated personal NO_2 when used as a surrogate, but when additional variables representing indoor exposures (such as exposure to gas cooking and unvented water heaters) were added to the model, R increased to 0.77, suggesting that indoor sources play a large role in NO_2 exposure among the study participants. Bellander et al. (2012) measured personal NO_2 exposure using 7-day integrated diffusion samplers and modeled it as a function of NO_2 concentrations measured at an urban area, rural area, roadside, and outside of the participants' homes and places of work in Stockholm County, Sweden. They observed slopes ranging from 0.25−0.37 ($R^2 = 0.01−0.20$). Kousa et al. (2001) developed a time-weighted microenvironmental model of NO_2 exposure based on time-activity data and 48-hour microenvironmental measurements. The microenvironmental model corresponded with personal exposure measurements ($\beta = 0.90$; $R^2 = 0.74$).

Meng et al. (2012b) performed a random effects meta-analysis of 15 studies that calculated slopes and correlations between personal NO_2 measurements of E_T and central site ambient NO_2 concentrations for 32 sample populations, of which 7 were from daily average analyses, 8 were from longitudinal panel analyses, and 17 were from analyses whose correlations were pooled over short time periods up to 1 week in length. Metaregression results are shown in Table 3-7. Meng et al. (2012b) found that the magnitude and correlation of associations between personal exposure and ambient concentration depended on several factors, including study design (pooled data across days, longitudinal panel, or daily average), season, meteorological conditions, ambient $PM_{2.5}$ level, and pre-existing cardiopulmonary disease of the exposure subjects. Together, the low associations reported in these studies indicate that most of the total personal NO_2 exposure measurements for these studies were influenced either by nonambient sources or by spatially variable NO_2 not well detected by the central site monitor. However, Meng et al. (2012b) also stated that the longitudinal panel studies included in their meta-analysis had several measurements below detection limit that could have erroneously reduced the correlations, which otherwise would be expected to be higher.

Table 3-7 Meta regression results from 15 studies examining the relationship between personal nitrogen dioxide exposure measurements and ambient concentrations.

Study Design	Slope	Correlation	Slope	Correlation
	Based on Original Studies		Corrected for Publication Bias	
Pooled[a]	0.40	0.42	0.30	0.37
Longitudinal panel[b]	0.14	0.16	0.14	0.16
Daily average[c]	0.29	0.72	0.20	0.45

[a]Pooled analyses: Piechocki-Minguy et al. (2006), Linn et al. (1996), Liard et al. (1999), Gauvin et al. (2001), Alm et al. (1998), Brown et al. (2009), Sarnat et al. (2006), Delfino et al. (2008).

[b]Longitudinal analyses: Sarnat et al. (2005), Sarnat et al. (2001), Sarnat et al. (2000), Linaker et al. (2000), Kim et al. (2006), Koutrakis et al. (2005).

[c]Daily average analyses: Mukala et al. (2000), Liard et al. (1999), and Alm et al. (1998)

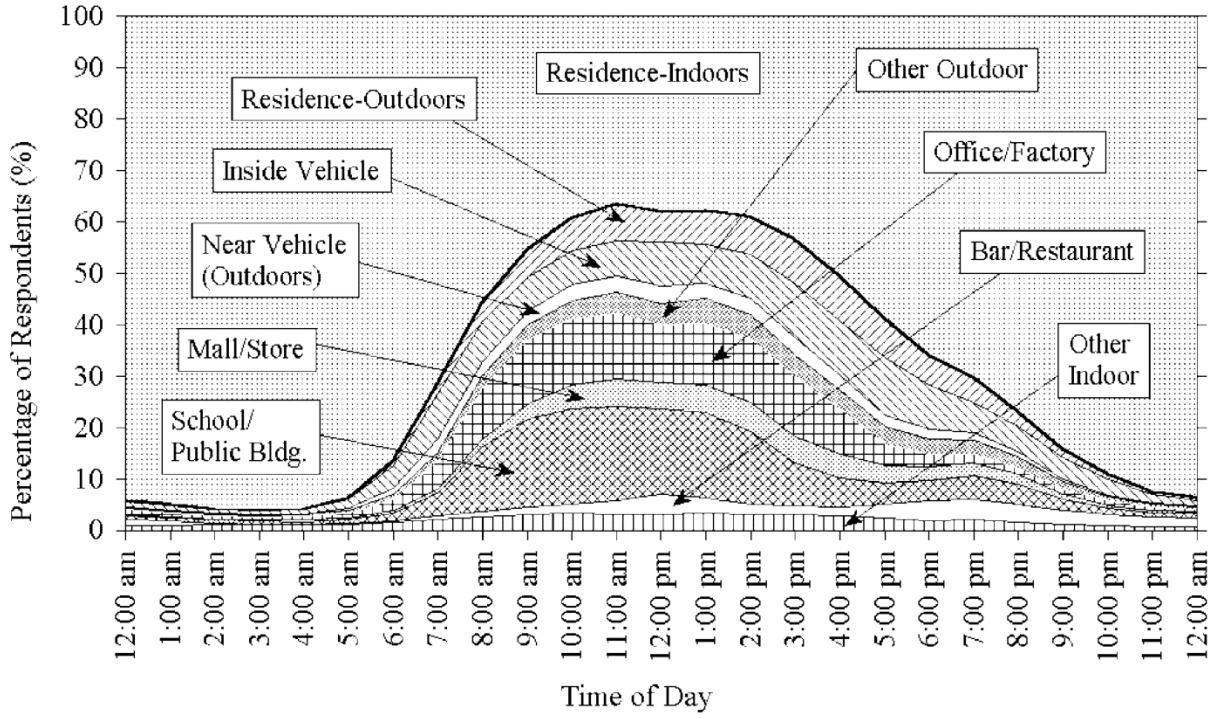
Source: Meng et al. (2012b).

3.4.3 Factors Contributing to Error in Estimating Exposure to Ambient Nitrogen Dioxide

Recent studies of factors influencing exposure error build from the existing literature presented in the 2008 ISA for Oxides of Nitrogen U.S. EPA (2008), which have focused on time-activity patterns, spatial variability, infiltration, nonambient exposures, and instrument accuracy and precision, as described in the subsequent subsections. These factors can influence epidemiologic results for studies of short-term and long-term NO_2 exposure, as detailed further in Section 3.4.5.

3.4.3.1 Time-Activity Patterns

The complex human activity patterns across the population (all ages) are illustrated in Figure 3-3 (Klepeis et al., 2001) for data from the National Human Activity Pattern Survey (NHAPS). This figure is presented to illustrate the diversity of daily activities among the entire population as well as a generalized proportion of time spent in each microenvironment. Time-activity data become an important source of uncertainty when considering that ambient exposures vary in different microenvironments (e.g., transit, residential), and that exposure assignment is typically based on the assumption that study participants are in one location (residential) for the study duration.



Source: Reprinted with permission of Nature Publishing Group, Klepeis et al. (2001).

Figure 3-3 Distribution of time sample population spends in various environments, from the U.S. National Human Activity Pattern Survey (all ages).

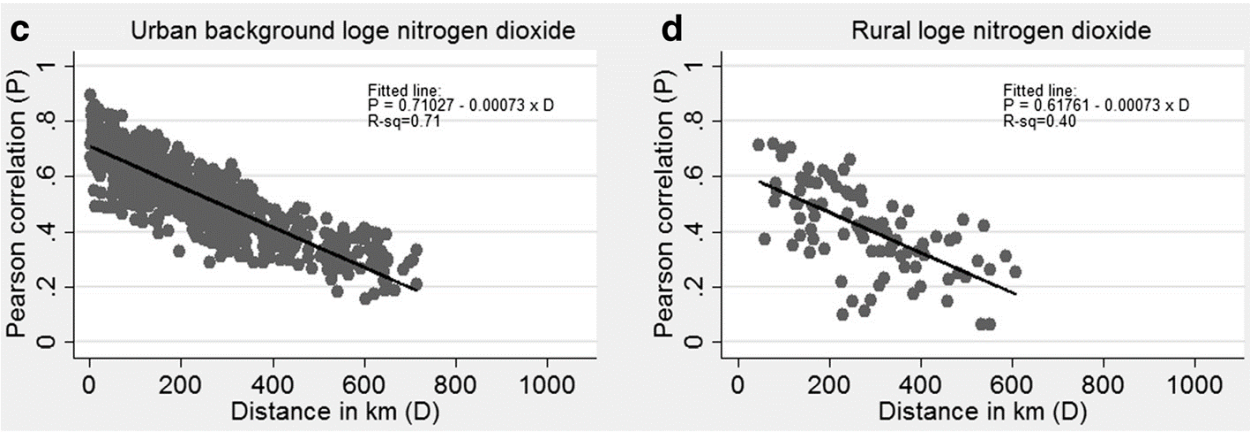
Different time-activity patterns have been found when analyzing activity patterns for different populations or life stages. For example, Wu et al. (2010) observed activity patterns for a panel of adults and children from Camden, NJ communities with larger percentages of nonwhites (85%) and those below the poverty line (33%) compared with NHAPS. The study participants spent more time outdoors compared with the nationwide cohort (3.8 hours vs. 1.8 hours nationally); note that Wu et al. (2010) undersampled participants ages 65+ years, and the median age of the population studied in Wu et al. (2010) was 27 years compared with 35 years nationwide. Other recent time-activity panel studies have included working adults (Isaacs et al., 2013; Bellander et al., 2012; Kornartit et al., 2010), pregnant women (Iñiguez et al., 2009), adolescents (DeCastro et al., 2007), and children (Mölter et al., 2012; Xue et al., 2004). In many cases, the time-activity data were limited to residential, occupational, and outdoor location categories to simplify assignment of concentrations to which the subjects were estimated to be exposed in each microenvironment. The implication of these findings is that, given that time-activity data vary among different populations, the one-location assumption used in many studies

varies in accuracy among those different populations. However, given that few studies are as large as NHAPS, it would be premature to make conclusions about time-activity data for smaller segments of the population.

Recently, Kornartit et al. (2010) tested the associations between time-weighted exposure estimates from area samples with personal sampling measurements for a London, U.K. panel study. Kornartit et al. (2010) measured NO_2 concentration for 1 week with passive Palmes tube samplers in several outdoor and indoor microenvironments for 55 subjects aged 21−60 years and correlated a time-weighted average of those microenvironmental NO_2 concentration measurements with personal NO_2 concentration measurements, also measured with Palmes tubes. They observed a slope of 0.94 for the relationship between time-weighted average and personal NO_2 concentrations (R^2 = 0.85) in winter and a slope of 0.59 (R^2 = 0.65) in summer. Higher levels of NO_2 were observed for both time-weighted average and personal concentrations in summer compared with winter. However, correlations between personal NO_2 exposure and time-weighted microenvironmental NO_2 concentrations were higher in winter, implying panel studies using personal NO_2 exposure measurements may be more dominated by indoor sources during cold-weather months. The authors concluded that the time-weighting approach provided a reasonable approximation of personal exposure but sometimes underestimated it.

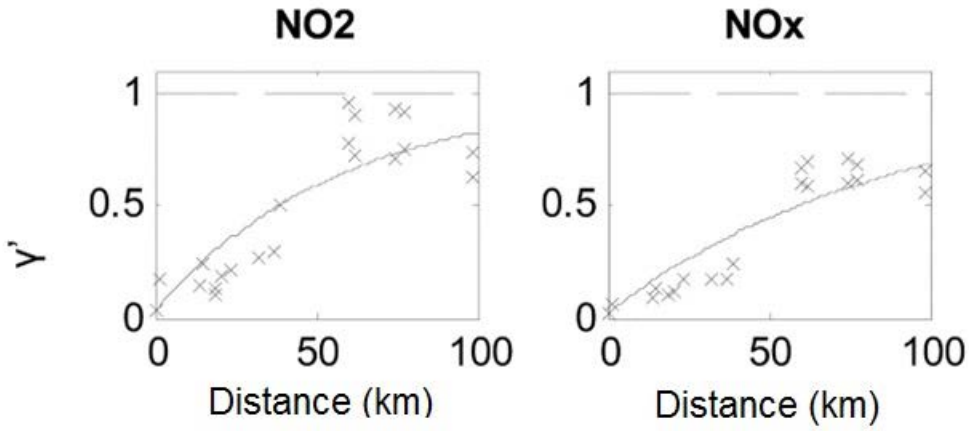
3.4.3.2 Spatial Variability in Nitrogen Dioxide Concentrations

Data for spatial variability in ambient NO, NO_2, and NO_X concentrations are provided in Section 2.5 for national, urban, neighborhood, and micro scales. The data illustrate that national variation in wintertime concentrations largely follows the degree of urbanization, while variation at urban and smaller scales is influenced by source location, source strength, meteorology, and natural and urban topography. Figure 3-4 illustrates regional-scale variability in background levels of daily 1-h max NO_2 based on Pearson correlation between monitor pairs for urban and rural monitors across the U.K. (Butland et al., 2013). Likewise, Figure 3-5 depicts urban-scale variability for NO_2 and NO_X, based on a semivariogram function (Goldman et al., 2010). Gradients in near-road concentrations of NO_2 and NO indicate spatial variability at finer scales within 300 m of the road (see Figures 2-16 and 2-17 in Section 2.5.3 and Figure 3-2 in Section 3.3.1.1). All of these data indicate that the magnitude of the error in exposure estimation increases with distance between the monitor and the subject. Hence, there is a potential for exposure misclassification if the ambient NO_2 concentration measured at a given site differs from the concentration at the location of an epidemiologic study participant, and this issue is present regardless of the spatial scale of the epidemiology study.



Source: Butland et al. (2013)

Figure 3-4 Regional-scale variability in nitrogen dioxide for urban and rural area data across the U.K.



Source: Goldman et al. (2010)

Figure 3-5 Urban-scale variability in nitrogen dioxide (NO_2) and the sum of nitric oxide and NO_2 (NO_X) in Atlanta, GA. On the *y*-axis, γ′ denotes the semivariogram, i.e., a unitless function that describes the ratio between spatial and temporal variance of the differences between two observations.

3.4.3.3 Infiltration and Building Ventilation

Given that people spend the majority of their time indoors, building air exchange rates influence exposure to ambient NO_2. In an analysis of daily average NO_2 data from the DEARS, Meng et al. (2012a) observed seasonal differences, with slopes of 0.24 ± 0.04 for E_T versus the concentration measured at a central site monitor, $C_{a,csm}$, and of 0.13 ± 0.06 for E_a versus $C_{a,csm}$ for summer measurements. For winter measurements, the associations were lower (E_T vs. $C_{a,csm}$: slope = 0.08 ± 0.05; E_a vs. $C_{a,csm}$: slope = 0.07 ± 0.07). Meng et al. (2012a) found that high air exchange rate (>1.3 air changes per hour), no central air conditioning, use and nonuse of window fans, and presence of old carpeting were determinants of α, the exposure factor defined in Equation 3-10 and approximated by the ratio of E_a to C_a, for NO_2 in summer; none of these factors were determinants of α for NO_2 in winter. In Mölter et al. (2012), outdoor exposures were calculated with LUR, while indoor exposures were calculated using the Probabilistic Model for Indoor Pollution Exposures (INDAIR) model that accounts both for infiltration due to home ventilation characteristics and indoor sources. Sensitivity to air exchange rate of INDAIR predictions of indoor NO_2 in the absence of indoor sources underscores potential for bias and uncertainty in α, which depends on air exchange rate, penetration, and indoor deposition (Dimitroulopoulou et al., 2006).

3.4.3.4 Instrument Accuracy and Precision

Instrument error occurs when the NO_2 measurements are subject to interferences that cause positive or negative biases or noise. NO_2 measurements are subject to positive bias from detection of other oxidized nitrogen compounds on the measurement substrate, and these errors are larger in warm seasons. See Section 2.4.1 for details on errors that affect FRMs and FEMs used for central site monitoring, and see Section 3.2.1.2 for a description of errors to which personal samplers are subject. Intermonitor comparison is often used to estimate instrument precision. For example, Goldman et al. (2010) investigated instrument precision error at locations where ambient monitors were colocated. Instrument precision error increased with increasing concentration. When instrument error and concentration are correlated, error in the exposure estimates will be larger in locations where there are more prevalent or stronger sources or at times when NO_2 emissions are higher for a given location. For example, it would be anticipated that the magnitude of the instrument error is largest at times of day when emissions are highest, such as rush hour. Instrument error was also observed to exhibit some autocorrelation at 1- and 2-day lags in the Goldman et al. (2010) study. Hence, the diurnal variability in relative NO_2 instrument error does not change substantially from day to day. For epidemiologic studies of short-term NO_2 exposure, the influence of

instrument error would not be expected to change if the health data were obtained on a daily basis.

3.4.4 Confounding

To assess the independent effects of NO_2 in an epidemiologic study of the health effects, it is necessary to identify (Bateson et al., 2007): (1) which copollutants (e.g., $PM_{2.5}$, UFP, BC) and additional exposures (e.g., noise, traffic levels) are potential confounders of the health effect-NO_2 relationship so that their correlation with NO_2 can be tested and, if needed, they are accounted for in the epidemiologic model; (2) the time period over which correlations might exist so that potential confounders are considered appropriately for the time period relevant for the epidemiologic study design (e.g., pollutants or other factors that are correlated over the long term might not be important for a short-term exposure epidemiologic study); and (3) the spatial correlation structure across multiple pollutants, if the epidemiologic study design is for long-term exposure. This section considers temporal copollutant correlations and how relationships among copollutants may change in space. Temporal copollutant correlations are computed from the time series of concentrations for two different collocated pollutants. Temporal correlations are informative for epidemiologic studies of short-term exposure when the sampling interval is a month or less for each of the copollutants. Temporal correlations are informative for epidemiologic studies of long-term exposures when sampling intervals are months-to-years. Spatial relationships are evaluated by comparing within-pollutant variation across space for different pollutants. The following sections review coexposures that can potentially confound the relationship between a health effect and NO_2 over different temporal and spatial resolutions.

3.4.4.1 Temporal Relationships among Ambient Nitrogen Dioxide and Copollutant Exposures

Studies and analyses reported in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) demonstrated that ambient NO_2 was correlated with several traffic-related pollutants in urban and suburban areas generally in the range of Pearson R = 0.5 to 0.75 for $PM_{2.5}$ and CO and R = 0.8 to 0.9 for EC. These results suggest that in some cases NO_2 can be a surrogate for traffic pollution (U.S. EPA, 2008). In contrast, correlations between NO_2 and O_3 were generally R = −0.71 to 0.1. Numerous air quality, exposure, and epidemiologic studies have more recently evaluated associations between concentrations of ambient NO_2 and those of other pollutants. Many of these studies report Pearson or Spearman correlations of ambient NO_2 with other criteria pollutants, mainly focusing on

those related to traffic sources ($PM_{2.5}$, CO, PM_{10}). A few studies have explored associations between NO_2 and other traffic-related pollutants, such as EC, UFP, and VOCs. Data for correlations between NO_2 and other criteria pollutants are summarized in Table 3-8, broken into short-term exposure and long-term exposure epidemiology studies. Figure 3-6 plots data for correlations between NO_2 and all copollutants for which data were available, including $PM_{2.5}$, PM_{10} $PM_{10-2.5}$, O_3, CO, SO_2, EC, OC, UFP, particle number concentration (PNC), toluene, and benzene. Figure 3-6 separates the data by averaging period. "Within-hourly" denotes averaging time ranging from 20 seconds to 1-h daily max. "Within daily" is noted for averaging time ranging from 3 to 24 hours. Three hour averaging times are typically applied during rush hour measurement periods. "Within monthly" refers to averaging times ranging from 84 hours to 1 month. "Annual or longer-term correlations" are for studies that averaged the data over a period of 1 to 5 years. The studies presented in Table 3-8 only include monitored data and not correlations computed from LUR studies. Some of these studies used personal or area sampling in lieu of central site monitoring. Note that, while Table 3-8 and Figure 3-6 are informative for considering the influence of averaging time on correlations, small sample sizes for any given pollutant and averaging period preclude making definitive conclusions about the observations. In particular, the number of near-road studies reporting correlations between NO_2 and copollutants was too small to make any conclusions about differences in NO_2-copollutant correlations between near-road and central site or personal measures.

The higher the copollutant correlation, the more difficult it is to disentangle the health effects from NO_2 from those of the copollutants. This is particularly true of traffic-related copollutants, and recent evidence indicates that copollutant confounding adds such uncertainty. Figure 3-6 shows the range of temporal NO_2 copollutant correlation coefficients among the studies in Table 3-8 plus one additional measurement study that did not include other criteria air pollutants (Williams et al., 2012a). Existing studies indicate that NO_2 has, in general, correlations over Pearson $R = 0.5$ with other NAAQS and traffic-related pollutants. Similar to findings in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) the strongest temporal correlations are typically observed for NO_2 with primary traffic-related pollutants, such as benzene, CO, EC, and PNC. A wide range of temporal correlations is observed for NO_2 with $PM_{2.5}$, PM_{10}, and SO_2. Correlations of NO_2 with $PM_{2.5}$ and PM_{10} tend to be positive for the within-hourly, within-daily, and long term metrics. For the within-monthly measures, median correlations are closer to zero. The reason for this difference is unknown, but fewer data are available for the within-monthly correlations. The lowest temporal correlations are typically observed for NO_2 with O_3 and $PM_{10-2.5}$, with correlations having a wide range in magnitude ($R = -0.71$ to 0.66; median $R = 0.15$). These observations are not surprising given the nonlinear relationship between NO_2 concentration and instantaneous O_3 production rate observed

close to the location of emission (Pusede and Cohen, 2012; LaFranchi et al., 2011; Murphy et al., 2007, 2006). Temporal correlations for near-road studies are highlighted in red for Figure 3-6. It is notable that the near-road correlations did not appear to be systematically different from the urban scale correlations. Statistical testing for near-road versus urban scale interpollutant correlations was not performed given the small number of near-road studies.

Short-Term Temporal Correlations

For the shorter time periods (within hourly and within daily), UFP, BC, CO, and EC tended to have higher correlations with NO_2, while O_3 had several negative correlations with NO_2. The within-daily category had the most data for $PM_{2.5}$ and PM_{10}, and a wide range of correlations was observed with NO_2 for each of those copollutants. Fewer data were available for within-monthly correlations. Black carbon, benzene, and toluene were observed to have the highest correlations with NO_2 in this temporal category. Across time-averaging periods, there is not a discernible pattern with respect to correlations of near-road measurements.

Table 3-8 Synthesis of nitrogen dioxide ambient-ambient copollutant correlations from measurements reported in the literature.

Study[a]	Averaging Time	Location	Scale	Correlation Measure	CO	O_3	SO_2	$PM_{2.5}$	PM_{10}
Short-term exposure epidemiologic studies									
Polidori and Fine (2012)	1-min	Los Angeles, CA (15 m downwind of I-710) summer	Near-road	Pearson	0.65	NR[b]	NR	NR	NR
		Los Angeles, CA (80 m downwind of I-710) summer	Near-road	Pearson	0.65	NR	NR	NR	NR
		Los Angeles, CA (background) summer	Urban	Pearson	0.66	NR	NR	NR	NR
		Los Angeles, CA (15 m downwind of I-710) winter	Near-road	Pearson	0.60	NR	NR	NR	NR
		Los Angeles, CA (80 m downwind of I-710) winter	Near-road	Pearson	0.62	NR	NR	NR	NR
		Los Angeles, CA (background) winter	Urban	Pearson	0.79	NR	NR	NR	NR
Levy et al. (2014)	<2 min	Montreal, Canada (all year)	Urban	Pearson	0.48	−0.45	0.11	0.29	0.39
		Montreal, Canada (summer)	Urban	Pearson	0.77	−0.74	0.17	0.34	0.35
		Montreal, Canada (fall)	Urban	Pearson	0.40	−0.33	0.25	0.26	0.30
		Montreal, Canada (winter)	Urban	Pearson	0.16	−0.36	0.04	0.34	0.35
Padró-Martínez et al. (2012)	2 min	Boston, MA	Urban	Spearman	0.51	NR	NR	0.21	NR
Chuang et al. (2008)	Hourly	Boston, MA	Urban	Pearson	NR	NR	NR	0.38	0.33

Table 3-8 (Continued): Synthesis of nitrogen dioxide ambient-ambient correlations from measurements reported in the literature.

Study[a]	Averaging Time	Location	Scale	Correlation Measure	CO	O_3	SO_2	$PM_{2.5}$	PM_{10}
Strickland et al. (2010)	1-h daily max	Atlanta, GA (cold season)	Urban	Spearman	0.59	0.11	0.36	0.37	0.46
		Atlanta, GA (warm season)	Urban	Spearman	0.54	0.42	0.37	0.36	0.44
Villeneuve et al. (2007)	1-h daily max	Edmonton, Canada	Urban	Pearson	0.74	NR	NR	NR	NR
Jalaludin et al. (2007)	1-h daily max	Sydney, Australia	Urban	NR	0.6	0.25	0.46	0.65	0.48
Mortimer et al. (2002)	1-h daily max	8 U.S. cities	Urban	NR	NR	0.27	NR	NR	NR
Burnett et al. (2000)	1-h daily max	8 Canadian cities	Urban	NR	0.65	0.12	0.49	0.53	0.53
Mar et al. (2000)	1-h daily max	Phoenix, AZ	Urban	NR	0.87	NR	0.57	0.77	0.53
Tolbert et al. (2007)	1-h daily max	Atlanta, GA	Urban	Spearman	0.7	0.44	0.36	0.47	0.53
Darrow et al. (2011)	1-h daily max	Atlanta, GA	Urban	Partial Spearman	0.61	0.40	NR	0.50	NR
	Morning commute (7:00 a.m.−10:00 a.m.)	Atlanta, GA	Urban	Partial Spearman	0.57	−0.16	NR	0.46	NR
	Daytime (8:00 a.m.−7:00 p.m.)	Atlanta, GA	Urban	Partial Spearman	0.53	−0.07	NR	0.41	NR
	Nighttime (12:00 a.m.−6:00 a.m.)	Atlanta, GA	Urban	Partial Spearman	0.66	−0.66	NR	0.52	NR
Moshammer et al. (2006)	8-h avg	Linz, Austria	Urban	Pearson	NR	NR	NR	0.54	0.62
Darrow et al. (2011)	24-h avg	Atlanta, GA	Urban	Partial Spearman	0.66	−0.15	NR	0.20	NR

Table 3-8 (Continued): Synthesis of nitrogen dioxide ambient-ambient correlations from measurements reported in the literature.

Study[a]	Averaging Time	Location	Scale	Correlation Measure	CO	O_3	SO_2	$PM_{2.5}$	PM_{10}
Faustini et al. (2011)	24-h avg	Milan	Urban	Pearson	NR	NR	NR	NR	0.79
		Mestre	Urban	Pearson	NR	NR	NR	NR	0.66
		Turin	Urban	Pearson	NR	NR	NR	NR	0.72
		Bologna	Urban	Pearson	NR	NR	NR	NR	0.66
		Florence	Urban	Pearson	NR	NR	NR	NR	0.65
		Pisa	Urban	Pearson	NR	NR	NR	NR	0.57
		Rome	Urban	Pearson	NR	NR	NR	NR	0.5
		Cagliari	Urban	Pearson	NR	NR	NR	NR	0.23
		Taranto	Urban	Pearson	NR	NR	NR	NR	0.19
		Palermo	Urban	Pearson	NR	NR	NR	NR	0.22
Samoli et al. (2011)	24-h avg	Athens, Greece	Urban	NR	NR	NR	0.55	NR	NR
Ko et al. (2007)	24-h avg	Hong Kong	Urban	Pearson	NR	0.34	0.66	0.44	0.4
Mehta et al. (2013)	24-h avg	Ho Chi Minh City, Vietnam (dry season)	Urban	NR	NR	0.44	0.29	NR	0.78
		Ho Chi Minh City, Vietnam (wet season)	Urban	NR	NR	0.17	0.01	NR	0.18
Andersen et al. (2008)	24-h avg	Copenhagen, Denmark	Near-road	Spearman	NR	−0.58	NR	0.41	0.43
Mannes et al. (2005)	24-h avg	Sydney, Australia	Urban	Pearson	0.57	0.29	NR	0.66	0.47

Table 3-8 (Continued): Synthesis of nitrogen dioxide ambient-ambient correlations from measurements reported in the literature.

Study[a]	Averaging Time	Location	Scale	Correlation Measure	CO	O_3	SO_2	$PM_{2.5}$	PM_{10}
Schildcrout et al. (2006)	24-h avg	Albuquerque, NM	Urban	NR	0.76	0.04	NR	NR	0.26
		Baltimore, MD	Urban	NR	0.69	0.44	0.49	NR	0.62
		Boston, MA	Urban	NR	0.8	0.47	0.68	NR	0.48
		Denver, CO	Urban	NR	0.85	0.24	0.56	NR	0.64
		San Diego, CA	Urban	NR	0.92	0.39	0.23	NR	0.55
		St. Louis, MO	Urban	NR	0.71	0.42	0.58	NR	0.45
		Toronto, Canada	Urban	NR	0.63	0.4	0.63	NR	0.64
Liu et al. (2009)	24-h avg	Ontario, Canada	Urban	Spearman	NR	−0.51	0.18	0.71	NR
Strak et al. (2013)	24-h avg	Locations across the Netherlands	Urban	Spearman	NR	−0.62	NR	0.45	0.49
O'Connor et al. (2008)	24-h avg	Inner-cities across the U.S.	Urban	NR	0.54	−0.31	0.59	0.59	NR
Timonen et al. (2006)	24-h avg	Amsterdam, the Netherlands	Urban	Spearman	0.76	NR	NR	0.49	NR
		Erfurt, Germany	Urban	Spearman	0.86	NR	NR	0.82	NR
		Helsinki, Finland	Urban	Spearman	0.32	NR	NR	0.35	NR
Guo et al. (2009)	24-h avg	Beijing, China	Urban	Pearson	NR	NR	0.53	0.67	NR
Rojas-Martinez et al. (2007)	24-h avg	Mexico City, Mexico	Urban	Pearson	NR	0.17	NR	NR	0.25
Sarnat et al. (2001)	24-h avg	Baltimore, MD (summer)	Urban	Spearman	0.75	0.02	NR	0.37	NR
		Baltimore, MD (winter)	Urban	Spearman	0.76	−0.71	−0.17	0.75	NR
Sarnat et al. (2005)	24-h avg	Boston, MA (summer)	Near-road	Spearman	NR	NR	NR	0.44	NR
		Boston, MA (winter)	Near-road	Spearman	NR	NR	NR	0.64	NR

Table 3-8 (Continued): Synthesis of nitrogen dioxide ambient-ambient correlations from measurements reported in the literature.

Study[a]	Averaging Time	Location	Scale	Correlation Measure	CO	O_3	SO_2	$PM_{2.5}$	PM_{10}
Kim et al. (2006)	24-h avg	Toronto, Canada	Near-road	Spearman	0.72	NR	NR	0.44	NR
Roberts and Martin (2006)	24-h avg	Cleveland, OH	Urban	NR-Pairwise	0.67	0.36	0.56	NR	0.63
		Nashville, TN	Urban	NR-Pairwise	0.36	0.26	0.08	NR	0.44
Andersen et al. (2007)	24-h avg	Copenhagen, Denmark	Urban	Spearman	0.74	NR	NR	NR	0.42
Chen et al. (2008)	24-h avg	Shanghai, China	Urban	NR	NR	NR	0.73	NR	0.71
Arhami et al. (2009)	24-h avg	San Gabriel Valley, CA (summer/fall)	Urban	Spearman	NR	NR	NR	0.1	0.31
		San Gabriel Valley, CA (fall/winter)	Urban	Spearman	NR	NR	NR	0.44	0.34
		Riverside, CA (summer/fall)	Urban	Spearman	NR	NR	NR	0.07	0.21
		Riverside, CA (fall/winter)	Urban	Spearman	NR	NR	NR	0.56	0.64
Delfino et al. (2009)	24-h avg	San Gabriel Valley and Riverside, CA (aggregated)	Urban	NR	0.79	−0.42	NR	0.19	NR
Baxter et al. (2013)	24-h avg	Boston, MA	Urban	Spearman	NR	NR	NR	0.41	NR
		Pittsburgh, PA	Urban	Spearman	NR	NR	NR	0.46	NR
		Memphis, TN	Urban	Spearman	NR	NR	NR	0.27	NR
		Detroit, MI	Urban	Spearman	NR	NR	NR	0.59	NR
		Milwaukee, WI	Urban	Spearman	NR	NR	NR	0.55	NR
		San Diego, CA	Urban	Spearman	NR	NR	NR	0.57	NR
		Riverside, CA	Urban	Spearman	NR	NR	NR	0.37	NR
Williams et al. (2012c)	24-h avg	Research Triangle Park, NC	Urban	Spearman	NR	−0.12	NR	0.03	NR

Table 3-8 (Continued): Synthesis of nitrogen dioxide ambient-ambient correlations from measurements reported in the literature.

Study[a]	Averaging Time	Location	Scale	Correlation Measure	CO	O_3	SO_2	$PM_{2.5}$	PM_{10}
Williams et al. (2012a)	24-h avg	Detroit, MI	Near-road	Spearman	NR	NR	NR	NR	NR
Delfino et al. (2008)	24-h avg	Los Angeles, CA	Urban	Spearman	NR	NR	NR	0.36	NR
Suh and Zanobetti (2010)	24-h avg	Atlanta, GA	Urban	Spearman	NR	NR	NR	0.47	NR
Schembari et al. (2013)	24-h avg	Barcelona, Spain	Urban	Spearman	NR	NR	NR	0.41	NR
Laurent et al. (2013)	24-h avg	Los Angeles and Orange counties, CA	Urban	Pearson	0.83	−0.81	NR	0.77	0.70
Peters et al. (2009)	24-h avg	Erfurt, Germany	Urban	Spearman	0.68	−0.55	0.54	0.63	0.64
Sánchez Jiménez et al. (2012)	24-h avg	Glasgow, U.K.	Near-road	Spearman	0.6	NR	NR	NR	0.83
		Glasgow, U.K.	Background	Spearman	0.4	NR	NR	NR	0.69
		Glasgow, U.K.	Background	Spearman	0.74	NR	NR	NR	NR
		London, U.K.	Near-road	Spearman	0.3	NR	NR	0.49	0.67
		London, U.K.	Background	Spearman	0.61	NR	NR	0.42	0.37
Steinvil et al. (2009)	24-h avg	Tel Aviv, Israel	Urban	Partial Pearson	0.75	−0.34	0.70	NR	0.076
Steinvil et al. (2008)	24-h avg	Tel Aviv, Israel	Urban	Partial Pearson	0.86	−0.78	0.72	NR	0.082
Tao et al. (2012)	24-h avg	Guangzhou, Foshan, Zhongshan, and Zhuhai, China	Urban-regional	Pearson	0.72	0.17	0.82	NR	0.82
Wichmann et al. (2012)	24-h avg	Copenhagen, Denmark (warm period)	Urban	Spearman	0.62	NR	NR	NR	0.47
		Copenhagen, Denmark (cold period)	Urban	Spearman	0.72	NR	NR	NR	0.46

Table 3-8 (Continued): Synthesis of nitrogen dioxide ambient-ambient correlations from measurements reported in the literature.

Study[a]	Averaging Time	Location	Scale	Correlation Measure	CO	O_3	SO_2	$PM_{2.5}$	PM_{10}
Dimitriou and Kassomenos (2014)	24-h avg	London, U.K. (cold period)	Urban	Pearson	NR	NR	NR	0.52	0.49
			Near road	Pearson	NR	NR	NR	0.49	0.70
		London, U.K. (warm period)	Urban	Pearson	NR	NR	NR	0.63	0.56
			Near road	Pearson	NR	NR	NR	0.60	0.67
		Paris, France (cold period)	Urban	Pearson	NR	NR	NR	0.65	0.71
			Near road	Pearson	NR	NR	NR	0.60	0.68
		Paris, France (warm period)	Urban	Pearson	NR	NR	NR	0.54	0.50
			Near road	Pearson	NR	NR	NR	0.75	0.83
		Copenhagen, Denmark (cold period)	Urban	Pearson	NR	NR	NR	0.31	0.35
			Near road	Pearson	NR	NR	NR	0.36	0.37
		Copenhagen, Denmark (warm period)	Urban	Pearson	NR	NR	NR	0.42	0.42
			Near road	Pearson	NR	NR	NR	0.53	0.55
		Hamburg, Germany (cold period)	Urban	Pearson	NR	NR	NR	0.21	0.23
			Near road	Pearson	NR	NR	NR	0.40	0.52
		Hamburg, Germany (warm period)	Urban	Pearson	NR	NR	NR	0.50	0.51
			Near road	Pearson	NR	NR	NR	0.69	0.70

Table 3-8 (Continued): Synthesis of nitrogen dioxide ambient-ambient correlations from measurements reported in the literature.

Study[a]	Averaging Time	Location	Scale	Correlation Measure	CO	O_3	SO_2	$PM_{2.5}$	PM_{10}
Dimitriou and Kassomenos (2014) (Continued)	24-h avg	Stockholm, Sweden (cold period)	Urban	Pearson	NR	NR	NR	0.20	0.24
			Near road	Pearson	NR	NR	NR	0.49	0.45
		Stockholm, Sweden (warm period)	Urban	Pearson	NR	NR	NR	0.38	0.45
			Near road	Pearson	NR	NR	NR	0.58	0.52
Clougherty et al. (2013)	84-h avg	New York, NY	Urban	Pearson	NR	NR	0.51	0.74	NR
Sarnat et al. (2012)	96-h avg	El Paso, TX (site A)	Urban	Spearman	NR	NR	NR	−0.39	−0.3
		El Paso, TX (site B)	Near-road	Spearman	NR	NR	NR	−0.28	−0.1
		Ciudad Juarez, Mexico (site A)	Urban	Spearman	NR	NR	NR	−0.28	−0.1
		Ciudad Juarez, Mexico (site B)	Near-road	Spearman	NR	NR	NR	0	0.11
Greenwald et al. (2013)	96-h avg	2 sites in El Paso, TX	Urban	Pearson	NR	NR	−0.22	0.2	0.31
Wheeler et al. (2008)	2-week	Windsor, ON, Canada (all year)	Urban	Spearman	NR	NR	0.85	NR	NR
		Windsor, ON, Canada (winter)	Urban	Spearman	NR	NR	0.84	NR	NR
		Windsor, ON, Canada (spring)	Urban	Spearman	NR	NR	0.61	NR	NR
		Windsor, ON, Canada (summer)	Urban	Spearman	NR	NR	0.51	NR	NR
		Windsor, ON, Canada (fall)	Urban	Spearman	NR	NR	0.66	NR	NR
Trasande et al. (2013)	1-month avg	United States	Varies	Pearson	0.12	−0.023	−0.10	−0.090	−0.011
Long-term exposure epidemiologic studies									
Dadvand et al. (2014)	9-mo	Barcelona, Spain	Urban	Spearman	NR	NR	NR	0.48	0.33

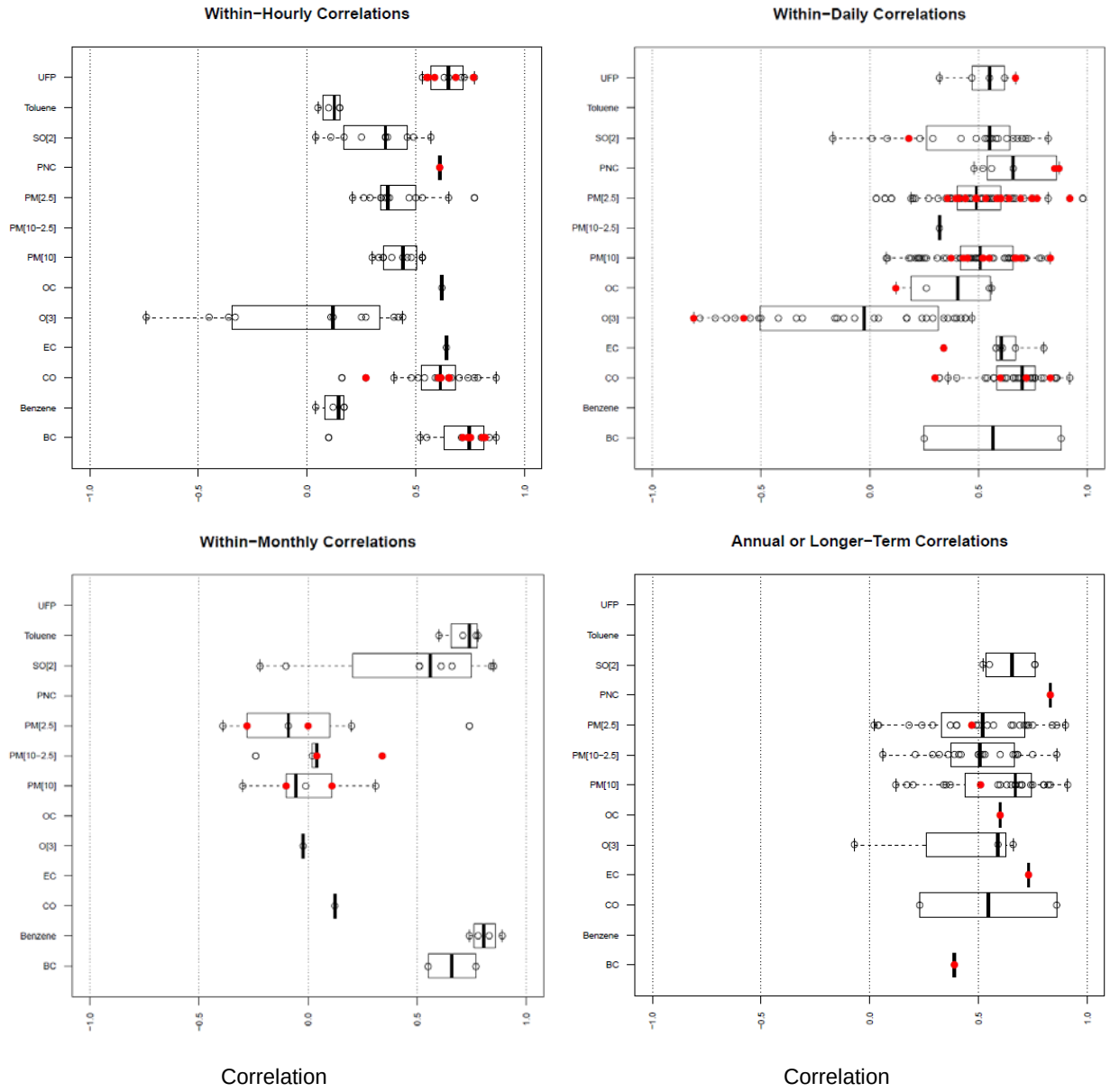
Table 3-8 (Continued): Synthesis of nitrogen dioxide ambient-ambient correlations from measurements reported in the literature.

Study[a]	Averaging Time	Location	Scale	Correlation Measure	CO	O_3	SO_2	$PM_{2.5}$	PM_{10}
Katanoda et al. (2011)	1-yr avg	Japanese cities	Urban	Pearson	NR	NR	0.76	NR	NR
Dong et al. (2011)	1-yr avg	7 cities across China	Urban	NR	0.23	0.66	0.52	NR	0.7
Hwang and Lee (2010)	1-yr avg	14 Taiwanese communities	Urban	NR	0.86	−0.07	0.55	0.37	NR
Heinrich et al. (2013)	1-yr avg	North Rhine-Westphalia, Germany	Urban	Spearman	NR	NR	NR	0.50	NR
Ducret-Stich et al. (2013)	1-yr avg	Swiss Alps	Near-road	Spearman	NR	NR	NR	NR	0.51
			On highway	Spearman	NR	NR	NR	NR	0.04−0.63
Eeftens et al. (2012)	1-yr avg	Oslo, Norway	Urban	Spearman	NR	NR	NR	0.24	0.34
		Stockholm County, Sweeden	Urban	Spearman	NR	NR	NR	0.75	0.80
		Helsinki/Turku, Finland	Urban	Spearman	NR	NR	NR	0.71	0.80
		Copenhagen, Denmark	Urban	Spearman	NR	NR	NR	0.40	0.60
		Kaunas, Lithuania	Urban	Spearman	NR	NR	NR	0.04	0.17
		Manchester, England	Urban	Spearman	NR	NR	NR	0.40	0.59
		London/Oxford, England	Urban	Spearman	NR	NR	NR	0.84	0.82
		Netherlands/Belgium	Urban	Spearman	NR	NR	NR	0.57	0.74
		Ruhr Area, Germany	Urban	Spearman	NR	NR	NR	0.69	0.65
		Munich/Augsberg, Germany	Urban	Spearman	NR	NR	NR	0.29	0.67
		Vorarlberg, Austria	Urban	Spearman	NR	NR	NR	0.04	0.35
		Paris, France	Urban	Spearman	NR	NR	NR	0.86	0.91

Table 3-8 (Continued): Synthesis of nitrogen dioxide ambient-ambient correlations from measurements reported in the literature.

Study[a]	Averaging Time	Location	Scale	Correlation Measure	CO	O_3	SO_2	$PM_{2.5}$	PM_{10}
Eeftens et al. (2012) (Continued)	1-yr avg (Continued)	Gyor, Hungary	Urban	Spearman	NR	NR	NR	0.02	0.12
		Lugano, Switzerland	Urban	Spearman	NR	NR	NR	0.66	0.83
		Turin, Italy	Urban	Spearman	NR	NR	NR	0.65	0.67
		Rome, Italy	Urban	Spearman	NR	NR	NR	0.73	0.75
		Barcelona, Spain	Urban	Spearman	NR	NR	NR	0.90	0.69
		Catalunya, Spain	Urban	Spearman	NR	NR	NR	0.72	0.63
		Athens, Greece	Urban	Spearman	NR	NR	NR	0.49	0.70
		Heraklion, Greece	Urban	Spearman	NR	NR	NR	0.18	0.37
McConnell et al. (2003)	4-yr avg	12 communities in southern California	Urban	Pearson	NR	0.59	NR	0.54	0.2
Gan et al. (2012a)	5-yr avg	Vancouver, Canada	Urban	Spearman	NR	NR	NR	0.47	NR

avg = average; CO = carbon monoxide; NR = not reported; O_3 = ozone; $PM_{2.5}$ = in general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 2.5 µm, a measure of fine particles; PM_{10} = in general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 10 µm, a measure of thoracic particles; SO_2 = sulfur dioxide.
[a]Correlation data computed from LUR studies are not included here.



BC = black carbon; CO = carbon monoxide; EC = elemental carbon; LUR = land use regression; O[3] = ozone; OC = organic carbon; PM[2.5] = in general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 2.5 µm, a measure of fine particles; PM[10] = in general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 10 µm, a measure of thoracic particles; PM[10−2.5] = in general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 10 µm and greater than a nominal 2.5 µm, a measure of thoracic coarse particles; PNC = particle number concentration; SO_2 = sulfur dioxide; UFP = ultrafine particulate matter.

Notes: Boxes represent the interquartile range of the data with the median line plotted, and 90th and 10th percentile of the data are plotted as the whiskers. Correlation data computed from LUR studies are not included here. Correlations shown by closed red circles come from near-road studies, and correlations shown by open black circles either come from urban-regional scale studies or do not specify the study's spatial scale.

Source: National Center for Environmental Assessment analysis of data from studies referenced in Table 3-8.

Figure 3-6 Summary of temporal nitrogen dioxide-copollutant correlation coefficients from measurements reported in studies listed in Table 3-8, sorted by temporal averaging period.

Fewer studies have explored seasonal correlations between NO_2 and copollutants. Among these, a majority of studies report correlations of NO_2 with $PM_{2.5}$ and PM_{10}. In general, studies show stronger correlations of NO_2 with $PM_{2.5}$ and PM_{10} during cooler seasons. Connell et al. (2005) investigated associations between $PM_{2.5}$ and gaseous copollutants in Steubenville, OH using linear regression. NO_2 was more strongly correlated with $PM_{2.5}$ during the fall (R^2 = 0.53) and winter (R^2 = 0.53) seasons compared with the spring (R^2 = 0.27) and summer (R^2 = 0.086) seasons. Similarly, Sarnat et al. (2005) found positive associations between $PM_{2.5}$ and NO_2 during both seasons (summer: β = 0.44; winter: β = 0.64), with stronger associations in the winter in Baltimore, MD. Arhami et al. (2009) evaluated relationships between ambient copollutants at two sites in southern California (San Gabriel Valley and Riverside) for warmer and cooler seasons. During the warm season, the Spearman correlation coefficient (average among sites) was r = 0.09 between NO_2 and $PM_{2.5}$, whereas during the winter the correlation was r = 0.50. However, they did not observe a consistent seasonal trend between NO_2 and PM_{10}. While associations between NO_2 and PM_{10} were substantially lower during the summer (r = 0.21) at the Riverside site, correlations were relatively similar during both seasons at the San Gabriel Valley site (summer PM_{10}: r = 0.31; winter PM_{10}: r = 0.34). In contrast, for a study of copollutant variation in Montreal, Canada, Levy et al. (2014) reported higher magnitude Pearson correlations for several copollutants in summer (CO: R = 0.77; O_3: R = −0.74; SO_2: R = 0.17; $PM_{2.5}$: R = 0.34; UFP: R = 0.77; BC: R = 0.80; PM_{10}: R = 0.35) compared with winter (CO: R = 0.16; O_3: R = −0.36; SO_2: R = 0.04; $PM_{2.5}$: R = 0.34; UFP: R = 0.71; BC: R = 0.0.55; PM_{10}: R = 0.35). The Levy et al. (2014) study measured the pollutants using near-real-time instrumentation with recording intervals ranging from 1 second to 2 minutes.

The relationship between NO_2 and O_3 may also have seasonal patterns, although limited seasonal data exist between these two pollutants. In the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008), ambient concentrations of NO_2 and O_3 from several sites across Los Angeles, CA were compared during a multiyear period. Slightly positive correlations between these two pollutants were observed during the summer (Spearman r = 0.0 to 0.4), while negative correlations were observed during the winter (r = −0.5 to −0.8). The slightly positive correlations during the summer can be attributed in part to increased photochemical activity, resulting in enhanced O_3 formation. Higher O_3 concentrations increase the ratio of NO_2 to NO due to enhanced oxidation, thereby resulting in a stronger correspondence between NO_2 and O_3 during the summer. The magnitude of the relationship between NO_2 and O_3 may be dampened by the nonlinear relationship between the two species (Pusede and Cohen, 2012). Only one study in Table 3-8 reported seasonal differences in the correlation between NO_2 and O_3. Sarnat et al. (2001) measured daily concentrations of gaseous and PM pollutants during different seasons in Baltimore, MD. Similar to the trends reported in the 2008 ISA for Oxides of Nitrogen, they observed

a negative correlation between NO_2 and O_3 during the winter ($r = -0.71$) and a near-zero correlation during the summer ($r = 0.02$). However, because there is a lack of studies reporting such correlations, it is uncertain whether or not this seasonal trend exists between the two pollutants in different locations.

Recent studies have also compared NO_2-copollutant temporal correlations across different regions in the U.S., based on central site monitoring data. Baxter et al. (2013) studied differences in air pollution for the Northeast (Boston, MA; Pittsburgh, PA), South (Memphis, TN; Birmingham, AL), Midwest (Milwaukee, WI; Detroit, MI), and West (San Diego, CA; Riverside, CA). Average Spearman correlation coefficients between $PM_{2.5}$ and NO_2 for each region were different (Northeast: $r = 0.44$; South [data available for Memphis only]: $r = 0.27$; Midwest: $r = 0.57$; West: $r = 0.47$). Schildcrout et al. (2006) compared a number of gaseous and particulate pollutants in different cities across the U.S., including Albuquerque, NM; Baltimore, MD; Boston, MA; and Denver, CO. While correlations between ambient NO_2 and CO were relatively similar in all four locations, larger differences were observed between NO_2 and PM_{10} correlations, ranging from a Spearman correlation of $r = 0.64$ in Denver to $r = 0.26$ in Albuquerque. Other multicity studies conducted outside of the U.S. show that NO_2 copollutant correlations are widely variable across cities (Faustini et al., 2011; Dales et al., 2010, 2009; Stieb et al., 2008; Timonen et al., 2006).

A small subset of studies investigated temporal correlations between NO_2 and traffic-related VOCs, such as BTEX. In these studies, correlations between NO_2 and VOCs are variable. Brook et al. (2007) demonstrated that benzo(e)pyrene and hopanes, specific mobile source tracers, were more strongly correlated with NO_2 (Spearman $r = 0.27-0.80$) compared to $PM_{2.5}$ ($r = 0.26-0.62$) at several urban sites in Canada. Beckerman et al. (2008) observed correlations between NO_2 and BTEX of Pearson $R = 0.46-0.85$ in a near-road field campaign. In a panel study, Greenwald et al. (2013) compared ambient concentrations of traffic pollutants monitored outside two schools in El Paso, TX, including one school within close proximity to a major roadway with heavy diesel truck traffic. A Spearman correlation of $r = 0.77$ was observed between NO_2 and BTEX, suggesting that both pollutants are related to traffic sources.

Long-Term Temporal Correlations

Long-term exposure epidemiology studies for which interpollutant correlations were computed were substantially less numerous than short-term exposure epidemiology studies (Atkinson et al., 2013; Heinrich et al., 2013; Gan et al., 2012a; Darrow et al., 2011; Dong et al., 2011; Katanoda et al., 2011; Hwang and Lee, 2010; Delfino et al., 2009; Delfino et al., 2008; McConnell et al., 2003). For long-term averages, the most data

were for $PM_{2.5}$ and PM_{10}. In each case, the median correlations were near 0.5, and the correlations were positive and ranging from near 0 to near 0.9. The sample size for other copollutants was low in the long-term averages. Median correlations were comparable between long-term exposure and short-term exposure epidemiology studies for CO, SO_2, $PM_{2.5}$, BC, and PM_{10}. The largest difference was for the correlation between NO_2 and O_3, which was 0.59 over the long-term exposure epidemiology studies and 0.17 for all studies pooled. However, given that only three long-term studies were available to compute correlation between NO_2 and O_3 and one of those three studies reported a negative correlation, there is insufficient information to make a conclusion regarding independence of the effects of NO_2 and O_3. Long-term correlations were not computed for UFP, EC, OC, PNC, $PM_{10-2.5}$, benzene, and toluene, and the small relative number of long-term exposure epidemiology studies compared with short-term exposure epidemiology studies reporting temporal correlations add uncertainty to these numbers.

3.4.4.2 Spatial Variability among Ambient Nitrogen Dioxide and Copollutants

When an epidemiologic study design relies on spatial contrasts to draw conclusions, such as for a long-term exposure epidemiologic study, unmeasured spatial correlation between copollutants can lead to positive bias in the health effect estimate for each of the pollutants included in the model. Moreover, bias related to confounding can only be addressed when the spatial scale of variability in the exposure metric is smaller than the spatial scale of variability in the confounder (Paciorek, 2010). Dionisio et al. (2013) compared the coefficient of variation (CV = σ/μ) of six air pollutants across space using a hybrid AERMOD-background model of concentrations in the Atlanta, GA metropolitan area. They observed the following ordinal relationship of the covariates' CVs: NO_X (0.88) > CO (0.58) > EC (0.50) > $PM_{2.5}$ (0.13) > O_3 (0.07) > SO_4 (0.05). Dionisio et al. (2013) did not report the CV of NO_2, which would be expected to have a lower CV than NO_X. Likewise, Goldman et al. (2012) and Ivy et al. (2008) both used monitoring data from the Atlanta, GA metropolitan area to estimate spatial correlation functions, and they observed that NO_2 and NO_X, along with CO, SO_2, and EC, had substantially steeper spatial correlograms than O_3, PM_{10}, $PM_{2.5}$, SO_4, NO_3, NH_4, and OC. Sajani et al. (2011) also observed that spatial correlation decreased more substantially with distance between monitoring sites for NO_2 compared with PM_{10} and O_3 when looking at six Italian cities.

Changes in correlations across space have been observed in a small number of studies. For their long-term near-road study, Ducret-Stich et al. (2013) point out that the temporal correlations of NO_2 with EC and PNC were high close to the highway where they obtained measurements and decreased with increasing distance from the road. This

suggests that the influence of NO_2 on health effects might be better detected in an epidemiologic study of long-term exposure when the participants are further from the road so that an independent effect can be detected. The next section examines spatial distributions of those copollutants to shed additional light on how copollutant relationships change in space. Atari et al. (2009) tested the relationship between NO_2 and SO_2 across individual-level and Census tract-level spatial resolutions, which were estimated by a LUR model developed for testing odor threshold in Sarnia, Canada. They observed higher spatial correlation when averaging over a Census tract (R = 0.65) compared with individual-level resolution (R = 0.49). These findings illustrate greater spatial variability for NO_2, NO_X, CO, SO_2, and EC compared with the other pollutants. Based on the conclusions of Paciorek (2010), the observations noted in Dionisio et al. (2013), Goldman et al. (2012), Ivy et al. (2008), Sajani et al. (2011), Atari et al. (2009), and Sánchez Jiménez et al. (2012) suggest that differences in the spatial variability of NO_2 compared with copollutants having different spatial variation make it unlikely that copollutant confounding will occur everywhere in space. This is consistent with the findings of Ducret-Stich et al. (2013) regarding differences in copollutant correlations over space.

3.4.4.3 Personal and Indoor Relationships between Nitrogen Dioxide and Copollutant Exposures

Many studies have investigated the relationship between personal and ambient measurements of NO_2 and other pollutants to evaluate the use of central site measurements as a proxy for personal exposure to pollution. Other studies have explored relationships between indoor NO_2 and copollutants to understand sources and personal exposure in an indoor environment. Tables 3-9, 3-10, 3-11, and 3-12 present correlations of ambient, personal, or indoor NO_2 with similar measurements of copollutants. A limited number of studies reported in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) investigated the relationship between personal NO_2 and personal or ambient measurements of other pollutants (e.g., $PM_{2.5}$, EC, CO, volatile organic compounds, and HONO). Short-term correlation of personal NO_2 with these pollutants ranged from Spearman r = 0.26 to r = 0.71. Similar to the results in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008), correlations of $r = -0.33$ to r = 0.44 were observed between personal NO_2 and personal or ambient measurements of other regional ($PM_{2.5}$) and traffic-related pollutants (e.g., EC, OC). Additionally, O_3 consistently showed a negative or no correlation with NO_2. More recent studies report indoor NO_2 copollutant correlations and observe a broader range of correlations between NO_2 and EC of $r = -0.37$ to r = 0.66.

Table 3-9 Pearson correlation coefficients between ambient nitrogen dioxide and personal copollutants.

Study	Location	*n*	Averaging Times	$PM_{2.5}$	EC	OC	O_3
Delfino et al. (2008)	Los Angeles, CA	≤170	All: 24-h	0.32	0.2	0.16	NR
Suh and Zanobetti (2010)	Atlanta, GA	≤277	All: 24-h	0.25	0.33	NR	−0.09
Williams et al. (2012a)	Chapel Hill, NC	≤357	All: 24-h	−0.19	−0.17	NR	−0.01
Schembari et al. (2013)	Barcelona, Spain	≤65	NO_2: 7-day; $PM_{2.5}$/EC: 2-day	0.21	0.44	NR	NR

EC = elemental carbon; NR = not reported; O_3 = ozone; OC = organic carbon; $PM_{2.5}$ = in general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 2.5 µm, a measure of fine particles.

Table 3-10 Pearson correlation coefficients between personal nitrogen dioxide and ambient copollutants.

Study	Location	*n*	Averaging Times	$PM_{2.5}$	EC	OC	O_3
Delfino et al. (2008)	Los Angeles, CA	≤170	All: 24-h	0.21	0.2	0.18	NR
Suh and Zanobetti (2010)	Atlanta, GA	≤277	All: 24-h	0.2	0.22	NR	NR
Williams et al. (2012a)	Chapel Hill, NC	≤326	All: 24-h	0.33	−0.3	NR	−0.26
Schembari et al. (2013)	Barcelona, Spain	≤65	NO_2: 7-day; $PM_{2.5}$/EC: 2-day	0.28	0.22	NR	NR

EC = elemental carbon; NR = not reported; O_3 = ozone; OC = organic carbon; $PM_{2.5}$ = in general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 2.5 µm, a measure of fine particles.

Table 3-11 Pearson correlation coefficients between personal nitrogen dioxide and personal copollutants.

Study	Location	*n*	Averaging Times	$PM_{2.5}$	EC	OC	O_3
Delfino et al. (2008)	Los Angeles, CA	≤486	All: 24-h	0.38	0.22	0.2	NR
Suh and Zanobetti (2010)	Atlanta, GA	≤277	All: 24-h	0.29	0.49	NR	−0.03
Williams et al. (2012a)	Chapel Hill, NC	≤326	All: 24-h	0.06	0.33	NR	−0.11
Schembari et al. (2013)	Barcelona, Spain	≤65	NO_2: 7-day; $PM_{2.5}$/EC: 2-day	0.11	0.3	NR	NR

EC = elemental carbon; NR = not reported; O_3 = ozone; OC = organic carbon; $PM_{2.5}$ = in general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 2.5 µm, a measure of fine particles.

Table 3-12 Correlation coefficients between indoor nitrogen dioxide and indoor copollutants.

Study	Location	*n*	Averaging Times	PM	EC	OC	O_3
Sarnat et al. (2012)[a]	El Paso, TX (Site A)	15	NO_2: 4-day; $PM_{2.5}$/EC:2-day	−0.35 ($PM_{2.5}$)	0.58	NR	NR
				−0.26 ($PM_{10-2.5}$)			
				−0.19 (PM_{10})			
	El Paso, TX (Site B)	15	NO_2: 4-day; $PM_{2.5}$/EC:2-day	0.06 ($PM_{2.5}$)	−0.37	NR	NR
				0.28 ($PM_{10-2.5}$)			
				0.12 (PM_{10})			
	Ciudad Juarez, Mexico (Site A)	15	NO_2: 4-day; $PM_{2.5}$/EC:2-day	−0.29 ($PM_{2.5}$)	0.66	NR	NR
				−0.58 ($PM_{10-2.5}$)			
				−0.5 (PM_{10})			
	Ciudad Juarez, Mexico (Site B)	15	NO_2: 4-day; $PM_{2.5}$/EC:2-day	−0.04 ($PM_{2.5}$)	0.45	NR	NR
				−0.5 ($PM_{10-2.5}$)			
				−0.34 (PM_{10})			
Greenwald et al. (2013)[b]	2 sites in El Paso, TX	18−26	All: 4-day	0.76 ($PM_{2.5}$)	0.45	NR	NR
				0.83 (PM_{10})			

EC = elemental carbon; NR = not reported; O_3 = ozone; OC = organic carbon; PM = particulate matter; $PM_{2.5}$ = $PM_{2.5}$ = in general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 2.5 µm, a measure of fine particles; PM_{10} in general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 10 µm, a measure of thoracic particles; $PM_{10-2.5}$ = in general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 10 µm and greater than a nominal 2.5 µm, a measure of thoracic coarse particles.

[a]Spearman correlation.

[b]Pearson correlation.

In addition to these findings, higher correlations were typically observed between ambient measurements of NO_2 and other traffic-related pollutants (see Section 3.4.3.1) compared to personal measurements [e.g., correlations among personal exposure measurements in Table 3-11; (Schembari et al., 2013; Williams et al., 2012a; Suh and Zanobetti, 2010; Delfino et al., 2008)]. For example, Suh and Zanobetti (2010) observed a stronger relationship between ambient NO_2:EC (r = 0.61) and ambient NO_2:$PM_{2.5}$ (r = 0.47) compared to personal NO_2:EC (r = 0.49) and personal NO_2:$PM_{2.5}$ (r = 0.29). Delfino et al. (2008) observed similar results in the NO_2:EC relationship in a health study investigating the relationship between traffic-related pollution and lung function decrements in Los Angeles, CA. While the ambient NO_2:EC correlation was r = 0.61, lower correlations were observed for personal NO_2:EC (r = 0.22). Additionally, a small number of time-series studies have used NO_2 in receptor models to relate health effects to sources/factors (Baxter et al., 2013; Cakmak et al., 2009; Halonen et al., 2009; Mar et al., 2000). Each of these studies used factor analysis, the EPA positive matrix factorization method[1], or PCA analysis and found high loadings of NO_2 and traffic-related copollutants (e.g., EC, OC, CO) on the same factor, which was attributed to traffic-related pollution.

Correlations between NO_2 and VOCs also suggest different sources for personal exposure. For example, Martins et al. (2012) estimated personal NO_2 and BTEX exposure during four 1-week periods using a microenvironmental approach that combined outdoor and indoor concentrations with time-activity patterns. It consistently observed correlations of $r = -0.423$ to $r = 0.138$ between NO_2 and BTEX during different seasons. The lack of correlation between these pollutants can be attributed in part to differences in sources between indoor and outdoor microenvironments. While exposure to VOCs, namely benzene, was attributed mainly to indoor sources, NO_2 was largely associated with traffic sources. These studies emphasize that proximity to roadways and time spent in various indoor and outdoor microenvironments can impact the relationship between NO_2 and traffic-related VOCs.

Weaker correlations observed between personal measurements of NO_2 and other traffic-related pollutants (compared to ambient measurement correlations) suggest that personal exposure to NO_2 may include a number of outdoor and indoor sources comprising traffic and nontraffic emissions (e.g., gas stoves, residential wood burning, biomass burning). These observations provide further evidence that nonambient sources of NO_2 provide noise to the ambient NO_2 signal. At the same time, the weaker correlations between total personal NO_2 exposures and copollutant exposures indicate that for panel studies of total NO_2 exposure, ambient copollutants would be unlikely to confound health effect estimates for NO_2 exposure. Titration conditions for NO, NO_2, and

[1] http://intranet.epa.gov/heasd/products/pmf/pmf.htm.

O_3 also likely differ from indoors to outdoors, given variation in solar radiation and other atmospheric factors that influence atmospheric chemistry. Additionally, personal exposures are influenced by building air exchange rate and time-activity patterns that differ among study participants. This is in contrast to ambient NO_2 concentrations, which appear to be largely driven by variability in traffic pollution in many areas. This type of exposure error associated with ambient concentrations is discussed in more detail in Section 3.4.3.3.

Few studies have reported indoor NO_2 copollutant correlations for short-term averaging times, focusing on correlations between NO_2 and PM in different size fractions as well as NO_2 and BC. In these studies, correlations of Spearman $r = -0.37$ to 0.66 were observed between indoor NO_2 and EC; however, lower correlations are observed for indoor NO_2 and PM compared with NO_2 and EC. Sarnat et al. (2012) measured indoor concentrations of NO_2, EC, $PM_{2.5}$, $PM_{10-2.5}$, and PM_{10} at four elementary schools in two cities near the U.S.-Mexico border: El Paso, TX and Ciudad Juarez, Mexico. NO_2 and PM showed weaker and/or inverse correlations at all four elementary schools ($r = -0.58$ to 0.12). Greenwald et al. (2013) later conducted a follow-up study to Sarnat et al. (2012) and measured similar pollutants at the same schools in El Paso, TX. Although Greenwald et al. (2013) reported similar NO_2-EC correlations to those reported in Sarnat et al. (2012), stronger correlations were observed between NO_2 and $PM_{2.5}$ ($r = 0.76$) and between NO_2 and PM_{10} ($r = 0.83$). Differences in the NO_2 and PM correlations between these two studies reflect that NO_2 and PM can have many different sources in indoor environments, which impact their temporal and spatial patterns. Moreover, the results of Greenwald et al. (2013) suggest the potential for confounding of NO_2 health effect estimates by PM based on indoor measurements. Taken together, the existence and extent of such confounding is uncertain.

In general, ambient NO_2 would not necessarily be expected to correlate well with personal measures of copollutants. For example, in the case where the exposed population spends time at residences or workplaces sufficiently far from the near-road environment, personal NO_2 exposure would not be expected to correlate with ambient copollutants of traffic-related origin. Low correlations between ambient NO_2 and personal measures of copollutants could support inferences regarding the independent effects of NO_2.

3.4.4.4 Traffic and Noise as Confounders

For the purpose of inferring causality from the body of epidemiologic studies of short-term and long-term exposure to traffic-related pollutants, the Health Effects

Institute Report on Traffic-Related Air Pollution (HEI, 2010) raised the concern that distance-to-road models are especially subject to confounding the associations between health effects and exposures because traffic indicators may encompass additional information, such as noise, other air pollutants, stress, and socioeconomic status, that may also be associated with the health effects of interest. However, recent evidence is mixed regarding the correlations of NO and NO_2 with traffic and noise levels. Most of these studies are for short-term exposure. Hence, the role of traffic and noise as confounders or independent variables in the relationship between health effects and NO or NO_2 is unclear.

Several studies have examined the relationship of traffic-related noise with NO and NO_2. Kheirbek et al. (2014) added noise level meters to the dense New York, NY monitoring project described in Ross et al. (2013) and observed that 1-week avg noise level, obtained at 60 locations during Fall 2012, correlated with Pearson $R = 0.59$ for NO_2 and $R = 0.61$ for NO. Davies et al. (2009) measured 2-week avg of NO_2 and NO_X concurrently with 5-minute noise samples at 103 sites and observed correlations of $R = 0.53$ for NO_2 and $R = 0.64$ for NO_X. Gan et al. (2012b) calculated the correlations among air pollutants and noise from road traffic and aircraft using 5-min data from 103 sites in Vancouver, BC, Canada during 2003 (dates not stated). They observed lower correlations for NO_2 with road traffic noise (Spearman $r = 0.33$) and aircraft noise ($r = 0.14$) compared with the correlation of NO with these two noise sources (road traffic: $r = 0.41$; aircraft: $r = 0.26$). For both NO_2 and NO, correlations were higher for road traffic noise than aircraft noise. Over a 5-yr avg, Gan et al. (2012a) reported the correlation between NO_2 and noise from road traffic of Spearman $r = 0.33$ from Gan et al. (2012b) as well as a correlation between NO and noise from road traffic of Spearman $r = 0.39$.

Ross et al. (2011) also examined relationships of different frequency noises with NO and NO_2 using continuous monitors collecting 48,000 samples per second for six 24-hour periods in August 2009. Ross et al. (2011) measured the relationships between traffic level, noise, and concentrations of NO_2 and NO in New York, NY as part of the Ross et al. (2013) study. Unweighted noise of all frequencies was uncorrelated with NO_2 (Spearman $r = -0.01$) but correlation increased for NO (Spearman $r = 0.43$) for all times. Correlations were higher for medium frequency noise (NO_2: $r = 0.22$; NO: $r = 0.57$). Correlations between noise and traffic counts segregated by fleet mix were generally higher for cars (unweighted noise: $r = 0.37$; medium frequency: $r = 0.33$), trucks (unweighted noise: $r = 0.64$; medium frequency: $r = 0.71$), and buses (unweighted noise: $r = 0.61$; medium frequency: $r = 0.60$) compared with the correlations with nonsegregated traffic data. Likewise, at night, high frequency noise was correlated with NO_2 ($r = 0.83$) and NO ($r = 0.73$).

Distance to road has also been observed to influence the relationship between noise and NO_2 for both long-term and short-term noise and NO_2 exposure studies. For the years 1987−1996, Beelen et al. (2009) estimated correlations among 1-yr avg NO_2, traffic level, and noise, and they observed correlations between traffic and noise depending on spatial designation (R = 0.30−0.38) and for the correlation of NO_2 and noise (R = 0.46). When segregating loud noise ≥65 dBA, correlation dropped (R = 0.22). Note that Beelen et al. (2009) did not specify whether Pearson or Spearman correlations were computed. Ross et al. (2011) noted within-day variability in these relationships, where truck and car traffic are correlated (r = 0.81) during the morning rush hour but inversely correlated at night (r = −0.67). Dadvand et al. (2014) measured 24-h avg noise, NO_X, and NO_2 at 50-m, 200-m, 500-m, and beyond 500-m buffers from the road in Barcelona, Spain from 2001−2005 and observed that all three decreased with increasing distance from the road. Measured temporal Spearman correlation of noise was r = 0.45 for NO_2 and r = 0.56 for NO_X. Allen et al. (2009) also studied the relationship between NO_2, UFP, and 5-min avg A-weighted equivalent noise for 105 locations in Chicago, IL and Riverside, CA using measurements taken in December 2006 and April 2007. After adjustment for regional unspecified air pollutant gradients, Pearson correlations with noise were R = 0.16−0.62 for NO_2 (winter Chicago: R = 0.16; spring Chicago: R = 0.41; spring Riverside: R = 0.62) and 0.49−0.62 for NO. In Chicago, correlations of noise with NO and NO_2 were higher within a 100-m buffer of the road, while correlations of noise with NO and NO_2 were lower within a 100-m buffer in Riverside.

For short-term exposure studies, more evidence is available to consider the relationship between traffic-related noise and NO_2 compared with long-term exposure studies. Collectively, these studies suggest that potential for confounding of NO_2 effects by noise may be influenced by temporal and spatial resolution of the data, noise frequency, and fleet mix. Specifically, confounding is less probable as distance from the road increases. However, overall noise may be unlikely to act as a confounder. It should be noted that noise would also have to be etiologically related to the health outcome under consideration to confound the relationship between the health effect and NO_2 exposure. When noise is decomposed by frequency, confounding is more likely.

3.4.5 Implications for Epidemiologic Studies of Different Designs

Human health effects related to ambient NO_2 exposure can be modeled by:

$$Y = \beta_0 + \beta_1 E_a + \beta_Z Z + \varepsilon$$

Equation 3-12

where Y = health effect of interest, β_0 = model intercept, β_1 = health effect estimate for the ambient exposure, E_a = ambient NO_2 exposure, β_Z = vector of slope related to each covariate, Z = covariate vector, and ε = random error. Y and E_a can sometimes have non-normal distributions, and hence normalization functions such as lognormal or logit may sometimes be applied. For simplicity, Equation 3-12 is presented as a linear function, which is appropriate because most epidemiologic studies assume normality of the data.

Estimates of NO_2 exposures are subject to errors that can vary in nature, as described in Section 3.4.3. Classical error is defined as error scattered around the true personal exposure and independent of the measured exposure. Classical error results in bias of the epidemiologic health effect estimate. Classical error can also cause inflation or reduction of the standard error of the health effect estimate. Berkson error is defined as error scattered around the exposure surrogate (in most cases, the central site monitor measurement) and independent of the true value (Goldman et al., 2011; Reeves et al., 1998). Recent studies demonstrate that exposure error is a combination of Berkson-like and classical-like errors and depends on how exposure metrics are averaged across space. Szpiro et al. (2011) defined Berkson-like and classical-like errors as errors sharing some characteristics with Berkson and classical errors, respectively, but with some differences. Specifically, Berkson-like errors occur when the measurement does not capture all of the variability in the true exposure. Berkson-like errors increase the variability around the health effect estimate in a manner similar to Berkson error, but Berkson-like errors are spatially correlated and not independent of predicted exposures, unlike Berkson errors. Classical-like errors can add variability to predicted exposures and can bias health effect estimates in a manner similar to classical errors, but they differ from classical errors in that the variability in estimated exposures is also not independent across space.

The results of Meng et al. (2012b), described in Section 3.4.2, illustrate that epidemiologic study design can influence the relationship between personal exposure measurements to NO_2 concentrations and ambient concentrations (see Table 3-7). This meta-analysis found that correlations were highest for short-term exposure community time-series epidemiology studies (designated as “daily average” in Table 3-7), and

correlations were lowest for longitudinal panel cohort studies. The following sections consider how exposure assessment errors may influence interpretation of health effect estimates for epidemiologic studies of different designs.

3.4.5.1 Community Time-Series Studies

In most short-term exposure epidemiologic studies of the health effects of NO_2, the health effect endpoint is modeled as a function of ambient exposure, E_a, which is defined as the product of ambient concentration, C_a, and α, a term encompassing time-weighted averaging and infiltration of NO_2 (Section 3.4.1). Community time-series epidemiologic studies capturing the exposures and health outcomes of a large cohort frequently use the concentration measured at a central site monitor ($C_{a,csm}$) as a surrogate for E_a in an epidemiologic model (Wilson et al., 2000). At times, an average of central site monitored concentrations is used for the E_a surrogate. For studies involving thousands of participants, it is not feasible to measure personal exposures. Moreover, for community time-series epidemiology studies of short-term exposure, the temporal variability in concentration is of primary importance to relate to variability in the health effect estimate (Zeger et al., 2000). $C_{a,csm}$ can be an acceptable surrogate if the central site monitor captures the temporal variability of the true air pollutant exposure. When averaging across large numbers of individuals, $\overline{\alpha}$, which varies between 0 and 1, may quantify the bias introduced by substituting $C_{a,csm}$ for $\overline{E_a}$ for the case where $\overline{\alpha}$ is absorbed into the health effect estimate. Spatial variability in NO_2 concentrations across the study area could attenuate an epidemiologic health effect estimate if the exposures are not correlated in time with $C_{a,csm}$ when central site monitoring is used to represent exposure. 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 health effect estimate may decrease. $C_{a,csm}$ may be an acceptable surrogate for E_a if the concentration time series at the central site monitor is correlated in time with the exposures.

Goldman et al. (2011) simulated the effect of classical-like and Berkson-like errors due to spatiotemporal variability among ambient or outdoor (i.e., a noncentral site monitor situated outside the home) air pollutant concentrations over a large urban area on health effect estimates of emergency department (ED) visits for a time-series study of cardiovascular disease. The relative risk (RR) per ppm was negatively biased in the case of classical-like error (1-h daily max NO_2: −1.3%; 1-h daily max NO_X: 1.1%) and negligibly positively biased in the case of Berkson-like error (1-h daily max NO_2: 0.0042%; 1-h daily max NO_X: 0.0030%). The 95% confidence interval range for RR per ppm was wider for Berkson-like error (1-h daily max NO_2: 0.028; 1-h daily max

NO_X: 0.023) compared with classical-like error (1-h daily max NO_2: 0.0025; 1-h daily max NO_X: 0.0043).

Recent studies have explored the effect of spatial exposure measurement error on health effect estimates to test the appropriateness of using central site monitoring for time-series studies. Goldman et al. (2010) simulated spatial exposure measurement error based on a semivariogram function across monitor sites with and without temporal autocorrelation at 1- and 2-day lags to analyze the influence of spatiotemporal variability among ambient or outdoor concentrations over a large urban area on a time-series study of ED visits for cardiovascular disease. A random term was calculated through Monte Carlo simulations based on the data distribution from the semivariogram, which estimated the change in spatial variability in exposure with distance from the monitoring site. The average of the calculated random term was added to a central site monitoring time series (considered in this study to be the base case) to estimate population exposure to NO_2 subject to spatial error. For the analysis with temporal autocorrelation considered, RR per ppm for 1-h daily max NO_2 dropped slightly to 1.0046 (95% CI: 1.0026, 1.0065), and RR per ppm for 1-h daily max NO_X dropped to 1.0079 (95% CI: 1.0057, 1.0100) when both were compared with the central site monitor RR per ppm = 1.0139 (for all air pollutants)[1]. When temporal autocorrelation was not considered, RR per ppm dropped to 1.0044 for 1-h daily max NO_2 and 1.0074 for 1-h daily max NO_X. The results of Goldman et al. (2010) suggest that spatial exposure measurement error from use of central site monitoring data results in biasing the health effect estimate towards the null, but the magnitude of the change in effect was small.

Goldman et al. (2012) also studied the effect of different types of spatial averaging on bias in the health effect risk ratio and the effect of correlation between measured and "true" ambient concentrations of NO_2, NO_X, and other air pollutant measures to analyze the influence of spatiotemporal variability among ambient or outdoor concentrations over a large urban area on health effect estimates. Concentrations were simulated at alternate monitoring locations using the geostatistical approach described above for 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 to be "true" in this study, and other exposure assessment methods were assumed to have some error. Five different exposure assessment approaches were tested: using a single central site monitor, averaging the simulated exposures across all monitoring sites, performing a population-weighted average across all monitoring sites, performing an area-weighted average across all monitoring sites, and population-weighted averaging of the geostatistical simulation (see Table 3-13). Goldman

[1]Note that 95% CIs were not reported for the central site monitor RR or for the cases where temporal autocorrelation was not considered.

et al. (2012) observed that the exposure measurement error was somewhat correlated with both the measured and true values, reflecting both Berkson-like and classical-like error components. For the central site monitor, the exposure measurement errors were somewhat inversely correlated with the true value but had relatively higher positive correlation with the measured value. For the other exposure estimation methods, the exposure measurement errors were inversely correlated with the true value, while they had positive but lower magnitude correlation with the measured value. At the same time, the exposure measurement bias, given by the ratio of the exposure measurement error to the measured value, was much higher in magnitude at the central site monitor than for the other estimation methods for NO_2 and for NO_X concentrations with the exception of the area-weighted average, which produced a large negative exposure measurement bias.

These findings suggest more Berkson-like error in the more spatially resolved exposure metrics compared with the central site monitor and more classical-like error for the converse (i.e., more classical-like error in the central site monitor estimate compared with the other exposure assessment techniques). Hence, more bias would be anticipated for the health effect estimate calculated from the central site monitor, and more variability would be expected for the health effect estimate calculated with the more spatially resolved methods. It was observed that the more spatially variable air pollutants studied in Goldman et al. (2012) also had more bias in the health effect estimates. This was noted across exposure assessment methods but was more pronounced for the central site measurement data.

Butland et al. (2013) conducted a simulation study to test how spatial resolution influences health effect estimates in a time-series epidemiologic model of mortality as a function of NO_2 exposure for urban and rural areas. The test domain was subdivided into squares ranging in area from 1 km^2 to 25 km^2. Health effect estimates simulated using the 1-km^2 resolution area were considered to be "true," and mortality estimates were sampled from a Poisson distribution of mortality data. Monitor data were simulated based on a lognormal distribution using the correlelogram among pairs of NO_2 monitors to establish the variability of the distribution as a function of distance. The error structure in the model was constructed to include both Berkson-like and classical-like components. Health effect estimates for mortality based on NO_2 exposures were attenuated by 29 and 38% for urban and rural areas, respectively, when reducing the spatial resolution from 1 km^2 to 25 km^2 over a 3-year time-series analysis.

Table 3-13 The influence of exposure metrics on error in health effect estimates.

Exposure Estimation Approach	Bias[$(Z - Z^*)/Z$][a]	$R^2(Z, Z^*)$[b]	$R[(Z - Z^*), Z^*]$[c]	$R[(Z - Z^*), Z]$[c]
NO_2				
Central site monitor	0.62	0.24	−0.46	0.61
Unweighted average	0.25	0.38	−0.73	0.20
Population-weighted average	0.18	0.38	−0.78	0.14
Area-weighted average	−0.07	0.38	−0.87	−0.04
Geostatistical model—population-weighted average	N/A	0.45	−0.82	0.0017
NO_X				
Central site monitor	0.71	0.33	−0.11	0.81
Unweighted average	0.31	0.45	−0.63	0.29
Population-weighted average	0.03	0.46	−0.81	0.02
Area-weighted average	−0.88	0.47	−0.96	−0.31
Geostatistical model—population-weighted average	N/A	0.52	−0.80	−0.00042

N/A = not applicable; NO_2 = nitrogen dioxide; NO_X =the sum of nitric oxide and NO_2.

Note: Model errors were based on comparisons between measured data and simulated data at several monitoring sites. Errors were estimated for a single central site monitor, various monitor averages, and values computed from a geostatistical model. Z denotes the measured concentration, and Z^* denotes the true concentration, considered here to be from the CTM. Bias in the exposure metric is given as the proportion of error between the measurement and true value to the measurement.

[a]Data provided by the authors for Figure 5 of Goldman et al. (2012).

[b]Data provided by the authors of Figure 4 of Goldman et al. (2012).

[c]Pearson correlation.

Source: Goldman et al. (2012).

Sarnat et al. (2010) studied the spatial variability of concentrations of NO_2, along with CO, O_3, and $PM_{2.5}$, in the Atlanta, GA metropolitan area and how spatial variability affects interpretation of epidemiologic results, using time-series data for circulatory disease ED visits. Sensitivity to spatial variability was examined at slightly greater than neighborhood scale (8 km) in this study. Interestingly, Sarnat et al. (2010) found that relative risk varied with distance between the monitor and study population when comparing urban to rural locations, but distance of the study population to the monitor was not an important factor when comparing urban population groups. This suggests that,

even for spatially heterogeneous NO_2, urban scale concentration measures may produce results comparable to neighborhood-scale concentration measures if the sites were comparable throughout the city, for example, as a result of similar traffic patterns. However, Sarnat et al. (2010) caution that, because their study was limited to 8-km radii, it is not possible to interpret this work with respect to near-road and on-road microscale concentrations.

In a study of the effect of concentration metric choice (central site, arithmetic average across space, or population-weighted average) used to represent exposure in a time-series epidemiologic model, Strickland et al. (2011) found that choice of the concentration metric resulted in large differences in the observed associations between ED visits for pediatric asthma and exposure for spatially heterogeneous NO_2 but not for spatially homogeneous $PM_{2.5}$ when using a unit standardization for computing the relative risk. However, when Strickland et al. (2011) used IQR for standardization, there were little differences among the relative risk estimates across the concentration metrics. The differences observed between unit and IQR standardization are due to the fact that the IQR reflects the spatial variability in the exposure metrics for the spatial and population-weighted averages.

Error type also influences the health effect estimate from time-series studies. Dionisio et al. (2014) decomposed the exposure measurement error into spatial and population-based components. Spatial error was defined as the difference between concentration simulated by an AERMOD dispersion model and concentration measured at a CSM, and population error was defined as the difference between the SHEDS exposure model (using only ambient sources) and the dispersion model. Errors were computed for each ZIP code centroid. Three pollutants with high spatial variability (NO_X, CO, EC) termed "local" by the authors and three pollutants with low spatial variability ($PM_{2.5}$, O_3, SO_4) termed "regional" by the authors were included in the study. Although NO_2 was not included explicitly, the local results are relevant. Dionisio et al. (2014) observed more variability in both the spatial and population components of the exposure measurement error across the ZIP codes for the local pollutants compared with the regional pollutants. Attenuation of the health effect estimate by the spatial error component was much larger for the local pollutants compared with the regional pollutants, and the amount of bias by the spatial error component was roughly the same for NO_X, CO, and EC. However, the population error component caused much more attenuation of the health effect estimate for NO_X compared with CO and EC. In fact, CO had negligible bias of the health effect estimate due to the population error component. This discrepancy is likely related to the deposition rate of NO_X compared with the zero deposition rate for CO modeled in SHEDS. Given that NO_2 has a higher deposition rate than NO, the results of Dionisio et al. (2014) suggest that health effect estimates modeled

in time-series studies of NO_X exposure are likely extendable to NO_2; see Section 3.3.2.1 for information related to deposition of indoor NO_2. Hence, it is likely that spatial variability and indoor deposition both cause bias in the health effect estimate for NO_2.

Analysis of time-series epidemiologic studies have suggested that nonambient contributions introduce Berkson error into the exposure term, where the error does not bias health effect estimates for ambient NO_2 assuming that nonambient NO_2 sources are independent of ambient sources, but it does cause the confidence intervals around the health effect estimates to widen (Sheppard, 2005; Wilson et al., 2000). No data from cohort studies are available to test if this theory can be applied more broadly to all epidemiologic studies. Sheppard et al. (2005) simulated the effect of nonambient sources for a time-series study of the health effects of PM exposure and found that, as long as the ambient and nonambient sources were uncorrelated, the nonambient exposures would widen the confidence interval around the health effect estimates but would not bias the health effect estimate. This result is generalizable to NO_2 because it did not depend on the particle size distribution.

Exposure measurement error related to instrument precision has a smaller effect on health effect estimates in time-series studies compared with error related to spatial gradients in the concentration because instrument precision would not be expected to modify the ability of the instruments to respond to changes in concentration over time. Goldman et al. (2010) investigated the influence of instrument error on health effect estimates in a time-series epidemiology study by studying differences in exposure estimates and health effect estimates obtained using colocated monitors. In this study, a random error term based on observations from colocated monitors was added to a central site monitor's time series to simulate population estimates for ambient air concentrations subject to instrument precision error in 1,000 Monte Carlo simulations. Very little changes in the risk ratios were observed for 1-h daily max NO_2 and 1-h daily max NO_X concentrations. For 1-h daily max NO_2 concentration, the RR per ppm of NO_2 concentration with simulated instrument precision error was 1.0133 compared with RR per ppm = 1.0139 for the central site monitor. For 1-h daily max NO_X concentration with simulated instrument precision error, RR per ppm = 1.0132 compared with the central site monitor's RR of 1.0139. The amount of bias in the health effect estimate related to instrument precision was very small.

3.4.5.2 Longitudinal Cohort Studies

For cohort epidemiologic studies of long-term human exposure to NO_2, where the difference in the magnitude of the concentration is of most interest, if $C_{a,csm}$ is used as a

surrogate for E_a, then α can be considered to encompass the exposure measurement error related to uncertainties in the time-activity data and air exchange rate. Spatial variability in NO_2 concentrations across the study area could lead to bias in the health effect estimate if $C_{a,csm}$ is systematically higher or lower than E_a. This could occur, for example, if the study participants are clustered in a location where their NO_2 exposure is higher or lower than the exposure estimated at a modeled or measurement site. $C_{a,csm}$ may be an acceptable surrogate for E_a if the central site monitor is located in close proximity to the study participants (e.g., in a dense urban setting) and spatial variability of the NO_2 concentration across the study area where the study participants are located is minimal in the vicinity of each sample group. There is limited information regarding whether $C_{a,csm}$ is a biased exposure surrogate in the near-road environment for epidemiologic studies of long-term exposure.

Sensitivity of the epidemiologic model to the temporal and spatial characteristics of exposure data depends on the temporal characteristics of the disease process. Birth outcome studies serve as an example where the exposure window becomes an important consideration that helps to delineate short-term exposure from long-term exposure epidemiologic study design. For example, Ross et al. (2013) studied the role of spatial and temporal resolution of NO_2 estimates in the application of LUR to study birth outcomes in New York City. Seasonal variability was more evident when averaging NO_2 estimates across the final 6 weeks of gestation compared with the entire gestation period, but temporal variation had less influence on NO_2 predictions compared with $PM_{2.5}$. This finding reflects the fact that variability in NO_2 concentrations is more prominent in space than in time compared with $PM_{2.5}$. Additionally, Brauer et al. (2008) studied the influence of NO_2 exposure models (IDW of central site monitoring data and LUR) on health effect estimates for birth outcomes and observed higher adjusted odds ratios for IDW compared with LUR (which produced health effect estimates closer to null, Section 6.4.3). Clark et al. (2010) compared IDW with LUR for the analysis of asthma risk from in utero and first-year-of-life exposure to NO_2, NO, and other pollutants. They observed comparable adjusted odds ratios for the first year of NO_2 exposure and higher adjusted odds ratio higher for IDW compared with LUR for in utero NO_2 exposures (Section 6.4.3). The biologically relevant time period in eliciting a birth outcome likely determines whether spatial or temporal variation in concentration is more important to the epidemiologic model. It is possible that, if the biologically relevant time period is short, then temporal variability may play a larger role. In that case, the seasonal differences in NO_2 concentration become more important for measuring an effect. If the biologically relevant time period is longer, then the spatial contrasts evident in concentration maps become more important so that exposure misclassification can lead to over- or under-estimation of the effect.

Spatial resolution of the exposure estimates has been evaluated to examine the influence of spatial exposure error in cohort studies. This has been considered with spatially-resolved alternatives to central site monitoring data, such as data from a LUR, to describe exposure of individuals within a cohort that is spatially dispersed within a study area (see Section 3.2.2). Sellier et al. (2014) and Lepeule et al. (2010) evaluated various approaches to estimate exposure (nearest central site monitor, geostatistical model, LUR model, dispersion model) in a study of birth weight among a French mother-child cohort in the French cities of Nancy and Poitiers. Correlations among the methods varied with respect to methodology, distance, and land use type. For example, the correlation between LUR and dispersion modeling had a minimum Pearson $R = 0.58$ (for urban locations), while the correlation between central site monitoring and LUR had a minimum $R = 0.20$ (also for urban locations). No effect of the method was observed on change in birth weight, but confidence intervals around the health effect estimate generally increased for dispersion models, which tended to be the most spatially heterogeneous among the four methods studied.

The influence of spatial exposure misclassification on health effect estimate varies with the particular study parameters, such as model selection and location. Madsen et al. (2010) compared odds ratios for birth weight per quartiles of NO_2 exposure estimated from a near-road monitoring station and a dispersion model. Higher exposure variability was captured by the dispersion model, but the adjusted odds ratio showed an effect only for the near-road monitoring station exposure data, where time-averaged or residential exposures were likely to be overestimated. Wu et al. (2011) compared health effect estimates obtained using nearest monitors and LUR for birth outcomes for NO_2 in Los Angeles County and Orange County, CA. Odds ratios for NO_2 were comparable for nearest monitor and LUR for Los Angeles County, where the LUR was fit, but the odds ratio decreased for Orange County in comparison with nearest monitor. This is consistent with studies reporting higher exposure error when LUR models are fit in one city and applied elsewhere, as described in Section 3.2.2.1. Ghosh et al. (2012) compared health effect estimates for low birth weight using NO_2 exposure estimates from LUR (scaled to account for seasonal fluctuations in concentration) to nearest monitoring station in Los Angeles County, CA and found negligible difference between the health effect estimates obtained with each exposure metric.

Paciorek (2010) performed simulations to test the effect of spatial errors on health effect estimates in long-term exposure epidemiologic studies. He identified unmeasured spatial confounding as a key driver in biasing health effect estimates in a spatial regression. Paciorek (2010) maintained that bias can be reduced when variation in the exposure metric occurs at a smaller spatial scale than that of the unmeasured confounder. Szpiro et al. (2011) also explored the effect of specification of spatial conditions on the exposure

metric used in a cohort epidemiologic study. They evaluated bias and uncertainty of the health effect estimate obtained when using correctly specified and misspecified exposure simulation conditions, where correct specification (i.e., predicting the "true" concentration) was considered for comparison purposes to be the use of three spatial prediction variables and misspecification implies unmeasured confounding in the model. LUR calculations were used to simulate exposure, and correct specification was considered when three spatial covariates were included in the model; the misspecified model omitted a geographic covariate in the LUR. Each case was compared with the reference case where the health effect estimate was obtained by using monitoring data for the exposure metric. Szpiro et al. (2011) also reduced the amount of variability in the third covariate of the correctly specified exposure model in an additional set of simulations. Prediction accuracy of the exposure estimate was higher for the correctly specified model compared with the misspecified model. However, bias in the health effect estimate was also slightly more negative for the correctly specified model, and the health effect estimate was more variable for the correctly specified model compared with the misspecified model when the variability in the exposure covariate decreased. The results of Szpiro et al. (2011) suggested that use of more accurately defined exposure metrics in a cohort study does not necessarily improve health effect estimates. The Szpiro et al. (2011) simulations were for a generic air pollutant. The results are relevant to NO_2 exposure, but the spatial scale issue raised by Paciorek (2010) was not considered in the Szpiro et al. (2011) analysis.

Basagaña et al. (2013) also investigated the effect of differences in LUR model fitting on error in the epidemiologic health effect estimates. In this study, Basagaña et al. (2013) fit three LUR models with 20, 40, or 80 measurement locations. For this simulation study, the model considered correctly specified contained five covariates. As a comparison case, Basagaña et al. (2013) fit misspecified models containing 20 or 100 covariates (including the five original covariates). The misspecification effectively added error to the model. The simulated exposure error produced a combination of Berkson-like and classical-like errors on the health effect estimate. Compared with the true health effect estimate, bias towards the null was observed to increase with decreasing number of measurement locations used to fit the LUR model. At the same time, the mean squared error of the health effect estimate increased with decreasing number of measurement locations. Moreover, bias towards the null and mean squared error also grew with increasing the number of covariates from 5 to 20 to 100. Notably, R^2 did not trend with the number of variables, suggesting that R^2 is not a sufficient measure of LUR model quality.

Under the assumption that the exposure estimates come from the same underlying distribution as the true exposures associated with each health effect, Szpiro and Paciorek (2013) performed several simulations to investigate the influence of the distribution and

variability of the exposure estimates on health effect estimates in longitudinal cohort studies, building upon their previous studies of the influence of model misspecification (Szpiro et al., 2011) and unmeasured confounding (Paciorek, 2010) on longitudinal health effect estimates. In one set of simulations, the distribution of the exposure was varied. When the assigned exposure measurements were set to be uniform across space, the health effect estimate was biased away from the null compared with the case when the exposure subjects are colocated with the study participants. When an additional spatial covariate was omitted, the health effect estimate was biased towards the null compared with the correctly specified model. Additional simulations by Szpiro and Paciorek (2013) based on the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA-Air) NO_X concentration and health effects data set for Baltimore showed that bias in the health effect estimate was subject to classical-like error and incorrectly estimated standard errors. Both factors biased the health effect estimate towards the null. Bias in the health effect estimate was minimized when a bias correction factor was introduced and a bootstrapping technique was employed to recalculate standard error based on the distribution of the exposures associated with the health effect data. Although this approach was criticized as regression calibration (Spiegelman, 2013), Szpiro and Paciorek (2013) illustrated the influence of classical-like and Berkson-like errors on long-term exposure cohort study health effect estimates through these simulations.

Not considering time-activity patterns of study participants adds uncertainty to exposure estimates obtained via spatial modeling such as LUR. Setton et al. (2011) investigated how both spatial variability and unaccounted study participant mobility bias health effect estimates in long-term exposure epidemiologic models of health effects from NO_2 exposure in a simulation study of cohorts in southern California and Vancouver, BC. In this case, concentration at each participant's home was modeled (using the Comprehensive Air Quality Model with Extensions (CAMx) for southern California and using LUR and IDW interpolation of monitoring data for Vancouver). Populations were simulated using human activity data for Vancouver and transportation survey data for southern California. Bias in the health effect estimate increased in magnitude towards the null with distance from home and time spent away from home. Moreover, when spatial variability increased (through comparison of spatially variable LUR-derived NO_2 concentrations with a smoother monitor-based approach for mapping NO_2 for the Vancouver data), the health effect estimate obtained from the IDW-based approach was closer to the null compared with the LUR-based health effect estimate. Setton et al. (2011) interpreted this finding as evidence of the influence of smoothing spatially heterogeneous concentration profiles on the health effect estimate.

Instrumentation bias could be anticipated to influence health effect estimates from epidemiologic studies of long-term NO_2 exposures in some situations. Section 3.2.1.2

describes how passive monitors are likely to overestimate exposure given the influences of ambient temperature, relative humidity, and presence of copollutants. Therefore, LUR exposure may be overestimated when the LUR is fit using passive monitoring data. Sections 2.4.1 and 3.2.1.1 describe how the presence of copollutants can also cause NO_2 concentrations measured using central site monitors to be overestimated. Overestimating exposure can drive health effect estimates towards the null. Ambient temperature and relative humidity would not be expected to vary greatly within a city. However, local copollutant concentrations may be spatially variable such that an LUR model fit, and resulting health effect estimates, could have some differential bias across a city related to instrument error. Because climate and ambient sources are more likely to differ among cities, instrumentation error leading to overestimates of exposure could have a larger influence on the comparison of health effect estimates among cities when LUR or central site monitors are used to estimate exposures.

In the case of long-term exposure cohort studies, nonambient contributions to the total personal exposure estimates would be expected to widen the confidence interval around the health effect estimates by adding noise to the exposure signal, as is the case for time-series studies of short-term exposure. Also, addition of any non-negative nonambient component to the personal exposure measurement, such that the average total personal NO_2 exposure would necessarily be equal to or greater than the average personal exposure to ambient NO_2, would result in an underestimate of exposure. This associated nondifferential exposure misclassification could bias the health effect estimate towards the null.

3.4.5.3 Panel Studies

Consideration of errors in use of $C_{a,csm}$ as a surrogate for E_a provides information on the impact of this proxy measure on health effect estimates in panel studies. Van Roosbroeck et al. (2008) evaluated health effect estimates among a panel of children for associations of four respiratory outcomes with 48-hour NO_2 data from a single monitor located at the children's school. These health effect estimates were compared with those obtained from personal NO_2 monitoring to capture spatial variability in NO_2 concentrations and time-activity data. Van Roosbroeck et al. (2008) observed that health effect estimates were biased towards the null by roughly one-half to one-third when using a single monitor outside the school in lieu of personal exposure monitors. In this case, bias in the single-monitor health effect estimate was likely influenced by the spatial variability of the NO_2 concentration profile, time-activity of the study participants, and infiltration of ambient NO_2 indoors. The authors also adjusted the health effect estimate for nonambient

sources, including parental smoking, gas cooking, and presence of an unvented water heater.

Sarnat et al. (2012) considered the influence of exposure metric on health effect estimates obtained for a panel of school children. This study was conducted along the U.S.-Mexico border in El Paso, TX, and Ciudad Juarez, Mexico, and 96-h avg concentrations measured from central site chemiluminescent monitors, passive monitors outside the children's schools, and passive monitors inside the children's schools were all used as surrogates for exposure to NO_2. The largest health effect estimate was observed for measurements outside the school. In comparison, the health effect estimates for NO_2 measured inside the schools and at central site monitors were several times smaller (see Table 5-20). Based on the comparison between outdoor and central site monitoring results, Sarnat et al. (2012) concluded that exposure misclassification from using central site measurements, in lieu of measurements at the site of exposure, could lead to biasing the health effect estimate towards the null. They proposed that this bias was related to the failure of central site monitors to capture intra-urban spatial variability. The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) also did not find conclusive evidence of the influence of exposure measurement error on health effect estimates from panel epidemiologic studies of NO_2 exposure. In general, there is uncertainty regarding the influence of NO_2 monitor placement on the magnitude and directionality of bias of the health effect estimate as related to use of central site monitors in lieu of localized monitors in panel studies. As for epidemiologic studies of long-term NO_2 exposure (see Section 3.4.5.2), instrumentation error leading to overestimates of exposure could have a differential influence on health effect estimates, especially for inter-city comparisons.

3.5 Conclusions

This chapter presents the current state of the science for assessment of human exposure to NO_2. It builds upon the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008), which concluded that errors associated with the use of NO_2 concentrations measured at central site monitors as exposure metrics for epidemiologic studies tended to bias the health effect estimate towards the null for both short-term exposure and long-term exposure epidemiologic studies. As detailed within this chapter, recent studies provide support for the conclusions presented in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) for short-term exposure studies but differ in some cases for long-term exposure studies.

Commonly used exposure assessment methods include central site monitors, passive monitors, LUR, CTM, and dispersion models (see Section 3.2). The influence of measurement errors from each of these techniques varies with study design. These

methods are listed in Table 3-1, along with their application (i.e., the design of the study in which they are used) and associated errors. Community time-series studies of short-term NO_2 exposure typically use central site monitoring. Panel studies tend to employ central site monitors or, in some cases, passive monitors. Studies of long-term NO_2 exposure often use a variety of methods, including central site monitors, LUR, dispersion models and spatial smoothing techniques. Errors associated with these methods vary in importance based on their application. Dispersion modeling can be subject to errors related to simplifying assumptions about the meteorology, urban or natural topography, or photoreactivity of NO to form NO_2. Additionally, NO_2 exposure estimates from inverse distance weighting or other spatial smoothing techniques can be subject to error if the spatial scale of monitoring does not capture all sources. Studies employing exposure estimates obtained using these methods often report R^2, bias, and/or mean squared error to describe the quality of the exposure estimates. Given that these metrics do not always correlate, caution must be taken to interpret the quality of exposure data from an individual study on the basis of one metric.

Factors contributing to error in NO_2 exposure assessment include temporal activity of epidemiologic study participants, spatial variability of NO_2 concentrations across the study area, infiltration of NO_2 indoors, and instrument accuracy and precision (see Section 3.4.3). With respect to time-activity data, variability within and among different populations causes the limitation of having only one monitoring location in many studies to have varying influence on exposure estimates within and among those different populations. In general, spatial misalignment can occur when the time-activity patterns of study participants are not factored into the study design or when the location where NO_2 exposure is estimated does not coincide with the residential, school, or work location of interest. Studies of spatial variability of human exposure indicate that the magnitude of the error in exposure estimation increases with distance between the monitor and the subject. As a result, there is a potential for exposure misclassification if the ambient NO_2 concentration measured at a given site differs from that at the location of an epidemiologic study participant, and this issue is present regardless of the spatial scale of the epidemiology study. At the same time, the influence of spatial variability depends strongly on the temporal design of the epidemiologic study, as described in the paragraphs below. Infiltration and air exchange rate influence indoor levels of NO_2 in the absence of indoor sources and hence presents the potential for bias and uncertainty in α, which depends on air exchange rate, penetration, and indoor deposition. NO_2 monitors are often subject to positive biases resulting from interference by NO_Y species.

Community time-series epidemiologic studies most commonly use central site monitors to estimate human exposure to ambient NO_2 (see Section 3.4.5.1). Temporal variability in exposure is the relevant feature of the exposure data in a community time-series study.

Additionally, personal exposure measurements cannot feasibly be obtained for health studies with large numbers of participants. There is some uncertainty associated with using central site measurements of NO_2 concentrations to represent personal exposure because the temporal variability of the central site exposure estimate may differ from the temporal variability of the true exposure. Exposure estimates using NO_2 concentration measurements from central site monitors do not capture the spatial variability of the concentration field, which becomes a more important source of error for time-series epidemiology studies if the NO_2 concentration at the site of the study participants is not well correlated with measurements at the central site monitor. Nonambient contributions and differential infiltration of NO_2 can also add error or uncertainty to a health effect estimate. Instrument precision and accuracy are not thought to have a substantial influence on health effect estimates in time-series studies. Simulation studies testing the influence of exposure error in time-series studies suggest that exposure error may widen the confidence intervals of the health effect estimate and bias the estimate towards the null. This implies that reported health effect estimates for time-series studies of NO_2 exposure are potentially lower than true health effect estimates or that the reported confidence intervals around those health effect estimates are wider than the true confidence intervals.

Long-term exposure epidemiology studies compare subjects or populations at different locations (see Section 3.4.5.2). Therefore, spatial, rather than temporal, contrasts are more important in long-term exposure studies. NO_2 concentrations measured at central site monitors are often used to represent exposures when human health cohorts are compared among cities. There is some uncertainty associated with using central site measurements of NO_2 concentrations to represent personal exposure because the central site exposure estimate likely varies in a positive or negative direction from the personal exposure. This condition adds uncertainty to epidemiologic health effect estimates that are derived from spatial contrasts, such as multicity or within-city studies. Moreover, positive biases from measurement of NO_Y artifacts have the potential to enhance spatial contrasts in exposure. LUR or dispersion models are often used to estimate exposure at the residential locations of study participants in long-term exposure epidemiologic studies because those models are designed to capture spatial variability within a geographic area such as a city. LUR has been demonstrated to provide reasonable estimates of NO_2 exposure if the model is trained and applied in the same general location such that the exposure estimates and true exposures are assumed to come from the same data distribution. Spatial misalignment of the participant locations with the participants' exposure estimates can increase the uncertainty around the health effect estimate. Moreover, if the misalignment is systematic, for example if the study participants are clustered in a location where their NO_2 exposure is higher or lower than the exposure estimated at a modeled or measurement site, the health effect estimate can be biased in

either direction depending on whether the modeled NO_2 exposure is higher or lower than the true exposure. This issue adds uncertainty to health effect estimates derived from any given individual long-term study.

Panel epidemiologic studies of NO_2 exposure using central site monitors or centrally located monitors are subject to exposure misclassification due to spatial misalignment between the monitored ambient NO_2 concentration and the true personal exposure to ambient NO_2 (see Section 3.4.5.3). Available panel studies that compare health effect estimates among exposure assessment techniques have suggested that such spatial misalignment leads to attenuating the health effect estimate. However, only a limited number of panel studies have studied the influence of exposure measurement error on health effect estimates. For this reason, it is difficult to reach a conclusion about the magnitude and direction of error in the health effect estimates related to exposure error.

Copollutant confounding can occur when common sources emit multiple pollutants and therefore can increase uncertainty in identifying whether the copollutants are independently associated with a health effect (see Section 3.4.4). For traffic, NO (reacting to NO_2), CO, EC, UFP, and benzene are commonly coemitted and can be highly correlated with NO_2 in time and space. During winter, NO_2 emitted from heating fuel sources can also be highly correlated with $PM_{2.5}$ and PM_{10}. For both short-term exposure and long-term exposure epidemiologic studies, it is difficult to distinguish the health effect associated with NO_2 exposure among health effects attributed to other highly correlated pollutants. The temporal correlations may vary over space given that different pollutants have different spatial scales over which they vary from peak to background levels. For long-term exposure epidemiologic studies, bias related to copollutant confounding can be reduced when the spatial scale of the NO_2 exposure metric is smaller than the spatial scale of the correlated copollutant. Bias related to copollutant confounding may be more likely for unstable copollutants (e.g., UFP) or air pollutants that disperse more quickly than NO_2 (e.g., CO), compared with more spatially homogeneous pollutants (e.g., $PM_{2.5}$).

References for Chapter 3

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CHAPTER 4 DOSIMETRY AND MODES OF ACTION FOR INHALED OXIDES OF NITROGEN

4.1 Introduction

This chapter has two main purposes. The first is to describe the principles that underlie the dosimetry of nitrogen dioxide (NO_2) and nitric oxide (NO) and to discuss factors that influence it. The second is to describe the modes of action that may lead to the health effects that will be presented in Chapter 5 and Chapter 6. This chapter is not intended to be a comprehensive overview, but rather, it updates the basic concepts derived from the NO_2 and NO literature presented in the 1993 Air Quality Criteria for Oxides of Nitrogen (AQCD) and the 2008 Integrated Science Assessment (ISA) for Oxides of Nitrogen—Health Criteria (U.S. EPA, 2008) (U.S. EPA, 1993) and introduces the recent relevant literature.

In Section 4.2, particular attention is given to chemical properties of inhaled NO_2 and NO that affect absorption, distribution, metabolism, and elimination. Inhaled NO_2 and NO and subsequent reaction products are discussed in relation to endogenous production of these chemical species. Because few NO_2 dosimetry studies have been published since the 1993 AQCD (U.S. EPA, 1993), much of the information from that report has been pulled forward into the current document and is discussed in the context of more recent research. The topics of dosimetry and modes of action are bridged by reactions of NO_2 with components of the epithelial lining fluid (ELF) and by reactions of NO with heme proteins, processes that play roles in both uptake and biological responses.

Section 4.3 highlights findings of studies published since the 2008 ISA (U.S. EPA, 2008) that provide insight into the biological pathways affected by exposure to NO_2 and NO. Earlier studies that represent the current state of the science are also discussed. Studies conducted at more environmentally relevant concentrations of NO_2 and NO (i.e., $\leq$5,000 ppb, see Section 1.1) are of greater interest because biological pathways responsible for effects at higher concentrations may not be identical to those occurring at lower concentrations. Some studies at higher concentrations are included if they were early demonstrations of key biological pathways or if they are recent demonstrations of potentially important new pathways. This information is used to develop a mode of action framework for inhaled NO_2 and NO, which serves as a guide to interpreting health effects evidence presented in subsequent chapters (Chapters 5 and 6).

4.2 Dosimetry of Inhaled Oxides of Nitrogen

4.2.1 Introduction

This section provides a brief overview of NO_2 and NO dosimetry and updates information provided in the 2008 ISA for Oxides of Nitrogen—Health Criteria (U.S. EPA, 2008). Dosimetry refers to the measurement or estimation of the amount of a compound, or its reaction products, absorbed and/or generated at specific sites in the respiratory tract during an exposure. New to this ISA is the inclusion of basic information regarding the endogenous production of NO_2 and NO. It is important to consider inhaled NO_2 and NO and their subsequent reaction products in relation to endogenous production of these chemical species. To establish an environmentally relevant context, ambient NO_2 and NO concentrations are briefly discussed below; more detail is provided in Chapter 2.

Ambient NO_2 concentrations are highest in the winter months, near major roadways, during weekday morning hours, and decrease moderately during the afternoon (see Atlanta, GA data in Figures 2-20 and 2-21). One-hour average, near-road (15 m) NO_2 concentrations in Los Angeles, CA ranged from 3 to 80 ppb with median values of about 40 ppb in the winter and 30 ppb in the summer months of 2009 (Polidori and Fine, 2012). Away from major roadways, 1-h avg NO_2 concentrations may still reach 50 to 70 ppb with median NO_2 concentrations between roughly 10 to 30 ppb depending on the season and distance from roadways (Polidori and Fine, 2012). As will be discussed, the uptake of inhaled NO_2 may potentially increase levels of NO_2-derived reaction products beyond levels endogenously occurring in the respiratory tract.

Similar to NO_2, ambient NO concentrations are highest in the winter months near major roadways during weekday morning hours, but decrease to very low levels during the afternoon (see Atlanta, GA data in Figures 2-20 and 2-21). One-hour average, near-road (15 m) NO concentrations in Los Angeles, CA ranged from 0 ppb to over 400 ppb with median values of about 50 ppb in the winter and 20 ppb in the summer months of 2009 (Polidori and Fine, 2012). Away from major roadways, 1-h avg NO concentrations may still reach 250 ppb, but median NO concentrations are 5 ppb or less (Polidori and Fine, 2012). For the same roadway (Interstate 710), (Zhu et al., 2008) reported on-road NO_X (i.e., the sum of NO and NO_2) concentrations of around 400 ppb (average of eight 2-hour samples collected between 10:00 a.m. to noon during the period from June 2006 to May 2007). As will be discussed, these ambient NO concentrations are generally in the range of those occurring endogenously in the respiratory tract.

4.2.2 Dosimetry of Nitrogen Dioxide

NO_2 is a highly reactive gas that occurs as a radical wherein, although technically a resonance structure, the unpaired electron is more localized to the nitrogen atom than either of the oxygen atoms. Once inhaled, NO_2 first encounters the aqueous phase of the ELF, which is a contiguous but biologically complex aqueous fluid layer that covers all of the respiratory tract surfaces (Bastacky et al., 1995). The ELF constituent composition shows appreciable heterogeneity with respect to anatomic site and species. The ELF of alveolar surfaces and conducting airway surfaces has a monomolecular layer of surface active lipids (Bernhard et al., 2004; Hohlfeld, 2002; Mercer et al., 1994), largely fully saturated, which reduces surface tension and may provide a resistive barrier to the interfacial transfer of NO_2 (see below). Upon dissolution into the ELF, NO_2 is converted from a gas to a nonelectrolyte solute, and thus becomes subject to partitioning and reaction/diffusion. Thus, the ELF represents the initial barrier between NO_2 contained within the intra-respiratory tract gas phase and the underlying epithelia (Postlethwait and Bidani, 1990). NO_2 chemically interacts with antioxidants, unsaturated lipids, and other compounds in the ELF. It preferentially reacts with one electron donors (e.g., small molecular weight antioxidants, protein thiols, etc.), undergoes radical-radical addition reactions, may also abstract allylic hydrogen atoms from polyunsaturated fatty acids and, through a complex series of reactions, can add to unsaturated fatty acids to generate nitrolipids (Bonacci et al., 2012; Rudolph et al., 2010; O'Donnell et al., 1999). The compounds thought responsible, in large part, for the respiratory effects of inhaled NO_2 are the reaction products themselves or the metabolites of these products in the ELF. Quantifications of absolute NO_2 absorption reported in the 1993 AQCD and the 2008 ISA (U.S. EPA, 2008) (U.S. EPA, 1993) are briefly discussed below for thoroughness.

4.2.2.1 Mechanisms of Absorption of Nitrogen Dioxide

At the time of the 1993 AQCD (U.S. EPA, 1993), it was thought that inhaled NO_2 probably reacted with the water molecules in the ELF to form nitrous acid (HNO_2) and nitric acid (HNO_3). However, some limited data suggested that the absorption of NO_2 was linked to reactive substrates in the ELF and subsequent nitrite (NO_2^-) production. By the time of the 2008 ISA (U.S. EPA, 2008), chemical reactions between NO_2 and ELF constituents were more readily recognized as governing NO_2 absorption in the respiratory tract.

4.2.2.1.1 Reaction with Epithelial Lining Fluid Water

Previous studies have demonstrated that it is not NO_2 but instead the NO_2 dimer, dinitrogen tetroxide (N_2O_4), that reacts with water to yield NO_2^- and nitrate [NO_3^- (Finlayson-Pitts et al., 2003; Schwartz and White, 1983; England and Corcoran, 1974)]. However, in aqueous solutions, NO_2 rapidly reacts with many solutes (e.g., ascorbate and urate), particularly those that are easily oxidized. Furthermore, at environmentally relevant concentrations of NO_2 (e.g., around 100 ppb), the direct reactions of NO_2 with dissolved substrates also become important because, at equilibrium, there is very little N_2O_4 compared to NO_2. For example, using the delta Gibbs energies of formation of gaseous NO_2 and N_2O_4 (Chase, 1998), one can calculate that at equilibrium, when the concentration of NO_2 is 1,000 and 100 ppb, there are 1.48×10^5 and 1.48×10^6, respectively, molecules of NO_2 for each molecule of N_2O_4. Thus, at environmental exposure levels there are approximately 1.5 million NO_2 molecules for each N_2O_4 molecule. At these concentrations, it is far more likely for NO_2 (compared to N_2O_4) to penetrate into the aqueous milieu of the ELF. Ensuing reactions of NO_2 with dissolved reactive substrates also become more likely than reaction with a second NO_2 molecule (to form N_2O_4). During reactive uptake by pure water, all reactions occur via N_2O_4 regardless of the concentration of NO_2. However, in the presence of dissolved reactive substrates and at low, environmentally relevant concentrations of NO_2, this process becomes unlikely, and reactive uptake instead occurs via direct reactions of NO_2. The latter conditions resemble reactive uptake of NO_2 by the ELF that would entail direct reactions of NO_2 with, for example, dissolved small molecular weight antioxidants like glutathione (GSH), ascorbate, or urate.

Enami et al. (2009) revisited the discussions regarding NO_2 reaction with water versus ELF solutes. Because the authors postulate that NO_2 effects are largely due to nitrate formation and acidification via proton production, this issue warrants some discussion. The claim by Enami et al. (2009) that "antioxidants catalyze the hydrolytic decomposition of NO_2…but are not consumed in the process" is problematic in view of the vast existing environmental health literature that regards NO_2 as an oxidant gas (Pryor et al., 2006; Augusto et al., 2002; Ford et al., 2002; Kirsch et al., 2002; Wardman, 1998; Postlethwait et al., 1995; Huie, 1994; Neta et al., 1988; Finlayson-Pitts et al., 1987; Kikugawa and Kogi, 1987; Prütz et al., 1985; Pryor and Lightsey, 1981). However, Enami et al. (2009) measured nitrate without measuring nitrite and therefore their data do not strongly support their contention, except to suggest that some hydrolysis of NO_2 may be occurring because nitrate was detected. Nitrite data are important because any excess nitrite found (reaction with water generally yields a 1:1 ratio of nitrite and nitrate; thus, a yield of nitrite above 1 would be considered in excess) would indicate that it is the main

product formed as a result of one-electron oxidations by NO_2. Thus, by not measuring nitrite, an important index to assess oxidation by NO_2 was missed.

Note that Enami et al. (2009) conducted their experiments in the absence of oxygen, which makes their model of dubious applicability to the lung. At environmentally relevant concentrations and physiologic temperatures, intra-pulmonary gas phase NO_2 will exist in its monomeric form. Furthermore, in the presence of aqueous-phase reactive substrates, nitrite, but little or no nitrate, is formed during controlled in vitro exposures. Thus, broad reactivity of NO_2 with a diversity of reactive substrates (solutes) within the ELF facilitates chemical interactions with antioxidants, lipids, and proteins/peptides/amino acids.

4.2.2.1.2 Governing Determinants of Nitrogen Dioxide Absorption within the Respiratory Tract

The absorption of inhaled NO_2 into the ELF is governed by a process termed "reactive absorption" that involves dissolution followed by chemical reaction with reactive substrates in the ELF (Postlethwait and Bidani, 1990), as well as reactions within the interfacial region. Due to the limited aqueous solubility of NO_2 and thus the rapid saturation of the aqueous phase interfacial thin film (Bidani and Postlethwait, 1998), the net flux of NO_2 into reactant-free water is constrained by the relatively slow direct reaction of NO_2 with water (see above) compared with its radical reactions with biological substrates (further discussion below). Thus, rapid reactions with ELF substrates provide the net driving force for NO_2 mass transfer from the intra-pulmonary gas phase into the ELF (Bidani and Postlethwait, 1998; Postlethwait and Bidani, 1994; Postlethwait et al., 1991a; Postlethwait and Bidani, 1990). Concentrations of "free" solute NO_2 are likely negligible due to reaction-mediated removal. Empirical evidence suggests that acute NO_2 uptake in the lower respiratory tract is rate governed by chemical reactions of NO_2 with ELF constituents rather than solely by gas solubility in the ELF, wherein the reaction between NO_2 and water does not significantly contribute to the absorption of inhaled NO_2 (Postlethwait and Bidani, 1994, 1990). Absorption was also observed to increase with increasing temperature, an indication of chemical reaction rather than aqueous solubility, where solubility increases with temperature decrements (Postlethwait and Bidani, 1990). Postlethwait et al. (1991b) proposed that inhaled NO_2 ($\leq$10,000 ppb) did not penetrate the ELF to reach underlying sites and suggested that cytotoxicity likely was initiated by products formed during NO_2 reactions with ELF constituents. Subsequently, the reactive absorption of NO_2 was examined in a number of studies that sought to identify the substrates that predominantly drive NO_2 reactive absorption and to quantify the mass transfer kinetics of NO_2 in the respiratory tract. Uptake was observed to be first-order with respect to NO_2 at concentrations less than

10,000 ppb, to be aqueous substrate dependent, and to be saturable, meaning that the absolute amount of NO_2 uptake would reach a maximum value even if reactive substrate concentrations were in significant excess (Postlethwait et al., 1991a, b).

The absorption of inhaled NO_2 is thought to be coupled with either radical-mediated hydrogen abstraction to form HNO_2 (Postlethwait and Bidani, 1994, 1989) or electron transfer from ELF anionic species that directly reduces NO_2 to nitrite (Adgent et al., 2012). Both mechanisms produce an organic radical from the initial ELF substrate. At physiologic pH, any formed HNO_2 subsequently dissociates to hydrogen ion (H^+) and nitrite. The concentration of the resulting nitrite is likely insufficient to alter physiological function because basal nitrite levels may not change appreciably in either the respiratory tract or the circulation due to ambient NO_2 exposure. This is, in part, because nitrite will diffuse into the underlying epithelial cells and vascular space where, in the presence of red blood cells, it is oxidized to nitrate [(Postlethwait and Bidani, 1989; Postlethwait and Mustafa, 1981) see also Section 4.2.2.4]. Consequently, by default, effects are probably attributable to the organic radical secondary oxidants formed (Adgent et al., 2012; Velsor et al., 2003; Velsor and Postlethwait, 1997) and/or to the proton load, although the ELF buffering capacity is anticipated to compensate for environmentally relevant exposure-related proton generation.

Postlethwait et al. (1995) sought to determine the preferential absorption substrates for NO_2 in the ELF lavaged from male Sprague-Dawley rats. Because bronchoalveolar lavage (BAL) fluid collected from rats may be diluted up to 100 times relative to the native ELF (the dilution will be procedure specific), the effect of concentrating the BAL fluid on NO_2 absorption was also investigated. A linear association was found between the first-order rate constant for NO_2 absorption and the relative concentration of the BAL fluid constituents. This suggested that concentration of the reactive substrates in the ELF determines, in part, the rate of NO_2 absorption. The absorption due to specific ELF constituents was also examined in chemically pure solutions. Albumin, reduced cysteine, glutathione, ascorbate, and urate were the hydrophilic moieties found to be the most active substrates for NO_2 absorption. Unsaturated fatty acids (such as oleic, linoleic, and linolenic) were also identified as active absorption substrates and thought to account for up to 20% of NO_2 absorption. Vitamins A and E exhibited the greatest reactivity of the substrates that were examined. However, the low concentrations of urate (the ELF of rodents and some primates contains significantly less urate than the ELF of humans due to differences in nitrogenous waste metabolism) and vitamins A and E were thought to preclude them from being appreciable substrates in vivo. The authors concluded that ascorbate and glutathione were the primary NO_2 absorption substrates in rat ELF. Postlethwait et al. (1995) also found that the pulmonary surfactant component, dipalmitoyl phosphatidylcholine (DPPC), was relatively unreactive towards NO_2, and

subsequent studies documented that compressed monomolecular interfacial films of DPPC inhibit NO_2 absorption in vitro (Connor et al., 2001). Documenting whether surface active phospholipids (which comprise surfactant) inhibit NO_2 mass transfer in vivo is extremely challenging because any in situ manipulations that disrupt the surface tension-lowering actions of a surfactant lead to a plethora of pathophysiologic sequelae. However, even though such potentially important influences on NO_2 mass transfer have not been verified in vivo, modeling studies could estimate how such effects would influence the intra-pulmonary distribution of inhaled NO_2, local mass transfer rates, and thus dosimetry.

4.2.2.1.3 Reaction/Diffusion of Nitrogen Dioxide in the Epithelial Lining Fluid, Potential for Penetration to Underlying Cells

Because the uptake of NO_2 from inhaled air into the ELF is governed by reactive absorption, it may be postulated that rapid ELF reactions prevent NO_2 from reaching underlying respiratory tract tissues. To evaluate this supposition, consideration must be given to the time required for NO_2 to diffuse through some thickness of the ELF versus the rate of NO_2 reactions with substrates in that ELF.

The ELF varies in composition and thickness with distal progression into the lung. The ELF of most of the tracheobronchial region may generally be described as consisting of two layers: an upper mucus layer and a periciliary layer, which surrounds the cilia (Button et al., 2012; Widdicombe, 2002; Widdicombe and Widdicombe, 1995; Van As, 1977). The length of human cilia is about 7 µm in the trachea and bronchi and around 5 µm in the bronchioles (Song et al., 2009; Clary-Meinesz et al., 1997; Widdicombe and Widdicombe, 1995). In the healthy lung, the thickness of the periciliary layer is roughly the length of the cilia (Song et al., 2009; Widdicombe and Widdicombe, 1995). This periciliary layer forms a continuous liquid lining over the tracheobronchial airways; whereas the upper mucus layer is discontinuous and diminishes or is absent in smaller bronchioles (Widdicombe, 2002; Van As, 1977). The periciliary layer may be the only ELF layer (i.e., there is little to no overlaying mucus) in the ciliated airways of infants and healthy adults who are unaffected by disease, infection, etc. (Bhaskar et al., 1985).

The ELF covering the alveolar surface is considerably thinner than the periciliary layer found in the tracheobronchial region. The alveolar ELF consists of two layers: an upper surfactant layer and a subphase fluid (Ng et al., 2004). Bastacky et al. (1995) conducted a low-temperature scanning electron microscopy analysis of rapidly frozen samples (9 animals; 9,339 measurements) of rat lungs inflated to approximately 80% total lung capacity. The alveolar ELF was found to be continuous, but of varied depth. Three distinct ELF areas were described: (1) a thin layer [0.1 µm median depth, geometric

standard deviation (GSD) ~2.16][1] over relatively flat areas and comprising 80% of the alveolar surface, (2) a slightly thinner layer (0.08 µm, GSD ~1.79) over protruding features and accounting for 10% of the surface, and (3) a thick layer (0.66 µm, GSD ~2.18) occurring at alveolar junctions and accounting for 10% of the surface. Based on these distributions of thicknesses, 10% of the alveolar region is covered by an ELF layer of 0.04 µm or less. Presuming that these depths would also occur in humans at 80% total lung capacity and assuming isotropic expansion and contraction, depths should be expected to be 20−40% greater during normal tidal breathing (rest and light exercise) when the lung is inflated to between 50−60% total lung capacity averaged across the respiratory cycle. During tidal breathing, a median ELF depth of 0.12−0.14 µm would be expected over 80% of the alveolar surface with 10% of the alveolar surface having a median depth of around 0.05 µm or less. Considering the entire distribution of depths during tidal breathing, about 30, 60, and 90% of the alveolar surface would be estimated to have a lining layer thickness of less than or equal to 0.1, 0.2, and 0.5 µm, respectively.

The root mean square distance (d) that NO_2 can diffuse in some time (t) is given by the Einstein-Smoluchowski equation:

$$d = \sqrt{2Dt}$$

Equation 4-1

where D is the molecular diffusion coefficient of NO_2. A D value for NO_2 in water at 25°C of 1.4×10^{-9} m^2/sec has been reported and will be used in the calculations (Ford et al., 2002). In the lung, the D for NO_2 would be increased by temperature and decreased by the higher viscosity of the ELF compared to water. The time available for diffusion can be estimated based on the half-time for reactions between NO_2 and reactive substrates, assuming pseudo first-order kinetics apply. This half-time (τ) has the form:

[1] Although the authors stated that the distributions appeared to be log-normal, they did not report the (GSD) for the three distinct areas they described. The GSD values were calculated from 25, 50, and 75th percentiles of the distributions.

$$\tau = \frac{\ln(2)}{\sum_i^n k_i c_i}$$

Equation 4-2

where $\sum_i^n k_i c_i$ is the summation of the products of the second-order rate constants (k_i) and substrate concentrations (c_i) for the primary reactive substances in the ELF.

Substituting τ for *t* in Equation 4-1 yields:

$$d = \sqrt{\frac{2D \ln(2)}{\sum_i^n k_i c_i}}$$

Equation 4-3

and approximates the distance NO_2 may diffuse before it chemically reacts with ELF constituent molecules (e.g., antioxidants, proteins, lipids, etc.). A similar approach of comparing the half-time in Equation 4-2 to the time for diffusion through the ELF or other phase boundaries such as a membrane bilayer (see Equation 4-1 and solve for *t*) was originally applied by Pryor (1992) and later by Ford et al. (2002).

In considering the classes of ELF biomolecules that react with NO_2, one may focus on the water-soluble small molecular weight antioxidants (e.g., ascorbate, urate, and glutathione), which exist in the ELF in high concentrations and are very reactive toward NO_2 and consequently have large $k_i c_i$ terms. Lipids, on the other hand, would not be expected to decrease considerably the transit time of NO_2 because only those lipids containing fatty acids with two or more double bonds have significant reactivity towards NO_2, and the lipids in the ELF are highly saturated.

The reaction rate constants of 3.5×10^7 $M^{-1}sec^{-1}$, 2×10^7 $M^{-1}sec^{-1}$, and 2×10^7 $M^{-1}sec^{-1}$ were assumed for the small molecular weight antioxidants ascorbate, urate, and glutathione, respectively (Ford et al., 2002). These rates were determined in solution using the pulse radiolysis fast kinetics technique. The kinetics of ascorbate and urate were directly monitored, while in the case of glutathione, $ABTS^{2-}$ [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] was used to produce the intense chromophore $ABTS^{\bullet-}$ (note, here and elsewhere the superscript $^\bullet$ designates a radical species) from its reaction with the glutathiyl radical.

Species and anatomical loci must be considered when selecting appropriate concentrations of reactive ELF biomolecules. Table 4-1 illustrates the small molecular weight antioxidant composition differences between human and rat bronchoalveolar ELF and the differences between human nasal and bronchoalveolar ELF (Squadrito et al., 2010; Van Der Vliet et al., 1999). Predicted by Equation 4-3 and shown in Table 4-1, NO_2 is predicted to penetrate 0.2 to 0.6 μm into the ELF and would not likely reach airway tissues in the bronchi or bronchioles. Even extending the time for diffusion to 5τ, NO_2 would only be predicted to penetrate 0.5 to 1.3 μm into the ELF, which does not approach the 5 μm depth expected in the ciliated airways. However, minimal NO_2 diffusion through the ELF in the bronchi and bronchioles does not preclude the potential importance of reaction products reaching the underlying tissues in these regions.

Table 4-1 Small molecular weight antioxidantconcentrations in epithelial lining fluid and predicted penetration distances for nitrogen dioxide.

Species—Site	Ascorbate	Urate	Glutathione	$\sum_{i}^{n} k_i c_i$ (sec^{-1})	ELF Penetration Distance μm
Substrate Concentration, c_i (μM)					
Human—nasal	28 ± 19	225 ± 105	<0.5	5.5×10^3	0.6
Human—bronchoalveolar	40 ± 18	207 ± 167	109 ± 64	7.7×10^3	0.5
Rat—bronchoalveolar	1,004 ± 325	81 ± 27	43 ± 15	3.8×10^4	0.2
Rate constant, k_i ($M^{-1}sec^{-1}$)					
	3.5×10^7	2×10^7	2×10^7		

Substrate concentrations from Van Der Vliet et al. (1999) for human and from Squadrito et al. (2010) for rat. Reaction rate constants from Ford et al. (2002).

In the alveolar region, the thickness of the ELF is sufficiently thin (≤0.2 μm over 60% of the alveolar surface) for NO_2 to diffuse through. There are some important differences between the ELF of the alveolar region and the ELF of the tracheobronchial airways. In studies modeling NO_2 and ozone (O_3) uptake, a first-order rate constant has been assumed for the alveolar ELF, which is 60-times slower than that of the tracheobronchial ELF (Miller et al., 1985; 1982). The slower reaction rate in the alveolar ELF would increase the estimated potential diffusion distance to nearly 4 μm, well beyond the depth of the alveolar ELF. Additionally, the presence of DPPC , a principle component of pulmonary surfactant, has been shown in vitro to reduce the uptake of NO_2 and O_3 by inhibiting their ability to reach and react with the underlying subphase fluid containing

ascorbate, glutathione, and uric acid (Connor et al., 2004; Connor et al., 2001). The physical properties of the interfacial saturated phospholipids may act to reduce the diffusivity of NO_2. Both the DPPC and the overall slower reaction rate in the alveolar ELF would increase diffusive resistance and increase the back diffusion of NO_2 from the surfactant into the gas phase. Nonetheless, the time for NO_2 diffusion through a 0.2-µm alveolar ELF is over two orders of magnitude faster than the NO_2 reaction rate half-time in the alveolar ELF. Thus, of the inhaled NO_2 reaching the alveolar region and diffusing into the ELF, an appreciable amount of NO_2 may reasonably be expected to diffuse through the ELF to reach underlying tissues over much of the alveolar surface. Reaction rates in these underlying tissues are expected to exceed those in the alveolar and tracheobronchial ELF and would more rapidly consume NO_2 (Pryor, 1992; Miller et al., 1985).

4.2.2.2 Epithelial Lining Fluid Interactions with Nitrogen Dioxide

Small molecular weight antioxidants vary appreciably across species. For example, due to the lack of urate oxidase, humans, primates, and select other species have increased levels of urate. Conversely, rodent concentrations of urate are small compared to humans. Such differences need to be recognized when considering preferential reactive absorption substrates and the profile of products formed via reaction with NO_2. Glutathione and ascorbate are the primary NO_2-absorption substrates in rat ELF with near 1:1 stoichiometric yields of NO_2 uptake: nitrite formation, suggesting that one-electron reduction of NO_2 is a predominant reaction pathway that also yields the corresponding organic radical (Postlethwait et al., 1995).

Beyond cell-specific differential susceptibility and the airway lumen concentration of NO_2, site-specific injury was proposed to depend on rate of bioactive reaction product formation relative to the extent of quenching (detoxification) of these products within the ELF. Velsor and Postlethwait (1997) investigated the mechanisms of acute cellular injury from NO_2 exposure. In an in vitro test system using red blood cells, the maximal levels of membrane oxidation were observed at low antioxidant levels versus null (absent antioxidants) or high antioxidant levels. Glutathione- and ascorbate-related membrane oxidation was superoxide- and hydrogen peroxide-dependent, respectively. The authors proposed that increased absorption of NO_2 occurred at the higher antioxidant concentrations, but little secondary oxidation of the membrane occurred because the reactive species (e.g., superoxide and hydrogen peroxide) generated during absorption were quenched. A lower rate of NO_2 absorption occurred at the low antioxidant concentrations, but oxidants were not quenched and so were available to interact with the cell membrane. Further in vitro analyses also suggested that exposure-related responses

may not be strictly linear with respect to the inhaled NO_2 dose (concentration and/or time) because the dependence of NO_2 absorption and biologic target oxidation demonstrated a bell-shaped function with respect to the initial antioxidant concentration (Adgent et al., 2012; Velsor et al., 2003). Because the ELF varies throughout the respiratory tract, the heterogeneous distribution of epithelial injury observed following NO_2 exposures may be explained, in part, by the ELF-dependent effects on local NO_2 uptake and product formation. However, it should be noted that while these dose-response relationships have been documented in vitro, in vivo validation has not yet been accomplished due to the complexities in reproducibly modulating in situ ELF compositions. Importantly, such in vitro results are difficult to directly extrapolate to the in vivo situation, as precise rates of NO_2 uptake, and thus product formation, are a function of many factors including gas-phase NO_2 concentration, aqueous substrate concentrations, surface area, gas flow, and pH of the ELF (Adgent et al., 2012; Bidani and Postlethwait, 1998). However, an in vivo study of healthy male albino mice (5 weeks old) suggested that a low dose of ascorbate (25 mg/kg) may exacerbate inflammatory responses in terminal bronchial tissues following NO_2 exposure (20,000 ppb; 4 h/day, 10 days); whereas at a higher dose of ascorbate (100 mg/kg), NO_2-exposed mice tissues were similar to tissues from filter air-exposed controls (Zhang et al., 2010b). These in vivo responses seem parallel to those observed in vitro.

Antioxidant levels also vary spatially between lung regions and temporally with NO_2 exposure. While in vitro studies have clearly illustrated the role of antioxidants in mediating NO_2 uptake and membrane oxidation, the temporal dynamics of biological responses to NO_2 that occur in vivo are far more complex. Given the rapid reactions of inhaled NO_2 with various biological substrates, the short half-life of some primary and secondary reaction products as well as the continuous turnover of the ELF, specific chemical species do not likely persist at any given anatomic locale for any appreciable time. Kelly et al. (1996a) examined the effect of a 4-hour NO_2 (2,000 ppb) exposure on antioxidant levels in bronchial lavage (BL) fluid and BAL fluid of 44 healthy nonsmoking adults (19−45 years, median 24 years). The baseline concentrations of urate and ascorbate were strongly correlated between the BL fluid and BAL fluid within individuals ($r = 0.88$, $p < 0.001$; $r = 0.78$, $p = 0.001$; respectively); whereas the concentrations of glutathione in the BL fluid and BAL fluid were not correlated. At 1.5 hours after the NO_2 exposure, urate and ascorbate were significantly reduced in both lavage fractions, while glutathione levels were significantly increased but only in BL fluid. By 6 hours post-exposure, ascorbate levels had returned to baseline in both lavage fractions, but urate had become significantly increased in both lavage fractions and glutathione levels remained elevated in BL fluid. By 24 hours post-exposure, all antioxidant levels had returned to baseline. The levels of glutathione in BAL fluid did not change from baseline at any time point in response to NO_2 exposure.

The depletion of urate and ascorbate, but not glutathione, has also been observed with ex vivo exposure of human BAL fluid to NO_2. Kelly et al. (1996b) collected BAL fluid from male lung cancer patients (n = 16) and exposed the BAL fluid ex vivo at 37°C to NO_2 (50 to 2,000 ppb; 4 hours) or O_3 (50 to 1,000 ppb; 4 hours). Kelly and Tetley (1997) also collected BAL fluid from lung cancer patients (n = 12; 54 ± 16 years) and exposed the BAL fluid ex vivo to NO_2 (50 to 1,000 ppb; 4 hours). Both studies found that NO_2 depletes urate and ascorbate, but not glutathione, from BAL fluid. Kelly et al. (1996b) noted a differential consumption of the antioxidants, with urate loss being greater than that of ascorbate, which was lost at a much greater rate than glutathione. Kelly and Tetley (1997) found that the rates of urate and ascorbate consumption were correlated with their initial concentrations in the BAL fluid, such that higher initial antioxidant concentrations were associated with a greater rate of antioxidant depletion. Illustrating the complex interaction of antioxidants, these studies also suggest that glutathione oxidized by NO_2 may be again reduced by urate and/or ascorbate.

Human and animal results stemming from samples obtained after exposure should be viewed with appropriate caution. As detailed below, secondary reactions within the ELF, sample handling and, importantly, the temporal sequence of exposure relative to sample acquisition may all confound data interpretation. Because the ELF is a dynamic compartment, samples obtained after exposure (>30 minutes) may not reflect biochemical conditions that were present during exposure. This is a critical point, as while there is some value in quantifying the net short-term effects on ELF composition due to exposure, the biological consequences of exposure are largely a function of the ELF conditions during exposure, which initiate a cascade of events leading to alterations in cell signaling, cell injury, inflammation, and so forth. Thus, measurements of ELF components should be interpreted in the context of ELF turnover time, clearance of "stable" reaction products, and species generated/regenerated as a consequence of secondary redox reactions. Reported measurements may reflect net effects on individual antioxidants but lend limited insights into the initial reactions of NO_2 within the ELF, and by extension, into what bioactive products may be formed and how differences in ELF constituent profiles govern biological outcomes. A clear example is evident in the work of Ford et al. (2002), who characterized the reaction of the GSH radical ($GS^{\bullet}$) with urate (UH_2^-) at a pH (6.0) slightly below the recognized ELF pH (~6.8 to 7.0). NO_2 more readily reacts with glutathione than urate, producing $GS^{\bullet}$ and NO_2^-. However, the subsequent reaction $GS^{\bullet} + UH_2^- \rightarrow GSH + UH^{\bullet -}$ has a rate constant of $\sim 3 \times 10^7$ M^{-1} sec^{-1}, which could translate to an initial NO_2 reaction with glutathione followed by reduction of the thiyl radical by urate. This could result in an apparent, but potentially inaccurate, conclusion of direct loss of urate during subsequent analyses. In addition, some reports have suggested observations that include significant levels of the ascorbate oxidation product dehydroascorbate (DHA). As with the example of secondary urate oxidation, such

observations need to be evaluated with caution as the half-life of DHA under biological conditions is very short (minutes; the ascorbyl radical dismutation produces reduced ascorbate and DHA; and DHA spontaneously decomposes to its keto acid). Furthermore, because high redox couples are maintained in the ELF, and the ELF is constantly turning over due to secretion and mucociliary clearance, it is unlikely that any appreciable accumulation of DHA would occur. Therefore, care must be taken to avoid introducing methodological artifacts (e.g., ascorbate oxidation during sample acquisition, handling, and/or storage) that could significantly confound data interpretation. Consequently, an understanding of the precise and preferential substrates is needed to discern the genesis of species differences and the products formed that account for NO_2 exposure-related cellular perturbations.

Thus, variability in antioxidant concentrations and reactions among species may affect NO_2 dose and health outcomes. Guinea pigs and mice have a lower basal activity of glutathione transferase and glutathione peroxidase and lower α-tocopherol levels in the lung compared to rats (Ichinose et al., 1988; Sagai et al., 1987). Human nasal lavage fluid has a high proportion of urate and low levels of ascorbate; whereas mice, rats, or guinea pigs have high levels of ascorbate and undetectable levels of urate. Glutathione is not detected in the nasal lavage fluid of most of these species, except monkeys. Guinea pigs and rats have a higher antioxidant to protein ratio in nasal lavage fluid and BAL fluid than humans (Hatch, 1992). The BAL fluid profile differs from that of the nasal lavage fluid. Humans have a higher proportion of glutathione and less ascorbate in their BAL fluid compared to guinea pigs and rats (Slade et al., 1993; Hatch, 1992). Rats have the highest antioxidant-to-protein mass ratio in their BAL fluid (Slade et al., 1993). Antioxidant defenses also vary with age (Servais et al., 2005) and exposure history (Duan et al., 1996). In the case of another reactive gas, O_3, some studies have found that differences in antioxidant levels among species and lung regions did not appear to be the primary factor affecting O_3-induced tissue injury (Duan et al., 1996; 1993). However, a close correlation between site-specific O_3 dose, the degree of epithelial injury, and depletion of reduced glutathione was observed in monkeys (Plopper et al., 1998). For both NO_2 and O_3, differences in reactive substrates among species and regions of the respiratory tract are recognized, but the importance of these differences in relation to tissue injury is not fully understood.

4.2.2.3 Regional and Total Respiratory Absorption of Nitrogen Dioxide

Very limited work related to the quantification of NO_2 uptake has been published since the 1993 AQCD (U.S. EPA, 1993) or the subsequent 2008 ISA (U.S. EPA, 2008). Consequently, only an abbreviated discussion of this is included.

4.2.2.3.1 Experimental Studies of Nitrogen Dioxide Uptake

Upper Respiratory Tract Absorption

The nasal uptake of NO_2 has been experimentally measured in dogs, rabbits, and rats under conditions of unidirectional flow. Yokoyama (1968) reported 42.1 ± 14.9% (mean ± SD) uptake of NO_2 in the isolated nasal passages of two dogs (3.5 L/min) and three rabbits (0.75 L/min) exposed to 4,000 and 41,000 ppb NO_2. Uptake did not appear to depend on the exposure concentration and was relatively constant over a 10- to 15-minute period. Cavanagh and Morris (1987) measured 28 and 25% uptake of NO_2 (40,400 ppb) in the noses of four naive and four previously exposed rats (0.10 L/min; 4-hours; 40,400 ppb), respectively, and uptake was constant over the 24-minute period during which it was monitored.

Kleinman and Mautz (1991) measured the penetration of NO_2 through the upper airways during inhalation in six tracheostomized dogs exposed to 1,000 or 5,000 ppb NO_2. Uptake in the nasal passages was significantly greater at 1,000 ppb than at 5,000 ppb, although the magnitude of this difference was not reported. The mean uptake of NO_2 (1,000 ppb) in the nasal passages decreased from 80 to 70% as the ventilation rate increased from about 3 to 7 L/min. During oral breathing, uptake was not dependent on concentration. The mean oral uptake of NO_2 (1,000 and 5,000 ppb) decreased from 60 to 30% as the ventilation rate increased from 3 to 7 L/min. Although nasal uptake tended to be greater than oral uptake, the difference was not statistically significant. The tendency for greater nasal than oral uptake on NO_2 is consistent with that observed for O_3 as described in Chapter 5 of the 2013 ISA for Ozone and Related Photochemical Oxidants (U.S. EPA, 2013).

Overall, NO_2 fractional absorption (uptake efficiency) in the upper respiratory tract is greater in the nasal passage than in the oral passage and decreases with increasing ventilation rates. As a result, a greater proportion of inhaled NO_2 is delivered to the lower respiratory tract at higher ventilation rates associated with exercise. In humans, exercise causes a shift in the breathing pattern from nasal to oronasal relative to rest. Because the nasal passages scrub gas-phase NO_2 more efficiently than the mouth and uptake

efficiency decreases with increasing flow, exercise delivers a disproportionately greater quantity of the inhaled mass to the lower respiratory tract, where the NO_2 is readily absorbed.

Additionally, children tend to have a greater oral breathing contribution than adults at rest and during exercise (Bennett et al., 2008; Becquemin et al., 1999). Chadha et al. (1987) found that the majority (11 of 12) of patients with asthma or allergic rhinitis also breathe oronasally at rest. Thus, compared to healthy adults, children and individuals with asthma might be expected to have greater NO_2 penetration into the lower respiratory tract. Furthermore, normalized to body mass, median daily ventilation rates (m^3/kg per day) decrease over the course of life (Brochu et al., 2011). This decrease in ventilation relative to body mass is rapid and nearly linear from infancy through early adulthood. Relative to normal-weight adults (25−45 years of age), ventilation rates normalized to body mass are increased 1.5-times in normal-weight children (7−10 years of age) and doubled in normal weight infants (0.22−0.5 years of age). Relative to their body mass, children respire greater amounts of air and associated pollutants than adults and have a greater portion of respired pollutants reaching the lower respiratory tract than adults.

Lower Respiratory Tract Absorption

Postlethwait and Mustafa (1989) investigated the effect of exposure concentration and breathing frequency on the uptake of NO_2 in isolated perfused rat lungs. To evaluate the effect of exposure concentration, the lungs were exposed to NO_2 (4,000 to 20,000 ppb) while ventilated at 50 breaths/min with a tidal volume (V_T) of 2.0 mL. To examine the effect of breathing frequency, the lungs were exposed to NO_2 (5,000 ppb) while ventilated at 30−90 breaths/min with a V_T of 1.5 mL. All exposures were for 90 minutes. The uptake of NO_2 ranged from 59 to 72% with an average of 65% and was not affected by exposure concentration or breathing frequency. A combined regression analysis showed a linear relationship between NO_2 dose to the lungs and total inhaled dose. Illustrating variability in NO_2 uptake measurements, Postlethwait and Mustafa (1989) observed 59% NO_2 uptake in lungs ventilated at 30 breaths/min with a V_T of 1.5 mL; whereas Postlethwait and Mustafa (1981) measured 35% NO_2 uptake for the same breathing condition. In another study, 73% uptake of NO_2 was reported for rat lungs ventilated at 50 breaths/min with a V_T of 2.3 mL (Postlethwait et al., 1992). It should be noted that typical breathing frequencies are around 80, 100, and 160 breaths/min for rats during sleep, rest, and light exercise, respectively (de Winter-Sorkina and Cassee, 2002). Hence, the breathing frequencies at which NO_2 uptake has been measured are lower than for rats breathing normally. Furthermore, one must consider the potential impacts of the methods used to measure NO_2 uptake (mass balance; wet chemical versus automated analyzer which may or may not include a dilution component due to the sampling rate)

and the lack of perfusion of the bronchial circulation in isolated rat lungs (Postlethwait et al., 1990). In addition to measuring uptake in the upper respiratory tract, Kleinman and Mautz (1991) also measured NO_2 uptake in the lower respiratory tract of tracheostomized dogs. In general, there was about 90% NO_2 uptake in the lung that was independent of ventilation rates from 3 to 16 L/min.

Total Respiratory Tract Absorption

Bauer et al. (1986) measured the uptake of NO_2 (300 ppb) in 15 adults with asthma exposed for 30 minutes (20 minutes at rest, then 10 minutes exercising on a bicycle ergometer) via a mouthpiece during rest and exercise. There was a statistically significant increase in uptake from 72% during rest to 87% during exercise. The minute ventilation also increased from 8.1 L/min during rest to 30.4 L/min during exercise. Hence, exercise increased the NO_2 dose rate by 4.5-times in these subjects. In an earlier study by Wagner (1970), seven healthy adults inhaled a NO_2/NO mixture containing 290 to 7,200 ppb NO_2 for brief (but unspecified) periods. The average NO_2 uptake during 4,100 ppb and 7,200 ppb exposures was 82% during normal respiration (V_T, 0.4 L) and 92% during maximal respiration (V_T, 2 to 4 L). Kleinman and Mautz (1991) also measured the total respiratory tract uptake of NO_2 (5,000 ppb) in nontracheostomized female beagle dogs standing at rest or exercising on a treadmill. The dogs breathed through a small face mask. Total respiratory tract uptake of NO_2 was 78% during rest and increased to 94% during exercise. This increase in uptake may, in large part, be due to the increase in V_T from 0.18 L during rest to 0.27 L during exercise. Coupled with an increase in minute ventilation from 3.8 L/min during rest to 10.5 L/min during exercise, the dose rate of NO_2 was 3.3-times greater for the dogs during exercise than rest.

4.2.2.3.2 Dosimetry Models of Nitrogen Dioxide Uptake

Few theoretical studies have investigated NO_2 dosimetry. The original seminal dosimetry model of Miller et al. (1982) were developed before much of the above information regarding NO_2 reaction/diffusion within the ELF had been obtained. In this model, there was a strong distinction between uptake and dose. Uptake referred to the amount of NO_2 being removed from gas phase per lung surface area ($\mu g/cm^2$); whereas dose referred to the amount of NO_2 per lung surface area ($\mu g/cm^2$) that diffused through the ELF and reached the underlying tissues.

Miller et al. (1982), and subsequently Overton (1984), did not attempt to predict the amount of reactants in the ELF or the transport of reaction products to the tissues. They assumed that reactions of NO_2 with constituents in the ELF were protective in that these reactions reduced the flux of NO_2 to the tissues. Others have postulated that NO_2 reaction

products formed in the ELF, rather than NO_2 itself, could mediate responses (Velsor and Postlethwait, 1997; Postlethwait and Bidani, 1994; Overton, 1984). Overall, these modeling studies predict that the net NO_2 uptake (NO_2 flux to air-liquid interface) is relatively constant from the trachea to the terminal bronchioles and then rapidly decreases in the pulmonary region. The pattern of net NO_2 uptake rate is expected to be similar among species and unaffected by age in humans. However, the NO_2 uptake per unit surface area may be several times higher in infants compared to adults, because children under age 5 have a much smaller surface area in the extrathoracic (nasal) and alveolar regions (Sarangapani et al., 2003).

The predicted tissue dose and dose rate of NO_2 (NO_2 flux to liquid-tissue interface) are low in the trachea, increase to a maximum in the terminal bronchioles and the first generation of the pulmonary region, and then decrease rapidly with distal progression. The site of maximal NO_2 tissue dose is predicted to be fairly similar among species, ranging from the first generation of respiratory bronchioles in humans to the alveolar ducts in rats. However, estimates of NO_2 penetration in Table 4-1 showed that NO_2 is not expected to go deeper than 0.2 to 0.6 μm into the ELF of the ciliated airways before reacting with substrates. The production of toxic NO_2 reaction products in the ELF and the movement of the reaction products to the tissues have not been modeled.

Contrary to what in vitro studies have shown (Velsor and Postlethwait, 1997), modeling studies have generally considered NO_2 reactions in the ELF to be protective. The complex interactions among antioxidants, spatial differences in antioxidants across respiratory tract regions, temporal changes in ELF constituent levels in response to NO_2 exposure, and species differences in antioxidant defenses need to be considered in the next generation of dosimetric models. Current NO_2 dosimetry models are inadequate to put response data collected from animals and humans on a comparative footing with each other and with exposure conditions in epidemiologic studies. Total dose or liquid dose of NO_2 could be used as a first approximation for inter-species dosimetric comparisons using currently available NO_2 models.

As stated above, the total dose or uptake (μg per cm^2 surface area) of NO_2 is predicted to be relatively constant across the tracheobronchial airways with a rapid decrease in dose with progression into the gas exchange region (Miller et al., 1982). The model used by Miller et al. (1982) for NO_2 was generally the same as that subsequently used by Miller et al. (1988) for O_3. Miller et al. (1988) predicted that the total dose of O_3 is relatively similar among several mammalian species (namely, the rabbit, guinea pig, rat, and human). The total dose of NO_2 would also be expected to be relatively similar among these mammalian species. Although it may not be strictly appropriate to apply identical reaction rates for each of these species, varying the reaction rate from zero to that of O_3

increased the predicted total dose of NO_2 by less than 5 times in the trachea and bronchi. This is small relative to the 400-time decrease in total dose from the first generation of respiratory bronchioles to the alveolar sacs (Miller et al., 1982).

Asgharian et al. (2011) recently developed a model for soluble and reactive gas uptake that applied many of the basic concepts described by Miller et al. (1985). Unlike Miller et al. (1985), who separately considered liquid and tissue layers, Asgharian et al. (2011) lumped the liquid layer lining the airways and the tissue layer together with the same diffusion and reaction rates. The model predicted that formaldehyde could penetrate to a maximum of 200 µm tissue in the trachea during inhalation before being removed by reactions. Because predictions were for a single breath, it is possible that deeper tissues may be reached during continuous breathing. Applying the model to experimental O_3 data, Asgharian et al. (2011) estimated a first-order reaction rate of 10^5 sec^{-1} (i.e., half-time of only 7 µsec). By comparison, the rate of 0.018 sec^{-1} (i.e., half-time of 39 sec) was used for formaldehyde. Lumping the liquid and tissue layers may be appropriate for the relatively slow-reacting formaldehyde, but it is perhaps less so for O_3 and NO_2, which are expected to be removed by reactions within the liquid layer of ciliated airways (see Table 4-1). For the rapidly reacting gases O_3 and NO_2, a distinction between liquid and tissue compartments may be mechanistically important to discern whether the gas itself or its reaction products are associated with health outcomes.

Existing dosimetric models can predict the total dose per surface area of distinct areas of the lungs (e.g., individual generations of the tracheobronchial airways and alveolar region). This total dose appears to be very similar among several mammalian species. Similarly, the site of maximal NO_2 tissue dose, near the beginning of the gas exchange region, is also predicted to be fairly similar among species. However, differences in potential NO_2 reactive substrates and reaction products among species have not been considered in modeling efforts. Thus, despite the predicted similarities in total NO_2 dose and site of maximal tissue dose, there is uncertainty related to inter-species differences in concentrations of reactive substrates and reaction products formed within the ELF and tissues. The importance of specific reaction products in mediating health effects in different species is similarly unclear. With regard to humans, individuals with asthma are more likely to experience health effects from ambient NO_2 exposures than healthy individuals (Section 7.3.1). Specific aspects of asthma pathology that may affect NO_2 uptake and disposition and that may be included in dosimetric models have not been identified. Furthermore, most models have focused on the lungs and have not considered the inter-species differences in the dose to nasal passages nor the potential importance of neural or other pathways in affecting health outcomes. Although total dose in the tracheobronchial airways and tissue dose in the alveolar region can be predicted,

modeling efforts do not sufficiently link these endpoints to subsequent downstream events.

4.2.2.4 Endogenous Generation, Metabolism, Distribution, and Elimination of Nitrogen Dioxide

Along with carbon monoxide (CO), NO_2 is a criteria pollutant believed to be produced endogenously in the lung. Evidence in support of a claim for endogenously produced O_3 [e.g., Babior et al. (2003) has received serious criticism (Pryor et al., 2006; Kettle et al., 2004; Sies, 2004; Smith, 2004) and is here considered controversial. A useful discussion of the issues can be found in Drahl (2009).

This endogenous production and function may have important implications for the interpretation of health effects studies. NO_2 may be produced endogenously by various processes, including the acidification of nitrite ($2\ H^+ + 2\ NO_2^- \rightarrow 2\ HNO_2 \rightarrow H_2O + N_2O_3 \rightarrow NO + NO_2 + H_2O$) (as can transpire in phagolysosomes), the decomposition of peroxynitrite and/or the nitrosoperoxylcarbonate anion ($ONOO^- + CO_2 \rightarrow ONOOCO_2^- \rightarrow CO_3^{\bullet -} + NO_2$), and the action of peroxidases when using nitrite and H_2O_2 as substrates. Nitrated proteins form when tyrosine residues are first oxidized to a tyrosyl radical intermediately followed by radical-radical addition of NO_2 to produce 3-nitrotyrosine. NO_2 is the terminal nitrating agent, and the presence of nitrated proteins provides solid evidence for the endogenous production of NO_2 per se. Endogenous NO_2 is expected to increase with dietary consumption of nitrite and nitrate (which occurs in substantial concentrations in some leafy vegetables, e.g., spinach) as well as during immune responses and inflammation. There is no known antioxidant enzymatic process for the decomposition of NO_2, but this is probably due to the spontaneous reactions that NO_2 undergoes with small molecular weight antioxidants, such as glutathione and ascorbate, which result in formation of nitrite and antioxidant radicals. These reactions are so fast that they only allow NO_2 to diffuse small distances in the submicrometer range before reacting (see above, Ford et al., 2002). NO_2 is slightly hydrophobic (Squadrito and Postlethwait, 2009) and faces no significant physical barriers to prevent it from readily traversing biological membranes. But in light of its high reactivity, NO_2 is unlikely to become systemically distributed, and therefore, its endogenous steady-state levels in distant tissues are unlikely to be affected, for example, by inhaled NO_2.

With regard to the lung, understanding the balance between endogenous products and those derived from inhaled ambient NO_2 is a complex and challenging issue. Because inhaled NO_2 predominantly undergoes univalent reduction to nitrite during reactive

absorption, changes in nitrite concentrations can be used as a surrogate for initial considerations of how inhaled NO_2 compares with that produced endogenously. As an example, rat lung ELF contains low µM to nM levels of nitrite, with nitrate being substantially more prevalent. Due to salivary and gut microflora nitrate reductase activity and to reactions of nitrite, especially with heme proteins which yield nitrate, there is a constant cyclic flux between nitrite and nitrate, with nitrate being the primary excretion product in urine. In a rat with numerous simplifying conditions, assuming a gas phase concentration of 200 ppb NO_2, a minute ventilation of 150 mL/min, an exposure time of 4 hours, quantitative conversion of NO_2 to nitrite, 100% uptake efficiency, an ELF volume of 150 µL, and no ELF clearance [even though nitrite has been shown to diffuse out of the ELF quickly (Postlethwait and Bidani, 1989)], there would be a net accumulation of approximately 0.3 µmol of nitrite. If the NO_2-derived nitrite were evenly distributed throughout the ELF pool, this would equate to an additional 2 mM concentration of nitrite. However, in vitro studies using isolated lungs have not reported increases of this magnitude consequent to 10,000−20,000 ppb NO_2 exposures, well above ambient concentrations, demonstrating that the ELF is a dynamic compartment and that small molecular weight reaction products (though charged) move readily from the respiratory tract surface to the vascular space.

Both nitrite and nitrate levels are very diet dependent, and diet represents the primary source for both ions. Although environmental exposures at current ambient NO_2 concentrations would likely have a minimal effect on the overall balance of nitrite and nitrate outside the respiratory tract, how inhaled NO_2 compares with endogenous production rates or amounts within the respiratory tract remains essentially unknown. However, the uptake of inhaled NO_2 may potentially increase levels of nitrite and/or other reaction products beyond levels endogenously occurring in the respiratory tract.

4.2.2.5 Metabolism, Distribution, and Elimination of Products Derived from Inhaled Nitrogen Dioxide

As stated earlier, NO_2 absorption may generate some HNO_2, which subsequently dissociates to H^+ and nitrite. Nitrite enters the underlying epithelial cells and subsequently the blood. In the presence of red blood cells and/or heme proteins, nitrite is oxidized to nitrate (Postlethwait and Mustafa, 1981). Nitrate is the primary stable oxide of nitrogen product and it is subsequently excreted in the urine. There has been concern that inhaled NO_2 may lead to the production of N-nitrosamines, many of which are carcinogenic, because NO_2 can produce nitrite and nitrate (in blood). Nitrate can be converted to nitrite by bacterial reduction in saliva, the gastrointestinal tract, and the urinary bladder. Nitrite has been found to react with secondary amines to form

N-nitrosamines. This remains speculative because nitrosamines are not detected in tissues of animals exposed by inhalation to NO_2 unless precursors to nitrosamines and/or inhibitors of nitrosamine metabolism are coadministered. Rubenchik et al. (1995) could not detect N-nitrosodimethylamine (NDMA) in tissues of mice exposed to 4,000 to 4,500 ppb NO_2 for 1 hour. However, NDMA was found in tissues when mice were simultaneously given oral doses of amidopyrine and 4-methylpyrazole, an inhibitor of NDMA metabolism. Nevertheless, endogenous NO_2 production and the cyclic inter-conversion of nitrite and nitrate may provide the precursors that drive nitrosamine formation. However, because ambient NO_2 contributes only modest amounts of nitrite/nitrate relative to dietary intake, any substantial contribution to systemic nitrosamine formation is not likely. Thus, the relative importance of inhaled NO_2 in endogenous N-nitrosamine formation has yet to be demonstrated. Metabolism of inhaled NO_2 may also, in some cases, transform other chemicals potentially present in the body into mutagens and carcinogens. Van Stee et al. (1983) reported N-nitrosomorpholine (NMOR) production in mice gavaged with 1 g of morpholine/kg body weight per day and then exposed (5−6 hours daily for 5 days) to 16,500−20,500 ppb NO_2. NMOR is a nitrosamine, which is a potent animal carcinogen. The single site containing the greatest amount of NMOR was the gastrointestinal tract, as would be expected due to the pH-dependent facilitation of N-nitrosation chemistry. Later, Van Stee et al. (1995) exposed mice to approximately 20,000 ppb $^{15}NO_2$ and 1 g/kg morpholine simultaneously. N-nitrosomorpholine was found in the body of the exposed mice. Of the NMOR in the body, 98.4% was labeled with ^{15}N which was derived from the inhaled $^{15}NO_2$, and 1.6% was derived presumably from endogenous sources. Inhaled NO_2 may also be involved in the production of mutagenic (and carcinogenic) nitroderivatives of other coexposed compounds, such as polycyclic aromatic hydrocarbon(s) (PAHs), via nitration reactions. Miyanishi et al. (1996) coexposed rats, mice, guinea pigs, and hamsters to 20,000 ppb NO_2 and various PAHs (pyrene, fluoranthene, fluorene, anthracene, or chrysene). Nitro derivatives of these PAHs, which were found to be highly mutagenic in the Ames/*S. typhimurium* assay, were excreted in the urine of these animals. Specifically, the nitrated metabolites of pyrene (1-nitro-6/8-hydroxypyrene and 1-nitro-3-hydroxypyrene) was detected in the urine. Further studies indicated that these metabolites are nitrated by an ionic reaction in vivo after the hydroxylation of pyrene in the liver.

4.2.3 Dosimetry of Nitric Oxide

NO occurs within the respiratory tract gas phase due to the following: (1) inhalation of ambient NO and (2) off-gassing from its endogenous production within pulmonary tissues, airspace surface inflammatory cells, and blood. The net uptake of NO within the

gas exchange regions depends on the balance between the intra-pulmonary gas phase concentration (discussed below) and the inhaled ambient concentration.

While NO exists as a radical species, it is much less reactive than many other radical species. However, it selectively participates in radical-radical reactions such as with superoxide radical anions [$O_2^{\bullet -}$, which produces peroxynitrite ($ONOO^-$)], thiyl radicals [e.g., cysteine ($Cys^{\bullet}$), glutathione ($GS^{\bullet}$), which produce S-nitrosothiols (RSNO)], and organic peroxyl radicals (Madej et al., 2008; Goldstein et al., 2004). In addition, NO reacts with heme-containing proteins such as hemoglobin (Pacher et al., 2007). Although the radical-based reactions generally occur at near diffusion-controlled rates, the prevalence of non-NO radical species at any given time is low. Thus, in terms of the overall uptake and tissue diffusion of NO within the lung, interception due to reactions is not expected to consume appreciable amounts of the total NO involved in mass transfer from the alveolar to the vascular space. Inhaled NO uptake occurs against the background of endogenous NO production, which is derived primarily from the catalytic activities of the several isoforms of nitric oxide synthase [NOS (Förstermann and Sessa, 2012)]. Estimates of nitrite and/or nitrate stemming from NO production via NOS suggest that endogenous NO production, even during inflammatory states, is at best modest compared to dietary intake, although, under specific conditions, plasma levels have been shown to transiently increase due to nondietary, endogenous biological activities. Additional endogenously generated NO may also occur from the acidification of nitrite in the presence of electron donors, such as within phagolysosomes, by dissociation of RSNO and by complex interactions within red blood cells that likely lead to the release of NO (Weitzberg et al., 2010). In combination, these processes result in the appearance of NO within the intra-pulmonary gas phase, which can be measured in expired breath and is routinely labeled as either “eNO” or expressed as the fractional amount of expired gas “FeNO.”

Reported eNO concentrations from the lower respiratory tract span a broad range (~5 to >300 ppb), with nasal/sinus concentrations generally accepted as being greater than what is measured from the lower respiratory tract [e.g., See and Christiani (2013), Alexanderson et al. (2012), Gelb et al. (2012), Noda et al. (2012), Taylor (2012), Bautista et al. (2011), Linhares et al. (2011), and Olin et al. (1998)]. eNO has been reported to be affected by a variety of factors including disease state, diet, sex (or height), species, smoking history, environmental exposures, and so forth. Although eNO from the lower respiratory tract is increased by asthma, this is not the case for nasal NO (ATS/ERS, 2005).

For the general U.S. population, results of the 2007−2011 National Health and Nutrition Examination Survey show a geometric mean eNO of 9.7 ppb in children (n = 1,855;

6−11 years of age; 10% with current asthma) and 13.3 ppb in teenagers and adults [n = 11,420; 12−80 years of age; 8% with current asthma (See and Christiani, 2013)]. In healthy, never-smokers [558 males (M), 573 females (F); 25−75 years of age], Olin et al. (2007) reported a geometric mean eNO of 16.6 ppb (95% reference interval, 6 to 47 ppb). The eNO levels increased with age and height of the individuals, but did not depend on sex. In healthy children (23 M, 28 F, 1−5 years of age), a geometric mean eNO of 7 ppb (95% CI: 3, 12) has been reported (van der Heijden et al., 2014). The eNO levels in these children were unrelated to age, height, weight, or sex. These eNO levels correspond to NO output rates of about 40−50 nL/min from the lower respiratory tract of healthy adults and about 20−30 nL/min for healthy children.

Kharitonov et al. (2005) reported nasal NO concentrations of 750 ppb (95% CI: 700, 810) in children [n = 20; 10 ± 3 (SD) years] and 900 ppb (95% CI: 870, 930) in adults (n = 29; 38 ± 11 years). Another study of healthy adults (n = 10; 18−35 years of age) found a nasal NO concentration of 670 ppb. Higher NO concentrations (9,100 ± 3,800 ppb; n = 5) have been reported for the paranasal sinuses of healthy adults (Lundberg et al., 1995). Asthma and current rhinitis do not appear to affect nasal NO concentrations (Alexanderson et al., 2012; Kharitonov et al., 2005). Nasal NO is reduced by exercise (ATS/ERS, 2005). The nasal NO concentrations described above correspond to NO output rates of about 300 nL/min for the nasal airways of adults with or without asthma and 230 nL/min for children with or without asthma. Nasal NO output rates of healthy primates are in the range of 200 to 450 nL/min (ATS/ERS, 2005). With a NO output of 730 nL/min, a large contribution to nasal NO appears to derive from the paranasal sinuses. Based on these NO output rates, the nasal passages may contribute, on average, roughly 15−20 ppb NO to the lower respiratory tract during rest.

The other primary approach to noninvasive assessment of the respiratory tract surface is expired breath condensate (EBC), which captures aerosolized materials contained in exhaled air, including those directly related to reactive nitrogen chemistry (e.g., nitrite, nitrate, 3-nitrotyrosine). Unfortunately, this relatively new field of analyzing exhaled constituents has encountered numerous situations where concentrations of eNO and EBC constituents are unrelated (Rava et al., 2012; Dressel et al., 2010; Malinovschi et al., 2009; Cardinale et al., 2007; Vints et al., 2005; Chambers and Ayres, 2001; Olin et al., 2001; Zetterquist et al., 1999; Olin et al., 1998; Jilma et al., 1996). Given the endogenous production of NO and the lack of a correlation between the two measurements, neither eNO nor EBC can be employed as a metric of exposure history with any significant degree of specificity for inhaled ambient NO.

The absorption of inhaled NO proceeds similarly to oxygen and carbon monoxide. In a study of seven healthy adults, Wagner (1970) observed an average NO (5,000 ppb)

uptake of 88% during normal respiration (V_T, 0.4 L) and 92% during maximal respiration (V_T, 2 to 4 L). Because blood acts as a near "infinite" sink for NO, it has been proposed as an alternative to CO for measuring pulmonary diffusing capacity [e.g., Chakraborty et al. (2004) and Heller et al. (2004)]. NO absorption follows Henry's law for dissolution into the aqueous phase, followed by diffusion into the vascular space where it interacts with red blood cell hemoglobin to ultimately form nitrate. Thus, due to its chemical conversion, NO net flux from alveolar gas phase to the blood occurs when the alveolar concentration exceeds that found in tissue and/or blood. Mass transfer resistances may be encountered (Borland et al., 2010; Chakraborty et al., 2004), but their combined effects are likely small due to the low (ppb) concentrations of NO.

The formation of RSNO within the ELF may contribute to the overall epithelial cell uptake via an L-type amino acid transporter [LAT (Torok et al., 2012; Brahmajothi et al., 2010)]. An in vitro study by Brahmajothi et al. (2010) showed that pre-incubation of cultured alveolar epithelial cells with L-cysteine increased intracellular RSNO concentrations by 3-times compared with diffusive transport. This increase involved transport via the LAT. LAT transport was further augmented by addition of glutathione and was independent of sodium transport. The authors concluded that NO gas uptake by alveolar epithelium occurred predominantly by forming extracellular S-nitroso-L-cysteine, which was then transported by LAT rather than by diffusion. Subsequently, Torok et al. (2012) also showed that LAT transport exceeded diffusive transport in isolated mice lungs. However, the precise extent of contribution of LAT transport remains unclear because formation of RSNO requires several steps due to the slow direct reactivity of NO with reduced thiols. In vivo, the time for these reactions may exceed the time for diffusion into and through alveolar epithelial cells. Furthermore, because blood acts as a sink for NO (i.e., a near zero boundary condition), lower intracellular concentrations of NO would occur in vivo compared to the nonzero boundary conditions in cell cultures and isolated lungs (Asgharian et al., 2011). While diffusive transport of NO is known and relatively well characterized, the importance of LAT transport in vivo has not been determined.

Ambient NO levels are likely similar to those endogenously occurring within the lung airspaces, except during morning commutes or near major roadways where they may possibly exceed endogenous levels. It is not known whether periods of high ambient NO exposure could alter endogenous NO production within the respiratory tract or pathways affected by endogenous NO. Importantly, it should be noted that in the clinical setting, therapeutic administration is a very different situation wherein >10,000 ppb NO may be administered continuously for prolonged periods.

4.2.4 Summary of Dosimetry

The uptake of inhaled NO_2 in the respiratory tract is governed by "reactive absorption," which involves chemical reactions with antioxidants, unsaturated lipids, and other compounds in the ELF. In vitro studies have clearly illustrated the role of antioxidants in mediating NO_2 uptake. The rapid reactions of NO_2 with tracheobronchial ELF substrates provides a net driving force for NO_2 mass transfer from the gas phase into the ELF. Concentrations of "free" solute NO_2 are likely negligible due to its reaction-mediated removal. Thus, it is not NO_2 itself, but rather its reaction products that are believed to interact with the apical surfaces of the tracheobronchial epithelial. At high substrate concentrations, oxidants/cytotoxic products are at least partially quenched due to secondary antioxidant reactions. At low substrate concentration, ELF-derived oxidants/cytotoxic products have a lower probability of being intercepted by unreacted antioxidants and instead may reach underlying targets.

Within the alveolar region, much of the inhaled NO_2 entering the ELF will diffuse through rapidly enough to avoid reactions and will reach underlying tissue surfaces. A principle component of pulmonary surfactant, DPPC, may partially reduce the uptake of NO_2 by slowing its diffusion and decreasing reaction with substrates in the subphase fluid. Reducing the reactive absorption increases diffusive resistance and back diffusion into the air phase, thereby reducing uptake from the gas phase. Nonetheless, rapid reactions of NO_2 with tissues will maintain a concentration gradient for NO_2 through the alveolar ELF to the underlying tissues.

Exercise, relative to rest, increases the dose rate of NO_2 to the respiratory tract because of greater NO_2 penetration through the extrathoracic airways and a greater intake rate of NO_2. The uptake of NO_2 by the upper respiratory tract decreases with increasing ventilation rates occurring with activity. This causes a greater proportion of inhaled NO_2 to be delivered to the lower respiratory tract. In humans, exercise results in a shift in the breathing pattern from nasal to oronasal relative to rest. Because the nasal passages scrub gas-phase NO_2 more efficiently than the mouth and because uptake efficiency decreases with increasing flow, exercise delivers a disproportionately greater quantity of the inhaled mass to the lower respiratory tract, where the NO_2 is readily absorbed. Experimental studies have shown that exercise increases the dose rate of NO_2 to the respiratory tract by 3- to 5-times compared to resting exposures.

Compared to healthy adults, children and individuals with asthma might be expected to have greater NO_2 penetration into the lower respiratory tract. Children tend to have a greater oral breathing contribution than adults at rest and during exercise. Limited data also suggest that patients with asthma or allergic rhinitis breathe oronasally at rest. Because the nasal passages scrub gas-phase NO_2 more efficiently, a greater quantity of

the inhaled NO_2 may reach the lower respiratory tract of oronasally breathing individuals. The dose rate to the lower airways of children compared to adults is increased further because children breathe at higher minute ventilations relative to their lung volumes.

Current dosimetry models for NO_2 do not adequately consider reactive absorption and secondary reactions that affect the probability of oxidants and/or cytotoxic products reaching target sites. Differences in potential NO_2 reactive substrates and reaction products among species have not been considered in modeling efforts. Although the models predict similar total NO_2 dose in the tracheobronchial airways and sites of maximal NO_2 tissue dose (i.e., near the beginning of the gas exchange region) among several mammalian species, the models do not sufficiently link these NO_2 doses to specific reaction products and downstream events. It is unclear to what extent environmental exposures at current ambient NO_2 concentrations might affect the overall balance of nitrite and nitrate or how ambient NO_2 uptake compares with endogenous production rates/amounts in the respiratory tract. However, the uptake of inhaled NO_2 could increase levels of nitrite and/or other reaction products beyond levels that are endogenously occurring in the respiratory tract.

The uptake of inhaled NO occurs against the background of endogenous NO production in the respiratory tract. In terms of the overall uptake and tissue diffusion of NO within the lung, interception due to reactions is not expected to consume appreciable amounts of the total NO involved in mass transfer from the alveolar to the vascular space. The absorption of inhaled NO proceeds similarly to oxygen and CO. Blood acts as a near "infinite" sink for NO. Absorption of NO follows Henry's law for dissolution into the aqueous phase, and is followed by diffusion into the vascular space, where it interacts with red blood cell hemoglobin to ultimately form nitrate. Ambient NO concentrations are likely similar to those endogenously occurring within the lung airspaces, except during morning commutes or near major roadways, where they may possibly exceed endogenous levels. It is not known whether periods of high ambient NO exposure could alter endogenous NO production within the respiratory tract or pathways affected by endogenous NO.

4.3 Modes of Action for Inhaled Oxides of Nitrogen

4.3.1 Introduction

The purpose of this section is to describe the biological pathways that underlie health effects resulting from short-term and long-term exposures to NO_2 and NO. Extensive research carried out over several decades in humans and in laboratory animals has yielded much information on these pathways. This section will discuss some of the representative studies with particular emphasis on studies published since the 2008 ISA for Oxides of Nitrogen—Health Criteria (U.S. EPA, 2008) and on studies in humans. This information will be used to develop a mode of action framework for inhaled NO_2 and NO.

Mode of action refers to a sequence of key events, endpoints, and outcomes that result in a given toxic effect (U.S. EPA, 2005). Elucidation of mechanism of action provides a more detailed understanding of key events, usually at the molecular level (U.S. EPA, 2005). The framework developed in this chapter will include some mechanistic information on initiating events at the molecular level, but will mainly focus on the effects of NO_2 and NO at the cellular, tissue, and organism level.

NO_2 is a radical species and a highly reactive oxidant gas [(Fukuto et al., 2012) Table 4-2]. It is well appreciated that oxidation and nitration reaction products, which are formed as a result of NO_2 exposure, initiate numerous responses at the cellular, tissue, and whole organ level of the respiratory system. Exposure to NO_2 may also have effects outside the respiratory tract. NO is a radical species and a gas that is more selective in its reactivity than NO_2 [(Fukuto et al., 2012) Table 4-2]. Once inhaled, NO rapidly crosses the alveolar capillary barrier into the vascular compartment and avidly binds to hemoglobin. Subsequent reactions with hemoglobin lead to the generation of circulating nitrate, nitrite, and methemoglobin.

Table 4-2 Chemical properties of nitrogen dioxide (NO_2) and nitric oxide (NO) that contribute to modes of action.

NO_2	NO
Radical species	Radical species
Somewhat hydrophobic	Very hydrophobic
Very reactive	Selectively reactive
Less diffusible	More diffusible
Reactions with unsaturated fatty acids, thiols, and low molecular weight antioxidants	Radical-radical reactions with (1) superoxide to form peroxynitrite (2) thiyl radicals to form RSNO (3) organic peroxyl radicals
Reacts with amino acids, proteins and lipids to form nitrated species	Reacts with heme-containing proteins, transition metals and oxygen
Initiates radical reactions and lipid peroxidation	Quenches radical reactions
Metabolites include nitrite and nitrate	Metabolites include nitrite and nitrate

RSNO = S-nitrosothiols.

Both NO_2 and NO are formed endogenously in cells and tissues (Sections 4.2.2.4 and 4.2.3). Formation of endogenous NO is catalyzed by NOS. Three pathways contribute to the formation of endogenous NO_2: (1) acidification of nitrite, usually occurring in the phagolysosomes; (2) reaction of peroxynitrite with carbonate to form nitrosoperoxylcarbonate anion, which decomposes to carbonate anion and NO_2; and (3) reaction of peroxidases using nitrite and hydrogen peroxide as substrates. These enzymatic and nonenzymatic pathways are increased during immune responses and inflammation, leading to higher endogenous levels of NO and NO_2. Furthermore, dietary consumption of nitrate leads to enhanced levels of NO in the stomach and to enhanced circulating levels of nitrite due to activity of the enterosalivary cycle (Weitzberg and Lundberg, 2013; Lundberg et al., 2011). The contribution of environmentally relevant concentrations of inhaled NO_2 and NO to levels of circulating nitrite and nitrate is thought to be minimal (Section 4.2.2.4). However, inhaled NO_2 may act on the same targets as endogenous NO_2 produced during inflammation in the respiratory tract (Ckless et al., 2011). Because endogenous NO_2 is thought to contribute to the development of lung disease, inhaled NO_2 may further this process.

The following subsections describe the current understanding of biological pathways that may be responsible for the pulmonary and extrapulmonary effects of inhaled NO_2 and NO. For NO_2, this includes the formation of oxidation and nitration reaction products (Section 4.3.2.1), activation of neural reflexes (Section 4.3.2.2), initiation of

inflammation (Section 4.3.2.3), alteration of epithelial barrier function (Section 4.3.2.4), enhancement of bronchial smooth muscle reactivity (Section 4.3.2.5), modification of innate/adaptive immunity (Section 4.3.2.6), and remodeling of airways and alveoli (Section 4.3.2.7). The potential induction of carcinogenesis is also briefly described (Section 4.3.2.8). While NO_2 exposure may result in effects occurring outside of the respiratory tract, biological pathways underlying extrapulmonary effects of NO_2 are not well understood (Section 4.3.2.9). Activation of neural reflexes and release of NO_2 metabolites or vasoactive mediators from the lung to the bloodstream are possibilities. Inhaled NO impacts the pulmonary and systemic vasculature mainly through interaction with heme proteins (Section 4.3.3). Other effects of NO may be due to circulating metabolites, such as nitrite, nitrate, and methemoglobin, to interactions with redox-active transition metals, and to reactions with thiyl and superoxide radicals. Because endogenous NO is an important mediator of cell signaling, inhaled NO has the potential to disrupt cell signaling.

4.3.2 Nitrogen Dioxide

4.3.2.1 Formation of Oxidation and Nitration Reaction Products

The 2008 ISA and the 1993 AQCD (U.S. EPA, 2008) (U.S. EPA, 1993) summarized biochemical effects observed in the respiratory tract after NO_2 exposure. These effects have been attributed to the strong oxidizing potential of NO_2, resulting in the formation of reactive oxygen species (ROS). Key responses include oxidation of membrane polyunsaturated fatty acids, thiol groups, and antioxidants. Chemical alterations of lipids, amino acids, proteins, and enzymes can lead to functional changes in membranes, enzymes, and oxidant/antioxidant status. For example, lipid peroxidation of unsaturated fatty acids in membranes may alter membrane fluidity and permeability. As a result, epithelial barrier functions may be impaired, and phospholipases may be activated leading to the release of arachidonic acid. In addition, oxidation of protein thiols may result in enzyme dysfunction. Further, consumption of low molecular weight antioxidants by NO_2 may result in decreased antioxidant defenses. Effects may occur directly through the action of NO_2 or secondarily due to its reaction products, such as organic radicals, ROS, or reactive nitrogen species (RNS). Later effects may occur due to release of ROS and/or RNS by leukocytes responding to cell damage.

As summarized in the 2008 ISA and the 1993 AQCD (U.S. EPA, 2008) (U.S. EPA, 1993), considerable attention has been paid to the effects of NO_2 on the antioxidant defense system in the ELF and in respiratory tract tissue. Studies in humans and animals

exposed to NO_2 have demonstrated changes in low molecular weight antioxidants such as glutathione, ascorbate, and α-tocopherol, and in the activities of enzymes responsible for glutathione synthesis or maintenance of redox status. For example, a controlled human exposure study found depletion of urate and ascorbate, but not glutathione, in BAL fluid 1.5 hours following a 4-hour exposure to 2,000 ppb NO_2 (Kelly et al., 1996a). While these results may be interpreted as evidence that NO_2 prefers to react with urate or ascorbate over glutathione, an alternative interpretation is that glutathione reacts with NO_2 and that the product of the reaction is reduced by urate or ascorbate. Other studies have found that antioxidant status modulates the effects of NO_2 inhalation. For example, in a controlled human exposure study, supplementation with ascorbate and α-tocopherol decreased the levels of lipid peroxidation products found in BAL fluid following a 3-hour exposure to 4,000 ppb NO_2 (Mohsenin, 1991). Additionally, changes in lung antioxidant enzyme activity have been reported in animals exposed to NO_2 (U.S. EPA, 2008). For example, long-term exposure to NO_2 resulted in decreased glutathione peroxidase activity in weanling mice that were α-tocopherol deficient, while supplementation with α-tocopherol resulted in an increase in glutathione peroxidase activity (Ayaz and Csallany, 1978). Thus, NO_2 inhalation is capable of perturbing glutathione-dependent reactions. These changes may reflect altered cell populations because injury induced by NO_2 exposure may result in the influx of inflammatory cells or the proliferation of resident epithelial or mesenchymal cells. Changes in cell populations due to proliferative repair may also account for the upregulation of Phase II, Phase I, and glycolytic enzymes, which have been observed following NO_2 exposure.

As discussed in Section 4.2.2.1.2, reactive absorption of NO_2 gas occurs by reactions with antioxidants and other components of the ELF. Studies employing in vitro and in vivo systems point to the ability of antioxidants to both react with NO_2 to form reactive intermediates and to quench those reactive intermediate species. NO_2 exposure in the presence of ELF antioxidants resulted in the formation of superoxide and hydrogen peroxide in an in vitro cell system (Velsor and Postlethwait, 1997). In this study, quenching of NO_2-derived secondary oxidants was dependent on antioxidant concentration, with lower concentrations promoting and higher concentrations reducing oxidative injury. A recent in vivo study provided additional support for this mechanism. Supplementation of mice with ascorbate had a biphasic effect, with a lower dose of ascorbate promoting and a higher dose of ascorbate reducing lung injury and inflammation induced by exposure to 20,000 ppb NO_2 (Zhang et al., 2010b). Thus, toxicity resulting from NO_2 exposure may be due to a product derived from the initial ELF substrate and/or to secondary reaction products formed. These reaction products may not be long-lived due to short half-lives and/or continuous turnover of the ELF. Further, quenching of reaction products by ELF antioxidants may limit damage to respiratory epithelium. The heterogeneous distribution of epithelial injury due to reactive

intermediates formed from inhaled NO_2 may reflect ELF-dependent local effects because the ELF is nonuniform in composition and quantity along the respiratory tract. Furthermore, localized endogenous formation of NO_2 in the respiratory tract could potentially overwhelm the antioxidant capacity and contribute to epithelial injury.

Nitrogen-based metabolites and RNS are also formed in the ELF as a result of NO_2 exposure. Nitrite is the primary product of the chemical reactions of NO_2 in the respiratory tract. As discussed in Section 4.2.2.1.2, nitrite formed in the ELF can diffuse into respiratory tract epithelial cells and subsequently into the vascular space. While the effects of nitrite on the epithelial cell are not well known, it is unlikely that nitrite is responsible for the toxicity of NO_2. Interestingly, numerous studies have explored the effects of increased systemic nitrite on various tissues and organs. Nitrite has been found to protect against ischemia-reperfusion injury in the heart and other organs (Weitzberg and Lundberg, 2013). In addition, systemic nitrite administration prevented airway and epithelial injury due to exposure to chlorine gas in rats (Yadav et al., 2011). Further, nitrite is known to have a direct relaxing effect on smooth muscle [(Folinsbee, 1992) see Section 4.3.4.1], suggesting that it may play a role in bronchodilation.

RNS, such as RSNO and nitrated proteins, fatty acids, and lipids, may be formed in the respiratory tract following NO_2 exposure. Evidence for these reaction products is mainly provided by in vitro cell systems and ex vivo systems (Section 4.3.4). However, Matalon et al. (2009) recently demonstrated the nitration of surfactant protein D (SP-D) in mice exposed to 20,000 ppb NO_2 for 4 hours. SP-D nitration was accompanied by protein cross-linking and a decrease in SP-D aggregating activity, which could potentially impact microbial clearance, immune regulation, and surfactant metabolism. In addition to inhibiting protein function, nitration of proteins may induce antigenicity or trigger immune reactions (Daiber and Muenzel, 2012). Further, the presence of nitrated amino acids, such as 3-nitrotyrosine, in cells or tissues is an indicator of endogenous NO_2 and peroxynitrite formation. Other potential RNS formed may have less deleterious effects. For example, nitrated (or nitro) fatty acids have a direct relaxing effect on smooth muscle, perhaps even on airway smooth muscle (Que et al., 2009; Lima et al., 2005). In addition, RSNOs are known to be bronchodilators (Que et al., 2009). Additional discussion of the biological effects of these products of NO_2 metabolism is found in Section 4.3.4.

4.3.2.2 Activation of Neural Reflexes

NO_2 is classified as a pulmonary irritant (Alarie, 1973). Pulmonary irritants stimulate afferent nerve endings in the lung, resulting in increased respiratory rate, decreased V_T,

and subsequent rapid shallow breathing. Sometimes pulmonary irritants also stimulate mild bronchoconstriction, bradycardia, and hypotension (Alarie, 1973). All of these pathways involve the vagus nerve.

In guinea pigs, NO_2 exposure (5,200−13,000 ppb; 2−4 hours) by nose-cone resulted in statistically significant increases in respiratory rate and decreases in V_T (Murphy et al., 1964). These responses were concentration and time dependent and were reversible when animals were returned to clean air. In contrast, no changes in these respiratory parameters were observed with 4-hour exposures to 16,000 and 50,000 ppb NO. In another study, guinea pigs exposed to 7,000−146,000 ppb NO_2 for 1 hour demonstrated a concentration-dependent increase in respiratory rate 10 minutes following exposure and a concentration-dependent decrease in V_T 10 minutes, 2 hours, and 19 hours following exposure (Silbaugh et al., 1981). NO_2 exposure-induced increases in respiratory rate have also been reported in rats (Freeman et al., 1966) and mice (McGrath and Smith, 1984). In mice, statistically significant increases in respiratory rate and decreases in V_T were found in response to an 8-minute exposure to 100,000 ppb NO_2, but not to 15,000 or 50,000 ppb NO_2 (McGrath and Smith, 1984). In this latter study, continuous pre-exposure to 5,000 ppb NO_2 for 3 days lessened the response to 100,000 ppb NO_2, suggesting the development of tolerance or an attenuated response to NO_2 (U.S. EPA, 1993). In rats, continuous exposure to 800 ppb and higher concentrations of NO_2 resulted in elevated respiratory rates throughout life (Freeman et al., 1966). However, no NO_2 exposure-induced increases in respiratory rate in human subjects have been reported. In fact, respiratory rates tended to decrease in humans exposed to 0−480 ppb for 20 minutes (Bylin et al., 1985). The authors proposed that NO_2 in this range of concentrations did not act as a pulmonary irritant in humans.

NO_2 has been shown to elicit a small increase in airway resistance, which is consistent with mild bronchoconstriction, in humans but not in rabbits or guinea pigs [Alarie (1973) and studies cited below]. One study in human subjects at rest found a nonmonotonic response to NO_2 in terms of airway resistance (Bylin et al., 1985). In this study, specific airway resistance was increased after 20 minutes of exposure to 250 ppb NO_2 and was decreased after 20 minutes of exposure to 480 ppb NO_2. The authors suggested that reflex bronchoconstriction occurred at the lower concentration and that other mechanisms counteracted this effect at the higher concentration. Other controlled human exposure studies found no change in airway resistance with acute exposures of 530−1,100 ppb NO_2 and increases in airway resistance with acute exposures above 1,600−2,500 ppb in healthy human subjects (U.S. EPA, 1993). Human subjects with chronic lung disease exposed for 5 minutes to 2,100 ppb NO_2 also exhibited increased airway resistance (von Nieding and Wagner, 1979). In addition, both forced expiratory volume in 1 second (FEV_1) and forced vital capacity were decreased in healthy human subjects exposed to

2,000 ppb NO_2 for 4 hours (Blomberg et al., 1999). These latter changes in pulmonary function are consistent with reflex bronchoconstriction. Because the response was lessened with each successive exposure on 4 consecutive days, the authors suggested the development of tolerance or an attenuated response.

Some evidence points to NO_2 exposure-induced histamine release from mast cells, rather than reflex bronchoconstriction, as the mechanism underlying changes in airway resistance. A study in rats showed that mast cell degranulation occurred after acute exposure to NO_2 [500 ppb for 4 hours; 1,000 ppb for 1 hour (Thomas et al., 1967)]. In addition, a histamine-suppressive agent, but not atropine or β-agonists, blocked NO_2-mediated increases in airway resistance in healthy humans and in humans with chronic lung disease exposed to 5,000−8,000 ppb NO_2 for 5 minutes (von Nieding and Wagner, 1979). Because atropine inhibits vagal responses, these findings indicate that neural reflexes were not involved in NO_2-induced changes in pulmonary function in human subjects. More recent studies in animals have provided experimental evidence for a relationship between lipid peroxidation/oxidative stress and the release of histamine by allergen-activated mast cells (Beaven, 2009; Gushchin et al., 1990). Taken together, these studies suggest that NO_2 exposure may lead to lipid peroxidation, which may promote mast cell-mediated changes in pulmonary function, albeit at high concentrations.

There is some experimental support for NO_2 exposure-induced cardiovascular reflexes. An acute exposure to NO_2 in an occupational setting resulted in tachycardia in one case report (U.S. EPA, 1993; Bates et al., 1971), while rats exposed acutely to 20,000 ppb or higher concentrations of NO_2 exhibited bradycardia (U.S. EPA, 1993; Tsubone et al., 1982). This latter response was abolished by injection of atropine, which inhibits vagal responses. Further, a decreased heart rate, which was not accompanied by an increase in respiratory rate, was observed in mice exposed to 1,200 and 4,000 ppb NO_2 for 1 month (Suzuki et al., 1981). The lack of respiratory rate response suggests that the decreased heart rate was due to a different mechanism than rapid stimulation of irritant receptors by NO_2. Controlled human exposure studies have also examined the effects of NO_2 on heart rate and heart rate variability (Section 5.3.11.1). Older studies and one recent study failed to find statistically significant changes in heart rate at ambient-relevant concentrations of NO_2. A recent controlled human exposure study involving a 1-hour exposure to 400 ppb NO_2 failed to find an effect on heart rate variability in subjects with coronary heart disease (Scaife et al., 2012). However, a second recent controlled human exposure study reported an effect on heart rate variability, which is a measure of autonomic tone, resulting from a 2-hour exposure to 500 ppb NO_2 (Huang et al., 2012). Altered heart rate variability found in epidemiologic studies (Section 5.3.11.1) is consistent with a possible effect of NO_2 exposure on autonomic tone.

In summary, NO_2 is a pulmonary irritant that may activate reflexes through vagal pathways to increase respiratory rate, decrease V_T, stimulate reflex bronchoconstriction, and induce bradycardia. Responses are rapid, concentration dependent, and variable among species. Evidence that reflex responses occur in humans is weak because no increases in respiratory rate have been reported as a result of NO_2 exposure. Further, modest increases in airway resistance in human subjects exposed to NO_2 were not blocked by atropine, which inhibits vagal responses. Findings attributed to reflex bronchoconstriction in humans may be due to alternative pathways such as mast cell degranulation. The recent demonstration that NO_2 exposure (500 ppb; 2 hours) results in altered heart rate variability suggests the possible activation of a neural reflex in humans. However, the clearest evidence for reflex responses mediated by the vagus nerve involved exposures of experimental animals to NO_2 at concentrations of at least 5,000 ppb.

4.3.2.3 Initiation of Inflammation

As summarized in the 2008 ISA and the 1993 AQCD (U.S. EPA, 2008) (U.S. EPA, 1993), NO_2 exposure-induced membrane perturbations resulted in the release of arachidonic acid and the formation of eicosanoid products (Sections 5.2.2.5 and 5.2.7). Animal toxicological and controlled human exposure studies have found increases in concentrations of eicosanoids in BAL fluid immediately following exposure to NO_2 (Jörres et al., 1995; Schlesinger et al., 1990). Eicosanoids play an important role in the recruitment of neutrophils. Interestingly, higher concentrations and longer durations of exposure to NO_2 employed in these studies resulted in inhibited eicosanoid production (Robison and Forman, 1993; Schlesinger et al., 1990).

Recently, acute exposure of mice to 10,000 ppb and higher concentrations of NO_2 was shown to activate the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) in airway epithelium (Ather et al., 2010; Bevelander et al., 2007). NFκB activation resulted in the production of pro-inflammatory cytokines. Inflammation and acute lung injury in this model were found to be dependent on an active NFκB pathway. Controlled human exposure studies demonstrated increased levels of cytokines IL-6 and IL-8 in BL fluid following NO_2 exposure. IL-8 levels were increased at 1.5 and 16 hours following a 4-hour exposure to 2,000 ppb NO_2, while levels of IL-6 were increased at 16 hours following the exposure [Section 5.2.7 (U.S. EPA, 2008) (Devlin et al., 1999; Blomberg et al., 1997)]. The cell signaling pathways responsible for upregulating cytokines at this lower level of exposure to NO_2 are not clear.

Airway inflammation often occurs following NO_2 exposure. Studies in rodents exposed acutely (1 hour to 3 days) to NO_2 (500−30,000 ppb) have demonstrated airway inflammation mainly consisting of neutrophils and macrophages, and sometimes of mast cells and lymphocytes, by histological technique or sampling of BAL fluid [as summarized in Sandstrom et al. (1990) (Poynter et al., 2006; Pagani et al., 1994)]. Numerous studies in healthy human subjects exposed to NO_2 have documented airway inflammation in endobronchial biopsy tissue and in sputum, BL fluid, and BAL fluid. Many of these studies were conducted while subjects were exercising intermittently and exposed to 1,500−4,000 ppb NO_2 for a few hours. Neutrophilia was a prominent feature (U.S. EPA, 2008) (Frampton et al., 2002; Devlin et al., 1999; Azadniv et al., 1998; Blomberg et al., 1997). In addition, other types of inflammatory cells, including macrophages, lymphocytes, and mast cells, have been demonstrated (Frampton et al., 2002; Sandström et al., 1991; Sandstrom et al., 1990).

Controlled human exposure studies have also evaluated the effects of repeated NO_2 exposure on airway inflammation in healthy adults. Persistent neutrophilic inflammation, demonstrated by increased numbers of neutrophils and increased levels of myeloperoxidase in the BL fluid, was observed following 4 consecutive days of 4-hour exposure to 2,000 ppb NO_2 (Blomberg et al., 1999). Repeated exposure also led to the upregulation of cytokines characteristic of the T helper cell 2 (Th2) inflammatory response and also intercellular adhesion molecule 1 (ICAM-1) in respiratory epithelium (U.S. EPA, 2008) (Pathmanathan et al., 2003). Upregulation of ICAM-1 suggests a potential mechanism for the persistent neutrophil influx that was observed (Blomberg et al., 1999). A study of repeated exposure to 4,000 ppb (exposure every other day for a total of six exposures) found inflammatory responses that differed from those observed after a single exposure (Sandström et al., 1992). In particular, numbers of mast cells and lymphocytes in the lavage fluid, which were increased following a single exposure, were not increased following repeated exposure. Furthermore, repeated exposure to 1,500 ppb NO_2 (by the same protocol) resulted in smaller numbers of some lymphocyte subpopulations in BAL obtained following exposure compared with numbers in BAL obtained prior to exposure (Sandstrom et al., 1992). In contrast, no changes in lymphocyte subpopulations were reported following repeated exposure to 600 ppb NO_2 (4 exposures over 6 days), with the exception of a slight increase in natural killer cells (Rubinstein et al., 1991).

Recently, a controlled human exposure study investigated the effects of repeated NO_2 exposure on eosinophilic airway inflammation in subjects with atopic asthma (Ezratty et al., 2014). Subjects were exposed to 200 or 600 ppb NO_2 for 30 minutes on the first day and twice for 30 minutes on the second day. Compared with baseline, the number and percentage of eosinophils and the amount of eosinophil cationic protein (ECP) in

sputum were significantly increased after the three exposures to 600, but not 200 ppb NO_2. Furthermore, ECP was highly correlated with eosinophil counts in sputum. No increases in either of these parameters were observed 6 hours after the first exposure to 600 ppb NO_2. While the design of this study did not include an allergen challenge, several other studies examined eosinophilic inflammation and other allergic responses to NO_2 and an allergen. These are discussed in Sections 4.3.2.6.2, 4.3.2.6.3, and 5.2.2.5. Collectively, these studies suggest that exposure to NO_2 may prime eosinophils for subsequent activation by allergens in previously sensitized individuals (Davies et al., 1997; Wang et al., 1995b).

4.3.2.4 Alteration of Epithelial Barrier Function

Lipid peroxidation and altered phospholipid composition in the respiratory tract following NO_2 exposure may affect membrane fluidity and airway epithelial barrier function. NO_2 exposure-induced inflammation may further impair epithelial barrier function. Increases in vascular permeability may occur, leading to the influx of plasma proteins such as albumin into the airway lumen.

As summarized in the 2008 ISA and the 1993 AQCD (U.S. EPA, 2008) (U.S. EPA, 1993), numerous studies have demonstrated increases in biomarkers of increased permeability, such as protein and albumin, as well as biomarkers of cellular injury, such as lactate dehydrogenase (LDH) and shed epithelial cells, in BAL fluid following exposure to NO_2 (Section 5.2.7). Because LDH can be oxidatively inactivated, use of this indicator may underestimate the extent of injury during oxidative stress. Many, but not all, of these effects were observed at NO_2 concentrations that are higher than ambient-relevant levels. Notably, one controlled human exposure study found increased albumin levels in BL fluid following 4 consecutive days of 4-hour exposure to 2,000 ppb NO_2 (Blomberg et al., 1999).

Several studies in experimental animals found that antioxidant deficiency worsened the cellular injury and/or impaired epithelial barrier function following NO_2 exposure. Ascorbate deficiency enhanced protein levels in the BAL fluid of NO_2-exposed guinea pigs, suggesting a role for BAL fluid ascorbate in preventing the deleterious effects of NO_2 (Hatch et al., 1986). Similarly, α-tocopherol deficiency enhanced lipid peroxidation in NO_2-exposed rats (Sevanian et al., 1982). Recently, selenium deficiency was found to enhance the injury response in rats exposed to 1,000−50,000 ppb (acute, subacute, and chronic exposures) NO_2 (de Burbure et al., 2007). Levels of both BAL fluid total protein and serum club cell secretory protein (CC16) were increased in selenium-deficient rats exposed to NO_2. Selenium supplementation diminished this response, which suggests that

the selenium-containing enzyme, glutathione peroxidase, may have played an important mitigating role.

Increases in lung permeability due to high concentrations of NO_2 (100,000 ppb and above) are known to cause death from pulmonary edema (Lehnert et al., 1994; Gray et al., 1954). At lower concentrations, more subtle effects have been reported. Exposure of rats to 5,000 and 10,000 ppb NO_2 for 3 or 25 days resulted in epithelial degeneration and necrosis and in proteinaceous edema (Barth et al., 1995), while exposure to 800−10,000 ppb NO_2 for 1 and 3 days resulted in concentration-dependent increases in BAL fluid protein (Müller et al., 1994). BAL fluid protein was also elevated in guinea pigs exposed for 1 week to 400 ppb NO_2 (Sherwin and Carlson, 1973).

High concentrations of NO_2 (70,000 ppb, 30 minutes) were found to enhance translocation of instilled antigen from the lung to the bloodstream of guinea pigs (Matsumura, 1970). More subtle increases in lung permeability due to NO_2 exposure could enhance the translocation of an antigen to local lymph nodes and subsequently to the circulation (U.S. EPA, 2008) (Gilmour et al., 1996) and/or to the immunocompetent and inflammatory cells underlying the epithelium that are involved in allergic reactions (Jenkins et al., 1999). However, increased lung permeability following exposure to NO_2 does not always lead to allergic sensitization (Alberg et al., 2011). Increased epithelial permeability may alternatively contribute to the activation of neural reflexes and the stimulation of smooth muscle receptors by allowing greater access of an agonist (Dimeo et al., 1981).

Susceptibility to NO_2 exposure-induced lung injury was investigated in several mice strains with differing genetic backgrounds (Kleeberger et al., 1997). Lavageable total protein, a biomarker for increased lung permeability, was variable among mouse strains following a 3-hour exposure to 15,000 ppb NO_2. In addition, repeated exposure to NO_2 (10,000 ppb, 6 h/day, 5 consecutive days) resulted in adaptation of the permeability response in one of the tested strains but not in the other. Although specific genes were not identified, this study provided evidence that genetic components conferred susceptibility to NO_2, at least in terms of lung permeability.

4.3.2.5 Enhancement of Bronchial Smooth Muscle Reactivity

Exposure to NO_2 enhanced the inherent reactivity of airway smooth muscle in human subjects with and without asthma [(Folinsbee, 1992) Section 5.2.2.1] and in animal models (see below). This “airway responsiveness” is defined as the sensitivity of airways to a variety of natural or pharmacological stimuli (O'Byrne et al., 2009). Airway hyperresponsiveness (AHR) is a key feature of asthma, which is a chronic inflammatory

disease of the airways. As summarized in the 2008 ISA (U.S. EPA, 2008) and in Section 5.2.2.1, numerous studies found that human subjects who were exposed to NO_2 were more sensitive to the nonspecific stimuli methacholine than human subjects who were exposed to air. Subjects with asthma exhibited greater sensitivity than subjects without asthma when similarly exposed. In addition, several studies found that NO_2 exposure enhanced airway responsiveness to specific stimuli, such as allergens, in subjects with mild allergic asthma.

Exercise during exposure to NO_2 appeared to modify airway responsiveness in subjects with asthma [(Folinsbee, 1992) Section 5.2.2.1]. Mechanisms by which this occurs are not understood, but two hypotheses have been postulated. First, exercise-induced refractoriness, which has been demonstrated in some subjects with asthma, may alter responsiveness to NO_2 (Magnussen et al., 1986). A second hypothesis is that nitrite formed by reactions of NO_2 in the ELF mediates compensatory relaxation of airway smooth muscle (Folinsbee, 1992). Exercise would increase the total dose of NO_2 to the respiratory tract, thus increasing nitrite formation. Recent studies have shown that RNS have bronchodilatory effects. For example, endogenous RSNOs are an important modulator of airway responsiveness in subjects with asthma and in eosinophilic inflammation (Lee et al., 2011; Que et al., 2009).

Animal toxicological studies have also demonstrated NO_2-induced AHR to nonspecific and specific challenges, as summarized in the 2008 ISA and the 1993 AQCD (U.S. EPA, 2008) (U.S. EPA, 1993) and in Sections 5.2.2.1 and 6.2.2.3. Exposures ranged from acute to subchronic in these studies, and results suggest that more than one mechanism may have contributed to the observed AHR. Acute exposure of guinea pigs to NO_2 (10 minutes, 7,000 ppb and higher) resulted in concentration-dependent AHR to histamine, which was administered immediately after exposure (Silbaugh et al., 1981). This response was short-lived because no enhanced responsiveness was seen at 2 and 19 hours post-exposure to NO_2. The rapidity of the response and the concomitant change in respiratory rate suggest enhanced vagally mediated reflex responses (Section 4.3.2.2) as a possible underlying mechanism. A 7-day exposure to 4,000 ppb NO_2 also induced AHR to histamine in guinea pigs (Kobayashi and Shinozaki, 1990). Eicosanoids were proposed to play a role in this response. In addition, a study in mice sensitized and challenged with ovalbumin found that short-term exposure to NO_2 (25,000 ppb, but not 5,000 ppb; 3 days) resulted in AHR to methacholine (Poynter et al., 2006). This enhanced sensitivity correlated with an increase in numbers of eosinophils, suggesting eosinophilic inflammation as a possible underlying mechanism in this model of allergic airway disease. A subchronic study demonstrated dose-dependent AHR to histamine in NO_2-exposed guinea pigs [1,000−4,000 ppb, 24 h/day, 6−12 weeks (U.S. EPA, 2008) (Kobayashi and Miura, 1995)]. Specific airway resistance in the absence of a challenge

agent was also increased, which indicates the development of airway obstruction. This finding suggests airway remodeling as a possible underlying mechanism for AHR. Another subchronic exposure study (5,000 ppb NO_2, 4 h/day, 5 days/week, 6 weeks) found a delayed bronchial response, which was measured as increased respiratory rate and was suggestive of AHR, in guinea pigs sensitized and challenged with *C. albicans* and exposed to NO_2 (Kitabatake et al., 1995).

Mechanisms underlying the effects of NO_2 on airway responsiveness are not well understood. Effects of NO_2 exposure on redox status in the respiratory tract should be considered because asthma pathogenesis, including airway inflammation, hyperresponsiveness, and remodeling, may be under redox control (Comhair and Erzurum, 2010; Kloek et al., 2010). In support of this mechanism, supplementation with the antioxidant ascorbate was found to prevent nonspecific AHR in subjects with asthma who were exposed to NO_2 (Mohsenin, 1987).

Several different inflammatory pathways may underlie the increased airway responsiveness following NO_2 exposure (Krishna and Holgate, 1999). First, mast cell activation may contribute to NO_2 exposure-induced AHR. As discussed in Section 4.3.2.2, acute exposure to NO_2 led to mast cell activation in rats and possibly in human subjects. Histamine released by mast cells can directly bind to receptors on smooth muscle cells and cause contraction. This response would have the appearance of reflex bronchoconstriction but would not involve neural pathways. Secondly, neutrophilic and eosinophilic inflammation, which have been demonstrated following single and repeated exposures to NO_2 (Section 4.3.2.3), may play a role. Neutrophils and other inflammatory cell types release mediators, such as IL-13, IL-17A, and tumor necrosis factor-α (TNF-α), which can alter the calcium sensitivity of the smooth muscle and enhance a contractile response to a stimulus (Kudo et al., 2013). Eosinophils can release ECP and other mediators involved in allergen-induced asthmatic responses. This pathway may contribute to the enhanced immune response to allergens demonstrated following NO_2 exposure (Section 4.3.2.6.2). Eosinophil release of ECP may also cause damage to the airway epithelium in allergic airway disease (Ohashi et al., 1994). This damage may result in epithelial shedding and mucociliary dysfunction, which may allow greater access of allergens to the airway epithelium and submucosa. In addition, epithelial shedding may lead to greater exposure of sensory nerve endings on nerve fibers and to enhanced activation of neural reflexes and airway smooth muscle contraction (Hesterberg et al., 2009; Cockcroft and Davis, 2006). These processes may explain the close relationship that has been observed between epithelial shedding and AHR (Ohashi et al., 1994). Thus, neutrophilic and/or eosinophilic airway inflammation following NO_2 exposure may contribute to AHR through the release of mediators or by impairing epithelial barrier function (Section 4.3.2.4). Thirdly, chronic airway inflammation may cause structural

changes in the airway walls that enhance the contractile response of the smooth muscle to a given stimuli (Cockcroft and Davis, 2006).

Evidence also supports a role for endogenous NO_2 in mediating AHR. Increased peroxynitrite formation occurs during inflammatory states, resulting from the reaction of NO and superoxide. Peroxynitrite subsequently reacts with CO_2 to form nitrosoperoxylcarbonate anion, which decomposes to carbonate radical and NO_2 (Section 4.2.2.4). Recent studies provided evidence that endogenous peroxynitrite contributes to AHR in animal models of allergic airway disease (Section 4.3.2.6.2). These studies demonstrate that NO metabolism is dysfunctional in inflamed lungs and enhances peroxynitrite formation. Amelioration of the dysfunction resulted in less nitrative stress, airway remodeling and airway responsiveness (Ahmad et al., 2011; Mabalirajan et al., 2010b; Maarsingh et al., 2009; Maarsingh et al., 2008).These studies highlight the possibility that inhaled NO_2 can add to the lung burden of endogenous NO_2, which contributes to AHR and allergic airway disease in animal models (Section 4.3.2.6.2).

4.3.2.6 Modification of Innate/Adaptive Immunity

Host defense depends on effective barrier function and on innate and adaptive immunity (Al-Hegelan et al., 2011). The effects of NO_2 on barrier function in the airways were discussed above (Section 4.3.2.4). This section focuses on the mechanisms by which exposure to NO_2 impacts innate and adaptive immunity. Both tissue damage and foreign pathogens are triggers for the activation of the innate immune system. Innate immune system activation results in the influx of inflammatory cells, such as neutrophils, mast cells, basophils, eosinophils, monocytes, and dendritic cells, and the generation of cytokines, such as TNF-α, IL-1, IL-6, keratinocyte chemoattractant, and IL-17. Further, innate immunity encompasses complement, collectins, and the phagocytic functions of macrophages, neutrophils, and dendritic cells. In addition, airway epithelium contributes to innate immune responses. Innate immunity is highly dependent on cell signaling networks involving toll-like receptor (TLR) 4 in airway epithelium and other cell types. Adaptive immunity provides immunologic memory through the actions of B and T lymphocytes. Important links between the two systems are provided by dendritic cells and antigen presentation.

4.3.2.6.1 Impairment of Host Defenses

As summarized in the 2008 ISA (U.S. EPA, 2008), potential mechanisms by which NO_2 exposure may impair host defenses include ciliary dyskinesis, damage to ciliated epithelial cells, and altered alveolar macrophage function, all of which may contribute to

altered mucociliary transport and/or clearing infectious and noninfectious particles from the lung. Altered alveolar macrophage function and other potential mechanisms, such as increases in pro-inflammatory mediators and cytokines, increased immunoglobulin E (IgE) concentrations, interactions with allergens, and altered lymphocyte subsets, reflect modification of innate and/or adaptive immunity. These changes may underlie susceptibility to infection, which has been observed in animals exposed to NO_2 (Section 5.2.5.1).

Controlled human exposure studies have demonstrated reduced mucociliary activity due to depressed ciliary function, depressed phagocytic activity, and superoxide production in alveolar macrophages, and altered humoral- and cell-mediated immunity following exposure to 1,500−4,000 ppb NO_2 for a few hours [(Frampton et al., 2002; Devlin et al., 1999; Helleday et al., 1995; Sandstrom et al., 1992; Sandström et al., 1992; Sandström et al., 1991) Section 5.2.5]. Studies involving repeated daily exposure to 1,500 ppb NO_2 (but not 600 ppb NO_2) found reductions in lymphocyte subpopulations (Sandstrom et al., 1992; Rubinstein et al., 1991; Sandstrom et al., 1990). Furthermore, repeated daily exposure to 2,000 ppb NO_2 resulted in upregulation of ICAM-1 in bronchial biopsy specimens (Pathmanathan et al., 2003). These findings suggest a potential mechanism underlying susceptibility to viral infection because ICAM-1 is a major receptor for rhino- and respiratory-syncytial viruses. Finally, enhanced susceptibility of airway epithelium to influenza viral infection was suggested in a study involving exposure to 1,000−3,000 ppb NO_2 over 3 days, although statistical significance was not achieved (Goings et al., 1989). Humans exposed to 600 and 1,500 ppb NO_2 for 3 hours exhibited an increased injury response, as measured in bronchial epithelial cells, resulting from influenza and respiratory syncytial virus (Frampton et al., 2002). Epidemiologic evidence for associations between exposure to NO_2 and increased respiratory infections is somewhat inconsistent (Sections 5.2.5.2 and 5.2.5.3).

As summarized in the 2008 ISA (U.S. EPA, 2008) and 1993 AQCD (U.S. EPA, 1993), studies in NO_2-exposed animals (500−10,000 ppb) have demonstrated altered mucociliary clearance and several changes in alveolar macrophages. These changes include morphological evidence of damage to alveolar macrophages (membrane bleb formation and mitochondrial damage), decreased viability, and decreased function [decreased superoxide production, decreased phagocytic capacity, and decreased migration towards a stimulus (Robison et al., 1993; Davis et al., 1992; Rose et al., 1989; Schlesinger et al., 1987; Schlesinger and Gearhart, 1987; Suzuki et al., 1986; Greene and Schneider, 1978; Dowell et al., 1971)]. A recent study involving exposure to 20,000 ppb NO_2 demonstrated nitration of SP-D, a surfactant protein that functions as a collectin (Matalon et al., 2009). This was accompanied by cross-linking and a decrease in SP-D aggregating activity, which could potentially impact the role of SP-D in microbial

clearance and surfactant metabolism. Infectivity models have shown increased mortality and decreased bactericidal activity (U.S. EPA, 2008) (Jakab, 1987; Miller et al., 1987; Ehrlich, 1980; Ehrlich et al., 1977), as a result of NO_2 exposure. Further discussion is found in Sections 5.2.4 and 6.2.9.

4.3.2.6.2 Exacerbation of Allergic Airway Disease

Inhaled allergens activate an acute immune response in allergen-sensitive individuals. This response is characterized by early and late phases. Key players in the early asthmatic response are mast cells and basophils, which release mediators following allergen binding to IgE receptors on their cell surfaces. These mediators include histamine and cysteinyl leukotrienes, which bind airway smooth muscle receptors and induce contraction. Mediators also activate T lymphocyte subsets (i.e., $CD4^+$ T-cells), resulting in the release of Th2 cytokines that can cause airway smooth muscle contraction and recruit mast cells. Th2 cytokines also promote the influx and activation of eosinophils and neutrophils. Airway mucosal eosinophilia is characteristic of asthma and rhinitis. Eosinophils exert their effects via degranulation and/or cytolysis, resulting in release of ECP and other mediators (Erjefält et al., 1999). Th2 cytokines also activate B lymphocytes, resulting in the production of allergen-specific IgE. These responses initiated by Th2 cytokines contribute to the late asthmatic response, which is characterized by airway obstruction generally occurring 3−8 hours following an antigen challenge (Cockcroft and Davis, 2006), and to other responses occurring greater than >8 hours following an antigen challenge.

Exogenous Nitrogen Dioxide

As summarized in the 2008 ISA (U.S. EPA, 2008) and in Section 5.2.2.1, exposure to NO_2 affects the acute immune response to inhaled allergens. Several controlled human exposure studies found that NO_2 exposure enhanced airway responsiveness to specific stimuli, such as house dust mite (HDM) allergen (Jenkins et al., 1999; Tunnicliffe et al., 1994) in subjects with mild allergic asthma. Further, repeated exposure to NO_2 resulted in an enhanced response to a dose of allergen that was asymptomatic when given alone (Strand et al., 1998). Airway responses were measured during the first 2 hours after allergen challenge which falls within the timeline of the early phase asthmatic response. These results provide evidence that NO_2 exposure exacerbates the early phase asthmatic response to allergen challenge, as measured by enhanced contraction of airway smooth muscle cell.

Controlled human exposure studies also demonstrated that NO_2 exposure exacerbated the late phase asthmatic response to allergen challenge in subjects with mild allergic asthma.

Airway obstruction, measured as a spontaneous fall in FEV_1 occurring after resolution of the early asthmatic response (generally 3−8 hours after an antigen challenge), was observed in subjects with asthma exposed to 400 ppb NO_2 for 1 hour (Tunnicliffe et al., 1994) and to 250 ppb NO_2 for 30 minutes for 4 consecutive days (Strand et al., 1998). Other studies measured cell counts and mediators characteristic of the late phase asthmatic response. Increased numbers of neutrophils and increased levels of ECP in BAL fluid and/or BL fluid, both indicators of inflammatory response to allergen challenge, were reported following exposure to 260 ppb NO_2 for 15−30 minutes (Barck et al., 2005; Barck et al., 2002). Furthermore, increased ECP levels were observed in sputum and blood, and an increase in myeloperoxidase (indicator of neutrophil activation) was seen in blood. In subjects with allergic rhinitis, NO_2 exposure (400 ppb for 6 hours) increased eosinophil activation, measured by ECP in nasal lavage, following nasal allergen provocation (Wang et al., 1995a). These studies suggest that exposure to NO_2 may prime eosinophils for subsequent activation by an allergen in previously sensitized individuals (Davies et al., 1997; Wang et al., 1995b). However, another study found decreased sputum eosinophils 6 hours after HDM challenge in subjects with HDM-sensitive allergic asthma exposed to 400 ppb NO_2 for 3 hours (Witten et al., 2005).

Late phase allergic responses were also investigated in animal models of allergic airway disease (see also Section 5.2.2.5). Increased specific immune response to HDM allergen, including enhanced antigen-specific serum IgE, and increased lung inflammation were demonstrated in Brown Norway rats sensitized to and challenged with HDM allergen followed by 3-hour exposure to 5,000 ppb NO_2 (Gilmour et al., 1996). Similarly, a recent study showed that NO_2 exposure (25,000 ppb, 6 h/day for 3 days) increased the degree and duration of the allergic inflammatory response in mice sensitized and challenged with ovalbumin (Poynter et al., 2006). Both neutrophilic and eosinophilic airway inflammation were found in these studies; exposure of mice to a lower concentration of NO_2 (5,000 ppb) failed to induce this response. Two other studies in ovalbumin-sensitized and ovalbumin-challenged mice found decreased eosinophilic inflammation in response to 5,000 ppb NO_2; however, one of these studies found an increase in eosinophils following exposure to 20,000 ppb NO_2 (Hubbard et al., 2002; Proust et al., 2002). This increase in eosinophils was accompanied by increased levels of eosinophil peroxidase, a marker of activation. Both responses were observed 3 days, but not 1 day, after the 3-hour NO_2 exposure. These results in animal models provide some evidence of NO_2-mediated enhancement of late phase allergic responses, albeit at higher concentrations than those considered environmentally relevant or following repeated exposures. It is important to note that eosinophil activation and eosinophil influx reflect different processes and that only the study by Hubbard et al. (2002) measured markers of activation. The ovalbumin-sensitized and ovalbumin-challenged mouse model may not mimic the eosinophil degranulation and/or cytolysis that are characteristic of asthma and allergic

rhinitis in humans (Malm-Erjefält et al., 2001). Hence species-related differences may account for the differences in results of animal and controlled human exposure studies.

Collectively, these studies demonstrate that inhaled NO_2 enhanced both early and late phase responses to inhaled allergens in humans with asthma and allergy. Furthermore, exposure to NO_2 augmented allergic inflammation in some rodent models of allergic airway disease. These results provide evidence for NO_2-induced exacerbation of allergic airway disease in the presence of an allergen challenge. Evidence for NO_2-induced airway eosinophilia in the absence of an allergen challenge was described in Section 4.3.2.3. Hence, NO_2 exposure may lead to asthma exacerbations by multiple pathways.

Endogenous Nitrogen Dioxide

Several recent animal toxicological studies have explored the role of endogenous NO and peroxynitrite, the latter of which decomposes to form NO_2, on allergic airway disease in animal models. In one study, upregulating the enzyme endothelial nitric oxide synthase (eNOS; and presumably NO production) decreased airway inflammation, airway remodeling, and airway responsiveness in a mouse model of asthma (Ahmad et al., 2011). Asthma phenotype-related features, such as cell infiltrates, mucus hypersecretion, peribronchial collagen, and Th2 cytokines, were also diminished. Further, decreased inducible nitric oxide synthase (iNOS) expression and 3-nitrotyrosine immunostaining in airway epithelium were reported, as were diminished epithelial injury and apoptosis. Because 3-nitrotyrosine is a marker of NO_2/peroxynitrite formation, these findings suggest that an increase in NO may have resulted in reduced peroxynitrite. While it is known that NO rapidly reacts with superoxide to form peroxynitrite and that superoxide levels are increased in inflammation, it is also known that an excess of NO will react with peroxynitrite and quench its reactivity. In fact, Stenger et al. (2010) found that high concentrations of inhaled NO (10,000 ppb) prevented the formation of 3-nitrotyrosine in the lungs of neonatal mice exposed to hyperoxia.

In a second set of studies, increased levels of the NOS substrate, L-arginine, were found to decrease airway inflammation and airway responsiveness in a guinea pig model of asthma (Maarsingh et al., 2009). Similarly, increased L-arginine levels reduced peroxynitrite formation and airway responsiveness in a mouse model of asthma (Mabalirajan et al., 2010b). Markers of allergic inflammation, such as eosinophilia and Th2 cytokines, markers of oxidative and nitrative stress, and markers of airway remodeling, such as goblet cell metaplasia and subepithelial fibrosis, were also decreased. Further, increased L-arginine levels reduced mitochondrial dysfunction and airway injury (Mabalirajan et al., 2010a). Limitation of L-arginine is known to uncouple NOS enzyme

activity, resulting in the production of superoxide in addition to NO. This situation is commonly found in disease models and leads to peroxynitrite formation. Increasing L-arginine availability is a common strategy used to prevent enzyme uncoupling and peroxynitrite formation. Another approach was employed in a study by North et al. (2009) where inhibition of the enzyme arginase 1 (arginase 1 decreases arginine availability) was found to decrease airway responsiveness in a mouse model of asthma. Similar findings were reported using arginase inhibition in a guinea pig model of allergic asthma where arginase was upregulated (Maarsingh et al., 2008). Inhibition of arginase resulted in amelioration of the asthma phenotype. These effects were attributed to decreased enzyme uncoupling, thus promoting the formation of NO, diminishing the generation of superoxide, and reducing the formation of peroxynitrite. In contrast, a different study found that arginase inhibition resulted in increased S-nitrosylated and nitrated proteins, increased inflammation, mucous cell metaplasia, NFκB activation, and increased airway responsiveness in a mouse model of asthma (Ckless et al., 2008). However, antigen-specific IgE and IL-4 levels were reduced. Thus, only some features of the asthma phenotype were ameliorated by arginase inhibition. The authors suggested that peroxynitrite, whose presence was indicated by the increase in nitrated proteins in mice treated with arginase, may have contributed to increased airway responsiveness in this model.

Evidence for similar pathways in humans is provided by a study in which endogenous markers of reactive nitrogen and oxygen chemistry were measured in individuals with and without asthma (Anderson et al., 2011). Levels of total nitrite and nitrate were higher in the BAL fluid of subjects with asthma compared to healthy subjects. In subjects with asthma, upregulation of iNOS was observed, and it was greater in distal airways compared with more proximal airways. In addition, levels of dihydroethidium-positive cells, which are capable of producing ROS (such as superoxide), were higher in both the BL fluid and BAL fluid of subjects with asthma compared with healthy subjects. Levels of arginase were also higher in BAL fluid of subjects with asthma compared with healthy subjects. These results suggest that uncoupling of NOS and/or NOS dysfunction, resulting in enhanced peroxynitrite/NO_2 formation, may contribute to the asthma phenotype in human subjects. They also provide biological plausibility for results of another study demonstrating a correlation between increased airway responsiveness and the induction of iNOS, the induction of arginase, and the production of superoxide in subjects with asthma.

Collectively, these studies provide evidence that the balance between endogenous NO and peroxynitrite influences features of the asthma phenotype in animal models of allergic airway disease and possibly in adults with asthma. Enhanced levels of superoxide, which are characteristic of asthma and other inflammatory states, favor the

formation of peroxynitrite at the expense of NO. Evidence from experimental studies indicates that peroxynitrite and other RNS are found in and contribute to allergic airway disease in animal models. Inhaled NO_2 may exacerbate allergic airway disease by adding to the lung burden of RNS in inflammatory states.

4.3.2.6.3 T-helper Cell 2 Skewing and Allergic Sensitization

A controlled human exposure study demonstrated that repeated daily exposures of healthy adults to NO_2 resulted in increased expression of IL-5, IL-10, IL-13, and ICAM-1 in respiratory epithelium following the last exposure [(Pathmanathan et al., 2003) Section 5.2.7]. These interleukins are characteristic of a Th2 inflammatory response. IL-5 is known to promote eosinophilia, while IL-13 is known to promote mucus production and AHR (Bevelander et al., 2007). These findings suggest a potential mechanism whereby repeated exposure to NO_2 may exert a pro-allergic influence. Further, upregulation of ICAM-1 suggests a potential mechanism for leukocyte influx. A separate study by these same investigators found persistent neutrophilic inflammation following the 4 days of repeated exposure (Blomberg et al., 1999).

In addition, two studies in animals examined the effects of longer term exposures to NO_2 on the development of allergic responses (Sections 5.2.7 and 6.2.5.2). In one study, exposure of guinea pigs to 3,000 or 9,000 ppb NO_2 increased the numbers of eosinophils in nasal epithelium and mucosa after 2 weeks (Ohashi et al., 1994). In the other, exposure to 4,000 ppb NO_2 for 12 weeks led to enhanced IgE-mediated release of histamine from mast cells isolated from guinea pigs (Fujimaki and Nohara, 1994). This response was not found in mast cells from rats similarly exposed. Both studies provide further evidence for NO_2 having a pro-allergic influence.

Furthermore, a recent study in mice provides evidence that NO_2 may act as an adjuvant promoting the development of allergic airway disease in response to a subsequent inhalation exposure to ovalbumin (Bevelander et al., 2007). Findings included AHR, mucous cell metaplasia, and eosinophilic inflammation, as well as ovalbumin-specific IgE and IgG1, $CD4^+$ T-cells biased toward Th2, and a T helper cell 17 (Th17) phenotype in the blood. These results are consistent with an allergic asthma phenotype in humans. The eosinophilic inflammation, mucus gene upregulation, and ovalbumin-specific IgE production were found to be dependent on TLR2 and myeloid differentiation primary response gene (88) pathways. TLR2 is known to promote maturation of dendritic cells, inflammation, and Th2 skewing. A subsequent study in the same model found that NO_2 exposure had several effects on pulmonary $CD11c^+$ dendritic cells, including increased cytokine production, upregulation of maturation markers, increased antigen uptake, migration to the lung-draining lymph node, and improved ability to stimulate naive $CD4^+$

T-cells (Hodgkins et al., 2010). Dendritic cells are key players in adaptive immune responses by regulating $CD4^+$-mediated T cell responses through the presentation of antigens in the draining lymph node. Further, dendritic cells can express a distinct pattern of costimulatory molecules and produce cytokines that create an environment for T cell polarization, thus skewing the T helper cell response. Changes reported in these two studies are consistent with the promotion of allergic sensitization and suggest a role for TLR2 in mediating this effect. A third study by these same investigators found that NO_2 exposure resulted in antigen-specific IL-17A generation from Th17 cells, which is characteristic of the severe asthma phenotype that is unresponsive to glucocorticoid treatment in humans (Martin et al., 2013). Although all studies involved 1-hour exposures to high concentrations of NO_2 (10,000−15,000 ppb), they are included here because they describe potentially new mechanisms by which inhaled NO_2 may exert its effects. It should additionally be noted that airway inflammation was seen in mice exposed to 15,000 ppb, but not to 10,000 ppb, NO_2 for 1 hour and that pulmonary damage was minimal in this model (Martin et al., 2013).

In contrast, a similar study failed to find that NO_2 acted as an adjuvant in a mouse model of allergic airway disease (Alberg et al., 2011). The exposure consisted of 5,000 or 25,000 ppb NO_2 for 4 hours and followed exposure to ovalbumin which was administered intranasally. Adjuvant activity was measured as the production of allergen-specific IgE antibodies. Methodological differences in study design with respect to the timing of ovalbumin and NO_2 exposures and the route of ovalbumin exposure may account for differences in findings between this study and others. In fact, Bevelander et al. (2007) found that NO_2 promoted allergic sensitization when exposure occurred prior (but not subsequent) to ovalbumin.

It has been hypothesized that both endogenous and exogenous ROS and/or RNS can alter the balance between tolerance and allergic sensitization due to an inhaled agent (Ckless et al., 2011). Some activities of dendritic cells and T-cells, such as maturation of the antigen presenting capacity of dendritic cells, dendritic cell stimulation of $CD4^+$ T-cells, and polarization of T-cells, are redox sensitive. Endogenous ROS and RNS are produced by a variety of respiratory tract cells, including epithelial cells, dendritic cells, T lymphocytes, macrophages, neutrophils, and eosinophils, especially during inflammation. Peroxynitrite formation, myeloperoxidase activity and/or nitrite acidification may also be enhanced during inflammation and may contribute to endogenous NO_2 levels. ROS and RNS are thought to promote the allergic phenotype. Air pollution-derived exogenous ROS and RNS can potentially contribute to oxidative and/or nitrative stress in the respiratory tract and influence the adaptive immune response that occurs once dendritic cells are activated. Thus, recent studies suggest the possibility of an interaction between inhaled NO_2 and NO_2 endogenously formed in the respiratory tract.

Collectively, these studies in humans and animals provide evidence that NO_2 exposure may lead to the development of allergic responses in nonallergic individuals or animals via Th2 skewing and allergic sensitization. Furthermore, they are consistent with a link between exposure to ambient NO_2 and increased prevalence of allergic sensitization found in a few epidemiologic studies discussed in Section 6.2.5.

4.3.2.7 Remodeling of Airways and Alveoli

As summarized in the 2008 ISA (U.S. EPA, 2008) and the 1993 AQCD (U.S. EPA, 1993), numerous studies have examined morphological changes in the respiratory tract resulting from chronic NO_2 exposure. The sites and types of morphological lesions produced by exposure to NO_2 were similar in all species when effective concentrations were used (U.S. EPA, 1993). The centriacinar region, including the terminal conducting airways, the alveolar ducts, and the alveoli, exhibited the greatest sensitivity to NO_2 exposure, while the nasal cavity was minimally affected. The cells most injured in the centriacinar region were the ciliated cells of the bronchiolar epithelium and the Type I cells of the alveolar epithelium. These were replaced with nonciliated bronchiolar cells and Type II cells, respectively, which were relatively resistant to continued NO_2 exposure. Some lesions rapidly resolved post-exposure. One study found that collagen synthesis rates were increased in NO_2-exposed rats. Because collagen is an important structural protein in the lung and because increased total lung collagen is characteristic of pulmonary fibrosis, it was proposed that NO_2 exposure may cause fibrotic-like diseases. Exposure to NO_2 also enhanced pre-existing emphysema-like conditions in animal models (U.S. EPA, 2008). Other studies demonstrated that NO_2 exposure induced air space enlargements in the alveolar region and suggested that chronic exposures could result in permanent alterations resembling emphysema-like diseases (U.S. EPA, 1993). A recent study confirmed and extended these findings. NO_2 exposure in rats (10,000 ppb for 21 days) caused increased apoptosis of alveolar epithelial cells and enlargement of air spaces (Fehrenbach et al., 2007). Further, alveolar septal cell turnover was increased, and changes in extracellular matrix were reported. However, there was no loss of alveolar walls (i.e., total alveolar wall volume or total alveolar surface area), indicating that the lesions induced did not meet the 1985 National Heart Lung and Blood Institute definition of human emphysema (U.S. EPA, 1993).

A chronic study in rats exposed to 9,500 ppb NO_2 for 7 h/day, 5 days/week for 24 months found an additional effect on morphology (Mauderly et al., 1990). Bronchiolar epithelium was observed in centriacinar alveoli, and this response progressed with increasing length of exposure. This has been termed “alveolar bronchiolization” (Nettesheim et al., 1970),

reflecting the replacement of one type of epithelium by another. Long-term consequences of alveolar bronchiolization are not known.

The relationship between NO_2 exposure-induced morphologic changes in animal models and impaired lung development seen in epidemiological studies is not clear. Effects of NO_2 exposure on lung morphology in rats have been shown to be age-dependent (U.S. EPA, 2008) (U.S. EPA, 1993). Six-week-old rats exposed to NO_2 for 6 weeks were more sensitive to the effects of NO_2 exposure than 1-day-old rats exposed for 6 weeks (Chang et al., 1986). In humans, the respiratory and immune systems are immature in newborns, and the respiratory system continues to develop until about 20 years of age. This suggests the potential for NO_2 exposure-induced permanent morphological changes in humans if exposure should occur during critical windows of development. However, experimental evidence to substantiate this claim is currently lacking.

Evidence from animal models of allergic airway disease suggests a role for endogenous NO_2 in airway remodeling. These studies, described above in Section 4.3.2.6.2, found that decreased NO bioavailability during inflammation favored the formation of peroxynitrite, which decomposes to NO_2. Interventions that reduced peroxynitrite formation, as evidenced by decreased 3-nitrotyrosine immunostaining, resulted in an amelioration of airway remodeling, as measured by mucus hypersecretion, peribronchial collagen, goblet cell metaplasia, subepithelial fibrosis, and epithelial apoptosis (Ahmad et al., 2011; Mabalirajan et al., 2010b). Exposure to inhaled NO_2 was found to enhance allergic airway inflammation and airway responsiveness in experimental animals previously sensitized and challenged with allergen (Poynter et al., 2006). Airway remodeling was not evaluated in this study which involved acute exposures to NO_2. Whether repeated or chronic exposures to NO_2 lead to airway remodeling in the context of allergic airway disease is not known. However, in nonallergic guinea pigs, subchronic exposure to NO_2 (60−4,000 ppb, 24 h/day, 6−12 weeks) enhanced both airway responsiveness and specific airway resistance, suggesting that airway remodeling may have contributed to the development of AHR (Kobayashi and Miura, 1995).

4.3.2.8 Potential Induction of Carcinogenesis

Some studies have explored the potential carcinogenicity of NO_2. There is no clear evidence that NO_2 acts as a carcinogen [(U.S. EPA, 2008) (U.S. EPA, 1993) Section 6.6.9]. However, NO_2 may act as a tumor promoter at the site of contact, possibly due to its ability to produce cellular damage and promote regenerative cell proliferation. In addition, it has been shown to be genotoxic and mutagenic in some systems, including human nasal epithelial mucosa cells ex vivo exposed to urban-level concentrations

[100 ppb (Koehler et al., 2011, 2010)]. Some studies demonstrated that inhaled NO_2 at high concentrations (e.g., 20,000 ppb) can contribute to the formation of mutagens and carcinogens if other precursor chemicals are found in the body, e.g., N-nitrosomorpholine from morpholine and nitro-pyrene from pyrene [(U.S. EPA, 2008) Section 4.2.2.5].

4.3.2.9 Transduction of Extrapulmonary Responses

While the respiratory tract has been viewed as the primary target of inhaled NO_2, effects outside the respiratory tract have been demonstrated in numerous controlled human exposure and toxicological studies (U.S. EPA, 2008) (U.S. EPA, 1993). These include hematological effects and effects on the heart, central nervous system, liver, and kidneys and on reproduction and development. Epidemiologic evidence of associations between NO_2 exposure and some extrapulmonary effects has also been described (Sections 5.3, 6.3, and 6.4).

Some NO_2-induced effects, which have been demonstrated, are briefly described here. Two controlled human exposure studies involving NO_2 inhalation over several hours found effects on circulating red blood cells, including reduced hemoglobin and hematocrit levels; one of these also found reduced acetylcholinesterase activity [(Frampton et al., 2002; Posin et al., 1978) Section 5.3.11]. Changes in lymphocyte numbers and subsets in the peripheral blood have been demonstrated in human subjects following exposure to NO_2 (Frampton et al., 2002; Sandström et al., 1992). A recent controlled human exposure study found altered blood lipids (Huang et al., 2012). Studies in experimental animals have demonstrated decreases in red blood cell number as well as increases in diphosphoglycerate, sialic acid, and methemoglobin following several days of NO_2 exposure (Section 5.3.11). However, changes in hematocrit and hemoglobin did not occur following longer term exposure to NO_2. Increases in blood glutathione levels and altered blood lipids resulting from NO_2 exposure have also been reported (U.S. EPA, 2008). More recent studies in rats exposed for 7 days to NO_2 (2,660 or 5,320 ppb NO_2) have shown mild pathology of brain and heart tissue, which was accompanied by markers of inflammation and/or oxidative stress [(Li et al., 2012; Li et al., 2011) Sections 5.3.11 and 6.4.4]. In addition, animal studies demonstrated reproductive and developmental effects resulting from exposure to NO_2 during gestation. These included decreased litter size and neonatal weight, and effects on post-natal development (Section 6.4). Many, but not all, of these extrapulmonary effects in animal models have been observed at concentrations of NO_2 that are higher than ambient-relevant concentrations.

Given the reactivity of NO_2, extrapulmonary effects are likely due to NO_2-derived reaction products rather than to NO_2 itself. One pathway by which a reaction product

could mediate extrapulmonary effects of NO_2 would be the activation of pulmonary irritant receptors that results in cardiovascular reflex responses (Section 4.3.2.2). Evidence suggests that the reduction in heart rate observed after acute exposure of experimental animals to high concentrations of NO_2 may be due to stimulation of pulmonary irritant receptors. However, much weaker evidence exists for activation of pulmonary irritant receptors in humans because studies observed no increases in respiratory rate or decreases in heart rate. A recent controlled human exposure study found altered heart rate variability following exposure to an ambient-relevant concentration of NO_2 [500 ppb (Huang et al., 2012)]; whether this effect was due to pulmonary irritant receptor stimulation is unclear.

Alternatively, NO_2-derived reaction products in the lung may "spillover" into the circulation. One reaction product of inhaled NO_2, nitrite, is known to gain access to the circulation. In the presence of red blood cell hemoglobin, nitrite is oxidized to nitrate (Postlethwait and Mustafa, 1981), and nitrosylhemoglobin and methemoglobin are formed. Nitrite has known effects on blood cells, vascular cells, and other tissues. Much recent attention has been paid to nitrite's systemic vasodilatory effects that occur under hypoxic conditions. As discussed in the 2008 ISA and the 1993 AQCD (U.S. EPA, 2008) (U.S. EPA, 1993), one controlled human exposure study demonstrated that NO_2 exposure (4,000 ppb, 75 minutes, intermittent exercise) resulted in a reduction in blood pressure (Linn et al., 1985), which is consistent with the systemic vasodilatory properties of nitrite under conditions of low oxygen. However, studies from other laboratories did not see this effect (Section 5.3.6.2). Furthermore, dosimetric considerations suggest that contributions of nitrite derived from ambient NO_2 to plasma levels of nitrite are small compared to nitrite derived from dietary sources (Section 4.2.2.4).

Besides nitrite and nitrate, other NO_2-derived reaction products may potentially translocate to the circulation. The formation of fatty acid epoxides followed by transport to the circulation and then to the liver was postulated to explain the effect of NO_2 exposure (250 ppb, 3 hours) on pentobarbital-induced sleeping time in mice (Miller et al., 1980). Findings of lipid peroxidation and markers of oxidative stress in some animal studies (Li et al., 2012; Li et al., 2011), which utilized much higher concentrations of NO_2 than the study by Miller et al. (1980), also suggest the presence of circulating ROS or RNS. However, there is no experimental evidence to date for the translocation of NO_2-derived ROS and/or RNS to the circulation following NO_2 exposure.

A third pathway by which a NO_2-derived reaction product may transduce extrapulmonary responses is the "spillover" of inflammatory or vasoactive mediators from the lung into the circulation. This possibility is consistent with changes in peripheral blood inflammatory cells and in tissue markers of inflammation that have been observed

following exposure to NO_2. Confirmation that this mechanism occurs in human subjects exposed to ambient-relevant concentrations of NO_2 was provided by a recent study [(Channell et al., 2012) Section 5.3.11]. Exposure of healthy human subjects to NO_2 (500 ppb for 2 hours) resulted in circulating pro-inflammatory factors in the plasma. Application of plasma to cultured endothelial cells resulted in upregulation of ICAM-1 and vascular cell adhesion molecule 1, as well as the release of IL-8 into the supernatant of the cultured cells. Furthermore, the amount of soluble lectin-like receptor for oxidized low-density lipoprotein was increased in plasma obtained 24 hours post-exposure. Changes in plasma high density lipoprotein levels were observed in a separate study employing the same exposure parameters (Huang et al., 2012). These findings point to a pathway by which inhaled NO_2 leads to circulating soluble factors that promote inflammatory signaling in the vasculature.

4.3.3 Nitric Oxide

As summarized in the 2008 ISA, the 1993 AQCD (U.S. EPA, 2008) (U.S. EPA, 1993), and a recent review (Hill et al., 2010), the synthesis of endogenous NO in cells is catalyzed by three different isoforms of NOS (eNOS, iNOS, neuronal NOS). NO is involved in intracellular signaling in virtually every cell and tissue. In general, low levels of endogenous NO play important roles in cellular homeostasis, while higher levels are important in cellular adaptation and still higher levels are cytotoxic. Further, signaling functions of NO may be altered in the presence of acute inflammation (Hill et al., 2010).

Like NO_2, NO is a radical species (Fukuto et al., 2012). However, it is more selectively reactive than NO_2 (Hill et al., 2010). In addition, it is more hydrophobic and can more easily cross cell membranes and diffuse much greater distances compared with NO_2. As a result, there may be overlap between endogenous and exogenous NO in terms of biological targets and pathways. The following discussion focuses on common mechanisms underlying the effects of both endogenous and exogenous NO.

Because NO has a high affinity for heme-bound iron, many of its actions are related to its interactions with heme proteins (Hill et al., 2010). For example, activation of the heme protein guanylate cyclase is responsible for smooth muscle relaxation and vasodilation of pulmonary and systemic vessels, and possibly for bronchodilator effects. Inhaled NO rapidly reacts with soluble guanylate cyclase in the pulmonary arterial smooth muscle. At the same time, inhaled NO rapidly diffuses into the circulation and reacts with red blood cell hemoglobin to form nitrosylhemoglobin, which is subsequently oxidized to methemoglobin and nitrate. Increased blood concentrations of nitrosylhemoglobin and methemoglobin have been reported in mice exposed for 1 hour to 20,000−40,000 ppb

NO, as well as in mice exposed chronically to 2,400 and 10,000 ppb NO (U.S. EPA, 1993). Some S-nitrosohemoglobin may be formed in partially deoxygenated blood (Wennmalm et al., 1993). NO can also disrupt iron-sulfur centers in proteins (Hill et al., 2010). Furthermore, redox reactions of NO and transition metals, such as iron and copper, facilitate S-nitrosylation of protein and nonprotein thiols. Binding of NO to iron- and copper-containing proteins in the mitochondria may play an important role in mitochondrial respiration. NO also rapidly reacts with superoxide, an oxygen-derived radical species, to produce the potent oxidant peroxynitrite (Hill et al., 2010). Peroxynitrite subsequently reacts with CO_2 to form the nitrosoperoxylcarbonate anion, followed by decomposition to carbonate radical and NO_2 (Section 4.2.2.4).

Endogenous NO is formed in the respiratory tract at high levels (Section 4.2.3), and it has physiologic functions. The paranasal sinuses are a major source of NO in air derived from the nasal airways, with average levels of 9,100 ppb NO (n = 5) measured in the sinuses (Lundberg et al., 1995). Expression of iNOS was found to be higher in epithelial cells of the paranasal sinuses than in epithelial cells of the nasal cavity. This NO produced by nasal airways is thought to play a role in sinus host defense through bacteriostatic activity. In addition, NO produced by nasal airways was found to modulate pulmonary function in humans through effects on pulmonary vascular tone and blood flow (Lundberg et al., 1996). In healthy subjects, a comparison of nasal and oral breathing demonstrated that nasal airway NO enhanced transcutaneous oxygen tension. In intubated patients, nasal airway NO increased arterial oxygenation and decreased pulmonary vascular resistance. Additionally, endogenous NO has been shown to act as a bronchodilator (Belvisi et al., 1992). Endogenous NO produced at high concentrations by phagocytic cells is also known to participate in the killing of bacteria and parasites; this contributes to host defense (U.S. EPA, 2008). Another effect of endogenous NO on host defense is modulation of ciliary beat frequency (Jain et al., 1993). Specifically, NO derived from more distal airways was found to increase ciliary beat frequency. Furthermore, endogenous NO production can be upregulated during inflammation (Anderson et al., 2011). In fact, induction of iNOS in proximal or distal airways of subjects with asthma results in levels of NO in exhaled breath as high as 20−50 ppb (Alving et al., 1993; Hamid et al., 1993).

Endogenous NO has known pro- and anti-inflammatory effects; thus its role in inflammatory lung disease is not clear. While both eNOS and iNOS contribute to NO production in the lung, the relatively low levels of NO produced by eNOS are thought to be more important in metabolic homeostasis (Ahmad et al., 2011). Some evidence points to a role of iNOS-derived NO in the pathogenesis of asthma because it has been correlated with inflammation, epithelial injury, and clinical exacerbations of asthma [(Anderson et al., 2011) Section 4.3.2.6.2]. Furthermore, preferential iNOS upregulation

was found in the distal airways compared with more proximal airways in subjects with asthma. This is of interest because asthma is a disease of the small airways. As mentioned above, signaling functions of NO may be altered in the presence of acute inflammation (Hill et al., 2010), which is characterized by enhanced levels of superoxide. Superoxide reacts with NO to form peroxynitrite, which has been shown in animal models to play a role in the pathogenesis of allergic airway disease (Section 4.3.2.6.2).

NO exposure has been shown to alter pulmonary function, morphology, and vascular function (U.S. EPA, 2008) (U.S. EPA, 1993). Studies in animals have demonstrated that inhaled NO reversed acute methacholine-induced bronchoconstriction (Hogman et al., 1993; Dupuy et al., 1992). This was observed with exposures of 5,000 ppb NO in guinea pigs and 80,000 ppb in rabbits. Chronic inhalation exposures have been found to alter the morphology of the alveolar septal units in rats (Mercer et al., 1995). This effect was not seen with chronic inhalation exposures to NO_2 at similar concentrations (500 ppb with twice daily spikes of 1,500 ppb). In addition, inhaled NO has been shown to alter transferrin and red blood cells in mice. Further, acute inhalation exposure of NO decreased pulmonary vascular resistance in pigs and reduced pulmonary arterial pressure in a rodent model of chronic pulmonary hypertension. A recent study also found that inhaled NO (1,000, 5,000, 20,000, and 80,000 ppb) selectively dilated pulmonary blood vessels, improved ventilation-perfusion mismatch, and reduced hypoxemia-induced pulmonary vascular resistance in a pig model (Lovich et al., 2011).

Inhaled NO is used clinically at concentrations higher than those that are environmentally relevant. Although it can cause both pulmonary and systemic vasodilation, effects on pulmonary vasculature occur at lower concentrations than those required for vasodilation of systemic vessels. This selectivity for pulmonary vasculature is likely due to the rapid scavenging of NO by hemoglobin in the blood. Hence, inhaled NO has been used to mitigate pulmonary hypertension in newborns and adults. High concentrations of inhaled NO are also known to alter ciliary beating and mucus secretion in the airways, to increase renal output, to alter distribution of systemic blood flow, to alter coagulation, fibrinolysis, and platelet functions, and to modulate the inflammatory response (U.S. EPA, 2008).

Endogenous NO is an important mediator of cardiovascular homeostasis. It has anti-inflammatory and antithrombotic effects, is cytoprotective, and induces antioxidant defenses (Wang and Widlansky, 2009). Two recent studies in animal models demonstrate that high concentrations of inhaled NO may result in vascular toxicity. One of these studies found rapid formation of plasma nitrites/nitrates in rats exposed for 1 hour to 3,000−10,000 ppb NO (Knuckles et al., 2011). Plasma nitrites/nitrates doubled after an hour of exposure to 3,000 ppb NO and tripled after an hour of exposure to 10,000 ppb NO. These changes were accompanied by an enhanced constriction response to

endothelin-1 in coronary arterioles, which reflected altered vasomotor tone. Although this latter effect appears to run counter to the vasodilator role of NO, it should be noted that high concentrations of NO, as were used in this study, are known to inhibit eNOS activity in other models (Griscavage et al., 1995). The increase in aortic eNOS content reported is consistent with enzyme inactivation and turnover. Another recent animal toxicological study conducted in $ApoE^{-/-}$ mice, a model of atherosclerosis, found that exposure to very high concentrations of inhaled NO over the course of a week (17,000 ppb NO for 6 h/day for 7 days) led to increases in messenger ribonucleic acid for aortic endothelin-1 and matrix metalloproteinase (MMP)-9, as well as to enhanced vascular gelatinase activity (Campen et al., 2010). These effects, which are biomarkers of vascular remodeling and plaque vulnerability, were not seen with 2,000 ppb NO_2. The authors suggested that the activity of eNOS was uncoupled, resulting in oxidative stress due to the production of superoxide instead of or in addition to NO. Both of these studies suggest that inhaled NO has the potential to disrupt normal signaling processes mediated by endogenous NO.

As mentioned above, endogenous NO plays key signaling roles in virtually every cell and tissue (Hill et al., 2010) and, as such, is an important mediator of homeostasis. Inhaled NO at high concentrations has the potential to have beneficial or deleterious effects on multiple organ systems. An important consideration is whether effects are mediated by an NO metabolite, by the release of NO from a metabolite that serves as a storage pool of NO, or through methemoglobin formation in the blood. Further discussion of the biological functions of NO metabolites is found below.

4.3.4 Metabolites of Nitric Oxide and Nitrogen Dioxide

4.3.4.1 Nitrites/Nitrates

Rapid appearance of nitrite and nitrate in the blood was demonstrated in rats exposed for 1−2 hours to 5,000−40,000 ppb NO_2 (Oda et al., 1981). Elevated levels of blood nitrite and nitrate were maintained as long as the exposure to NO_2 continued. A small increase in levels of nitrosylhemoglobin, but not methemoglobin, was detected in blood. The lack of accumulation of methemoglobin was likely due to reduction of methemoglobin to hemoglobin catalyzed by methemoglobin reductase. Two other studies measured methemoglobin in the blood of mice exposed to NO_2, with conflicting results (U.S. EPA, 1993). Rapid formation of plasma nitrites/nitrates has also been demonstrated in rats exposed for 1 hour to 3,000−10,000 ppb NO (Knuckles et al., 2011).

Recently, it has been proposed that nitrite is a storage form of NO because it can be reduced back to NO under conditions of low oxygen tension in a reaction catalyzed by

deoxyhemoglobin (Gladwin et al., 2005). In addition, nitrite is a signaling molecule in its own right and does not require conversion to NO for this activity (Bryan, 2006). Nitrite can increase cyclic guanosine monophosphate (cGMP) levels and heat shock protein 20 expression, decrease cytochrome P450 activity and alter heme oxygenase-1 expression (Bryan et al., 2005). Nitrite is also bactericidal (Major et al., 2010). Furthermore, under acidic conditions, nitrite can react with thiols to form RSNOs. Nitrite also reacts with hemoglobin to form iron-nitrosyl-hemoglobin and with oxyhemoglobin to form nitrate. Nitrite acts as a vasodilator under hypoxic conditions, through a reaction catalyzed by deoxyhemoglobin (Cosby et al., 2003). The venous circulation may be more sensitive to nitrite than the arterial circulation (Maher et al., 2008).

A recent study found that inhaled nitrite decreased pulmonary blood pressure in newborn lambs with hemolysis-induced pulmonary vasoconstriction (Blood et al., 2011). Nitrite was converted to NO in lung tissue by a mechanism that did not require reaction with deoxyhemoglobin in the circulation. This mechanism resulted in increased exhaled NO gas as well as the relaxation of vascular smooth muscle, which led to pulmonary vasodilation. Although concentrations of inhaled nitrite employed were high (0.87 mol/L sodium nitrite), this study is discussed here because it illustrates a novel biological activity of lung nitrite that is normally formed by reactions of NO_2 and NO in the ELF and/or the blood.

4.3.4.2 S-Nitrosothiols

RSNOs are found endogenously in tissues and extracellular fluids. High concentrations of RSNOs are found in the lung, and their levels may vary depending on disease status (Que et al., 2009). For example, levels of RSNOs in BAL fluid were higher in individuals with asthma compared with healthy subjects (Que et al., 2009). Transport of RSNOs from extracellular compartments into isolated perfused lungs and cultured alveolar epithelial cells occurs via a specific amino acid transport pathway (Torok et al., 2012; Brahmajothi et al., 2010).

Some S-nitrosohemoglobin may be formed in partially deoxygenated blood following inhalation of NO (Wennmalm et al., 1993). However, inhaled NO mainly reacts with red blood cell hemoglobin to form nitrosylhemoglobin, which is subsequently oxidized to methemoglobin and nitrate (Hill et al., 2010). The exact mechanisms by which RSNO formation occurs are not completely clear (Fukuto et al., 2012). NO does not react directly with thiol groups, but it can form RSNOs via reactions with thiyl groups and through intermediate formation of N_2O_3 or metal nitrosyls, such as nitrosylhemoglobin (Fukuto et al., 2012; Hill et al., 2010). Recent evidence suggests that NO may diffuse into

extracellular fluid and be transformed to RSNOs (Torok et al., 2012; Brahmajothi et al., 2010). These experiments were conducted ex vivo in isolated perfused lungs and in vitro in cultured lung epithelial cells, neither of which is a blood-perfused system. Hence it is not clear whether this mechanism contributes to RSNO formation in vivo where the majority of inhaled NO diffuses rapidly across the alveolar capillary barrier and binds to hemoglobin.

RSNOs are thought to serve as a storage or delivery form of NO and to play a role in cell signaling (Fukuto et al., 2012; Hill et al., 2010). They may mediate protein S-glutathionylation and thiol oxidation reactions that can act as redox switches to initiate cell signaling events or alter enzyme activity (Hill et al., 2010).

In the lung, RSNOs act as endogenous bronchodilators (Que et al., 2009) and suppress inflammation by decreasing activation of the transcription factor NFκB (Marshall and Stamler, 2001). Furthermore, augmentation of airway RNSOs by ethyl nitrite inhalation protected against lipopolysaccharide-induced lung injury in an animal model (Marshall et al., 2009). Several findings suggest an inverse relationship between endogenous airway RSNO levels and airway responsiveness. First, levels of airway S-nitrosoglutathione levels were decreased in children with asthmatic respiratory failure and in adults with asthma (Que et al., 2009; Gaston et al., 1998). Second, the enzyme nitrosoglutathione reductase (GSNOR), which regulates airway S-nitrosoglutathione content, was expressed at higher levels in BAL cell lysates in human subjects with asthma than in healthy subjects (Que et al., 2009). GSNOR expression was inversely correlated with S-nitrosoglutathione content. In addition, GSNOR activity in BAL fluid was increased and was inversely correlated with airway responsiveness in human asthma (Que et al., 2009). Third, levels of airway RSNOs were inversely correlated with airway responsiveness in human subjects with eosinophilic inflammation (Lee et al., 2011).

4.3.4.3 Nitrated Fatty Acids and Lipids

Nitration of unsaturated fatty acids and lipids can occur during inflammation and ischemia/reperfusion by reactions with NO and nitrite-derived species. (Higdon et al., 2012; Khoo et al., 2010). However, there is no firm evidence that these reactions occur following exposure to inhaled NO_2. Nitrated fatty acids (also known as nitro-fatty acids) can release NO, which stimulates vascular smooth muscle relaxation through cGMP-dependent pathways in vitro (Lima et al., 2005). However, most of the cell signaling effects of nitrated fatty acids in vivo are likely due to post-translational modification of proteins (Khoo et al., 2010). These electrophilic species react with

susceptible thiol groups in transcription factors (Higdon et al., 2012; Bonacci et al., 2011).

Nitro-fatty acids, such as nitro-oleic acid and nitro-linoleic acid, are anti-inflammatory (Bonacci et al., 2011) and vasculoprotective (Khoo et al., 2010). These effects are mediated via activation of the peroxisome proliferator-activated receptor gamma (PPARγ) and antioxidant response element (ARE) pathways and suppression of NFκB and signal transducer and activator of transcription 1 pathways (Bonacci et al., 2011). In a mouse model, nitro-oleic acid upregulated vascular eNOS and heme oxygenase-1 and inhibited angiotensin II-induced hypertension (Khoo et al., 2010; Zhang et al., 2010a). Nitro-oleic acid protected against ischemia/reperfusion injury in a mouse model (Rudolph et al., 2010). Nitro-oleic acid also activated MMPs (a pro-inflammatory effect) through thiol alkylation in vitro and inhibited MMP expression in macrophages through activation of PPARγ (Bonacci et al., 2011). Expression of MMP was also suppressed in a mouse model of atherosclerosis.

4.3.4.4 Nitrated Amino Acids and Proteins

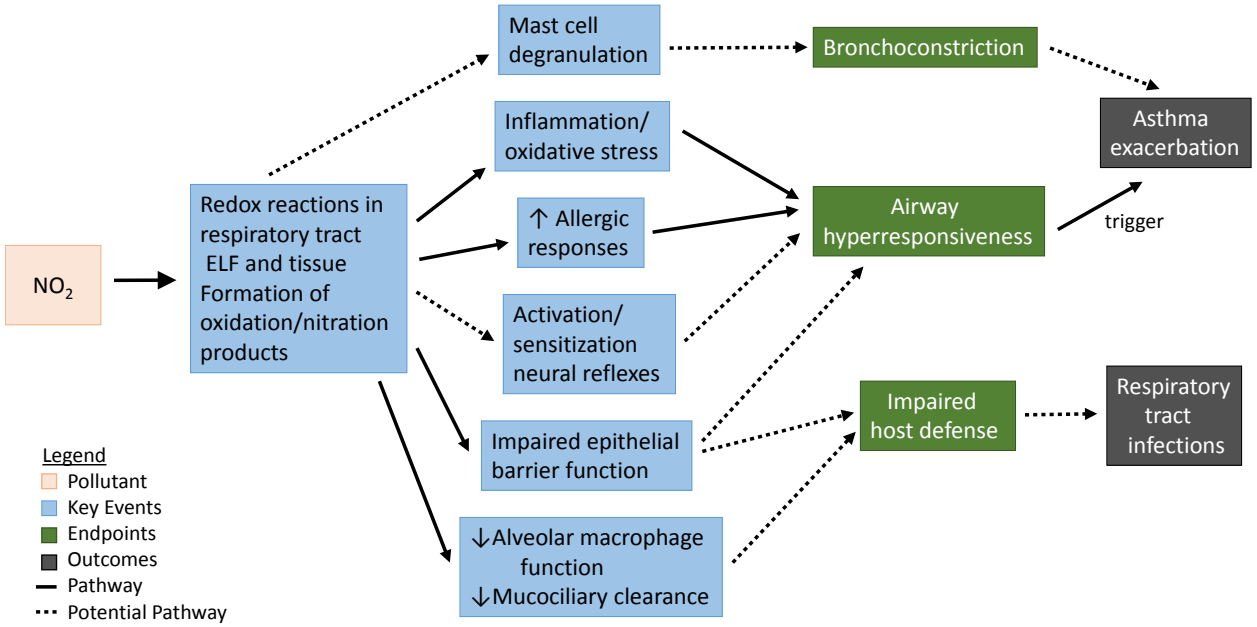
Peroxynitrite and NO_2 can react with amino acids to produce nitrated amino acids and proteins (Hill et al., 2010). These products can also be formed from nitrite and peroxide in a reaction catalyzed by myeloperoxidase. Nitration of proteins may cause inhibition of protein function and/or induce antigenicity. Specific antibodies formed against nitrated proteins may potentially trigger immune reactions (Daiber and Muenzel, 2012). The presence of nitrated amino acids, such as 3-nitrotyrosine, in cells or tissues is an indicator of NO_2 and/or peroxynitrite formation. A recent study reported formation of nitrated SP-D resulting from in vivo exposure to 20,000 ppb NO_2 (Matalon et al., 2009). This modification was accompanied by cross-linking and loss of aggregating activity.

4.3.5 Mode of Action Framework

This section describes the key events, endpoints, and outcomes that comprise the modes of action of inhaled NO_2 and NO. Biological pathways discussed above that may contribute to health effects resulting from short-term and long-term exposures to NO_2 and NO (Chapters 5 and 6) are summarized as a part of this analysis.

Because inhalation of NO_2 results in redox reactions in the respiratory tract, the initiating event in the development of respiratory effects is the formation of oxidation and/or nitration products in the ELF and possibly in airway or alveolar epithelium (Figure 4-1). Reactive intermediates thus formed are responsible for a variety of downstream key

events, which may include respiratory tract inflammation and/or oxidative stress, impaired epithelial barrier function, altered mucociliary clearance, activation and/or sensitization of neural reflexes, mast cell degranulation, and increased allergic responses. These key events may lead to several endpoints including bronchoconstriction, AHR, and impaired host defenses. The resulting outcomes of short-term NO_2 exposure may thus be asthma exacerbation (Section 5.2.2) and respiratory tract infections (Section 5.2.5).



Source: National Center for Environmental Assessment.

Figure 4-1 Mode of action of inhaled nitrogen dioxide (NO_2): short-term exposure and respiratory effects.

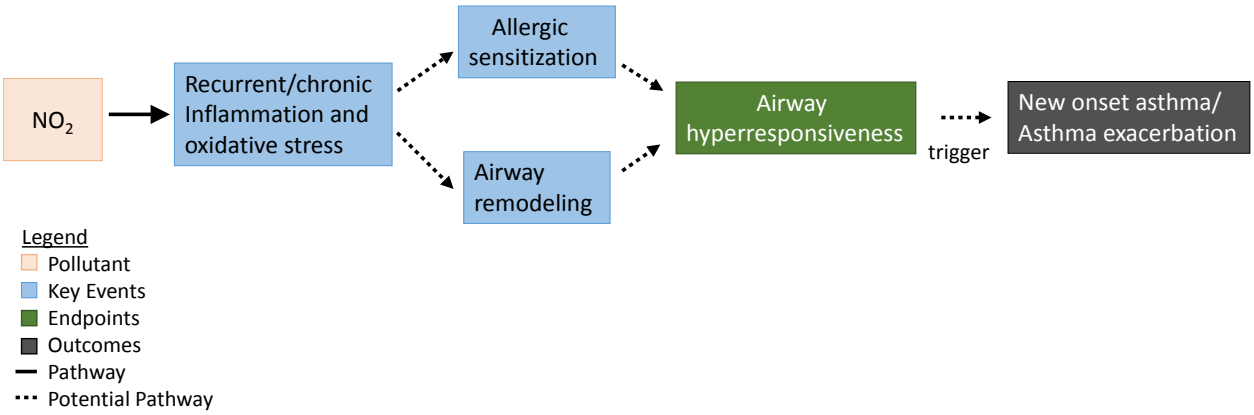
The strongest evidence for this mode of action comes from controlled human exposure studies. NO_2 exposure resulted in enhanced inflammatory mediators (e.g., eicosanoids, interleukins) and neutrophils in BL and/or BAL fluid of healthy subjects. Repeated exposure of healthy subjects led to increased albumin levels in BL fluid, suggesting impaired epithelial barrier function. In addition, repeated exposure of subjects with asthma to NO_2 enhanced eosinophils and a biomarker of eosinophil activation in sputum. Increased airway resistance was demonstrated following NO_2 exposure in healthy human subjects; this response did not involve vagally mediated neural reflexes. However, there appeared to be a role for mast cell degranulation. NO_2 exposure also enhanced airway responsiveness to nonspecific challenges, especially in subjects with asthma. Antioxidant

supplementation dampened NO_2 exposure-induced lipid peroxidation and airway responsiveness, providing support for in vitro findings implicating redox reactions in the ELF. In addition, both early and late asthmatic responses to an allergen challenge (e.g., AHR, neutrophil, and eosinophil activation) were enhanced by NO_2 exposure. NO_2-induced impairment of ciliary function and alveolar macrophage phagocytic activity suggested impairment of host defenses.

Experimental studies in animals suggest that vagally mediated neural reflexes, mast cell degranulation, and production of eicosanoids, which may sensitize receptors on nerve fibers and signal the influx of neutrophils, may contribute to NO_2 exposure-induced AHR. Exposure to NO_2 also enhanced allergic responses (e.g., IgE, eosinophilic, and neutrophilic inflammation). Nitration of the collectin protein SP-D and inhibition of its aggregating activity were also observed in NO_2-exposed animals. This may potentially impact microbial clearance and surfactant metabolism. NO_2 exposure-induced alteration of mucociliary clearance and alveolar macrophages also suggested impairment of host defenses.

Furthermore, there is some evidence for enhanced endogenous formation of peroxynitrite, which decomposes to NO_2, in both human subjects with asthma and animal models of allergic airway disease. In experimental animals, endogenous peroxynitrite/NO_2 formation was associated with AHR and allergic inflammatory responses. Reduction of peroxynitrite formation lessened airway responsiveness, allergic inflammation, and airway remodeling. These findings raise the possibility that inhaled NO_2 can add to the lung burden of endogenous NO_2 which is found in and contributes to AHR and allergic airway disease.

The initiating events in the development of respiratory effects due to long-term NO_2 exposure are recurrent and/or chronic inflammation and oxidative stress (Figure 4-2). These are the driving factors for potential downstream key events, allergic sensitization, and airway remodeling, which may lead to the endpoint AHR. The resulting outcome may thus be new asthma onset, which presents as an asthma exacerbation that leads to physician-diagnosed asthma (Section 6.2.2.1).



Source: National Center for Environmental Assessment.

Figure 4-2 Mode of action of inhaled nitrogen dioxide (NO_2): long-term exposure and respiratory effects.

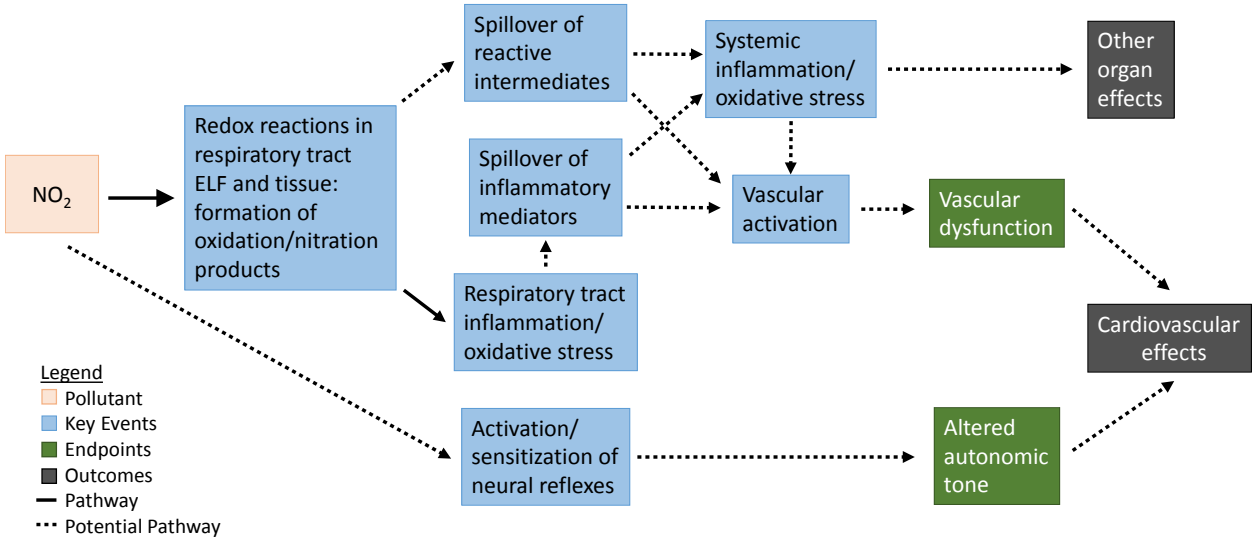
The strongest evidence for this mode of action in humans comes from controlled human exposure studies involving repeated exposures of healthy subjects to NO_2 over several days. Findings included upregulation of Th2 cytokines in respiratory epithelium, which is characteristic of allergic skewing and part of the allergic sensitization pathway. In addition, persistent airway neutrophilia and upregulation of ICAM-1 in airway epithelium were observed. Reductions in lymphocyte subpopulations, suggesting impaired host defense, also occurred. Although these were short-term exposure studies, findings suggest that cumulative effects may occur over time and the possibility that chronic or recurrent exposure to NO_2 may lead to the development of asthma.

Studies in experimental animals exposed to NO_2 for several weeks found nasal eosinophilia and enhanced mast cell responses. Other evidence suggests that endogenous NO_2 acts as an adjuvant promoting the development of allergic airway disease in response to an inhaled allergen. This is consistent with mechanistic studies that suggest that allergic sensitization involves several redox-sensitive steps and that ROS and/or RNS promote the development of an allergic phenotype.

Findings that reduction of endogenous peroxynitrite production decreased airway remodeling in animal models of allergic airway disease suggest that endogenous NO_2 may contribute to airway remodeling. Subchronic exposure to NO_2 enhanced both airway responsiveness and specific airway resistance, suggesting that airway remodeling may have contributed to the development of AHR in this nonallergic animal model. Thus, evidence points to the possibility that inhaled NO_2 can add to the lung burden of endogenous NO that contributes to airway remodeling. Mechanistic studies indicate that

inflammatory mediators and structural changes occurring due to airway remodeling can alter the contractility of airway smooth muscle. Thus, persistent inflammation, allergic sensitization, and airway remodeling due to enhanced endogenous NO_2 production or to long-term NO_2 exposure may contribute to the development of AHR.

There is more uncertainty regarding the mode of action for extrapulmonary effects of inhaled NO_2 (Figure 4-3). However, evidence suggests the following. The initiating events occur in the respiratory tract, where redox reactions lead to the formation of oxidation and/or nitration products. Reactive intermediates thus formed are responsible for downstream key events, which may include activation and/or sensitization of neural reflexes, spillover of reactive intermediates into the circulation, and respiratory tract inflammation and/or oxidative stress. This latter key event may lead to the spillover of inflammatory mediators into the circulation. Circulating reactive intermediates or inflammatory mediators may potentially result in systemic inflammation and/or oxidative stress, which may mediate distal effects in other organs. Alternatively, circulating soluble factors may result in vascular activation, which may lead to the endpoint vascular dysfunction. Activation of neural pathways could lead to the endpoint altered autonomic tone. The resulting outcomes might include cardiovascular effects or other organ effects (Sections 5.3, 6.3, and 6.4).



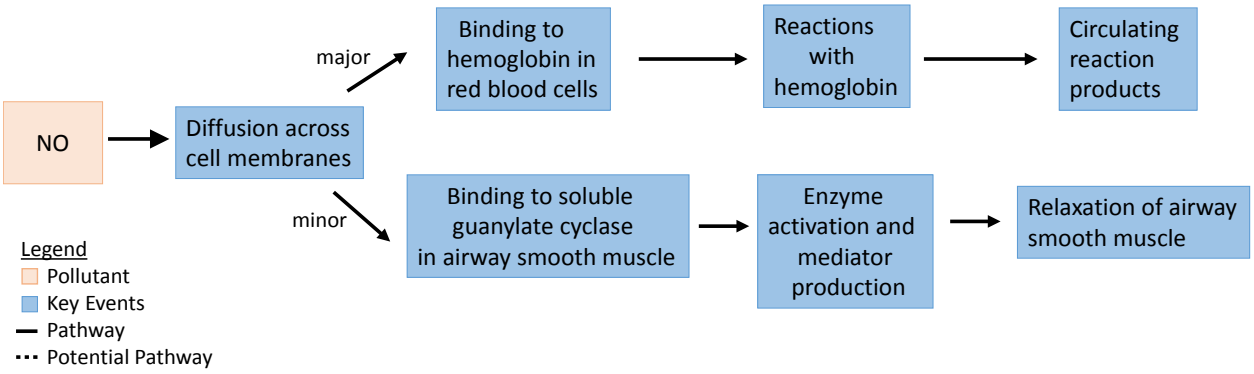
Source: National Center for Environmental Assessment.

Figure 4-3 Mode of action of inhaled nitrogen dioxide (NO_2): short-term and long-term exposure and extrapulmonary effects.

The strongest evidence for this mode of action in humans comes from recent controlled human exposure studies. One study found altered heart rate variability, which is a measure of autonomic tone, and altered blood lipids. Whether altered heart rate variability was due to stimulation of pulmonary irritant receptors is unclear because no studies in humans exposed to NO_2 have observed increases in respiratory rate or decreases in heart rate. The other study found that plasma from human subjects exposed to NO_2 contained increased levels of a soluble factor (soluble lectin-like oxidized low density lipoprotein receptor-1) compared with plasma from control subjects. In addition, this plasma stimulated vascular activation in an in vitro assay. These results indicate that spillover of a reactive intermediate or inflammatory mediator into the circulation occurred, which may transduce a downstream effect in the vasculature or in other organs. This possibility is consistent with changes in peripheral blood lymphocyte number and subsets, as well as with altered blood lipids, which have been observed in humans following exposure to NO_2. These findings point to a pathway by which inhaled NO_2 leads to circulating soluble factors that promote inflammatory signaling in the vasculature and/or other organs.

In experimental animal studies, findings of altered blood glutathione levels and lipids, decreased pentobarbital sleeping time, and mild pathology in brain and heart, which was accompanied by tissue markers of oxidative stress and inflammation, are consistent with the possibility that exposure to NO_2 results in circulating soluble factors that promote inflammatory signaling and/or oxidative stress. There is also support for NO_2 exposure-induced cardiovascular reflexes because one study showed bradycardia in experimental animals that were exposed to high concentrations of NO_2. Evidence indicated that this response in experimental animals was mediated by pulmonary irritant receptors and the vagus nerve, which is consistent with NO_2-induced changes in respiratory rate demonstrated in several studies.

Because NO has a high affinity for heme proteins and because there is no barrier to its diffusion across membranes, it rapidly crosses cell membranes and binds to heme proteins (Figure 4-4). For inhaled NO, that involves diffusing across the alveolar capillary barrier and binding to hemoglobin in red blood cells and, to a lesser extent, diffusing across airway epithelium to react with soluble guanylate cyclase in airway smooth muscle. These comprise the initiating events in the mode of action for inhaled NO. The resulting key events include reactions with hemoglobin to form nitrosylhemoglobin, methemoglobin, nitrate, and possibly S-nitrosohemoglobin, and activation of soluble guanylate cyclase, which produces mediators that relax airway smooth muscle. Because health effects of inhaled NO have not been identified in Chapters 5 and 6, no endpoints or outcomes have been included in this analysis.



Source: National Center for Environmental Assessment.

Figure 4-4 Mode of action of inhaled nitric oxide (NO).

4.4 Summary

This chapter provides a foundation for understanding how exposure to the gaseous air pollutants NO_2 and NO may lead to health effects. This encompasses the many steps between uptake into the respiratory tract and the biological responses that ensue. While NO_2 reacts with components of the ELF and with respiratory epithelium, NO reacts with heme proteins in the circulation. These chemical interactions are responsible for targeting these oxides of nitrogen species to different tissues, that is, the NO_2 to the respiratory tract and NO to the circulation. Biologic responses to inhaled NO_2 and NO were organized into a mode of action framework that may be used to guide interpretation of health effects evidence presented in subsequent chapters.

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CHAPTER 5 INTEGRATED HEALTH EFFECTS OF SHORT-TERM EXPOSURE TO OXIDES OF NITROGEN

5.1 Introduction

5.1.1 Scope of Chapter

The preceding chapters describe the widespread potential for human exposure to ambient oxides of nitrogen (Chapters 2 and 3) and the capability for ambient-relevant concentrations of inhaled NO_2 to initiate a cascade of molecular and cellular responses, particularly in the airways (Chapter 4). These lines of evidence point to the potential for ambient exposure to oxides of nitrogen to induce health effects. However, the preceding chapters also identify the importance of assessing exposure measurement error due to heterogeneity in ambient concentrations of oxides of nitrogen, effects of other correlated pollutants, and the extent to which mode-of-action information is available to support biological plausibility. With consideration of these issues, this chapter summarizes, integrates, and evaluates the evidence for relationships between various health effects and short-term (i.e., minutes up to 1 month, Section 1.5) exposure to oxides of nitrogen. The chapter sections comprise evaluations of the epidemiologic, controlled human exposure, and animal toxicological evidence for the effects of short-term exposure to oxides of nitrogen on health outcomes related to respiratory effects (Section 5.2), cardiovascular and related metabolic effects (Section 5.3), and total mortality (Section 5.4). Reproductive and developmental effects also have been examined in relation to short-term exposure to oxides of nitrogen. However, this evidence is evaluated with studies of long-term exposure in Chapter 6 because associations are often compared among various short- and long-term exposure periods that are difficult to distinguish.

Individual sections for broad health categories (i.e., respiratory, cardiovascular, mortality) begin with a summary of conclusions from the 2008 ISA for Oxides of Nitrogen followed by an evaluation of recent (i.e., published since the completion of the 2008 ISA for Oxides of Nitrogen) studies that builds upon evidence from previous reviews. Within each of these sections, results are organized into smaller outcome groups [e.g., asthma exacerbation, myocardial infarction (MI)] that comprise a continuum of subclinical to clinical effects. The discussion of individual events and outcomes is then organized by specific scientific discipline (i.e., epidemiology, controlled human exposure, toxicology). This organization permits clear description of the extent of coherence and biological

plausibility for the effects of oxides of nitrogen on a group of related outcomes, and in turn, transparent characterization of the weight of evidence in drawing conclusions.

Sections for each of the broad health categories conclude with an integrated assessment of the evidence and a conclusion regarding causality. A determination of causality was made for each broad health category by evaluating the evidence for each category independently with the causal framework (described in the Preamble to the ISA). Findings for mortality informed multiple causal determinations. Findings for cause-specific mortality (i.e., respiratory, cardiovascular) were used to assess the continuum of effects and inform the causal determinations for respiratory and cardiovascular and related metabolic effects. A separate causal determination was made for total mortality (Section 5.4) based on the evidence for non-accidental causes of mortality combined and also informed by the extent to which evidence for the spectrum of cardiovascular and respiratory effects provides biological plausibility for NO_2-related total mortality. Judgments regarding causality were made by evaluating the evidence over the full range of exposures in animal toxicological, controlled human exposure, and epidemiologic studies defined in this ISA to be relevant to ambient exposure [i.e., up to 5,000 ppb NO_2 or NO] as described in Section 1.2. Experimental studies that examined higher NO_2 or NO concentrations were evaluated particularly to inform mode of action.

5.1.2 Evidence Evaluation and Integration to Form Causal Determinations

5.1.2.1 Evaluation of Individual Studies

As described in the Preamble to the ISA (Section 5.a), causal determinations were informed by the integration of evidence across scientific disciplines (e.g., exposure, animal toxicology, epidemiology) and related outcomes and judgments of the quality of individual studies. Table 5-1 describes aspects considered in evaluating the quality of controlled human exposure, animal toxicological, and epidemiologic studies. These aspects aid in the assessment of various sources of bias and uncertainty within a study and in turn, judgments about the strength of inference from their results. This evaluation was applied to studies included in this ISA from previous assessments and those published since the 2008 ISA for Oxides of Nitrogen. The study aspects are based on the ARRIVE (Kilkenny et al., 2010) and STROBE (von Elm et al., 2007) guidelines for animal experiments and epidemiologic studies, respectively. These guidelines were developed to improve standards of reporting and ensure that the data from animal experiments and epidemiologic studies can be fully evaluated. The aspects found in Table 5-1 are consistent with the questions and criteria that have been proposed by

previous approaches for evaluating health science data.[1] Additionally, the aspects are compatible with published EPA guidelines related to cancer, neurotoxicity, reproductive toxicity, and developmental toxicity (U.S. EPA, 2005, 1998, 1996, 1991).

The study aspects described in Table 5-1 are not intended to be a complete list that informs the evaluation of study quality but they comprise the major aspects of study evaluation considered in this ISA. Where possible, study quality considerations, for example, exposure assessment and confounding (i.e., bias due to a relationship with the outcome and correlation with exposures to oxides of nitrogen), are framed to be specific to oxides of nitrogen. Thus, judgments of the quality of a study can vary depending on the specific pollutant being assessed. Importantly, these aspects were not used as a checklist. Particular aspects or the absence of some of these features in a study did not necessarily define a less informative study or exclude a study from consideration in the ISA. Further, these aspects were not criteria for a particular determination of causality in the five-level hierarchy. As described in the Preamble, causal 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 5-1 Summary and description of scientific considerations for evaluating the quality of studies on the health effects of oxides of nitrogen.

NOTE: Study aspects of interest are reported in gray boxes (e.g., Study Design). Summary bullets are provided in the top sections and are described in more detail in the text immediately following.

Study Design		
Controlled Human Exposure	**Animal Toxicology**	**Epidemiology**
• Clearly defined hypotheses/aims • Appropriately matched control exposures • Randomization and allocation concealment • Balanced crossover (repeated measures) or parallel design studies	• Clearly defined hypotheses/aims • Appropriately matched control exposures • Randomization and allocation concealment • All groups handled and cared for equally	• Clearly defined hypotheses/aims • Key designs for short-term exposure: time series, case crossover, panel • Key designs for long-term exposure: prospective cohort, nested case-control • High power studies key: large sample sizes, multiple years, multicity studies

[1] For example, NTP OHAT approach (Rooney et al., 2014), IRIS Preamble (U.S. EPA, 2013b), ToxRTool (Klimisch et al., 1997).

Table 5-1 (Continued): Summary and description of scientific considerations for evaluating the quality of studies on the health effects from oxides of nitrogen.

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. Similarly, 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 and care of animals, including housing, husbandry, etc. 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:
Studies should 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). Studies with large sample sizes and conducted over multiple years are considered to produce more reliable results. 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 also 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	**Animal Toxicology**	**Epidemiology**
• Similarly matched control and exposed subjects • Subject characteristics reported • Clearly indicated inclusion and exclusion criteria • Independent, clinical assessment of the health condition • Loss or withdrawal of subjects should be reported with rationale	• Animal characteristics reported • Studies testing and reporting both sexes and multiple life stages preferred • Loss or exclusion of animals should be reported with rationale	• Representative of population of interest • High participation and low drop-out over time that is not dependent on exposure or health status • Clearly indicated inclusion and exclusion criteria • Independent, clinical assessment of health condition • Groups are compared if from same source population

Table 5-1 (Continued): Summary and description of scientific considerations for evaluating the quality of studies on the health effects from oxides of nitrogen.

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 diseases and history of cardiovascular events.[a] 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, sub-strain, genetic background, age, sex, and weight. However, differences in these parameters across studies do not make the studies incomparable. Unless data indicate otherwise, all animal species and strains are considered appropriate for evaluating effects of NO_2 or NO exposure. It is preferred that the authors test for effects in both sexes and multiple life stages, 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:
The ideal study population is recruited from and is representative of the target population. Studies with high participation and low drop-out over time that is not dependent on exposure or health status are considered to have low potential for selection bias. Criteria for including and excluding subjects should be clearly indicated. For populations with an underlying health condition, independent, clinical assessment of the health condition is ideal, but self-report of physician diagnosis generally is considered to be reliable for respiratory diseases and history of cardiovascular events.[a] Groups with and without an underlying health condition should be compared if they 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	**Animal Toxicology**	**Epidemiology**
• Studies of NO_2 are emphasized	• Studies of NO_2 are emphasized	• NO_2 emphasized over NO, NO_X • Comparisons of health effect associations among gaseous oxides of nitrogen species ideal

Controlled Human Exposure:
The focus is on studies testing NO_2 exposure.
Animal Toxicology:
The focus is on studies testing NO_2 exposure.
Epidemiology:
Health effects are evaluated mostly for NO_2, and less so for NO or NO_X. Studies that compare health effect associations among these species are informative. Typically, one species is examined, and studies of NO_2 are emphasized. It is not clear that ambient-relevant NO exposures induce negative health effects (Section 4.2.3). The relationship of NO_X to NO_2 varies with distance from roads, and thus, may vary among subjects. Hence, there is uncertainty about the extent to which associations with NO_X reflect those for NO_2 vs. other pollutants from traffic.

Table 5-1 (Continued): Summary and description of scientific considerations for evaluating the quality of studies on the health effects from oxides of nitrogen.

Exposure Assessment or Assignment		
Controlled Human Exposure	**Animal Toxicology**	**Epidemiology**
• Well characterized and reported exposure conditions • Limited to studies that utilize NO_2 and/or NO concentrations less than or equal to 5,000 ppb • Preference is given to studies that include exposure control groups • Randomized exposure groups	• Well characterized and reported exposure conditions • Inhalation exposure • Limited to studies that utilize NO_2 and/or NO concentrations less than or equal to 5,000 ppb • All studies should include exposure control groups • Randomized exposure groups	• Exposure metrics that accurately represent temporal or spatial variability for study area • Comparisons of exposure measurement methods • Indoor and total personal exposures can inform independent effects of NO_2 • Lag/duration of exposure metric correspond with time course for health effect
Controlled Human Exposure:		
Studies should well characterize pollutant concentration, temperature, and relative humidity and/or have measures in place to adequately control the exposure conditions for subject safety. For this assessment, the focus is on studies that utilize NO_2 and/or NO concentrations less than or equal to 5,000 ppb (Section 1.2). Studies that utilize higher exposure concentrations may provide information relevant to MOA, dosimetry, or at-risk human populations. 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:		
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. Non-inhalation exposure experiments may provide information relevant to MOA. In vitro studies may be included if they provide mechanistic insight or examine similar effects as in vivo, but are generally not included. All studies should include exposure control groups (e.g., clean filtered air). For this assessment, the focus will be on studies that utilize NO_2 and/or NO concentrations less than or equal to 5,000 ppb (Section 1.2). Studies that utilize higher exposure concentrations may provide information relevant to MOA, dosimetry, interspecies variation, or at-risk human populations.		

Table 5-1 (Continued): Summary and description of scientific considerations for evaluating the quality of studies on the health effects from oxides of nitrogen.

Epidemiology:

Of primary relevance are relationships of health effects with the ambient component of exposure to oxides of nitrogen. 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. The duration or lag of the exposure metric should correspond 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).

Given the spatial heterogeneity in ambient oxides of nitrogen and variable relationships between personal exposures and ambient concentrations (Section 3.4.3), validated methods that capture the extent of variability for the particular study design (temporal vs. spatial contrasts) and location carry greater weight. Central site measurements, whether averaged across multiple monitors or assigned from the nearest or single available monitor, have well-recognized limitations in capturing spatial variation in oxides of nitrogen. Inference from central site measurements can be adequate if correlated with personal exposures, closely located to study subjects, highly correlated across monitors within a location, used in locations with well-distributed sources, or combined with time-activity information.

In studies of short-term exposure, metrics that may capture variation in ambient oxides of nitrogen and strengthen inference include concentrations in subjects' microenvironments (e.g., outdoor home, school, in-vehicle) and individual-level outdoor concentrations combined with time-activity data. Results for total personal and indoor NO_2 exposure are other lines of evidence that inform judgments about causality of NO_2 because inference is based on an individual's microenvironmental exposures and potential for copollutant confounding may be lower or different than that for ambient concentrations. Results for total personal exposure can inform the effects of ambient exposure when well correlated with ambient concentrations. For long-term exposures, LUR models that well represent spatial variation in ambient NO_2 can provide estimates of individual exposure. Less weight is placed on NO_X from dispersion models because of limitations in accurate estimation of within-community conditions (Section 3.2.1.2). And because NO_X from dispersion models often shows near perfect correlations (r = 0.94−0.99) with EC, $PM_{2.5}$, and CO, the effects of NO_X cannot be distinguished from traffic-related copollutants.

Exposure measurement error often attenuates health effect estimates or increases the precision of the association (i.e., width of 95% CIs), particularly associations based on temporal variation in short-term exposure (Section 3.4.5.1). However, exposure measurement error can bias estimates away from the null, particularly for long-term exposures.

Outcome Assessment/Evaluation

Controlled Human Exposure	Animal Toxicology	Epidemiology
• Same manner of outcome assessment for all groups • Validated, reliable methods • Reporting of outcome assessment details • Blinding of endpoint evaluators • Appropriate timing of endpoint evaluation	• Same manner of outcome assessment for all groups • Validated, reliable methods • Reporting of outcome assessment details • Blinding of endpoint evaluators • Appropriate timing of endpoint evaluation	• Same manner of outcome assessment for all groups • Validated, reliable methods • Assessment is blind to exposure status • Appropriate timing of endpoint 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 the 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 the endpoint evaluated. Endpoints should be assessed at time points that are appropriate for the research questions.

Table 5-1 (Continued): Summary and description of scientific considerations for evaluating the quality of studies on the health effects from oxides of nitrogen.

Epidemiology:

Outcomes should be assessed or reported without knowledge of exposure status. Such bias could produce artifactual associations. Outcomes assessed by interview, self-report, clinical examination, or analysis of biological indicators should be defined by consistent criteria and collected by validated, reliable methods. Independent, clinical assessment is ideal for outcomes such as lung function or incidence of disease, but report of physician diagnosis has shown good reliability.[a] Outcomes should be assessed at time intervals that correspond with the time course for physiological changes (e.g., up to a few days for symptoms). 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,[b] 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	Animal Toxicology	Epidemiology
• Well-characterized exposure	• Well-characterized exposure	• Traffic-related copollutants are key: CO, $PM_{2.5}$, BC/EC, OC, UFP, metal PM components, VOCs • Also considered: PM_{10}, SO_2, O_3

Controlled Human Exposure:

Exposure should be well characterized to evaluate independent effects of NO_2.

Animal Toxicology:

Exposure should be well characterized to evaluate independent effects of NO_2.

Epidemiology:

Not accounting for copollutant confounding can produce artifactual associations; thus, studies that examine copollutant confounding carry greater weight. The predominant method is copollutant modeling, which is especially informative when measurement error is comparable for copollutants and correlations are not high. Interaction and joint effect models are examined to a lesser extent. Copollutant confounding also can be informed by evaluating correlations between oxides of nitrogen and copollutants and comparing health associations between gaseous oxides of nitrogen and copollutants in single-pollutant models if exposure measurement error is comparable among pollutants. Studies that examine only gaseous oxides of nitrogen are considered poorly to inform the potential for copollutant confounding. Copollutant confounding is evaluated based on the extent of their correlations typically observed with oxides of nitrogen and relationships observed with health effects.
Among copollutants, of primary concern are traffic-related pollutants, which include CO, $PM_{2.5}$, BC/EC, OC, UFP, metal PM components such as copper, zinc, and iron, as well as VOCs such as benzene, acetaldehyde, toluene, ethylbenzene, and xylene. Short-term and long-term metrics for these pollutants consistently show moderate to high correlations with oxides of nitrogen (Figure 3-6). Many traffic-related pollutants also are characterized to have common modes of action.[c] Common key events include formation of secondary oxidation products, inflammation, and for respiratory effects, increases in airway responsiveness. They also show relationships with many of the health effects evaluated in this ISA[d] except as follows. For long-term exposure, there is uncertainty regarding confounding by UFP because of their short atmospheric lifetime. Also for long-term exposure, CO is not considered to be an important confounding copollutant for mortality or lung cancer.[d]
Of less concern is confounding by PM_{10}, SO_2, and O_3 because they show varying and often lower correlations with NO_2 (Figure 3-6). O_3 generally is negatively or weakly positively correlated with NO_2 but may be a confounding copollutant where moderate positive correlations are observed. O_3 and SO_2 in particular show similarities with NO_2 in mode of action. PM_{10}, SO_2, and O_3 show relationships with the health effect evaluated in this ISA[d] except as follows. For short-term exposure, SO_2 is not considered to be a strong confounding copollutant for cardiovascular effects. For long-term exposure, neither O_3 nor SO_2 is considered to be a strong confounding copollutant.

Table 5-1 (Continued): Summary and description of scientific considerations for evaluating the quality of studies on the health effects from oxides of nitrogen.

Other Potential Confounding Factors[e]		
Controlled Human Exposure	**Animal Toxicology**	**Epidemiology**
• Preference given to studies with adequate control of factors influencing health response	• Preference given to studies with adequate control of factors influencing health response	• Potential confounders related to health effect and correlated with oxides of nitrogen should be examined • Potential confounders vary by study design (temporally vs. spatially correlated) and by health effects
Controlled Human Exposure:		
Preference is given to studies utilizing experimental and control groups that are matched for individual level characteristics (e.g., body weight, smoking history, age) and time varying factors (e.g., seasonal and diurnal patterns).		
Animal Toxicology:		
Preference is given to studies utilizing experimental and control groups that are matched for individual level characteristics (e.g., 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 oxides of nitrogen and/or traffic indicators. 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 oxides of nitrogen and health effects, which can bias results toward the null. In the absence of information linking health risk factors to oxides of nitrogen or traffic indicators, a factor may be evaluated as a potential effect measure modifier, but uncertainty is noted as to its role as a confounder. Confounders vary according to study design, exposure duration, and health effect and include the following: For time-series and panel studies of short-term exposure: • Respiratory Effects—meteorology, day of week, season, medication use, allergen exposure (potential effect modifier) • Cardiovascular Effects—meteorology, day of week, season, medication use • Total Mortality—meteorology, day of week, season, long-term temporal trends For studies of long-term exposure: • Respiratory Effects—socioeconomic status, race, age, medication use, smoking, stress • Cardiovascular, Reproductive, and Development Effects—socioeconomic status, race, age, medication use, smoking, stress, noise • Total Mortality—socioeconomic status, race, age, medication use, smoking, comorbid health conditions • Cancer—socioeconomic status, race, age, occupational exposure		
Statistical Methodology		
Controlled Human Exposure	**Animal Toxicology**	**Epidemiology**
• Clearly described and appropriate statistical methods for the study design and research question • Preference given to adequately powered studies • Consideration given to trends in data and reproducibility	• Clearly described and appropriate statistical methods for the study design and research question • Preference given to adequately powered studies • Consideration given to trends in data and reproducibility	• Multivariable regression adjusting for potential confounders ideal • Exception is multipollutant models. Multicollinearity can produce unreliable results • Results based on small sample sizes can be unreliable

Table 5-1 (Continued): Summary and description of scientific considerations for evaluating the quality of studies on the health effects from oxides of nitrogen.

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. 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. 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 because of 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 of 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 should be appropriate for the power of the study. 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 pollutant-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.

BC = black carbon, CI = confidence interval, CO = carbon monoxide, EC = elemental carbon, ISA = Integrated Science Assessment, LUR = land use regression, MOA = mode of action, NO = nitric oxide, NO_2 = nitrogen dioxide, NO_X = sum of NO and NO_2, O_3 = ozone, OC = organic carbon, PM = particulate matter, SES = socioeconomic status, SO_2 = sulfur dioxide, UFP = ultrafine particles, VOC = volatile organic compound.

[a]Toren et al. (1993); (Murgia et al. (2014); Weakley et al. (2013); Yang et al. (2011); Heckbert et al. (2004); Barr et al. (2002); Muhajarine et al. (1997))

[b]Burney et al. (1989)

[c]Information on modes of action for NO_2 is described in Section 4.3. The characterization of similar modes of action for many traffic-related pollutants is based on information described in the most recently completed ISAs (U.S. EPA, 2013a, 2010, 2009, 2008b) and the Health Effects Institute's 2010 review of Traffic-related air pollution (HEI, 2010).

[d]Judgments regarding potential confounding by other criteria pollutants are based on studies evaluated in this ISA, causal determinations made in the most recently completed ISAs (U.S. EPA, 2013a, 2010, 2009, 2008b), as well as recent reviews published by the Health Effects Institute. Judgments regarding potential confounding by the PM components EC/BC, OC, metals, and UFP as well as VOCs should not be inferred as conclusions regarding causality. Their consideration is based on associations with oxides of nitrogen and health effects observed in the studies examined in this ISA and reviews conducted by the Health Effects Institute (HEI Review Panel on Ultrafine Particles, 2013; HEI, 2010). Judgments regarding potential confounding by PM_{10} should not be inferred as conclusions regarding causality specifically for that size fraction. The 2009 ISA for PM evaluated PM_{10} studies but did not form individual causal determinations for that size fraction because PM_{10} comprises both fine and thoracic coarse particles.

[e]Many factors evaluated as potential confounders can be effect measure modifiers (e.g., season, comorbid health condition) or mediators of health effects related to oxides of nitrogen (comorbid health condition).

1

5.1.2.2 Evaluation of Confounding in Epidemiologic Studies

Epidemiologic studies of short-term exposure to oxides of nitrogen relied primarily on temporal variation in exposure (e.g., day-to-day changes in ambient NO_2 concentrations) and health effects. Other risk factors for health effects also exhibit similar temporal trends as oxides of nitrogen and include meteorological variables, season, long-term time trends, medication use, and copollutant exposures. These factors and others specified in Table 5-1 are important to evaluate as potential confounders of associations for oxides of nitrogen, particularly given the small effect sizes typically observed. Epidemiologic studies reviewed in this ISA varied in the extent to which they considered potential confounding. Because no single study considered all potential confounders, and not all factors were examined in the collective body of studies, residual confounding by unmeasured factors is possible. Residual confounding also is possible by poorly measured factors. In this ISA, potential confounding was assessed as the extent to which the collection of studies examined factors well documented in the literature to be associated with exposure to oxides of nitrogen and health outcomes.

In epidemiologic studies evaluated in this ISA, confounding was assessed primarily using multivariable models that include NO_2 concentrations and the putative confounder in the same model. The NO_2 effect estimate represents the effect of NO_2 keeping the level of the covariate constant. In the ISA, confounding is assessed by examining the change in the magnitude of the effect estimate and width of the 95% confidence interval (CI) for NO_2 in multivariable models, not just a change in statistical significance. The limitations of multivariable models are well recognized. If NO_2 and the potential confounder are highly correlated, the collinearity (i.e., covariates predict each other) introduced by including them in the same model can misleadingly decrease or increase the magnitude or precision of the effect estimates for NO_2 or the potential confounder. Collinearity can occur, for example, if pollutants are from the same sources or are derived from NO_2 [e.g., ozone (O_3)], or if meteorology affects formation of both pollutants. Adding correlated but non-causal variables can produce models that fit the data poorly, and residual confounding is possible if confounders are excluded or poorly measured.

For evaluation of copollutant confounding, the predominant method of studies reviewed in this ISA was copollutant models (NO_2 plus one copollutant). Inference about the independent effects of NO_2 from copollutant models can be limited because the varying spatial distributions of NO_2 and the copollutant may not satisfy the assumptions of equal measurement error or constant correlations for NO_2 and the copollutant (Gryparis et al., 2007). Further, copollutant models for NO_2 assumed linear relationships with copollutants, and nonlinear relationships are possible because of varying near road

gradients (Figure 3-2). Other methods for evaluating copollutant confounding do not require the aforementioned assumptions, including a hierarchical Bayesian approach that estimates single-pollutant effects in a particular location then combines these single-pollutant effects across locations in a model as the predictor and outcome, respectively (Gryparis et al., 2007; Schwartz and Coull, 2003). Such Bayesian models are unavailable for NO_2. Models examining joint effect or interaction terms for NO_2 and a copollutant also can inform potential confounding and synergistic effects. These are available only to a limited extent, particularly for traffic-related copollutants. Because examination of copollutant confounding is based largely on copollutant models, their limitations are considered in drawing inferences about independent associations for NO_2. Emphasis is placed on results based on exposure assessment methods that likely produce comparable measurement error for NO_2 and copollutants such as ambient or total personal and microenvironmental exposure assessment.

5.1.2.3 Additional Considerations for Epidemiologic Studies

The ISA presents epidemiologic effect estimates for associations with health outcomes scaled to the same increment of oxide of nitrogen concentration. This standardization increases comparability among studies that scale effect estimates to various changes in concentrations, e.g., interquartile range (IQR) for the study period or an arbitrary unit such as 10 ppb. The increments for standardization vary by averaging time (e.g., 24-h avg, 1-h max) and oxide of nitrogen. For 24-h avg, effect estimates were scaled to a 20-ppb increase for NO_2 or NO and a 40-ppb increase for NO_X. For 1-h max, effect estimates were scaled to a 30-ppb increase for NO_2, an 80-ppb increase for NO, and a 100-ppb increase for NO_X. For 8-h max, the increments for standardization are 25 ppb for NO_2, 45 ppb for NO, and 65 ppb for NO_X. These increments were derived by calculating the U.S.-wide percentile distributions for various averaging times and then calculating the approximate difference between the median (a typical pollution day) and the 95th percentile (a more polluted day) for a given averaging time [see Table 2-1 for 1-h max percentiles and Table S5-1 for 24-h avg and 8-h max percentiles; (U.S. EPA, 2014f)].

There were common exceptions to this standardization method. Averaging times other than 24-h avg or 1-h max were examined, for example, 2-h to 15-h avg. Effect estimates based on these averaging times were not standardized but are presented in the ISA as reported in their respective studies. Some studies reported effect estimates in terms of $\mu g/m^3$ increases in oxides of nitrogen, which could be converted to ppb and standardized for NO_2 and NO but not NO_X. Because the proportions of NO_2 and NO are unknown for the various NO_X metrics, NO_X concentrations could not be converted from $\mu g/m^3$ to ppb. Also, data are not available to calculate the percentiles of NO_X concentrations in $\mu g/m^3$ at

a national scale for the U.S. or other countries. Therefore, the ISA presents effect estimates based on μg/m^3 of NO_X as they are reported in their respective studies.

5.1.2.4 Integration of Scientific Evidence

In addition to quality and strength of inference from individual studies, causal determinations were based on the integration of multiple lines of evidence, which included evaluation of the consistency and coherence of findings across disciplines and related outcomes and the extent to which chance, confounding, and other biases could be ruled out with reasonable confidence. Aspects considered in evidence integration are described in detail in the Preamble, and examples are summarized below. Controlled human exposure and animal toxicological studies can provide direct evidence for health effects related to NO_2 or NO exposures. Coherence between experimental and epidemiologic findings can address uncertainties within the collective body of evidence. For example, experimental evidence for effects from a controlled exposure could address whether epidemiologic associations with health outcomes plausibly reflect an independent effect of ambient NO_2 exposure or could be confounded by other factors. Experimental studies additionally can provide biological plausibility for observed effects by describing key events within the modes of action. Thus, the integration of evidence across a spectrum of related outcomes and across disciplines was used to inform uncertainties for any particular outcome or discipline due to factors such as chance, publication bias, selection bias, and confounding by copollutant exposures or other factors. The evaluation of health effects also drew upon information on potential error associated with various exposure assessment methods and the uptake and distribution of oxides of nitrogen in the body. The subsequent sections assess study quality and strength of inference and integrate multiple lines of evidence to characterize relationships between oxides of nitrogen and various health effects.

5.2 Respiratory Effects

5.2.1 Introduction

The 2008 ISA for Oxides of Nitrogen concluded that evidence was sufficient to infer a likely to be causal relationship between short-term exposure to NO_2 and respiratory effects (U.S. EPA, 2008a), based heavily on a large body of epidemiologic evidence. In studies that were not available until after the completion of the 1993 AQCD for Oxides of Nitrogen (U.S. EPA, 1993), short-term increases in ambient NO_2 concentrations were

consistently associated with increases in respiratory-related hospital admissions and emergency department (ED) visits. The coherence of these findings with NO_2-related increases in respiratory symptoms in children with asthma supported an effect of NO_2 exposure on asthma exacerbation. NO_2 was not consistently related to lung function decrements across epidemiologic and controlled human exposure studies and populations with varying respiratory conditions such as asthma or chronic obstructive pulmonary disease (COPD). However, epidemiologic studies of children and adults with asthma observed associations with lung function measured by supervised spirometry (U.S. EPA, 2008a).

The 2008 ISA identified multiple lines of evidence as supporting an independent relationship between short-term NO_2 exposure and respiratory effects. Controlled human exposure studies demonstrated NO_2-induced increases in airway responsiveness in adults with asthma. These findings for increased airway responsiveness, a characteristic feature of asthma, provided biological plausibility for epidemiologic evidence for asthma exacerbation. Further, airway responsiveness was increased following <1 to 6-hour exposures to NO_2 at concentrations in the range of 100 to 300 ppb, which are not much higher than peak ambient concentrations (Section 2.5).

Previous epidemiologic studies also indicated independent associations for NO_2. Personal and indoor NO_2 were associated with respiratory effects, and associations with both personal and ambient NO_2 were observed in copollutant models that adjusted for another traffic-related pollutant such as carbon monoxide (CO) or fine particulate matter ($PM_{2.5}$). In the few available results, NO_2-related respiratory effects were observed with adjustment for elemental carbon (EC), organic carbon (OC), or ultrafine particles (UFP); other traffic-related copollutants were not examined for potential confounding. Controlled human exposure and animal toxicological studies also demonstrated NO_2-induced impairments in host defense, changes in the oxidant/antioxidant balance, and increases in pulmonary inflammation at concentrations of 1,500 to 5,000 ppb NO_2, higher than those demonstrated to increase airway responsiveness (U.S. EPA, 2008a). The 2008 ISA did not explicitly link these NO_2-induced biochemical and immunological changes to lines of evidence for asthma exacerbation. Although there was coherence of evidence across related outcomes and disciplines supporting a relationship between short-term ambient NO_2 exposure and respiratory effects, due to the high correlations of NO_2 with other traffic-related pollutants and limited analysis of potential confounding, sufficient uncertainty was noted regarding the role of NO_2 as an indicator for another traffic-related pollutant or a mixture of such pollutants.

As will be described in the following sections, consistent with the body of evidence presented in the 2008 ISA for Oxides of Nitrogen, recent studies continue to demonstrate

respiratory effects related to short-term NO_2 exposure. The majority of the recent evidence is from epidemiologic studies, which expand on findings for associations between ambient NO_2 and a broad array of respiratory effects from subclinical increases in pulmonary inflammation to respiratory mortality, but particularly for effects related to asthma exacerbation. Because there are few recent controlled human exposure and animal toxicological studies, previous findings are a large basis of the characterization and integration of evidence. Where available, results from recent studies are evaluated in the context of results from previous studies. To clearly characterize differences in the weight of evidence and the extent of coherence among disciplines and related outcomes, the discussion of scientific information is organized by respiratory outcome group, e.g., asthma exacerbation, allergy exacerbation, respiratory infection.

5.2.2 Asthma Exacerbation

As detailed in the preceding section, previous studies provided several lines of evidence in support of a relationship between short-term NO_2 exposure and asthma exacerbation, represented as respiratory effects in populations with asthma. This evidence is corroborated by recent studies. In characterizing the current state of the evidence, this section begins with effects on increasing airway responsiveness and decreasing lung function. These are indications of bronchoconstriction and airway obstruction, which can lead to poorer control of asthma symptoms and potentially hospital admissions or ED visits for asthma. The evaluation of clinical indicators of asthma exacerbation follows with discussion of pulmonary inflammation and oxidative stress, which are part of the mode of action for asthma exacerbation and mediate decreases in lung function and increases in airway responsiveness (Figure 4-1).

5.2.2.1 Airway Responsiveness in Individuals with Asthma

Overview

Controlled human exposure studies evaluating the effect of inhaled NO_2 on the inherent responsiveness of the airways to challenge by bronchoconstricting agents have had mixed results. In general, existing meta-analyses show statistically significant effects of NO_2 on the airway responsiveness of individuals with asthma. However, no meta-analysis has provided a comprehensive assessment of the clinical relevance of changes in airway responsiveness, the potential for methodological biases in the original papers, and the distribution of responses. This section provides analyses showing that a statistically significant fraction (i.e., 70% of individuals with asthma exposed to NO_2 at rest)

experience increases in airway responsiveness following 30-minute exposures to NO_2 in the range of 200 to 300 ppb and following 60-minute exposures to 100 ppb. The distribution of changes in airway responsiveness is log-normally distributed with a median change of 0.75 [provocative dose (PD) following NO_2 divided by PD following filtered air exposure] and geometric standard deviation (GSD) of 1.88. About a quarter of the exposed individuals experience a clinically relevant reduction in their provocative dose due to NO_2 relative to air exposure. The fraction experiencing an increase in responsiveness was statistically significant and robust to exclusion of individual studies. The results of the meta-analysis showed minimal change in airway responsiveness for individuals exposed to NO_2 during exercise. A variety of factors that may affect the assessment of airway responsiveness and how those factors may directionally bias the results of individual studies and the analyses in this current assessment are considered.

Background

Bronchial challenge agents can be classified as nonspecific [e.g., histamine, sulfur dioxide (SO_2), cold air] or specific (i.e., allergen). Nonspecific agents can be differentiated between "direct" stimuli [e.g., histamine, carbachol, and methacholine] which act on airway smooth muscle receptors and "indirect" stimuli (e.g., exercise, cold air) which act on smooth muscle through intermediate pathways, especially via inflammatory mediators (Cockcroft and Davis, 2006c). Specific allergen challenges [e.g., house dust mite, cat allergen] also act "indirectly" via inflammatory mediators to initiate smooth muscle contraction and bronchoconstriction. This section focuses on changes in airway responsiveness to bronchial challenge attributable to NO_2 in individuals with asthma. Discussed in Section 4.3.2.5, toxicological studies have demonstrated increased airway responsiveness to nonspecific challenges following short-term exposure. Described in Sections 5.2.2.5 and 4.3.2.6, altered responses to specific allergens following NO_2 exposure have also been demonstrated in human and animal studies.

Responses to bronchial challenge are typically quantified in terms of the PD or provocative concentration (PC) of an agent required to produce a 20% reduction in forced expiratory volume in 1 second (FEV_1) (PD_{20} or PC_{20}, respectively) or a 100% increase in specific airway resistance (sRAW) (PD_{100} or PC_{100}, respectively). There is a wide range in airway responsiveness that is influenced by many factors, including medications, cigarette smoke, air pollutants, respiratory infections, occupational exposures, disease status, and respiratory irritants. In the general population, airway responsiveness is log-normally distributed with individuals having airway hyperresponsiveness (AHR) tending to be those with asthma (Postma and Boezen, 2004; Cockcroft et al., 1983). Along with symptoms, variable airway obstruction, and airway inflammation, AHR is a primary feature in the clinical definition and characterization of asthma severity (Reddel

et al., 2009). However, not all individuals with asthma experience airway hyperresponsiveness. The range in airway responsiveness among individuals with asthma extends into the range of healthy individuals without asthma (Cockcroft, 2010). In asthma, there is a strong relationship between the degree of nonspecific airway responsiveness and the intensity of the early airway response to specific allergens to which individuals have become sensitized (Cockcroft and Davis, 2006a).

In studies investigating the effect of NO_2 exposure on airway responsiveness, individuals with asthma generally have a lower PD of a bronchial challenge agent than healthy individuals to produce a given reduction in lung function. In Morrow and Utell (1989a), the average PD of carbachol producing a given change in lung function in individuals with mild-to-moderate asthma was 16 times lower than in age-matched healthy controls. Similarly, Hazucha et al. (1983) reported a 10−12 times lower average baseline PD_{100} to methacholine in individuals with mild asthma than healthy age-matched controls. The PDs for asthma in Morrow and Utell (1989a) did not overlap with those of the healthy controls, whereas Hazucha et al. (1983) observed an overlap with 2 of 15 subjects with asthma being relatively unresponsive to bronchial challenge. The bronchoconstrictive response to indirect acting agents (especially specific allergens) can be more difficult to predict and control than the bronchoconstrictive response to non-specific agents that act directly on airway smooth muscle receptors (O'Byrne et al., 2009). Consequently, most of the available literature relevant to the evaluation of the effects of NO_2 on airway responsiveness has focused primarily on the responses of individuals with asthma to bronchial challenge with "nonspecific" bronchoconstricting agents (e.g., methacholine, SO_2, cold air).

In healthy adults without asthma or AHR, there is likely little or no clinical significance of transient, small increases in airway responsiveness following low-level NO_2 inhalation exposures. In individuals with asthma, however, transient changes in airway responsiveness in response to inhaled pollutants may have clinical consequences. Increased airway responsiveness is linked with airway inflammation and airway remodeling (Chetta et al., 1996), increased risk for exacerbation (Van Schayck et al., 1991), reduced lung function (Xuan et al., 2000), and increased symptoms (Murray et al., 1981). A variety of environmental challenges can transiently increase AHR and worsen asthma control, including allergen exposures (Strand et al., 1997; Brusasco et al., 1990), viral infections (Cheung et al., 1995; Fraenkel et al., 1995), cigarette smoke (Tashkin et al., 1993), O_3 (Kehrl et al., 1999), and other respiratory irritants (Kinsella et al., 1991). An exposure that worsens airway responsiveness to one agent in individuals with asthma may also enhance airway responsiveness to other challenge agents. Transient increases in airway responsiveness following NO_2 or other pollutant exposures have the potential to

increase symptoms and worsen asthma control, even if the pollutant exposure does not cause acute decrements in lung function.

Three meta-analyses in the peer-reviewed literature have assessed the effects of NO_2 exposure on airway responsiveness in individuals with asthma (Goodman et al., 2009; Kjaergaard and Rasmussen, 1996; Folinsbee, 1992). Kjaergaard and Rasmussen (1996) reported statistically significant effects of NO_2 exposure on the airway responsiveness of subjects with asthma exposed to less than or equal to 300 ppb NO_2 but not for exposures in excess of 300 ppb NO_2. With consideration given to activity level during exposure, Folinsbee (1992) found statistically significant increases in airway responsiveness of subjects with asthma exposed to NO_2 at rest across all concentration ranges (namely, <200 ppb, 200 to 300 ppb, and >300 ppb). However, there was no statistically significant effect of NO_2 exposures on responsiveness during exercise. For instance, following exposures between 200 and 300 ppb NO_2, 76% of subjects exposed at rest had increased responsiveness which was statistically significant, whereas only 52% of subjects exposed while exercising had increased responsiveness, which was not a statistically significant change. The analyses of Folinsbee (1992) and Kjaergaard and Rasmussen (1996) in effect assessed nonspecific responsiveness because few studies of allergen responsiveness were available.

The analyses conducted by Folinsbee (1992) were detailed in Chapter 15 of the 1993 AQCD for Oxides of Nitrogen (U.S. EPA, 1993). Results of these analyses appeared in Table 15-10 of the 1993 AQCD and supported the conclusion that NO_2 exposure increases airway responsiveness in individuals with asthma. The results of a slightly modified analysis focusing exclusively on non-specific responsiveness appeared in Tables 3.1-3 of the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a). The overall conclusion of that modified analysis was that NO_2 exposures as low as 100 ppb (the lowest concentration experimentally evaluated) conducted during rest, but not exercise, increased non-specific responsiveness of individuals with asthma. Due to differences in study protocols (e.g., rest vs. exercise) in the NO_2-airway responsiveness literature, the original (Folinsbee, 1992) and updated meta-analyses in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a) assessed only the fraction of individuals experiencing increased or decreased airway responsiveness following NO_2 exposure.

Goodman et al. (2009) provided meta-analyses and meta-regressions evaluating the effects of NO_2 exposure on airway responsiveness in subjects with asthma. By considering studies of specific allergen and nonspecific responsiveness following NO_2 exposure, Goodman et al. (2009) evaluated a larger number of studies than the analysis in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a), which was limited to nonspecific responsiveness in subjects with asthma in an attempt to reduce the

heterogeneity among studies. Goodman et al. (2009) evaluated changes in three endpoints following NO_2 exposure relative to a control air exposure: (1) the fraction of subjects with asthma experiencing increases in responsiveness, (2) the PD of the bronchial challenge agent, and (3) the FEV_1 response to the challenge agent. Overall, statistically significant effects of NO_2 exposure on each of these three endpoints were observed. Consistent with the meta-analysis provided in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a), Goodman et al. (2009) found 64% (95% CI: 58, 71%) of subjects with asthma exposed at rest to NO_2 experienced an increase in airway responsiveness, whereas there was no effect of NO_2 exposure during exercise with 52% (95% CI: 43, 60%) having an increase in responsiveness. Additionally, NO_2 exposure resulted in statistically significant reductions in PD as well as increases in the FEV_1 decrement following bronchial challenge.

Goodman et al. (2009) concluded that, "NO_2 is not associated with clinically relevant effects on AHR at exposures up to 600 ppb based primarily on the small magnitude of effects and the overall lack of exposure-response associations." Relative to therapeutic agents used to treat airway responsiveness, which may be considered effective if they more than double the PD for methacholine, the authors concluded that a −50% change in the PD due to NO_2 exposure would be considered adverse. Using the summary statistics provided in individual studies, the effect of NO_2 exposure was a −27% (95% CI: −37, −18%) change in the PD. Stratifying by rest and exercise exposure, the NO_2-induced changes in PD were −30% (95% CI: −38, −22%) and −24% (95% CI: −40, −7%), respectively. Thus, the authors concluded that the effects of NO_2 exposure on airway responsiveness were sufficiently small so as not to be considered adverse. The appropriateness of weighing the deleterious effects of a generally unavoidable ambient exposure using the criteria for judging the efficacy of beneficial therapeutic agents is not clear. Based on the lack of a monotonic increase in responsiveness with exposure, the authors also suggested that NO_2 is not a causal factor. However, as airway responsiveness data is log-normally distributed (Postma and Boezen, 2004; Cockcroft et al., 1983), use of arithmetic mean PD data may affect the validity of some analyses in the Goodman et al. (2009) study. For example, in the study by Bylin et al. (1988) following exposure to 140 ppb NO_2, there was an arithmetic mean increase of 17% in the PD relative to filtered air, which was driven by a few individuals; whereas, the median and geometric mean show a 24% and 16% decrease, respectively, in the PD following NO_2 relative to filtered air exposure.

None of the above described meta-analyses provided a comprehensive assessment of the clinical relevance of changes in airway responsiveness, the potential for methodological biases in the original papers, or the distribution of responses. This section provides such analyses of airway responsiveness data and a discussion of factors that may have affected

the experimental determination of airway responsiveness as presented by Brown (2015). Detailed descriptions of individual studies are provided in the 1993 AQCD for Oxides of Nitrogen (U.S. EPA, 1993) and 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a). As done in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a), the fraction of individuals having an increase in airway responsiveness following NO_2 exposure was assessed. Due to considerable variability in exposure protocols and the potential for this variability in protocols to affect estimates of PD (see Factors Affecting Airway Hyperresponsiveness and Dose-response), the magnitude of NO_2-induced changes in PD was not evaluated in the original work by Folinsbee (1992) or in related EPA documents (U.S. EPA, 2008a) (U.S. EPA, 1993). Herein, the magnitude of the PD change for nonspecific agents is evaluated in studies that presented individual subject data for persons with asthma exposed to NO_2 at rest. The focus on resting exposures and nonspecific challenges when assessing the magnitude of change in PD (dPD) was due to the statistically significant effects of NO_2 exposure on airway responsiveness for these conditions as reported in the 2008 ISA for Oxides of Nitrogen [see Section 3.1.3.2 of`(U.S. EPA, 2008a)]. In assessing the magnitude of PD change, additional consideration was given to individuals experiencing a doubling-dose change in PD following exposure to NO_2 relative to filtered air. In a joint statement of the American Thoracic Society (ATS) and European Respiratory Society, one doubling dose change in PD is recognized as a potential indicator, although not a validated estimate, of clinically relevant changes in airway responsiveness (Reddel et al., 2009). Additional analyses also evaluate the distribution of PD responses to NO_2 and the concentration/dose-response relationship.

Methods

Study and Data Selection

Studies included in the meta-analyses were identified from the meta-analysis by Goodman et al. (2009), the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a), and from a literature search for controlled human exposure studies of individuals with asthma exposed to NO_2 that were published since the 2008 ISA. For inclusion into the meta-analyses, studies were required to provide data on the number of subjects whose airway responsiveness increased or decreased following exposure to NO_2 and filtered air. Only one new controlled human exposure study (Riedl et al., 2012) investigating the effect of NO_2 on airway responsiveness was published since the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a). The location and type of airway responsiveness data extracted from papers using both resting and exercising exposures is provided in Supplemental Table 5-2 (U.S. EPA, 2014g).

As an update to Table 3.1-2 in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a), Tables 5-2 and 5-3 present studies selected for inclusion into the meta-analyses. Relative to Table 3.1-2 in the 2008 ISA, Tables 5-2 and 5-3 include data that are either new or previously excluded (namely, specific allergen challenges had been intentionally excluded) for 155 subject exposures from nine studies (Riedl et al., 2012; Witten et al., 2005; Barck et al., 2002; Jenkins et al., 1999; Strand et al., 1998; Strand et al., 1997; Tunnicliffe et al., 1994; Morrow and Utell, 1989a; Orehek et al., 1976). In general, the subjects recruited for these studies ranged in age from 18 to 50 years with the exception of Avol et al. (1989) who studied children aged 8−16 years. The disease status of subjects was mild asthma in most studies, but ranged from inactive asthma up to severe asthma in a few studies.

Table 5-2 Resting exposures to nitrogen dioxide (NO_2) and airway responsiveness in individuals with asthma.

Reference	N	NO_2 ppb	Exp. (min)	Chall-enge Type	End Point	Time Post-exp min	Change in AR[a]		Average PD ± SE[b]		*p*-value[c]
							+	–	Air	NO_2	
Ahmed et al. (1983a)	20	100	60	CARB	sGaw	NA	13	7	6.0 ± 2.4	2.7 ± 0.8	NA
Ahmed et al. (1983b)	20	100	60	RAG	sGaw	IM	10	8	9.0 ± 5.7	11.7 ± 7.6	n.s.
Hazucha et al. (1983)	15	100	60	METH	sRaw	20	6	7	1.9 ± 0.4	2.0 ± 1.0	n.s.
Orehek et al. (1976)	20	100	60	CARB	sRaw	IM	14	3	0.56 ± 0.08	0.36 ± 0.05	<0.01[d]
Tunnicliffe et al. (1994)	8	100	60	HDM	FEV_1	IM	3	5	−14.62 ΔFEV_1	−14.41 ΔFEV_1	n.s.
Bylin et al. (1988)	20	140	30	HIST	sRaw	25	14	6	0.39 ± 0.07	0.28 ± 0.05	n.s.
Orehek et al. (1976)	4	200	60	CARB	sRaw	IM	3	0	0.60 ± 0.10	0.32 ± 0.02	n.s.
Jörres and Magnussen (1990)	14	250	30	SO_2	sRaw	27	11	2	46.5 ± 5.1	37.7 ± 3.5	<0.01
Barck et al. (2002)	13	260	30	BIR, TIM	FEV_1	240	5	7	−5 ± 2 ΔFEV_1	−4 ± 2 ΔFEV_1	n.s.
Strand et al. (1997)	18	260	30	BIR, TIM	sRaw	240	9	9	860 ± 450	970 ± 450	n.s.
Strand et al. (1998)	16	260	30	BIR	FEV_1	240	11	4	−0.1 ± 0.8 ΔFEV_1	−2.5 ± 1.0 ΔFEV_1	0.03
Bylin et al. (1988)	20	270	30	HIST	sRaw	25	14	6	0.39 ± 0.07	0.24 ± 0.04	<0.01

Table 5-2 (Continued): Resting exposures to nitrogen dioxide (NO2) and airway responsiveness in individuals with asthma

Reference	N	NO_2 ppb	Exp. (min)	Chall-enge Type	End Point	Time Post-exp min	Change in AR[a]		Average PD ± SE[b]		*p*-value[c]
							+	–	Air	NO_2	
Tunnicliffe et al. (1994)	8	400	60	HDM	FEV_1	IM	8	0	−14.62 ΔFEV_1	−18.64 ΔFEV_1	0.009
Bylin et al. (1985)	8	480	20	HIST	sRaw	20	5	0	>30	>20	0.04
Mohsenin (1987a)	10	500	60	METH	pEF	IM	7	2	9.2 ± 4.7	4.6 ± 2.6	0.042
Bylin et al. (1988)	20	530	30	HIST	sRaw	25	12	7	0.39 ± 0.07	0.34 ± 0.08	n.s.

AR = airway responsiveness; BIR = birch; CARB = carbachol; Exp. = exposure, FEV_1 = forced expiratory volume in 1 s; HDM = house dust mite allergen; HIST = histamine; IM = immediately after exposure; METH = methacholine; NA = not available; NO_2 = nitrogen dioxide; n.s. = less than marginal statistical significance, $p > 0.10$; pEF = partial expiratory flow at 40% vital capacity; RAG = ragweed; SO_2 = sulfur dioxide; sGaw = specific airway conductance; sRaw = specific airway resistance; TIM = timothy.

[a]Change in AR: number of individuals showing increased (+) or decreased (−) airway responsiveness after NO_2 exposure compared to air.

[b]PD ± SE: arithmetic or geometric mean provocative dose (PD) ± standard error (SE). See individual papers for PD calculation and dosage units. ΔFEV_1 indicates the change in FEV_1 response at a constant challenge dose.

[c]Statistical significance of increase in AR to bronchial challenge following NO_2 exposure compared to filtered air as reported in the original study unless otherwise specified. Statistical tests varied between studies, e.g., sign test, t-test, analysis of variance.

[d]Statistical significance for all individuals with asthma from analysis by Dawson and Schenker (1979). Orehek et al. (1976) only tested for differences in sub-sets of individuals classified as “responders” and “non-responders.”

Table 5-3 Exercising exposures to nitrogen dioxide (NO_2) and airwayresponsiveness in individuals with asthma.

Reference	n	NO_2 ppb	Exp. min	Challenge Type	End Point	Time Post-exp min	Change in AR[a]		Average PD ± SE[b]		*p*-value[c]
							+	−	Air	NO_2	
Roger et al. (1990)	19	150	80	METH	sRaw	120	10[d]	7[d]	3.3 ± 0.7	3.1 ± 0.7	n.s.
Kleinman et al. (1983)	31	200	120	METH	FEV_1	IM	20	7	8.6 ± 2.9	3.0 ± 1.1	<0.05
Jenkins et al. (1999)	11	200	360	HDM	FEV_1	IM	6	5	2.94	2.77	n.s.
Jörres and Magnussen (1991)	11	250	30	METH	sRaw	60	6	5	0.41 ± 1.6	0.41 ± 1.6	n.s.
Strand et al. (1996)	19	260	30	HIST	sRaw	30	13	5	296 ± 76	229 ± 56	0.08
Avol et al. (1988)	37	300	120	COLD	FEV_1	60	11[d]	16[d]	−8.4 ± 1.8 ΔFEV_1	−10.7 ± 2.0 ΔFEV_1	n.s.
Avol et al. (1989)	34	300	180	COLD	FEV_1	60	12[d]	21[d]	−5 ± 2 ΔFEV_1	−4 ± 2 ΔFEV_1	n.s.
Bauer et al. (1986)	15	300	30	COLD	FEV_1	60	9	3	0.83 ± 0.12	0.54 ± 0.10	<0.05
Morrow and Utell (1989a)	20	300	240	CARB	FEV_1	30	7[e]	2[e]	3.31 ± 8.64[e] ΔFEV_1	-6.98 ± 3.35[e] ΔFEV_1	n.s.
Roger et al. (1990)	19	300	80	METH	sRaw	120	8[d]	9[d]	3.3 ± 0.7	3.3 ± 0.8	n.s.

Table 5-3 (Continued): Exercising exposures to nitrogen dioxide (NO_2) and airway responsiveness in individuals with asthma.

Reference	n	NO_2 ppb	Exp. min	Challenge Type	End Point	Time Post-exp min	Change in AR[a]		Average PD ± SE[b]		*p*-value[c]
							+	−	Air	NO_2	
Rubinstein et al. (1990)	9	300	30	SO_2	sRaw	60	4	5	1.25 ± 0.23	1.31 ± 0.25	n.s.
Riedl et al. (2012)	15	350	120	METH	FEV_1	90	6	7	7.5 ± 2.6	7.0 ± 3.8	n.s.
Riedl et al. (2012)	15	350	120	CA	FEV_1	90	4	11	−6.9 ± 1.7 ΔFEV_1	−0.5 ± 1.7 ΔFEV_1	<0.05[f]
Jenkins et al. (1999)	10	400	180	HDM	FEV_1	IM	7	3	3.0	2.78	0.018
Witten et al. (2005)	15	400	180	HDM	FEV_1	IM	8	7	550 ± 240	160 ± 60	n.s.
Avol et al. (1988)	37	600	120	COLD	FEV_1	60	13[e]	16[e]	−8.4 ± 1.8 ΔFEV_1	−10.4 ± 2.2 ΔFEV_1	n.s.
Roger et al. (1990)	19	600	80	METH	sRaw	120	11[d]	8[d]	3.3 ± 0.7	3.7 ± 1.1	n.s.

AR = airway responsiveness; CARB = carbachol; CA = cat allergen; COLD = cold-dry air; Exp. = exposure; FEV_1 = forced expiratory volume in 1 s; HDM = house dust mite allergen; HIST = histamine; IM = immediately after exposure; METH = methacholine; NO_2 = nitrogen dioxide; n.s. = less than marginal statistical significance, $p > 0.10$; SO_2 = sulfur dioxide; sRaw = specific airway resistance.
[a]Change in AR: number of individuals showing increased (+) or decreased (−) airway responsiveness after NO_2 exposure compared to air.
[b]PD ± SE: arithmetic or geometric mean provocative dose (PD) ± standard error (SE). See individual papers for PD calculation and dosage units. ΔFEV_1 indicates the change in FEV_1 response at a constant challenge dose.
[c]Statistical significance of increase in AR to bronchial challenge following NO_2 exposure compared to filtered air as reported in the original study. Statistical tests varied between studies, e.g., sign test, t-test, analysis of variance.
[d]Number of individuals having an increase or decrease in airway responsiveness is from Folinsbee (1992).
[e]Data for 0.25% carbachol challenge from Appendix H of Morrow and Utell (1989b).
[f]Significantly greater ΔFEV_1 in response to a constant challenge dose following exposure to filtered air than NO_2, i.e., a protective effect of NO_2 exposure.

For studies that assessed airway responsiveness at multiple time points post-exposure or over repeated days of exposure, the data from the first time point and first day of exposure were selected for inclusion in Tables 5-2 and 5-3 to reduce the heterogeneity among studies. Selection of the earliest time point assessing airway responsiveness was, in part, due to late phase responses (3−8 hours post-allergen challenge) being mechanistically different from early phase responses (<30 minutes post-allergen challenge) (O'Byrne et al., 2009; Cockcroft and Davis, 2006c). It should be noted that Tables 5-2 and 5-3 are sorted by NO2 exposure concentration and, as such, studies that evaluated multiple NO2 exposure concentrations appear in multiple rows.

Fraction of Individuals with Nitrogen Dioxide-Induced Increase in Airway Responsiveness

Based on the summary data in Tables 5-2 and 5-3, the fraction of individuals experiencing an NO_2-induced increase in airway responsiveness was assessed in a manner consistent with the analysis conducted by Folinsbee (1992). Specifically, a two-tailed sign test was used to assess the statistical significance of directional changes in airway responsiveness between the NO_2 and filter air exposure days. The nonparametric

sign test, which assumes only that the responses of each subject are independent and makes no assumptions about the distribution of the response data, is appropriate to test the null hypothesis that observed values have the same probability of being positive or negative. This test allows estimation of whether a statistically significant fraction of individuals experience an increase or decrease in airway responsiveness, but does not provide information on the magnitude of the change in that endpoint. The significance of a two-tailed sign test was calculated in Microsoft® Office Excel® 2013 (subsequently, Excel®) as described by Currell and Dowman (2014).

Magnitude and Distribution of Nitrogen Dioxide-Induced Increase in Airway Responsiveness

Individual subject airway responsiveness data for non-specific challenges following resting exposures to filtered air and NO_2 were available for extraction from five studies (Jörres and Magnussen, 1990; Bylin et al., 1988; Mohsenin, 1987a; Bylin et al., 1985; Orehek et al., 1976). Data were obtained for 72 individuals and 116 NO_2 exposures. Twenty individuals in the Bylin et al. (1988) study were exposed to three NO_2 concentrations and four individuals in the Orehek et al. (1976) study were exposed to two NO_2 concentrations. The dPD due to NO_2 for each individual was assessed as:

$$\mathrm{dPD} = \frac{\mathrm{PD}_{\mathrm{NO_2}}}{\mathrm{PD}_{\mathrm{air}}}$$

Equation 5-1

where: PD_{NO_2} and PD_{air} are the PD following NO_2 and air exposures, respectively. Given that airway responsiveness is recognized as being log-normally distributed (Postma and Boezen, 2004; Cockcroft et al., 1983), this method of assessing dPD provides non-negative values for log transformation and plotting.

The distribution of dPD data (median and GSD) was determined for each study and overall for all subjects. To assess the distribution of dPD, the cumulative percentile for each datum was determined using the Excel® PERCENTRANK function. The lowest and highest dPD were assigned the cumulative probabilities of 0.1% and 99.9% rather than the 0 and 1 assigned by Excel®. Next, the standard normal deviate (z) for each cumulative percentile was determined using the Excel® NORMSINV function. The natural logarithms of the dPD were subsequently regressed against their corresponding z-values using the Excel® INTERCEPT and SLOPE functions. The median equals e (base of natural logarithms, 2.71828) raised to the power of the intercept and the GSD equals e raised to the power of the slope.

To assess the potential "adversity" or clinical relevance of changes in dPD, a sign test was utilized to determine whether there were a statistically greater number of individuals

experiencing a doubling dose reduction in dPD (<0.5) versus those having a doubling dose increase in dPD (>2). The equations used for this sign test are the same as those described above for determining the fraction of individuals with an NO_2-induced increase in airway responsiveness. A sensitivity analysis was performed to ensure that no single study or group of exposures affected the distribution of dPD and assessment of a doubling dose change. The sensitivity analysis was performed by removing entire studies or repeated subject exposures to multiple concentrations in two studies. Additionally, broad ranges of NO_2 exposure concentrations (e.g., the upper or lower half of the data) were excluded for the sensitivity analyses to see if specific exposure concentrations were driving results. Finally, dose-response was assessed by regressing the logarithms of dPD against NO_2 exposure concentration and against the product of NO_2 exposure concentration and duration using the Excel® Regression Data Analysis tool.

Results

Fraction of Individuals with Nitrogen Dioxide-Induced Increase in Airway Responsiveness

Tables 5-2 and 5-3 provide all studies with data on the fraction of individuals experiencing a change (increase or decrease) in airway responsiveness following both NO_2 and filtered air exposures. The statistical significance reported in the original publications for changes in airway responsiveness following NO_2 exposure compared to filtered air is also provided in these tables. Based on all listed studies, the general tendency of most studies is toward increased airway responsiveness following NO_2 exposure with some studies reaching statistical significance. Fewer studies showed no effect or a tendency for decreased airway responsiveness following NO_2. Published since the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a), one study reported a statistically significant decrease in airway responsiveness following NO_2, but the authors attributed the protective effect of NO_2 to chance (Riedl et al., 2012).

Tables 5-4, 5-5, and 5-6 present the fraction of individuals experiencing an NO_2-induced increase in airway responsiveness to non-specific agents, specific allergens, and all challenge types, respectively. Footnotes for these tables indicates the group from Tables 5-2 and 5-3 that were included in the analyses. For example, in Table 5-4 Footnote C, the results for resting exposures (see Table 5-2) to 100 ppb NO_2 are for the 33 individuals having an increase in nonspecific responsiveness and the 17 individuals having a decrease in nonspecific responsiveness in the studies by Ahmed et al. (1983a), Hazucha et al. (1983), and Orehek et al. (1976). Table 5-4 updates Table 3.1-3 of the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a) and is consistent with the prior conclusion that statistically significant increases in nonspecific airway responsiveness

(following resting NO_2 exposures) occur in the range of 200 and 300 ppb NO_2 for 30-minute exposures and at 100 ppb NO_2 for 60-minute exposures in individuals with asthma. Increases in airway responsiveness were not observed following the exercising exposures to NO_2. In general, Table 5-5 does not show statistically significant effects of NO_2 exposure on airway responsiveness to allergen challenge, except at NO_2 concentrations over 300 ppb. This may be, in part, due to the small number of individuals in the analysis. Considering both specific and nonspecific challenges, Table 5-6 shows statistically significant effects of NO_2 on airway responsiveness for resting but not exercising exposures as was also shown for nonspecific challenges in Table 5-4. However, given differing mechanisms of effect (see discussion of Bronchial Challenge Agent later in this section), preference should be given to the analysis of nonspecific responsiveness (Table 5-4) over the combined analysis of specific and nonspecific agents (Table 5-6).

Table 5-4 Fraction of individuals with asthma having nitrogen dioxide (NO_2)-induced increase in airway responsiveness to a non-specific challenge.

NO_2 Concentration (ppb)	All Exposures[a,b]	Exposure with Exercise[a,b]	Exposure at Rest[a,b]
$[NO_2] = 100$	0.66 (50; $p = 0.033$)	-	0.66 (50; $p = 0.033$)[c]
$100 \leq [NO_2] < 200$	0.66 (87; $p = 0.005$)	0.59 (17; n.s.)[d]	0.67 (70; $p = 0.006$)[e]
$200 \leq [NO_2] \leq 300$	0.59 (199; $p = 0.011$)	0.55 (163; n.s.)[f]	0.78 (36; $p = 0.001$)[g]
$[NO_2] > 300$	0.57 (94; n.s.)	0.49 (61; n.s.)[h]	0.73 (33; $p = 0.014$)[i]
All $[NO_2]$	0.60 (380; $p < 0.001$)	0.54 (241; n.s.)	0.71 (139; $p < 0.001$)

n.s. = less than marginal statistical significance ($p > 0.10$).
[a]Data are the fraction of subjects with asthma having an increase in airway responsiveness following NO_2 vs. air exposure. Values in parentheses are number of individuals with asthma having a change (±) in responsiveness and the *p*-value for a two-tailed sign test.
[b]Analysis is for the 380 subjects with asthma in Tables 5-2 and 5-3 having a change (+/−) in non-specific airway responsiveness.
[c]33 increases, 17 decreases; 100 ppb data from Ahmed et al. (1983a), Hazucha et al. (1983), and Orehek et al. (1976).
[d]10 increases, 7 decreases; 150 ppb data from Roger et al. (1990).
[e]47 increases, 23 decreases; 100 ppb data from Ahmed et al. (1983a), Hazucha et al. (1983), and Orehek et al. (1976); 140 ppb data from.Bylin et al. (1988).
[f]90 increases, 73 decreases; 200 ppb data from Kleinman et al. (1983); 250 ppb data from Jörres and Magnussen (1991); 260 ppb data from Strand et al. (1996); 300 ppb data from Avol et al. (1988), Avol et al. (1989), Bauer et al. (1986), Morrow and Utell (1989a), Roger et al. (1990), and Rubinstein et al. (1990).
[g]28 increases, 8 decreases; 200 ppb data from Orehek et al. (1976); 250 ppb data from Jörres and Magnussen (1990); 270 ppb data from Bylin et al. (1988).
[h]30 increases, 31 decreases; 350 ppb data from Riedl et al. (2012); 600 ppb data from Avol et al. (1988) and Roger et al. (1990).
[i]24 increases, 9 decreases; 480 ppb data from Bylin et al. (1985); 500 ppb data from Mohsenin (1987a); 530 ppb data from Bylin et al. (1988).

Table 5-5 Fraction of individuals with asthma having nitrogen dioxide (NO_2)-induced increase in specific airway responsiveness to an allergen challenge.

NO_2 Concentration (ppb)	All Exposures[a,b]	Exposure with Exercise[a,b]	Exposure at Rest[a,b]
$[NO_2] = 100$	0.50 (26; n.s.)	-	0.50 (26; n.s.)[c]
$100 \leq [NO_2] < 200$	0.50 (26; n.s.)	-	0.50 (26; n.s.)[c]
$200 \leq [NO_2] \leq 300$	0.55 (56; n.s.)	0.55 (11; n.s.)[d]	0.56 (45; n.s.)[e]
$[NO_2] > 300$	0.56 (48; n.s.)	0.48 (40; n.s.)[f]	1.00 (8; $p = 0.008$)[g]
All $[NO_2]$	0.55 (130; n.s.)	0.49 (51; n.s.)	0.58 (79; n.s.)

n.s., less than marginal statistical significance ($p > 0.10$), NO_2 = nitrogen dioxide.
[a]See Footnote a of Table 5-4.
[b]Analysis is for the 130 subjects with asthma in Tables 5-2 and 5-3 having a change (+/−) in specific allergen airway responsiveness.
[c]13 increases, 13 decreases; 100 ppb data from Ahmed et al. (1983b) and Tunnicliffe et al. (1994).
[d]6 increases, 5 decreases; 200 ppb data from Jenkins et al. (1999).
[e]25 increases, 20 decreases; 260 ppb data from Barck et al. (2002), Strand et al. (1997), and Strand et al. (1998).
[f]19 increases, 21 decreases; 350 ppb data from Riedl et al. (2012); 400 ppb data from Jenkins et al. (1999) and Witten et al. (2005).
[g]8 increases, 0 decreases; 400 ppb data from Tunnicliffe et al. (1994).

Table 5-6 Fraction of individuals with asthma having nitrogen dioxide (NO_2)-induced increase in airway responsiveness regardless of challenge types.

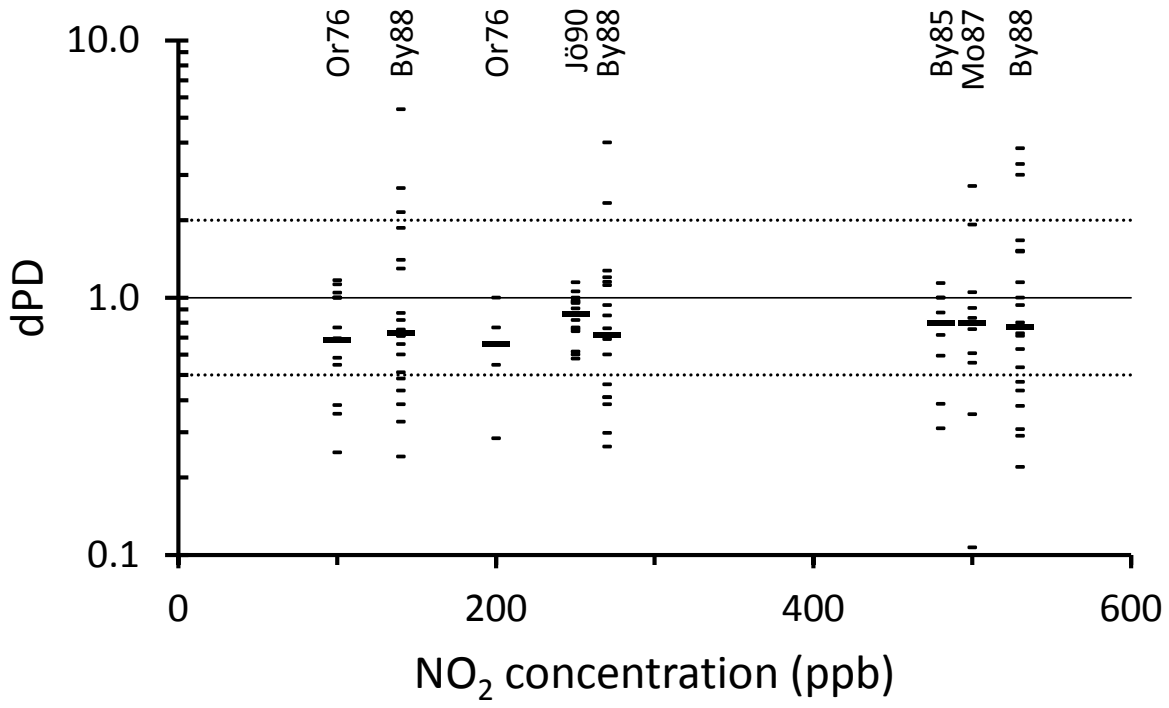
NO_2 Concentration (ppb)	All Exposures[a,b]	Exposure with Exercise[a,b]	Exposure at Rest[a,b]
$[NO_2] = 100$	0.61 (76; $p = 0.08$)	-	0.61 (76; $p = 0.08$)[c]
$100 \leq [NO_2] < 200$	0.62 (113; $p = 0.014$)	0.59 (17; n.s.)[d]	0.63 (96; $p = 0.018$)[e]
$200 \leq [NO_2] \leq 300$	0.58 (255; $p = 0.008$)	0.55 (174; n.s.)[f]	0.65 (81; $p = 0.007$)[g]
$[NO_2] > 300$	0.57 (142; n.s.)	0.49 (101; n.s.)[h]	0.78 (41; $p < 0.001$)[i]
All $[NO_2]$	0.59 (510; $p < 0.001$)	0.53 (292; n.s.)	0.67 (218; $p < 0.001$)

n.s., less than marginal statistical significance ($p > 0.10$), NO_2 = nitrogen dioxide.
[a]See Footnote a of Table 5-4.
[b]Analysis is for the 510 subjects with asthma in Tables 5-2 and Table 5-3 having a change (+/−) in airway responsiveness.
[c]46 increases, 30 decreases; 100 ppb data from Ahmed et al. (1983a), Ahmed et al. (1983b) Hazucha et al. (1983), Orehek et al. (1976), and Tunnicliffe et al. (1994).
[d]10 increases, 7 decreases; 150 ppb data from Roger et al. (1990).
[e]60 increases, 36 decreases; 100 ppb data from Ahmed et al. (1983a), Hazucha et al. (1983), Orehek et al. (1976), Ahmed et al. (1983b), and Tunnicliffe et al. (1994); 140 ppb data from.Bylin et al. (1988).
[f]96 increases, 78 decreases; 200 ppb data from Kleinman et al. (1983) and Jenkins et al. (1999); 250 ppb data from Jörres and Magnussen (1991); 260 ppb data from Strand et al. (1996); 300 ppb data from Avol et al. (1988), Avol et al. (1989), Bauer et al. (1986), Morrow and Utell (1989a), Roger et al. (1990), and Rubinstein et al. (1990).
[g]53 increases, 28 decreases; 200 ppb data from Orehek et al. (1976); 250 ppb data from Jörres and Magnussen (1990); 260 ppb data from Barck et al. (2002), Strand et al. (1997), and Strand et al. (1998); 270 ppb data from Bylin et al. (1988).
[h]49 increases, 52 decreases; 350 ppb data from Riedl et al. (2012); 400 ppb data from Jenkins et al. (1999) and Witten et al. (2005); 600 ppb data from Avol et al. (1988) and Roger et al. (1990).
[i]32 increases, 9 decreases; 400 ppb data from Tunnicliffe et al. (1994); 480 ppb data from Bylin et al. (1985); 500 ppb data from Mohsenin (1987a); 530 ppb data from Bylin et al. (1988).

Magnitude and Distribution of Nitrogen Dioxide-Induced Increase in Airway Responsiveness

The dPD for each individual was calculated as the PD following NO_2 divided by the PD following air exposure. Hence, a dPD greater than one suggests reduced responsiveness, whereas a dPD less than one suggests increased responsiveness following NO_2 exposure. The dPD from the five studies providing individual PD data following resting exposures to NO_2 and filtered air are illustrated in Figure 5-1. All of the median responses illustrated in Figure 5-1 show increased responsiveness following NO_2 exposure, i.e., an NO_2-induced reduction in the PD. It should be noted in Figure 5-1 that the dPD are on a log scale. The untransformed dPD data from Bylin et al. (1988) and Mohsenin (1987a) were positively skewed with a few individuals having large values of dPD. This results in a large difference between the median dPD and arithmetic mean dPD. For example, at the 140 ppb concentration in the Bylin et al. (1988) study, the median dPD of 0.73 suggests NO_2 increased responsiveness, which is consistent with 14 individuals having an increase in responsiveness versus 6 having a decrease, whereas the arithmetic mean dPD of 1.15 suggests a reduction in responsiveness. The untransformed dPD data from Bylin et al. (1985), Jörres and Magnussen (1990), and Orehek et al. (1976) were more symmetrical

than Bylin et al. (1988) and Mohsenin (1987a). For the full data set in Figure 5-1, un-transformed dPD had a skew of 3.0 (using Excel® SKEW function), whereas the log-transformed data had a skew of only 0.2.



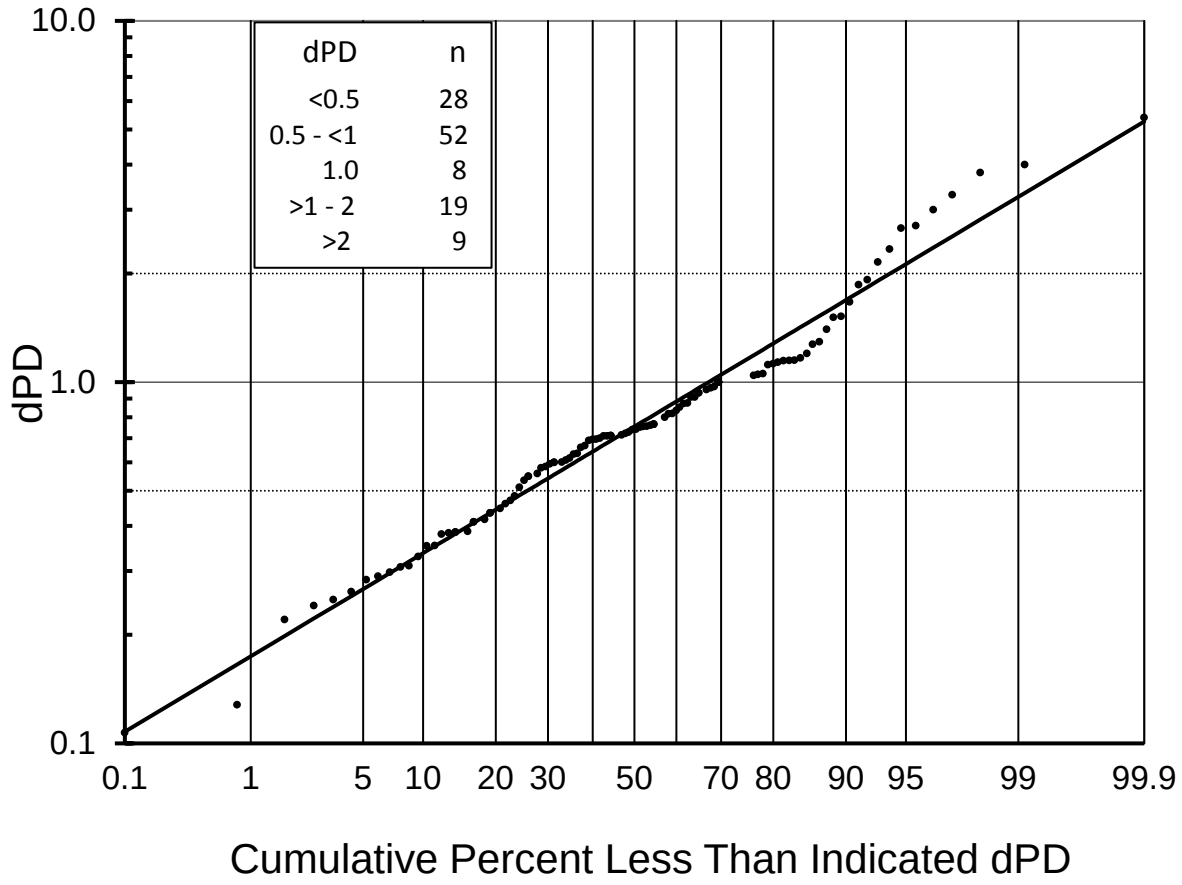
Note: Points illustrate the responses of 72 individual subjects, and bars are median responses. Doubling dose changes are illustrated by horizontal dotted lines. Data are from Or76 (Orehek et al., 1976), By88 (Bylin et al., 1988), Jö90 (Jörres and Magnussen, 1990), By85 (Bylin et al., 1985), and Mo87 (Mohsenin, 1987a).

Figure 5-1 Change in provocative dose (dPD) due to exposure to nitrogen dioxide (NO_2) in resting individuals with asthma.

A clinically relevant change in dPD is indicated by a doubling dose change, i.e., dPD >2 or <0.5. A clinically relevant, doubling dose, NO_2-induced increase in responsiveness (dPD <0.5) was observed in 24% of the data, while 8% had a double dose decrease in responsiveness (dPD >2). Of the 28 responses where dPD was <0.5, 17 were from Bylin et al. (1988). Of the nine responses where dPD was >2, eight were again from the Bylin et al. (1988) study. Subject 1 in the Bylin et al. (1988) study had the three highest dPD in Figure 5-1, which generally reflects the reproducibility of response. For all subjects in the Bylin et al. (1988) study, the Spearman's rank correlation between the 140 and 530 ppb exposures was 0.56 (p = 0.01) and was 0.48 (p = 0.03) between the 270 ppb

exposure and both the 140 and 530 ppb exposures. Clearly this study has the potential to affect both the assessment of a doubling dose change in dPD as well as the distribution of responses.

Figure 5-2 illustrates a log-probability plot of the dPD data. The data are log-normally distributed with an estimated (from fitted line on plot) median of 0.75 and a GSD of 1.88. The lowest and highest dPD were assigned the cumulative probabilities of 0.1% and 99.9%. Removing these two values did not affect the median and only slightly reduced the geometric standard deviation from 1.88 to 1.87. Most of the data (namely 69%) suggests an NO_2-induced increase in responsiveness (dPD <1) due to NO_2 exposure, while 24% of the data suggests decrease responsiveness (dPD >1). Consistent with the results in Table 5-4, a two-tailed sign test shows a statistically significant ($p < 0.001$) reduction in the dPD in 74% of the 108 dPD responses not equal to one. Of the 37 dPD having more than a doubling dose change, 76% show a clinically relevant NO_2-induced reduction in dPD ($p = 0.003$; two-tailed sign test).



Note: Data are for 72 individuals and 116 NO_2 exposures illustrated in Figure 5-1. Line is log-normal fit (0.75, median dPD; 1.88, geometric standard deviation). Table within figure is the number of observations within intervals of dPD. Doubling dose changes are illustrated by horizontal dotted lines. The discontinuity between the 70th and 77th percentiles is due to 8 of the 116 dPD being equal to one.

Figure 5-2 Log-normal distribution of change in provocative dose (dPD) due to exposure to nitrogen dioxide in resting individuals with asthma.

Table 5-7 provides a sensitivity analysis for the distribution of responses and NO_2-induced increases in responsiveness. The first row of the table provides the results based on all dPD for all 72 individuals and 116 NO_2 exposures in five studies (Jörres and Magnussen, 1990; Bylin et al., 1988; Mohsenin, 1987a; Bylin et al., 1985; Orehek et al., 1976). Subsequent rows show results with specific studies excluded. Both Bylin et al. (1988) and Orehek et al. (1976) included multiple exposure concentrations. For rows examining results with exclusion of these two studies, the first row excludes the entire study (all exposure concentrations) with subsequent rows excluding data for specific exposure concentrations from these studies. The last row of Table 5-7 provides results excluding all but the lowest exposure concentration from both Bylin et al. (1988) and Orehek et al. (1976). The sensitivity analysis shows that the NO_2-induced increase in airway responsiveness overall and the clinically relevant, doubling dose increase in responsiveness were robust to exclusion of individual studies and subparts of studies with multiple exposures. Also evaluated in this sensitivity analysis, the concentration range of the dataset was split into roughly halves and thirds to determine if effects were more marked for a specific range of concentrations. Dividing the dataset in half, effects were slightly stronger when concentrations >250 ppb were excluded than when concentrations ≤250 ppb were excluded. When dividing the dataset in thirds, effects were least evident when excluding concentrations <480 ppb and doubling dose changes were found only for the lowest concentration range (i.e., >140 ppb excluded), although those doubling dose changes were only marginally significant ($p = 0.057$). These findings suggest more of an NO_2 effect on airway responsiveness following lower concentration exposures.

Using the full dPD dataset of 116 exposures, linear regression did not show an association between log-transformed dPD and either NO_2 concentration ($p = 0.44$) or concentration × exposure duration ($p = 0.89$).

Table 5-7 Sensitivity analysis for distribution of responses and nitrogen dioxide (NO_2)-induced increase in responsiveness to a nonspecific challenge.

Population	Distribution[a]	All dPD Responses[b]	Doubling Dose dPD Only[c]
All 5 studies[d]	0.75 (1.88)	0.74 (108; $p < 0.001$)	0.76 (37; $p = 0.003$)
Studies excluded from analysis:			
Bylin et al. (1985)[e]	0.76 (1.89)	0.74 (102; $p < 0.001$)	0.74 (35; $p = 0.006$)
Bylin et al. (1988) NO_2 of 140, 270, and 270 ppb[e]	0.70 (1.64)	0.82 (49; $p < 0.001$)	0.92 (12; $p = 0.006$)
NO_2 of 140 and 270 ppb[f]	0.73 (1.81)	0.76 (68; $p < 0.001$)	0.81 (21; $p = 0.007$)
NO_2 of 140 and 530 ppb[f]	0.71 (1.72)	0.78 (69; $p < 0.001$)	0.85 (20; $p = 0.003$)
NO_2 of 270 and 530 ppb[f]	0.73 (1.78)	0.78 (69; $p < 0.001$)	0.80 (20; $p = 0.012$)
Jörres and Magnussen (1990)[e]	0.74 (1.94)	0.73 (95; $p < 0.001$)	0.76 (37; $p = 0.003$)
Mohsenin (1987a)[e]	0.76 (1.83)	0.74 (98; $p < 0.001$)	0.76 (34; $p = 0.003$)
Orehek et al. (1976) NO_2 of 100 and 200 ppb[e]	0.80 (1.89)	0.72 (88; $p < 0.001$)	0.70 (30; $p = 0.043$)
NO_2 of 100 ppb[f]	0.79 (1.89)	0.73 (91; $p < 0.001$)	0.71 (31; $p = 0.029$)
NO_2 of 200 ppb[f]	0.76 (1.89)	0.73 (105; $p < 0.001$)	0.75 (36; $p = 0.004$)
Bylin et al. (1988) NO_2 of 270 and 530 ppb[f] and Orehek et al. (1976) NO_2 of 200 ppb[f]	0.74 (1.78)	0.77 (66; $p < 0.001$)	0.79 (19; $p = 0.019$)
Concentrations excluded from analysis:			
>140 ppb	0.71 (1.81)	0.77 (37; $p = 0.003$)	0.79 (14; $p = 0.057$)
≤140 or >270 ppb	0.77 (1.56)	0.78 (36; $p = 0.001$)	0.78 (9; n.s.)
<480 ppb	0.78 (1.93)	0.69 (35; $p = 0.041$)	0.71 (14; n.s.)
>250 ppb	0.73 (1.71)	0.79 (53; $p < 0.001$)	0.80 (15; $p = 0.035$)
≤250 ppb	0.77 (1.93)	0.69 (55; $p = 0.006$)	0.73 (22; $p = 0.052$)

n.s. = less than marginal statistical significance ($p > 0.10$), NO_2 = nitrogen dioxide, dPD = change in provocative dose.

[da]Data are for 72 individuals and 116 NO_2 exposures illustrated in Figures 5-1 and 5-2 and from Orehek et al. (1976), Bylin et al. (1988), Jörres and Magnussen (1990), Bylin et al. (1985), and Mohsenin (1987a).

[b]Median (geometric standard deviation) of dPD data.

[c]Data are the fraction of subjects with asthma having an increase in airway responsiveness following NO_2 vs. air exposure. Values in parentheses are number of individuals with asthma having a change (±) in non-specific airway responsiveness and the *p*-value for a two-tailed sign test.

[d]Data are the fraction of subjects with asthma having a doubling dose reduction in dPD due to NO_2 exposure. Values in parentheses are number of individuals with asthma having at least a doubling dose change (±) in non-specific airway responsiveness and the *p*-value for a two-tailed sign test.

[e]Entire study deleted.

[f]Specific concentrations deleted from study with multiple exposure concentrations.

Discussion

The analyses conducted here show that the airway responsiveness of individuals with asthma is increased by brief exposures to NO_2. There was a statistically significant fraction of individuals with asthma exposed to NO_2 at rest, which experienced an increase in responsiveness. About 70% had an increase in nonspecific airway responsiveness following 30-minute exposures to NO_2 in the range of 200 to 300 ppb and following 60-minute exposures to 100 ppb. The median response of these individuals is an NO_2-induced reduction in dPD to 0.75 (1.88, geometric standard deviation). About a quarter of the exposed individuals experienced a clinically relevant, doubling dose reduction in their dPD due to NO_2 exposure. The fraction experiencing a doubling dose increase in responsiveness was also statistically significant and robust to exclusion of individual studies. Results showed minimal change in airway responsiveness for individuals exposed to NO_2 during exercise. The remainder of this discussion considers a variety of factors that may affect the assessment of airway responsiveness and how those factors may have directionally biased the results of individual studies and the analyses conducted as part of this assessment.

Exercise

In considering why increases in airway responsiveness occurred only after resting exposure to NO_2, Folinsbee (1992) and Bylin (1993) suggested that exercise itself may affect the mechanisms responsible for increased responsiveness. Based on the literature at that time, both of these authors noted that exercise may cause a refractory period during which airway responsiveness to challenge is diminished. Specifically, airway responsiveness to methacholine had been observed to be reduced following exercise (Inman et al., 1990). A more rapid reversal of methacholine-induced bronchoconstriction had also been observed following periods of exercise as compared to rest (Freedman et al., 1988). Additionally, the refractory period from exercise had been found to correlate with the responsiveness to methacholine; i.e., individuals who experienced a smaller bronchoconstrictive response following repeated bouts of exercise subsequently also had a smaller response to methacholine challenge (Magnussen et al., 1986). Recent literature continues to support the possibility that exercise may lead to a period of reduced airway responsiveness. The review by O'Byrne et al. (2009) noted with repeated bouts of exercise, the bronchoconstrictive response to exercise can be abolished in many individuals with asthma. The most probable mechanism explaining this exercise refractory period is the release of inhibitory prostaglandins that partially protect the airways. There may also be changes in eicosanoids associated with NO_2 exposure itself (Sections 4.3.2.3 and 5.2.2.5). Refractory periods following exercise of 40 minutes to 3 hours has been reported (Dryden et al., 2010).

A comparison of two studies that utilized the same challenge agent following the same duration of NO_2 exposure and nearly the same exposure concentration supports the conclusion that exercise may diminish the subsequent responsiveness to bronchial challenge. Jörres and Magnussen (1990) found a statistically significant increase in airway responsiveness to a SO_2 challenge in subjects with asthma following exposure to 250 ppb NO_2 for 30 minutes at rest; whereas, Rubinstein et al. (1990) found no change in responsiveness to a SO_2 challenge following exposure of subjects with asthma to 300 ppb NO_2 for 30 minutes with 20 minutes of exercise.

Overall, the literature on airway responsiveness supports the development of a refractory period following bouts of exercise. An effect of exercise refractoriness is consistent with greater increases in airway responsiveness following resting than exercising exposures to NO_2 as shown in Table 5-4.

Bronchial Challenge Delivery and Assessment

Variations in methods for administering the bronchoconstricting agents may substantially affect the results (Cockcroft and Davis, 2006b; Cockcroft et al., 2005). A repeated measures study of 55 subjects with asthma evaluating two ATS-recommended methods of methacholine delivery found a highly statistically significant ($p < 0.00001$), twofold difference in PC_{20} which was attributable to the delivery method (Cockcroft and Davis, 2006b). Even in the same subjects exposed by the same investigators in the same facility to the same bronchial challenge agent, there can be a doubling dose difference due to the delivery method. The difference observed by Cockcroft and Davis (2006b) may, in part, be due to the use of full vital capacity inspirations with breath-hold as part of the delivery technique that yielded the higher PC_{20}. The maximal lung inflations are recognized to induce bronchodilation.

The full vital capacity inspiration required for FEV_1 measurements when assessing airway response to challenge may cause a partial reversal of bronchospasm versus the use of other measures such as specific airway resistance or conductance (Jackson et al., 2004; Beaupré and Orehek, 1982; Orehek et al., 1981). It is likely that the use of forced vital capacity (FVC) maneuvers contributed to the lack of statistically significant effects in NO_2 studies employing exercising exposures and specific allergen challenges. For nonspecific challenges (Table 5-4), responsiveness was assessed using FVC maneuvers in only 6% of 139 individuals exposed at rest versus 62% of 241 individuals exposed during exercise. For specific allergen challenges (Table 5-5), responsiveness was assessed using FVC maneuvers in 54% of 79 individuals exposed at rest and 100% of 51 individuals exposed during exercise. Thus, the preferential use of FVC maneuvers in studies exposing individuals to NO_2 during exercise as well as in studies evaluating responsiveness to specific allergens could have contributed to not finding statistically

significant effects of NO_2 exposure on airway responsiveness. Where statistically significant effects were observed, generally the studies using resting exposures and nonspecific challenge agents, FVC maneuvers were seldom used to assess responsiveness. Consistent with the results in Tables 5-4 and 5-5, the use of FVC maneuvers may have biased NO_2 studies using exercise and specific allergen challenges toward the null.

Bronchial Challenge Agent

Bronchial challenge agents differ in the mechanisms by which they cause bronchoconstriction, acting either "directly" or "indirectly" on bronchial smooth muscle receptors. Even similarly delivered nonspecific, direct acting agents may affect the lung differently. In a comparison of responses to methacholine and histamine in healthy volunteers not having AHR, Verbanck et al. (2001) reported that histamine caused an overall narrowing of the airways (i.e., similar between parallel lung regions), whereas methacholine caused a differential narrowing of parallel airways, which altered ventilation distribution. The differential effects of these two direct acting agents may, in part, be due to their differing target receptors and the distribution of these receptors in the airways (O'Byrne et al., 2009). Comparison of the airway responsiveness among bronchial challenge agents is complicated by the differing mechanisms by which they initiate bronchoconstriction.

The lack of statistical significance in Table 5-5 does not necessarily diminish the potential importance of allergen exposures. First, as described above, use of FVC maneuvers in NO_2 studies may have biased results toward not finding an effect on airway responsiveness. Second, 80% of children with asthma are thought to be sensitized to common household allergens (O'Byrne et al., 2009). Third, individuals with asthma may experience an early phase response to allergen challenge with declines in lung function within 30 minutes, which primarily reflects release of histamine and other mediators by airway mast cells. Approximately half of those individuals having an early phase response also have a late phase response with a decline in lung function 3−8 hours after the challenge, which reflects enhanced airway inflammation and mucous production (O'Byrne et al., 2009; Cockcroft and Davis, 2006c). The early response may be reversed with bronchodilators, whereas the late response requires steroidal treatment. Studies have reported NO_2-induced effects on allergen responsiveness for both the early phase (Jenkins et al., 1999; Strand et al., 1998; Tunnicliffe et al., 1994) and late phase (Strand et al., 1998; Tunnicliffe et al., 1994). These effects were observed following 30-minute resting exposures to concentrations as low as 260 ppb NO_2. Finally, the response to an allergen is not only a function of the concentration of inhaled allergen, but also the degree of sensitization as measured by the level of allergen-specific IgE and responsiveness to

nonspecific agents (Cockcroft and Davis, 2006a). These factors make it difficult to predict the level of responsiveness to an allergen, and although rare, severe bronchoconstriction can occur with inhalation of very low allergen concentrations (O'Byrne et al., 2009). Given the ubiquity of allergens and potential severity of responses to allergen inhalation, that NO_2 exposure might augment these responses is of concern. The responsiveness to allergens in animals and humans is also addressed in Sections 4.3.2.6 and 5.2.2.5.

Subject Inclusion/Exclusion

Exercise is a major trigger of asthma symptoms in between 60 and 90% of people with asthma (Dryden et al., 2010). In their study of NO_2 effects on airway responsiveness, Roger et al. (1990) reported that all of their volunteers with asthma experienced either cold air or exercise-induced bronchoconstriction. Morrow and Utell (1989a) reported that, "Many of the asthmatic subjects were unable to undertake the carbachol challenge after either NO_2 or air exposures, presumably because of pre-existing exercise-induced bronchoconstriction." Consequently, in their study, data on changes in airway responsiveness were only available for 9 of 20 subjects. Thus, the existence of exercise-induced bronchospasm and symptoms may have caused an underlying difference in the health status of subjects for which airway responsiveness was evaluated between studies involving resting versus exercising exposures. Assessing those individuals with less responsive airways could bias results toward not finding an effect of NO_2 on airway responsiveness in studies utilizing exercising exposures.

Medication Usage

There was a wide range in restrictions on asthma medication usage among NO_2 studies. It is recommended that short-acting bronchodilators be stopped 8 hours before and long-acting bronchodilators 36 hours before the bronchial challenge (Reddel et al., 2009). Even after withholding salmeterol (a long-acting bronchodilator) for 24 hours, there is still a greater than twofold reduction in airway responsiveness relative to an unmedicated baseline (Reddel et al., 2009). In their NO_2 study, Hazucha et al. (1983) required that subjects not receive steroid therapy or daily bronchodilator therapy for a month prior to bronchial challenge testing. Other NO_2 study investigators recorded asthma medication usage and asked subjects to refrain from usage for defined periods of time depending on the medication, such as 8 hours for short-acting bronchodilators [e.g., (Witten et al., 2005; Avol et al., 1988)]. Restrictions were far less in some studies, for example, Kleinman et al. (1983) asked subjects to withhold bronchodilators for at least 4 hours prior to exposure, but subjects were not excluded for non-compliance because medication usage was generally balanced between filtered air and NO_2 exposure days. Still other studies provided no indication of asthma medications or prohibitions for study inclusion

[e.g., (Bylin et al., 1988)]. Pretreatment (500 mg, 4 times per day for 3 days) with ascorbic acid was shown to prevent NO_2-induced increases in airway responsiveness of healthy individuals (Mohsenin, 1987b). Thus, the use of asthma medications or dietary supplements may have reduced the ability of studies to identify an effect of NO_2 on airway responsiveness and may have affected observed provocative doses.

Airway Caliber

Bylin (1993) suggested that NO_2 may have a direct effect on airway smooth muscle, possibly relaxing and inducing mild bronchodilation at higher NO_2 doses. Consistent with this supposition, Bylin et al. (1985) reported statistically significant decreases in sRaw following exposure to 480 ppb NO_2 in healthy individuals, and a similar trend for sRaw decreases in individuals with asthma. Bronchoconstriction shifts the deposition site of challenge agents proximally, whereas bronchodilation shifts the deposition site more distally. Decreasing the surface dose in the bronchi may in turn decrease the responsiveness to the challenge.

The importance of particle dosimetry (which is affected by factors such as inhaled particle size, airway dimensions, and breathing rates) on airway responsiveness has been investigated in numerous studies. Some of the more conclusive findings are described here. Moss and Oldham (2006) found that the dose of methacholine producing a 200% increase in airway resistance in Balb/c mice and B6C3F1 mice was equivalent in terms of the amount deposited within the first six generations of airways. Wanner et al. (1985) found a strong correlation between the decrease in FEV_1 following histamine challenge and the estimated histamine dose to the airways of 10 smokers ($r = -0.82$, $p < 0.005$) and 10 nonsmokers ($r = -0.83$, $p < 0.005$). In a study of 19 individuals with asthma, Casset et al. (2007) found that the PD_{20} of house dust mite (HDM) allergen decreased with increasing inhaled particle size from 1 μm to 10 μm (mass median aerodynamic diameter). As inhaled particle size was increased, the pattern of particle deposition would be expected to move toward the larger more central airways. These studies demonstrate lower airway responsiveness for distal versus proximal deposition of challenge agents; and thus, are supportive of the supposition proposed by Bylin (1993).

Simply considering airway caliber may not adequately capture the complexity and anatomical heterogeneity of lung disease from asthma. In a comparison of individuals with asthma and healthy controls, Laube et al. (1992) reported that increasing heterogeneity in particle deposition was significantly associated with decreasing PD_{20} to methacholine. Heterogeneity in deposition is, in part, due to heterogeneity in ventilation distribution. In another study of individuals with asthma, Downie et al. (2007) found heterogeneity in ventilation distribution to be a predictor of airway responsiveness independent of airway inflammation and airway caliber.

The literature supports an effect of the surface dose of challenge agents to the conducting airways on airway responsiveness. The dose of bronchial challenge agents to the conducting airways may have been affected by numerous factors within and among studies evaluating the effect of NO_2 on airway responsiveness. Although it is clear that such factors could contribute to variability within and among studies, the available information is insufficient to support an effect such as decreased airway responsiveness at higher NO_2 concentrations due to bronchodilation.

Effect of Challenge Time Following Nitrogen Dioxide Exposure

With respect to the data in Tables 5-2 and 5-3, bronchial challenges were delivered an average of 60 minutes post-exposure. For non-specific agents, on average, challenges were delivered 16 minutes following resting exposures and 67 minutes following exercise exposures ($p < 0.01$). Although challenges may take upwards of 40 minutes to complete (Mohsenin, 1987a), the difference in the time when challenge agents were delivered could plausibly affect differences in airway responsiveness among studies.

Strand et al. (1996) exposed exercising adults with asthma to 260 ppb NO_2 for 30 minutes. Responsiveness to histamine was assessed at 30-minutes, 5-hours, 27-hours, and 7-days post-exposure. The PD_{100} tended ($p = 0.08$) to decrease after 30 minutes, became statistically significantly decreased by 5 hours ($p = 0.03$), and returned to baseline by 27-hours post NO_2 exposure compared to filtered air. Although the PD_{100} following NO_2 exposure was fairly constant between 30 minutes and 5 hours, the PD_{100} following filtered air was increased at the 5-hour time point, which may have contributed to the statistically significant difference between NO_2 and filtered air after 5 hours. This 5-hour time point is just beyond reported refractory periods following exercise of 40 minutes to 3 hours (Dryden et al., 2010). A comparison across other NO_2 studies of human subjects for an effect of challenge delivery timing is not possible due to differences in NO_2 concentration and exposure duration. Silbaugh et al. (1981) found a rapid return to baseline responsiveness in guinea pigs by 2 hours post exposure.

Although there is strong evidence for a refractory period following exercise and the preferential use of full vital capacity maneuvers, which may relax constricted airways in studies using exercise, the existing data on airway responsiveness following NO_2 exposure are insufficient to assess the influence of challenge delivery timing on airway responsiveness in those studies.

Effect of Repeated Nitrogen Dioxide Exposures

To mimic a daily commute, Strand et al. (1998) exposed adults with asthma on 4 sequential days to either filtered air or 260 ppb NO_2 for 30 minutes during rest. The early phase response to allergen challenge was statistically significantly increased by

NO_2 exposure; the 4-day mean change in FEV_1 was −2.5 after NO_2 versus −0.4% after air (p = 0.018). The late phase response to allergen challenge was also significantly greater after NO_2 with a 4-day avg change in FEV_1 after NO_2 of −4.4 versus −1.9% after air (p = 0.009). This study suggests that the effect of NO_2 exposure on airway responsiveness to allergen challenge is relatively constant over several contiguous days of repeated NO_2 exposure. Recently, Ezratty et al. (2014) demonstrated increases in eosinophils and eosinophil cationic protein (ECP) after repeated NO_2 exposures which could increase airway responsiveness. Repeated ambient NO_2 exposures could potentially attenuate or augment responses observed in the controlled exposure studies.

Extraneous Factors

Although some early studies progressively increased NO_2 exposure concentrations for safety purposes, the majority of controlled human exposure studies investigating the effects of NO_2 are of a randomized, controlled, crossover design in which subjects were exposed, without knowledge of the exposure condition and in random order to clean filtered air (the control) and, depending on the study, to one or more NO_2 concentrations. The filtered air control exposure provides an unbiased estimate of the effects of the experimental procedures on the outcome(s) of interest. Comparison of responses following this filtered air exposure to those following NO_2 exposure allows for estimation of the effects of NO_2 itself on an outcome measurement while controlling for independent effects of the experimental procedures. Furthermore, the studies by Hazucha et al. (1983) and Strand et al. (1997) provided airway responsiveness data at the time of enrollment in their study and airway responsiveness data following resting exposures to filtered air. Little to no discernible change was observed between airway responsiveness at inclusion and following the resting exposure which suggests that experimental procedures (other than exposure to NO_2) did not affect airway responsiveness.

Dose Response

Folinsbee (1992) noted that greater NO_2 doses occur with exercise due to both the increased ventilation rates and a tendency for increased exposure duration. However, in his meta-analyses, the effects of NO_2 exposure on airway responsiveness were found following resting, but not exercising, exposures to NO_2.

The dose-response of NO_2 on airway responsiveness may be modulated by a number of factors that have been described in this section. The finding of greater airway responsiveness following exposures at rest compared to exercise, despite a lower intake dose of NO_2 during the resting exposures, is consistent with an effect of exercise refractoriness. Greater airway responsiveness following exposures at rest compared to exercise is also consistent with the preferential usage of full vital capacity maneuvers in

studies having exercise to assess airway responsiveness. Issues related to subject selection and medication may have reduced observed effects of NO_2 on airway responsiveness and contributed to variability within and among studies. Both the choice of bronchial challenge agent and method of delivery would have likely contributed to variability among studies. Limited evidence also suggests airway dilation at higher intake doses could reduce airway responsiveness. Overall, the effects of exercise refractoriness, use of vital capacity maneuvers, and potential for some individuals with asthma with exercise-induced bronchoconstriction to be excluded from the evaluation of airway responsiveness appear to be the most likely contributors to not readily finding effects of NO_2 on airway responsiveness at higher intake doses occurring with exercise. Other methodological differences, if randomly occurring, among studies such as the choice of challenge agents, challenge delivery method, and asthma medication usage would likely add variability to assessment of airway responsiveness and thereby bias data toward the null of no discernible dose-response.

A few studies have investigated the effects of NO_2 exposure on airway responsiveness at more than one concentration. Intra-study evaluation of a potential dose-response reduces the inherent variability and uncertainty occurring with inter-study comparisons. Tunnicliffe et al. (1994) found a statistically significant and larger increase in airway responsiveness at 400 ppb as compared to tendency for increased responsiveness at 100 ppb. Orehek et al. (1976) provided responsiveness data for four individuals following exposure to both 100 and 200 ppb NO_2. Of these four individuals, three had similar PD_{100} between the two exposures, one individual had a doubling difference in the PD_{100} (0.42 mg at 200 ppb vs. 0.94 mg at 100 ppb). Bylin et al. (1988) found statistically significant effects of NO_2 on airway responsiveness at 270 ppb, but not at 140 ppb or 530 ppb. These three studies (Tunnicliffe et al., 1994; Bylin et al., 1988; Orehek et al., 1976) for resting exposure to NO_2 provide limited support for increasing airway responsiveness with increasing NO_2 concentration in individuals with asthma. Additionally, conducted as part of this assessment, the regression of individual log-transformed dPD data against dose in terms of both concentration and concentration × exposure duration did not show a dose-response relationship (Figure 5-1). The dose-response evidence from studies that used exercising protocols is less compelling. Roger et al. (1990) did not find a change in airway responsiveness at either 150 or 300 ppb NO_2. Jenkins et al. (1999) found statistically significant increases in airway responsiveness to allergens following a 3-hour exposure to 400 ppb NO_2, but not following a 6-hour exposure to 200 ppb NO_2 despite equivalence in terms of the total intake dose (concentration × exposure duration).

Several inter-study differences likely contribute to variability and uncertainty in cross study comparisons of provocative dose and lung function response to bronchial challenge

agents. Evaluation of the proportional change in these outcomes following NO_2 and filtered air exposure as performed by Goodman et al. (2009) and herein should allow for a valid comparison across studies because the air control would, theoretically, adjust for many methodological differences among studies. However, even after this adjustment, clear differences between resting and exercising exposures exist. Exercise itself, the preferential use of full vital capacity maneuvers to assess responsiveness, and exclusion of individuals with exercise-induced bronchospasm would all act to reduce the measured NO_2 effect on airway responsiveness in the studies with exercise. Not using log-transformed data may also affect the validity of statistical analysis requiring homoscedasticity and normally distributed data. It may not be possible to adequately remove the influence of some methodological factors that so substantially affect the airways or the determination of airway responsiveness in individuals with asthma. Thus, it is not clear to what extent inter-study assessments of the dose-response relationship between NO_2 exposure and airway responsiveness are affected by methodological biases of studies. The few studies having evaluated effects at multiple NO_2 concentrations, using resting exposure, are somewhat supportive of a dose-response relationship showing increasing airway responsiveness with increasing NO_2 exposure concentration. However, linear regression did not show an association between log-transformed dPD in Figure 5-1 and either NO_2 concentration ($p = 0.44$) or concentration × exposure duration ($p = 0.89$).

Summary and Conclusions

There is a wide range of airway responsiveness influenced by many factors, including exercise, medications, cigarette smoke, air pollutants, respiratory infections, disease status, and respiratory irritants. In the general population, airway responsiveness is log-normally distributed with individuals having asthma generally being more responsive than healthy age-matched controls. Nonspecific bronchial challenge agents causing bronchoconstriction may act directly (i.e., histamine, carbachol, and methacholine) on airway smooth muscle receptors or act indirectly (i.e., exercise, cold air) though intermediate pathways, especially via inflammatory mediators. Specific challenge agents (i.e., allergens) also act indirectly on smooth muscle to initiate bronchoconstriction.

Likely affecting the observed changes in airway responsiveness due to NO_2 exposure, there are methodological differences among NO_2 studies including subject activity level (rest vs. exercise) during NO_2 exposure, asthma medication usage, choice of airway challenge agent (e.g., direct and indirect non-specific stimuli), method of administering the bronchoconstricting agents, and physiological endpoint used to assess airway responsiveness. Most of these intra-study differences likely contributed to variability and uncertainty in comparison among studies of provocative doses and lung function responses to bronchial challenge agents. A few factors such as exercise, the use of full

vital capacity maneuvers to deliver challenge agents or measure airway responsiveness to challenge, and exclusion of subjects with exercise-induced bronchospasm may have preferentially biased studies to toward observing minimal NO_2 effect on airway responsiveness.

The analyses provided here show that in individuals with asthma exposed to NO_2 at rest, statistically significant increases in nonspecific airway responsiveness occur in the range of 200 and 300 ppb NO_2 for 30-minute exposures and at 100 ppb NO_2 for 60-minute exposures. Following exposure to NO_2, relative to filtered air exposure, there was a median decrease of 25% (1.88 geometric standard deviation) in the provocative dose. A clinically relevant, doubling dose increase (halving of the provocative dose) due to NO_2 occurred in a quarter of these individuals with asthma exposed to NO_2 during rest. A sensitivity analysis showed these findings to be robust and not driven by individual studies. Consistent with the majority of studies which did not find statistically significant changes in airway responsiveness when exposing individuals to NO_2 during exercise, the meta-analyses of data also showed no effect. Effects of exercise refractoriness and methodological aspects of these studies likely contributed to not readily finding effects of NO_2 on airway responsiveness in these studies. Analyses of the available data show clinically relevant and statistically significant effects of NO_2 on the airway responsiveness of individuals with asthma exposed to NO_2 during rest but not exercise.

5.2.2.2 Lung Function Changes in Populations with Asthma

Compared with evidence for airway responsiveness, the 2008 ISA for Oxides of Nitrogen reported weak evidence in controlled human exposure studies for the effects of NO_2 exposure on changes in lung function in adults with asthma in the absence of a challenge agent (U.S. EPA, 2008a). Epidemiologic evidence also was weak in adults with asthma. In previous epidemiologic studies, the evidence in children with asthma was based on unsupervised lung function measurements and was inconsistent. Most recent studies were epidemiologic and support associations between ambient NO_2 concentrations and lung function decrements in children with asthma.

Epidemiologic Studies

Collectively, previous and recent studies found associations between increases in ambient NO_2 concentrations and decrements in supervised spirometry measures (primarily FEV_1) in children with asthma. Across the various populations examined, results are less consistent for lung function measured under unsupervised conditions, primarily peak expiratory flow (PEF) at home. Results also are inconsistent for NO and NO_X. Ambient

concentrations of NO_2, locations, and time periods for epidemiologic studies of lung function are presented in Table 5-8.

Table 5-8 Mean and upper percentile concentrations of nitrogen dioxide (NO_2) in epidemiologic studies of lung function in populations with asthma.

Study[a]	Location	Study Period	NO_2 Metric Analyzed	Mean/Median Concentration (ppb)	Upper Percentile Concentration (ppb)
Delfino et al. (2008a)	Riverside, CA Whittier, CA	July–Dec 2003 July–Dec 2004	24-h avg total personal	28.6	Max: 105.7
			24-h avg central site	25.0	Max: 29.2
Smargiassi et al. (2014)	Montreal, Canada	Oct 2009–Apr 2010	24-h avg total personal	6.3	75th: 7.4 Max: 70.6
O'Connor et al. (2008)	Boston, MA; Bronx, NY; Chicago, IL; Dallas, TX; New York, NY; Seattle, WA; Tucson, AZ	Aug 1998–July 2001	24-h avg	NR	NR
Gillespie-Bennett et al. (2011)	Bluff, Christchurch, Dunedin, Porirua, Hutt Valley, New Zealand	Sept 2006	4-week avg	3.9	NR
Wiwatanadate and Trakultivakorn (2010)	Chiang Mai, Thailand	Aug 2005–June 2006	24-h avg	17.2	90th: 26.5 Max: 37.4
Mortimer et al. (2002)	Bronx and East Harlem, NY; Chicago, IL; Cleveland, OH; Detroit, MI; St. Louis, MO; Washington, DC	June–Aug 1993	4-h avg (6 a.m.–10 a.m.)	NR	NR
Just et al. (2002)	Paris, France	Apr–June 1996	24-h avg	28.6[c]	Max: 59.0[c]
Odajima et al. (2008)	Fukuoka, Japan	Apr–Sept 2002	3-h avg (7 p.m.–10 p.m.)	20.0	Max: 51.3
		Oct 2002–Mar 2003		11.0	Max: 49.0
Delfino et al. (2003)	Los Angeles, CA (Huntington Park area)	Nov 1999–Jan. 2000	1-h max 8-h max	7.2 5.9	90th: 9 Max: 14 90th: 8 Max: 11
Holguin et al. (2007)	Ciudad Juarez, Mexico	2001–2002	1-week avg	18.2	NR
Barraza-Villarreal et al. (2008)	Mexico City, Mexico	June 2003–June 2005	8-h max	37.4	Max: 77.6

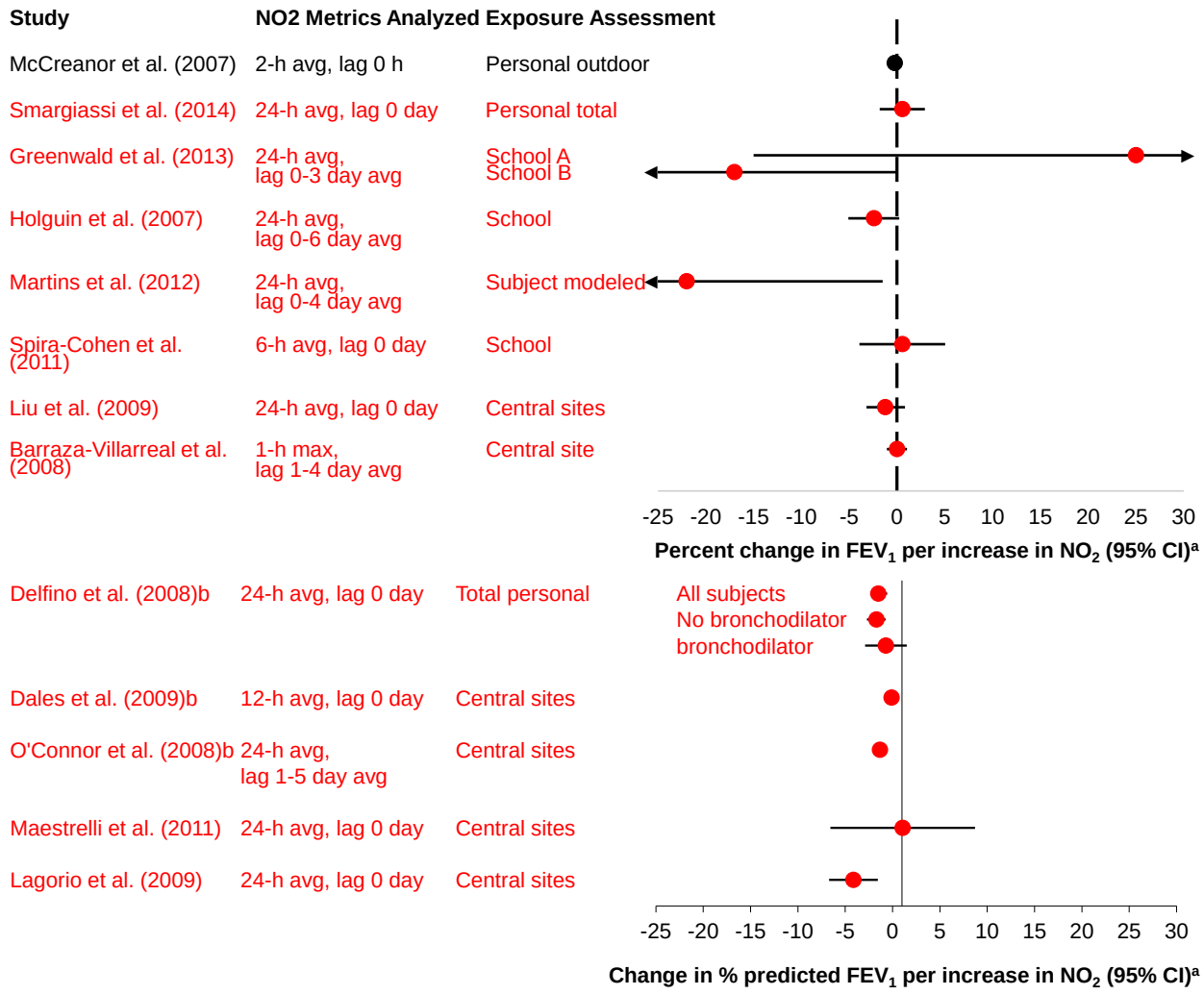
Table 5-8 (Continued): Mean and upper percentile concentrations of nitrogen dioxide (NO_2) in epidemiologic studies of lung function in populations with asthma.

Study[a]	Location	Study Period	NO_2 Metric Analyzed	Mean/Median Concentration (ppb)	Upper Percentile Concentration (ppb)
Liu et al. (2009) Dales et al. (2009)	Windsor, ON, Canada	Oct–Dec 2005	24-h avg	19.8	95th: 29.5
Hernández-Cadena et al. (2009)	Mexico City, Mexico	May–Sept 2005	1-h max	57	75th: 69 Max: 116
Martins et al. (2012)	Viseu, Portugal	Jan and June 2006 and 2007	1-week avg[b]	Across 4 periods: 4.5, 3.5, 9.8, 8.2[c]	Max across 4 periods: 4.6, 4.0, 10.9, 9.4[c]
Greenwald et al. (2013)	El Paso, TX	Mar–June 2010	96-h avg	School A: 6.5 School B: 17.5	NR
Spira-Cohen et al. (2011)	Bronx, NY	Spring 2002, Spring/Fall 2004, Spring 2005	6-h avg (9 a.m.–3 p.m.)	NR	NR
Yamazaki et al. (2011)	Yotsukaido, Japan	Oct–Dec 2000	1-h avg (6 p.m.–7 p.m.)	32.6	NR
Qian et al. (2009a)	Boston, MA; New York, NY; Madison, WI; Denver, CO; Philadelphia, PA; San Francisco, CA	Feb 1997–Jan 1999	24-h avg	20.8	75th: 25.5 Max: 60.7
Lagorio et al. (2006)	Rome, Italy	May–June, Nov–Dec 1999	24-h avg	37.6[c]	Max: 54.3[c]
McCreanor et al. (2007)	London, U.K.	Nov–Mar 2003–2005	2-h avg (10:30 a.m.–12:30 p.m.)	Oxford St: 75.5[c]	Max: 154[c]
				Hyde Park: 11.5[c]	Max: 77.7[c]
Maestrelli et al. (2011)	Padua, Italy	1999–2003	24-h avg	Across seasons and years: 20.9−37.0[c]	Range of 75th: 23.0–42.5[c]
Canova et al. (2010)	Padua, Italy	Summer/Fall 2004, Winter/ Summer/Fall 2005	24-h avg	27.2[c]	48.1[c]
Hiltermann et al. (1998)	Bilthoven, the Netherlands	July–Oct 1995	24-h avg	11.2[c]	22.5[c]
Wiwatanadate and Liwsrisakun (2011)	Chiang Mai, Thailand	Aug 2005–June 2006	24-h avg	17.2	90th: 26.5 Max: 37.4
Park et al. (2005)	Incheon, South Korea	Mar–June 2002	24-h avg	Control days: 31.6 Dust days: 20.7	NR

avg = average; NR = not reported, NO_2 = nitrogen dioxide.
[a]Studies presented in order of first appearance in the text of this section.
[b]Subject-level exposure estimates calculated from outdoor NO_2 at schools and other locations plus time-activity patterns.
[c]Concentrations converted from µg/m^3 to ppb using the conversion factor of 0.532 assuming standard temperature (25°C) and pressure (1 atm).

Children with Asthma

In contrast with studies reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a), several recent studies of children with asthma conducted spirometry under supervised conditions, and most indicate a relationship with short-term NO_2 exposure (Figure 5-3 and Table 5-9). Studies of supervised spirometry measured lung function daily, weekly, biweekly, or seasonally. Evidence for lung function measured daily by subjects at home is less consistent. Among these studies, some reported an association with NO_2 (Gillespie-Bennett et al., 2011; Delfino et al., 2008a; O'Connor et al., 2008), whereas others did not (Wiwatanadate and Trakultivakorn, 2010; Odajima et al., 2008; Just et al., 2002; Mortimer et al., 2002). Results were inconsistent between U.S. multicity studies [National Cooperative Inner-city Asthma Study (NCICAS), Inner City Asthma Study (ICAS)] (O'Connor et al., 2008; Mortimer et al., 2002). Several studies that reported no association with home lung function measurements did not provide quantitative results, including NCICAS (Odajima et al., 2008; Delfino et al., 2003; Just et al., 2002; Mortimer et al., 2002). Thus, the relative magnitude and precision of their results cannot be assessed. However, a relationship between ambient NO_2 and PEF is indicated in children with asthma in a recent meta-analysis (Weinmayr et al., 2010) that included mostly European studies as well as some studies reviewed in the 2008 ISA for Oxides of Nitrogen.



Note: Results are separated into two plots for the two most common indices of FEV_1 examined in studies. Results from more informative studies in terms of the exposure assessment method and potential confounding considered are presented first in each plot. Red = recent studies, black = previous studies. Study details and quantitative results are reported in Table 5-9. Table 5-9 presents results for an array of lung function indices; some of these did not have sufficient numbers to present in a figure.

[a]Effect estimates are standardized to a 20-ppb increase for 24-h avg NO_2 and a 30-ppb increase in 1-h maxium NO_2. Effect estimates for 1-h average to 12-h average NO_2 are not standardized but presented as they are reported in their respective studies (Section 5.1.2.3).

[b]Studies with home-based FEV_1 measurements. All other studies conducted supervised spirometry.

Figure 5-3 Associations of nitrogen dioxide (NO_2) ambient concentrations or personal exposure with percentage change in forced expiratory volume (FEV_1) (top plot) and change in percent predicted FEV_1 (bottom plot) in children and adults with asthma.

Table 5-9 Epidemiologic studies of lung function in children and adults with asthma.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Children with asthma: studies with small spatial scale exposure assessment and/or examination of copollutant confounding				
†Delfino et al. (2008a) Riverside, Whittier, CA n = 53, ages 9–18 yr, persistent asthma and exacerbation in previous 12 mo Repeated measures. Home spirometry. Examined daily for 10 days. 519 observations. Recruitment by referral from school nurses. Parent report of physician-diagnosed asthma. Non-smokers from nonsmoking homes. No information on participation rate. Mixed effects model with random effect for subject with pollutant concentrations centered on subject mean and adjusted for personal relative humidity, personal temperature, and follow-up period. Adjustment for city, beta agonist use, weekend, gas stove use did not alter results.	NO_2-total personal 24-h avg Monitoring checked daily Central site and personal NO_2 moderately correlated. r = 0.43.	0−1 avg	% predicted FEV_1 All subjects –1.68 (–3.17, –0.19)	with 1-h max $PM_{2.5}$: –1.27 (–2.77, 0.22) Moderate correlation with NO_2. Spearman r = 0.38 for personal $PM_{2.5}$, 0.36 for central site. $PM_{2.5}$ not altered by adjustment for NO_2. EC, OC not associated with FEV_1. Central site NO_2 with personal $PM_{2.5}$: –0.86 (–2.60, 0.89).
		0	All subjects −1.45 (–2.33, –0.57)	
			No bronchodilator, n = 37 –1.72 (–2.69, –0.75)	
			Bronchodilator use, n = 16 –0.70 (–2.90, 1.50)	
	NO_2-central site 24-h avg Site within 8 or 16 km of homes.	0	All subjects –1.30 (–2.44, –0.15)	
†Smargiassi et al. (2014) Montreal, Canada n = 72, ages 7−12 yr, 29% with ED visit in previous 12 mo, 43% using steroid medication during study Repeated measures. Supervised spirometry. Examined daily for 10 days. 700 observations. Residence near oil refineries & high traffic areas. Recruitment from asthma clinic or schools. Asthma ascertained by respirologist or parental report of physician diagnosis. No information on participation rate. Linear mixed effects models with random effect for subject, random and fixed effect for study day and adjusted for age, sex, height, month, day of week, asthma medication use, parental education, ethnicity, personal temperature, personal humidity.	NO_2-total personal 24-h avg 99% samples above limit of detection 65% time spent indoors	0	FEV_1: 0.56% (−1.80, 2.92) FVC: 0.36% (−1.44, 2.15) $FEF_{25-75\%}$: 0.35% (−4.7,5.4)	No copollutant model. No consistent associations with personal $PM_{2.5}$, benzene, total polycyclic aromatic hydrocarbons. Correlations among pollutants = 0.11 to 0.11.

Table 5-9 (Continued): Epidemiologic studies of lung function in children and adults with asthma.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
†Martins et al. (2012) Viseu, Portugal n = 51, mean age 7.3 (SD: 1.1) yr, 53% with atopy. Repeated measures. Supervised spirometry. Four measurements over two different seasons. Recruitment from urban and suburban schools. ~66% participation rate. Parental report of wheeze in previous 12 mo. GEE adjusted for age, sex, parental smoking, parental education, atopy, time of visit, average temperature, relative humidity. Also included height, weight, older siblings, mold or dampness in home, fireplace in home, pets in home because changed at least one pollutant effect estimate >10%.	NO_2-modeled personal outdoor 24-h avg Estimated from school outdoor NO_2, 20 city locations, MM5/CHIMERE modeling, and daily activity patterns. 20% time spent at school, 65% at home.	0−4 avg	FEV_1: −22% (−38, −1.49) FEV_1/FVC: −10% (−20, 0.83) $FEF_{25-75\%}$: −33% (−54, −2.57) FEV_1 after bronchodilator: 19% (3.46, 37)	For FEV_1: with PM_{10}: −27% (−67, 60) with benzene: −3.60% (−29, 31) with ethylbenzene: −17% (−41, 17) Benzene unaltered by adjustment for NO_2. Ethylbenzene & PM_{10} attenuated. Correlations with NO_2 negative or weakly positive. Spearman r = −0.72 to −0.55 for PM_{10}, −0.43 to 0.14 for VOCs.
†Greenwald et al. (2013) El Paso, TX n = 38, mean age 10 yr, 76% Mexican-American Repeated measures. Supervised spirometry. Examined weekly for 13 weeks. 413−441 observations. Recruitment from schools in low and high traffic area. No information on participation rate. School record of physician-diagnosed asthma. GLM with subject as random effect and adjusted for school, temperature, relative humidity, indoor NO.	NO_2-school outdoor School A: residential area School B: 91 m from major road.	0−3 avg	FEV_1: School A: 25% (−15, 84) School B: −17% (−32, 0.12)	No copollutant model. BC, SO_2 (central site) associated with FEV_1. Moderate correlation with NO_2. Pearson r = 0.62, −0.22. School BTEX associated with FEV_1, highly correlated with NO_2. r = 0.77.
	NO_2-school indoor All 24-h avg		School A: 38% (−12, 116) School B: −14% (−32, 7.2)	
†Holguin et al. (2007) Ciudad Juarez, Mexico n = 95, ages 6−12 yr, 78% mild, intermittent asthma, 58% atopy Repeated measures. Supervised spirometry. Examined biweekly for 4 mo. 87% participation. Self-report of physician-diagnosed asthma. Linear and non-linear mixed effects model with random effect for subject and school adjusted for sex, body mass index, day of week, season, maternal and paternal education, passive smoking exposure.	NO_2-school outdoor 24-h avg Schools located 239−692 m from homes.	0−6 avg	FEV_1: −2.4% (−5.09, 0.24)	No copollutant model. No association with $PM_{2.5}$, EC. Weak to moderate correlations with NO_2. Spearman r = 0.30 for $PM_{2.5}$, 0.49 for EC. Road density at home not school associated with lung function.

Table 5-9 (Continued): Epidemiologic studies of lung function in children and adults with asthma.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
†Spira-Cohen (2013) Spira-Cohen et al. (2011) Bronx, NY n = 40, ages 10−12 yr, 100% non-white, 44% with asthma ED visit or hospital admission in previous 12 mo Repeated measures. Supervised spirometry. Examined daily for 1 mo. 454 observations. No information on participation rate. Recruitment from schools by referrals from school nurses. Parental report of physician-diagnosed asthma. Mixed effects model with subject as random effect adjusted for height, sex, temperature. Adjustment for school (indicator of season) did not alter results.	NO_2-school outdoor 6-h avg (9 a.m.−3 p.m.) Schools 53−737 m from highways with varying traffic counts. Most children walk to school. 89% time spent indoors.	0	FEV_1: 0.56% (−3.93, 5.05) PEF: 2.20% (−2.41, 6.81) Per 60-ppb increase NO_2 (5th−95th percentile change)	NO_2 effect estimate adjusted for personal EC not reported. Personal EC associated with lung function and not altered by NO_2 adjustment. Personal EC-School NO_2 correlation NR. School EC-School NO_2 moderately correlated. r = 0.36.
†Gillespie-Bennett et al. (2011) Bluff, Dunedin, Christchurch, Porirua, Hutt Valley, New Zealand n = 358, ages 6−13 yr Cross-sectional. Home spirometry. Multiple measures of lung function, one NO_2 measurement. Recruitment from a home heating intervention. 77% participation. Mixed effects model with log-transformed NO_2 and random effect for subject. Adjustment for age, sex, region, ethnicity, intervention, income, temperature did not alter results.	NO_2-home outdoor	4-week avg	Per log increase NO_2: Evening FEV_1 −88 (−191, 15) mL	No copollutant model. No other pollutants examined.
	NO_2-home indoor		Evening FEV_1 −13 (−26, −0.38) mL	
†Liu et al. (2009) Windsor, ON, Canada n = 182, ages 9−14 yr Repeated measures. Supervised spirometry. Examined weekly for 4 weeks, same day of week. 672 observations. Recruitment from schools. No information on participation rate. Parental report of physician-diagnosed asthma. Mixed effect model with random effect for subject and adjusted for testing period, temperature, relative humidity, daily medication use.	NO_2-central site 24-h avg Average of 2 sites 99% subjects live within 10 km of sites.	0	FEV_1: –1.22% (–3.2, 0.84) $FEF_{25-75\%}$: –4.8% (–8.6, –0.94)	For lag 0−2 avg NO_2 and FEV_1 with $PM_{2.5}$: 1.2% (−3.8, 6.4) with SO_2: −1.5% (−4.9, 2.2) $PM_{2.5}$ association not altered by NO_2 adjustment, SO_2 attenuated. NO_2 highly correlated with $PM_{2.5}$. Spearman r = 0.71 for $PM_{2.5}$, 0.18 for SO_2.
		0−2 avg	FEV_1: −2.3% (−5.5, 0.92) $FEF_{25-75\%}$: −8.0 (−14, −1.6)	
†Dales et al. (2009) Windsor, ON, Canada n = 182, ages 9−14 yr Repeated measures. Same cohort as above. Unsupervised peak flow. Examined daily for 4 weeks, same day of week. 672 observations. Recruitment from schools. No information on participation rate. Parental report of physician-diagnosed asthma. Mixed effect model with random effect for subject and adjusted for sex, testing period, day of week, daily mean temperature, relative humidity, time spent outdoors.	NO_2-central site 12-h avg (8 a.m.−8 p.m.) Average of 2 sites; 99% subjects live within 10 km of sites. Mean 1.6 and 2.2 h/day spent outdoors.	0	Evening % predicted FEV_1: −0.10 (−0.31, 0.10) Diurnal change FEV_1: −0.34% (−0.64, −0.04) Per 9.8 ppb increase in NO_2 (interquartile range)	Copollutant model results only in figure. Evening FEV_1: NO_2 becomes positive with $PM_{2.5}$ adjustment. Diurnal change FEV_1: NO_2 not altered by adjustment for $PM_{2.5}$ or SO_2. SO_2 and $PM_{2.5}$ not altered by adjustment for NO_2. NO_2 highly correlated with $PM_{2.5}$. Pearson r = 0.68.

Table 5-9 (Continued): Epidemiologic studies of lung function in children and adults with asthma.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Children with asthma: studies with central site exposure assessment and no examination of copollutant confounding				
Delfino et al. (2003) Los Angeles, CA (Huntington Park area) n = 22, ages 10−16 yr, 100% Hispanic, 27% on anti-inflammatory medication Repeated measures. Home peak flow. Recruitment from schools. 92% follow-up participation. Non-smoking children from non-smoking homes. Self or parental report of physician-diagnosed asthma. General linear mixed effects model with autoregressive parameter and subject specific intercept and adjusted for respiratory infections. Adjustment for weekend or max temperature did not alter results.	NO_2-central site 8-h max 1 site within 4.8 km of home and school.	0 1	No quantitative data. Only reported no statistically significant association with PEF.	No copollutant model. Associations found with EC, OC, PM_{10} but not VOCs. Moderate to high correlations with 8-h max NO_2. Spearman r = 0.38 (PM_{10}) to 0.62 (OC). For VOCs, r = 0.57 (benzene) to 0.72 (xylene).
†Barraza-Villarreal et al. (2008) Mexico City, Mexico n = 163−179, ages 6−14 yr, 54% persistent asthma, 89% atopy Repeated measures. Supervised spirometry. Examined every 15 days for mean 22 weeks. 1,503 observations. No information of participation rate. Recruitment from pediatric clinic. Asthma severity assessed by pediatric allergist. Linear mixed effects model with random effect for subject and adjusted for minimum temperature, time, sex, body mass index, ICS use. Adjustment for outdoor activity, smoking exposure, allergy medication, season did not alter results.	NO_2-central site 1-h max Site within 5 km of school or home. Low correlation for school vs. central site: Spearman r = 0.2.1	1−4 avg	FEV_1: 0% (1.05, 1.04) FVC: −0.11% (−1.17, 0.95)	No copollutant model $PM_{2.5}$ associated with FEV_1 and FVC. Moderate correlation with NO_2. Pearson r = 61.
†Hernández-Cadena et al. (2009) Mexico City, Mexico n = 85, ages 7−12 yr, 62% mild, intermittent asthma, 90% atopy Cross-sectional. Supervised spirometry. Recruitment from asthma and allergy clinic. Atopy and asthma severity assessed at clinic. Linear regression adjusted for sex, pet ownership in previous 12 mo, visible mold in home, lag 1 max temperature. Adjustment for age and passive smoking exposure did not alter results. Did not examine potential confounding by SES.	NO_2-central site 1-h max Site within 5 km of home or school. 24-h avg and 8-h max similar results but less precise.	0	FEV_1 response to bronchodilator: −39% (−64, 5.4)	No copollutant model. O_3, not $PM_{2.5}$ associated with FEV_1 response. O_3 moderately correlated with NO_2. r = 0.35.

Table 5-9 (Continued): Epidemiologic studies of lung function in children and adults with asthma.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Mortimer et al. (2002) Bronx and East Harlem, NY; Chicago, IL; Cleveland, OH; Detroit, MI; St. Louis, MO; Washington, DC (NCICAS) n = 846, ages 4−9 yr Repeated measures. Home peak flow. Examined daily for four 2-week periods. Recruitment from ED visits and clinics. Parent report of physician-diagnosed asthma and symptoms in previous 12 mo or asthma symptoms for >6 weeks, or family history of asthma. Participation from 55% full cohort. Sample representative of full cohort except for greater asthma medication use. Mixed effects model adjusted for city, follow-up period, day of study, 24-h rainfall, 12-h avg temperature.	NO_2-central site 4-h avg (6 a.m.−10 a.m.) Average of all city monitors.	Single-day lags 1 to 6 1−5 avg 1−4 avg 0−4 avg 0−3 avg	No quantitative data. Only reported no association with PEF.	No copollutant model. O_3 associated with PEF. Weak correlation with NO_2. r = 0.27.
†O'Connor et al. (2008) Boston, MA; Bronx, NY; Chicago, IL; Dallas, TX; New York, NY; Seattle, WA; Tucson, AZ (ICAS) n = 861, ages 5−12 yr, persistent asthma and atopy, 82% black or Hispanic Repeated measures. Home spirometry. Examined for 2 weeks every 6 mo for 2 yr. 70% of maximum data obtained. Recruited from intervention study. Mixed effects model adjusted for site, month, site*month interaction, temperature, intervention group.	NO_2-central site 24-h avg All monitors close to home and not near industry. Median distance to site = 2.3 km.	1−5 avg	% predicted FEV_1: −1.33 (−1.88, −0.78) % predicted PEF: −1.63 (−2.20, −1.06)	Only three-pollutant model analyzed with $PM_{2.5}$ and O_3. Associations also found with $PM_{2.5}$, CO, SO_2, and O3. Moderate correlations with NO_2. r = 0.59 for $PM_{2.5}$, 0.54 for CO, 0.59 for SO_2. Weak correlation with O_3. r = −0.31.
†Yamazaki et al. (2011) Yotsukaido, Japan n = 17, ages 8−15 yr, 100% atopy Repeated measures. Supervised peak flow before medication use. Examined daily during long-term hospital stay. No air conditioning in hospital. Permitted to go outside if asthma stable. Poor generalizability. 1,198 observations. GEE adjusted for sex, age, height, temperature, day of week, temporal trends.	NO_2-central site 1-h avg (6 p.m.−7 p.m.) Monitor adjacent to hospital. No major roads nearby.	0	No quantitative data. PEF decreases with increasing NO_2 0 to 23 hours before measurement. Strongest associations at 0 h and 12 h.	Only three-pollutant model analyzed with $PM_{2.5}$ and O_3. $PM_{2.5}$, not O_3, also associated with evening PEF. $PM_{2.5}$ moderately correlated with NO_2. r = 0.62.
Just et al. (2002) Paris, France n = 82, ages 7−15 yr, asthma attack in previous 12 mo and daily asthma medication use, 90% atopy Repeated measures. Home peak flow. Examined daily for 3 mo. 82% participation. Recruitment from hospital outpatients. GEE adjusted for time trend, day of week, pollen, temperature, humidity.	NO_2-central site 24-h avg Average of 14 sites	NR	No quantitative data. Only reported no relationship with PEF.	No copollutant model. O_3 associated with PEF. No correlation with NO_2. Pearson r = 0.09.

Table 5-9 (Continued): Epidemiologic studies of lung function in children and adults with asthma.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
†Odajima et al. (2008) Fukuoka, Japan n = 70, ages 4−11 yr, 66% with asthma exacerbation Repeated measures. Home peak flow. Examined daily for 1 yr. >15,000 observations. Participation rate not reported. Recruitment from hospital where received treatment. GEE adjusted for age, sex, height, growth of child, temperature.	NO_2-central site 3-h avg 24-h avg 1 site		No quantitative data. Only reported no association with PEF.	Only three-pollutant model analyzed with suspended PM and O_3. Suspended PM associated with PEF in warm season. Weak correlation with NO_2. r = 0.30 for 24-h avg.
†Wiwatanadate and Trakultivakorn (2010)[b] Chang Mai, Thailand n = 31, ages 4−11 yr, asthma plus symptoms in previous 12 mo, 52% mild intermittent Repeated measures. Home peak flow. Examined daily for 1 yr. 97% participation. Recruitment from allergy clinic. GLM with random effect for subject and adjusted for time trend, day of week, height, weight, atmospheric pressure, temperature, sunshine duration.	NO_2-central site 24-h avg 1 site within 25 km of homes.	 0 1	PEF −1.80 (−5.40, 1.80) L/min 2.60 (−1.20, 6.40) L/min	No copollutant model. No consistent (across various lags of exposure) associations found for $PM_{2.5}$, CO, PM_{10}, SO_2, or O_3.
Adults with Asthma: studies with small spatial scale exposure assessment and/or examination of copollutant confounding				
McCreanor et al. (2007) London, U.K. n = 31 mild asthma, 32 moderate asthma, ages 19−55 yr, all with airway hyperresponsiveness, 84% with atopy Randomized cross-over natural experiment. Supervised spirometry. Exposure on busy road and park. 55 observations. Participation rate not reported. Recruitment from advertisements and volunteer databases. Mixed effects model with random effect for subject and adjusted for temperature, relative humidity.	NO_2-personal outdoor 2-h avg Measured next to subjects during outdoor exposures.	0-h 22-h Post-exposure	FEV_1: −0.22% (−0.40, −0.05) $FEF_{25-75\%}$: −0.78% (−1.4, −0.13) FEV_1: −0.13% (−0.35, 0.10) $FEF_{25-75\%}$: −0.75% (−1.6, 0.10) per 5.3 ppb increase in NO_2	For $FEF_{25-75\%}$ with UFP: −0.47% (−1.3, 0.39) with EC: −0.43% (−1.1, 0.26) with $PM_{2.5}$: −0.48% (−1.3, 0.25) Moderate correlations with NO_2. Spearman r = 0.58 for UFP and EC, 0.60 for $PM_{2.5}$.
†Qian et al. (2009b) Boston, MA; New York, NY; Denver, CO; Philadelphia, PA; San Francisco, CA; Madison, WI n = 119, ages 12−65 yr, persistent asthma, nonsmokers	NO_2-central site 24-h avg Average of all monitors within 32 km of subject ZIP code centroid.	0	PEF All subjects −0.68% (−1.30, −0.06) Placebo −0.29% (−.35, 0.80) Beta-agonist −1.08% (−2.18, −0.05) ICS	with SO_2: −0.11% (−0.87, 0.64) with PM_{10}: −0.80% (−1.7, 0.10) with O_3: −0.68% (−1.3, −0.05) SO_2 slightly attenuated with NO_2 adjustment. PM_{10}, O_3 not associated with PEF. Correlations NR.

Table 5-9 (Continued): Epidemiologic studies of lung function in children and adults with asthma.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Repeated measures. Home PEF. No information on participation rate. Study population representative of full cohort. Examined daily for 16 weeks. >14,000 observations. Trial of asthma medication, a priori comparison of medication regimens. Linear mixed effects model adjusted for age, sex, race/ethnicity, center, season, week, daily average temperature, daily average humidity. Adjustment for viral infections did not alter results.			−0.61% (−1.67, 0.39)	
		0–2 avg	All subjects −0.58% (−1.43, 0.27)	
Adults with Asthma: studies with central site exposure assessment and no examination of copollutant confounding				
†Maestrelli et al. (2011) Padua, Italy n = 32, mean age 39.6 (SD: 7.5) yr, 81% persistent asthma Repeated measures. Supervised spirometry. 6 measures over 2 yr. 166 observations. Selected from database of beta-agonist users (>6/yr for 3 yr), diagnosis clinically confirmed. 76% follow-up participation. Drop outs did not differ from participants. GEE adjusted for daily average temperature, humidity, atmospheric pressure, asthma medication use, current smoking status.	NO_2-central site 24-h avg Average of 2 city sites	0	% predicted FEV_1: 1.07 (−6.57, 8.71)	No copollutant model. CO associated with FEV_1. No association with personal or central site $PM_{2.5}$. No association for central site PM_{10}, SO_2, O_3. Correlations with NO_2 NR.
Lagorio et al. (2006) Rome, Italy n = 11, ages 18−64 yr, 100% mild, intermittent asthma Repeated measures. Supervised spirometry. Examined 2/week for two 1-mo periods. Mean 9, 15 observations/subject. Participation rate not reported. Recruitment of non-smokers from outpatient clinic. GEE adjusted for season, temperature, humidity, beta-agonist use.	NO_2-central site 24-h avg Average of 5 city sites	 0 0–1 avg	% predicted FEV_1: −4.14 (−6.71, −1.56) –4.81 (−7.54, −2.09)	No copollutant model. CO at lag 0−2 avg associated with FEV_1. No association for $PM_{2.5}$, PM_{10}, $PM_{10-2.5}$, O_3. Low to moderate correlations with NO_2. Spearman r = 0.05 for CO, 0.17 for O_3, 0.43−0.51 for PM.
†Canova et al. (2010) Padua, Italy n = 19, ages 15−44 yr, 81% moderate/severe asthma Repeated measures. Home PEF/FEV_1. Examined for five 30-day periods for 2 yr. Recruitment from prescription database of subjects with mean >6 prescription/yr for 3 yr. 50% subjects provided fewer than 33% maximum observations. GEE adjusted for temperature, humidity, atmospheric pressure, ICS use, smoking status.	NO_2-central site 24-h avg 2 city sites	0, 1, 2, 3 (single-day) 0−1 avg 0−3 avg	Results reported only in a figure NO_2 shows null associations with PEF and FEV_1.	No copollutant model. CO associated with PEF. Moderate correlation with NO_2. Spearman r = 0.48.

Table 5-9 (Continued): Epidemiologic studies of lung function in children and adults with asthma.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Park et al. (2005) Incheon, Korea n = 64 with asthma, ages 16−75 yr, 31% with severe asthma Repeated measures. Home PEF. Examined daily for 3−4 mo. Recruited from medical center. GEE model, covariates NR.	NO_2-central site 24-h avg 10 city sites	0	PEF 0.45 (−1.01, 1.90) L/min	No copollutant model. CO, PM_{10}, O_3 associated with PEF. No association for SO_2.
†Wiwatanadate and Liwsrisakun (2011) Chiang Mai, Thailand n = 121 with asthma and symptoms in previous 12 mo, ages 13−78 yr, 48% moderate/severe persistent asthma Repeated measures. Home PEF. Examined daily for 10 mo. Recruited from allergy clinic patients. GLM with random effect for subject and adjusted for sex, age, asthma severity, day of week, weight, pressure, temperature, sunshine duration, rain.	NO_2-central site 24-h avg 1 city site	5	Evening PEF: 1.00 (0.0, 2.00) Average PEF: 1.6 (0.60, 2.60) Units of PEF not reported.	Only multipollutant models analyzed. No associations with PM_{10}, SO_2, O_3. Interactions between NO_2 and copollutants or meteorological variables reported not to be statistically significant.
Hiltermann et al. (1998) Bilthoven, the Netherlands n = 60 with asthma, ages 18−55 yr, all with airway hyperresponsiveness, 87% with atopy Repeated measures. Home PEF. Examined daily for 4 mo. Recruitment from outpatient clinic. Model adjusted for allergen concentrations, smoking exposure, day of week, temperature, linear and quadratic term for study day	NO_2-central site 24-h avg 1 city site Site within 20 km of subjects' homes. 3 city sites highly correlated. r = 0.88.	 0 0−6 avg	Diurnal change PEF −0.75 (−8.12, 6.62) L/min −3.01 (−16, 10) L/min	No copollutant model. BS at lag 0 associated with PEF. No association with PM_{10} or O_3.

Note: More informative studies in terms of the exposure assessment method and potential confounding considered are presented first.

avg = average; BTEX = benzene, toluene, ethylbenzene, xylene; GEE = generalized estimating equations; GLM = generalized linear model; ICAS = Inner City Asthma Study; ICS = inhaled corticosteroid; NCICAS = National Cooperative Inner-city Asthma Study; NR = not reported; SES = socioeconomic status, BS = black smoke, CI = confidence interval, CO = carbon monoxide, EC = elemental carbon, ED = emergency department, $FEF_{25-75\%}$ = forced expiratory flow from 25% to 75% of vital capacity, FEV_1 = forced expiratory volume in 1 second, FVC = forced vital capacity, NO_2 = nitrogen dioxide, O_3 = ozone, OC = organic carbon, PEF = peak expiratory flow, PM = particulate matter, SD = standard deviation, SO_2 = sulfur dioxide, UFP = ultrafine particles, VOC = volatile organic compound.

[a]Effect estimates were standardized to a 20-ppb increase in 24-h avg NO_2, a 25-ppb increase in 8-h max NO_2, and a 30-ppb increase 1-h max NO_2. Effect estimates for other averaging times (1-h avg to 12-h avg) are not standardized but presented as they are reported in their respective studies (Section 5.1.2.3).

†Studies published since the 2008 ISA for Oxides of Nitrogen.

With respect to the populations examined, most studies assessed asthma as parental report of physician-diagnosed asthma. Children were recruited mostly from schools, supporting the likelihood that study populations were representative of the general population of children with asthma. Study populations represented a range of asthma severity, as ascertained by Global Initiative for Asthma guidelines or medication use, ED visit, or hospital admission for asthma in the previous year. Based on a priori hypotheses, results did not demonstrate larger NO_2-associated decrements in lung function in children with asthma than children without asthma (Barraza-Villarreal et al., 2008; Holguin et al., 2007). Post-hoc analyses pointed to stronger associations among children with asthma not taking inhaled corticosteroid (ICS) (Hernández-Cadena et al., 2009; Liu et al., 2009) or not taking controller bronchodilators (Delfino et al., 2008a). The limited results for larger associations in ICS non-users together with observations for NO_2-associated lung function decrements in populations with high prevalence of atopy (53%, 58%) (Martins et al., 2012; Holguin et al., 2007) are supported by findings for NO_2-induced increases in allergic inflammation (Section 5.2.2.5) and findings for mast cell degranulation (which leads to histamine release) in mediating NO_2-induced lung function decrements (Section 4.3.2.2). Bronchodilator use has been shown to reduce airway responsiveness in response to a challenge agent (Section 5.2.2.1).

For children with asthma, key evidence for NO_2-associated lung function decrements was provided by studies with exposure assessment in subjects' locations: total personal NO_2 (Smargiassi et al., 2014; Delfino et al., 2008a), personal outdoor NO_2 estimated from measurements at school and other locations and time-activity data (Martins et al., 2012) or outdoor school NO_2 (Greenwald et al., 2013; Spira-Cohen et al., 2011; Holguin et al., 2007). Among these studies, few reported participation rates (66%, 87%; Table 5-9); however, none reported issues with selective participation according to a specific subject characteristic or NO_2 exposure. These studies examined limited lags of NO_2 exposure but were similar in finding associations with multiday (i.e., lag 0−1 avg, 0−4 avg) averages of 24-h avg NO_2. Studies that measured or estimated personal exposures provide evidence of an effect of outdoor NO_2 exposure on decreasing lung function. Among children with wheeze in Portugal, indoor school and home NO_2 concentrations were below the limit of detection (Martins et al., 2012), and time-weighted average of microenvironmental NO_2 concentrations have shown agreement with personal NO_2 (Section 3.4.4.1).

Studies of total personal NO_2 produced contrasting results. Among children with asthma in the Los Angeles, CA area, slightly larger decrements in percent predicted FEV_1 were found with total personal NO_2 (−1.5 [95% CI: −2.3, −0.57] per 20-ppb increase in lag 0 day NO_2) than central site NO_2 (−1.3 [95% CI: −2.4, −0.15]) (Delfino et al., 2008a). A Spearman correlation of 0.43 between personal and central site NO_2 indicated that

ambient NO_2 had some influence on personal exposures. In contrast, total personal NO_2 exposure was not associated with lung function among children with asthma living near NO_2 emissions sources (i.e., oil refineries, high traffic roads) in Montreal, Canada (Smargiassi et al., 2014). Both studies of total personal exposure examined children for 10 consecutive days, and the total number of observations was higher in Smargiassi et al. (2014) than in Delfino (2006) (~700 vs. ~500). However, it is uncertain whether the Montreal study was sufficiently powered to detect associations with NO_2 exposures, which were far lower than in the Los Angeles study (mean: 6.3 ppb vs. 28.6 ppb) and showed low variability among children and days (IQR: 2.9 ppb vs. 16.8 ppb). The Montreal study did not provide strong evidence that lung function was associated with total personal exposures to $PM_{2.5}$, VOCs, or polycyclic aromatic hydrocarbons either (Smargiassi et al., 2014).

Among studies of outdoor school NO_2, associations with FEV_1 were found in populations in El Paso, TX, and Ciudad Juarez, Mexico, which are located along the U.S./Mexico border (Greenwald et al., 2013; Holguin et al., 2007) (Figure 5-3 and Table 5-9). Between two El Paso schools, associations were limited to the school located near a major road and characterized by higher outdoor pollutant concentrations and a larger percentage of Mexican-American children (Greenwald et al., 2013). No association with FEV_1 was found in children with asthma in Bronx, NY for school NO_2 averaged over the 6-h school day (Spira-Cohen et al., 2011). An effect of outdoor NO_2 is supported by similar FEV_1 decrements for outdoor and indoor NO_2 in an El Paso school (Greenwald et al., 2013) and larger lung function decrements for home outdoor than indoor NO_2 among children in five New Zealand towns (Gillespie-Bennett et al., 2011). The latter results have weaker implications as multiple daily FEV_1 measures were related to a single 4-week average of NO_2.

Compared with NO_2 exposures estimated for subjects' locations, evidence for associations with lung function decrements is more uncertain for NO_2 measured at central sites. Among studies that measured ambient NO_2 at central sites, some found associations with lung function decrements (Yamazaki et al., 2011; Dales et al., 2009; Hernández-Cadena et al., 2009; Liu et al., 2009; O'Connor et al., 2008). Many studies reported lack of association (Wiwatanadate and Trakultivakorn, 2010; Barraza-Villarreal et al., 2008; Odajima et al., 2008; Just et al., 2002; Mortimer et al., 2002). Among children in Mexico City, Mexico, and Thailand, various lung function parameters showed no or imprecise associations with NO_2 (Wiwatanadate and Trakultivakorn, 2010; Barraza-Villarreal et al., 2008) (Table 5-9). Other studies did not report quantitative results, and it was not possible to assess the extent to which their findings did or did not suggest associations. Across the studies examining central site NO_2, exposures were assigned as ambient measurements from a site located within 5 or 10 km of subjects' homes or schools,

measurements averaged among city monitors, or measurements from one site. The central site NO_2 assessment method did not appear to influence results; however, in Mexico City, a low correlation (r = 0.21) between central site and school NO_2 suggests that the central site may not have adequately represented the variability within the study area (Barraza-Villarreal et al., 2008).

Compared with NO_2 exposures estimated for subjects' locations, evidence for associations with lung function decrements is more uncertain for NO_2 measured at central sites. Among studies that measured ambient NO_2 at central sites, some found associations with lung function decrements (Yamazaki et al., 2011; Dales et al., 2009; Hernández-Cadena et al., 2009; Liu et al., 2009; O'Connor et al., 2008). Many studies reported lack of association (Wiwatanadate and Trakultivakorn, 2010; Barraza-Villarreal et al., 2008; Odajima et al., 2008; Just et al., 2002; Mortimer et al., 2002). Among children in Mexico City, Mexico, and Thailand, various lung function parameters showed no or imprecise associations with NO_2 (Wiwatanadate and Trakultivakorn, 2010; Barraza-Villarreal et al., 2008) (Table 5-9). Other studies did not report quantitative results, and it was not possible to assess the extent to which their findings did or did not suggest associations. Across the studies examining central site NO_2, exposures were assigned as ambient measurements from a site located within 5 or 10 km of subjects' homes or schools, measurements averaged among city monitors, or measurements from one site. The central site NO_2 assessment method did not appear to influence results; however, in Mexico City, a low correlation (r = 0.21) between central site and school NO_2 suggests that the central site may not have adequately represented the variability within the study area (Barraza-Villarreal et al., 2008).

Adjustment for potential confounding varied among studies but in most cases included temperature. Several studies adjusted for (or considered in preliminary analyses) relative humidity; a few studies adjusted for day of the week, smoking exposure, or asthma medication use (Table 5-9). Few studies analyzed copollutant models, and while Holguin et al. (2007) found that neither $PM_{2.5}$ nor EC was associated with FEV_1 among children with asthma in Ciudad Juarez, Mexico, most studies found associations with the traffic-related pollutants $PM_{2.5}$, BC/EC, or VOCs as well as with PM_{10}, SO_2, or O_3. A wide range of correlations with NO_2 were reported for $PM_{2.5}$ (r = 0.30−0.71). Negative or weakly positive correlations were reported for other pollutants (e.g., −0.72 for PM_{10} to 0.18 for SO_2). In copollutant models, NO_2 effect estimates were attenuated in some cases and unchanged in others. Copollutant effect estimates adjusted for NO_2 generally were not altered. Among children with wheeze in Portugal, the association of modeled outdoor NO_2 with FEV_1 was attenuated (−3.7% [95% CI: −33, 25%] per 20-ppb increase in 1-week avg NO_2) with adjustment for benzene (Spearman r = −0.42 to 0.14). Among children with asthma in Windsor, Ontario, Canada, associations of 12-h avg and 24-h avg

NO_2 with FEV_1 became positive with adjustment for highly correlated (r = 0.71) $PM_{2.5}$ (Dales et al., 2009; Liu et al., 2009) (Table 5-9). NO_2 associations with FEV_1 diurnal change were unchanged by $PM_{2.5}$ or SO_2 adjustment (Dales et al., 2009). In a more detailed copollutant analysis of personal and central site measures, Delfino et al. (2008a) found the association of personal NO_2 with FEV_1 to be robust (−1.3-point [95% CI: −2.8, 0.22] change in percent predicted FEV_1 per 20-ppb increase in NO_2) to adjustment for personal $PM_{2.5}$, which was weakly correlated with personal NO_2 (Spearman r = 0.38). Adjustment for personal $PM_{2.5}$ (r = 0.32) reduced the association of central site NO_2 with FEV_1 (−0.86-point [95% CI: −2.6, 0.89] change per 20-ppb increase in NO_2). The attenuation could indicate that ambient NO_2 serves as an indicator of personal $PM_{2.5}$ but could also result from less exposure measurement error for personal $PM_{2.5}$ than central site NO_2. Nonetheless, the moderate personal-ambient NO_2 correlation (r = 0.43) and the copollutant model results for personal NO_2 provide evidence for independent effects on FEV_1 of ambient NO_2.

Adults with Asthma

Most previous and recent studies of lung function in adults with asthma were based on PEF measured at home and indicated no association or inconsistent associations among the various lung function parameters or NO_2 exposure lags examined (Wiwatanadate and Liwsrisakun, 2011; Canova et al., 2010; Park et al., 2005; Hiltermann et al., 1998). Most of these studies recruited subjects from outpatient clinics or doctors' offices, and the nonrandom selection of the general population may produce study populations less representative of the asthma population. Ambient NO_2-associated decreases in PEF were found in a recent multicity U.S. study of adults with asthma (Qian et al., 2009b). The few studies with supervised spirometry also produced inconsistent evidence overall (Maestrelli et al., 2011; McCreanor et al., 2007; Lagorio et al., 2006) (Table 5-9). Most studies examined 24-h NO_2 that was assessed primarily from central site measurements, and results were equally inconsistent for NO_2 exposures assigned from one site or averaged from multiple city sites.

The strongest study with personal outdoor NO_2 measurements and examination of traffic-related copollutants provides evidence for an independent association for NO_2. Among adults in London, U.K. with mild to moderate asthma, NO_2 measured next to subjects while walking next to a high-traffic road (allowing only diesel buses and taxis) and while in a park was associated with decrements in FEV_1 and forced expiratory flow from 25% to 75% of vital capacity ($FEF_{25–75\%}$) (McCreanor et al., 2007). NO_2-related decrements occurred 2 to 22 hours after exposure. A 5.3-ppb increase in 2-h avg NO_2 was associated with a −0.22% (95% CI: −0.40, −0.05) change in FEV_1 and −0.78% (95% CI: −1.4, −0.13) change in $FEF_{25–75\%}$. In the London walking study and other studies, lung

function also was associated with the traffic-related pollutants EC, UFP, $PM_{2.5}$, or CO, which were moderately correlated with NO_2 (Spearman r = 0.43−0.60) (McCreanor et al., 2007; Lagorio et al., 2006). Associations also were observed with PM_{10} and SO_2. In the U.S. multicity study of adults, NO_2-PEF effect estimates were attenuated with adjustment for SO_2 (Qian et al., 2009b). Copollutant effect estimates were unaltered or less attenuated with adjustment for NO_2. The London walking study, with pollutants measured on site of outdoor exposures, provided some evidence for an independent association for NO_2. NO_2-associated decrements in FEV_1 were attenuated to near null with adjustment for UFP, EC, or $PM_{2.5}$ (McCreanor et al., 2007). Associations with $FEF_{25–75\%}$ decreased in magnitude and precision with copollutant adjustment but remained negative (e.g., −0.45% [95% CI: −0.73, 0.17%] per 30-ppb increase in 2-h avg NO_2 with adjustment for UFP, Spearman r = 0.58). These results indicate that the decrements in some lung function parameters associated with near-road exposures of relatively short duration (2 hours) were attributable to NO_2.

Controlled Human Exposure Studies

Most controlled human exposure studies examined adults and were reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a). Consistent with epidemiologic findings, numerous controlled human exposure studies generally did not report effects on lung function in adults with asthma. As detailed in Table 5-10, exposures ranged from 200 to 4,000 ppb NO_2 for 30 minutes to 6 hours, and most studies incorporated exercise in the exposure period to assess lung function during various physiological conditions.

Whereas NO_2 consistently induced increases in airway responsiveness in adults with asthma (Section 5.2.2.1), direct changes in lung function or airway resistance were not consistently found. Linn et al. (1985b) exposed adults with asthma and healthy adults to 4,000 ppb NO_2 for 75 minutes and reported no changes in airway resistance after NO_2 exposure in either group. Kleinman et al. (1983) found no statistically significant changes in forced expiratory flows or airway resistance after exposure to 200 ppb NO_2 for 2 hours with light exercise; however, Bauer et al. (1986) reported statistically significant decrements in forced expiratory flow rates in adults with asthma after exposure to 300 ppb NO_2 for 30 minutes. Jörres and Magnussen (1991) found no changes in lung function in adults with asthma exposed to 250 ppb NO_2 for 30 minutes; however, exposure to 1,000 ppb NO_2 for 3 hours with intermittent exercise (adjusted to individual maximum workload) resulted in small reductions in FEV_1 in adults with asthma (Jörres et al., 1995). Koenig et al. (1987) exposed healthy adolescents and those with asthma to 120 or 180 ppb NO_2 and did not find any changes in lung function measured during or after exposure relative to air exposures, nor were there differences between subjects with asthma and healthy subjects.

There is no strong evidence for interactions between NO_2 and O_3 in controlled human exposure studies. Jenkins et al. (1999) found no change in lung function in adults with asthma following exposure to 200 ppb NO_2 for 6 hours (with or without 200 ppb O_3) or 400 ppb NO_2 for 3 hours (without 400 ppb O_3). Statistically significant decreases in FEV_1 were found following the 3-hour exposure to O_3 and O_3 + NO_2.

Table 5-10 Controlled human exposure studies of individuals with asthma.

Study	Disease Status[a]; n, Sex; Age (mean ± SD)	Exposure Details (Concentration; Duration)	Outcomes Examined
Bauer et al. (1986)	Asthma; n = 15; 33 ± 7.8 yr	300 ppb for 30 min (20 min at rest, 10 min of exercise at $\dot{V}_E$ > 3 times resting	Pulmonary function before, during, and after exposure.
Jenkins et al. (1999)	Asthma; n = 9 M, 2 F; 31.2 ± 6.6 yr	(1) 200 ppb NO_2 for 6 h (2) 200 ppb NO_2 + 100 ppb O_3 for 6 h (3) 400 ppb NO_2 for 3 h (4) 400 ppb NO_2 + 200 ppb O_3 for 3 h (1−4) Exercise 10 min on/40 min off at $\dot{V}_E$ = 32 L/min)	Pulmonary function tests before and after exposure.
Jörres et al. (1995)	Healthy; n = 5 M, 3 F; 27 yr (range: 21−33) Asthma; n = 8 M, 4 F; 27 ± 5 yr	1,000 ppb for 3 h; Exercise 10 min on/10 min off at individual's maximum workload	Pulmonary function tests before, during, and after exposure. Symptoms immediately, 6 h, and 24 h after exposure. BAL fluid analysis 1 h after exposure (cell counts, histamine, prostaglandins).
Jörres and Magnussen (1991)	Asthma; n = 9 M, 2 F; 29 yr (range: 17−55)	250 ppb for 1 h; Rest for 20 min followed by 10 min of exercise ($\dot{V}_E$ = 30 L/min)	Airway resistance measured before, during, and after exposure.
Kleinman et al. (1983)	Asthma; n = 12 M, 19 F; 31 ± 11 yr	200 ppb for 2 h; Exercise 15 min on/15 min off at $\dot{V}_E$ = ~2 times resting	Pulmonary function testing before and after exposure. Symptoms before, immediately after, and day after exposure.

Table 5-10 (Continued): Controlled human exposure studies of individuals with asthma.

Study	Disease Status[a]; n, Sex; Age (mean ± SD)	Exposure Details (Concentration; Duration)	Outcomes Examined
Koenig et al. (1987)	Healthy; (1) n = 3 M, 7 F (2) n = 4 M, 6 F; Asthma; (1) n = 4 M, 6 F (2) n = 7 M, 3 F; 14.4 yr (range: 12−19 yr)	(1) 120 ppb NO_2, (2) 180 ppb NO_2; (1−2) Exposures were 30 min at rest with 10 min of exercise at $\dot{V}_E$ = 32.5 L/min	Pulmonary function tests before, during, and after exposure. Symptoms immediately after and 1 day after.
Linn et al. (1985b)	Healthy; n = 16 M, 9 F; (range: 20−36 yr) Asthma; n = 12 M, 11 F; (range: 18−34 yr)	4,000 ppb for 75 min; Two 15-min periods of exercise at $\dot{V}_E$ = 25 L/min and 50 L/min	Airway resistance before, during, and after exposure. Symptoms before, during, immediately after, 1 day after and 1 week after exposure.

Riedl et al. (2012)	Asthma
	Phase 1: methacholine challenge;
	n = 10 M, 5 F;
	37.3 ± 10.9 yr
	Phase 2: cat allergen challenge;
	n = 6 M, 9 F;
	36.1 ± 12.5 yr
		350 ppb for 2 h;
		Exercise 15 min on/15 min off at $\dot{V}_E$ = 15−20 L/min
			Symptoms before, during, 1−22 h after exposure.

Vagaggini et al. (1996)	Healthy; n = 7 M; 34 ± 5 yr Asthma; n = 4 M, 4 F; 29 ± 14 yr COPD; n = 7 M; 58 ± 12 yr	300 ppb for 1 h; Exercise at $\dot{V}_E$ = 25 L/min	Symptoms before and 2 h after exposure. Cell counts in sputum 2-h post-exposure.

BAL = bronchoalveolar lavage, COPD = chronic obstructive pulmonary disease, F = female, M = male, NO_2 = nitrogen dioxide, O_3 = ozone, SD = standard deviation.

5.2.2.3 Respiratory Symptoms and Asthma Medication Use in Populations with Asthma

The preceding epidemiologic evidence describing associations between short-term increases in ambient NO_2 concentrations and decreases in lung function in children with asthma, particularly those with atopy, supports evidence for NO_2-related increases in respiratory symptoms in children with asthma. Decreased lung function can indicate airway obstruction (Section 4.3.2), which can cause symptoms. Further characterizing the mode of action by which NO_2 exposure may induce respiratory symptoms is evidence for

NO_2-induced increases in airway responsiveness (Section 5.2.2.1) and pulmonary inflammation (Section 5.2.2.5) (Figure 4-1). Epidemiologic studies reviewed in the 2008 ISA for Oxides of Nitrogen consistently found increased respiratory symptoms in children with asthma in association with increases in indoor, personal, and ambient NO_2 concentrations (U.S. EPA, 2008a). There is weak support from a controlled human exposure study of symptoms in adolescents with asthma. NO_2-related increases in respiratory symptoms in adults with asthma were found in previous epidemiologic studies but not in controlled human exposure studies. Recent studies, most of which were epidemiologic, continue to indicate associations between short-term increases in ambient NO_2 concentration and increases in respiratory symptoms in children with asthma.

Epidemiologic Studies

Epidemiologic studies examined respiratory symptoms in relation to ambient NO_2 concentrations rather than NO or NO_X, and evidence is stronger for children with asthma than adults with asthma. Across the various populations examined, symptom data were collected by having subjects or their parents complete daily diaries for periods of 2 weeks to several months. Heterogeneity in the number of consecutive days of follow-up and the frequency of diary collection from study subjects do not appear to influence results. Ambient NO_2 concentrations, locations, and time periods for epidemiologic studies of respiratory symptoms are presented in Table 5-11.

Table 5-11 Mean and upper percentile concentrations of nitrogen dioxide (NO_2) in epidemiologic studies of respiratory symptoms in populations with asthma.

Study[a]	Location	Study Period	NO_2 Metric Analyzed	Mean/Median Concentration ppb	Upper Percentile Concentrations ppb
Schildcrout et al. (2006)	Albuquerque, NM Baltimore, MD Boston, MA Denver, CO San Diego, CA St. Louis, MO Toronto, Canada	Nov 1993–Sept 1995	24-h avg NO_2	Across cities: 17.8−26.0	90th: across cities 26.7–36.9 ppb
Romieu et al. (2006)	Mexico City, Mexico	Oct 1998–Apr 2000	1-h max NO_2	66	Max: 298
Segala et al. (1998)	Paris, France	Nov 1992–May 1993	24-h avg NO_2	30.3[b]	Max: 64.9[b]
Patel et al. (2010)	New York City and nearby suburb, NY	2003–2005, months NR	24-h avg NO_2	NR	NR

Table 5-11 (Continued): Mean and upper percentile concentrations of nitrogen dioxide (NO_2) in epidemiologic studies of respiratory symptoms in populations with asthma.

Study[a]	Location	Study Period	NO_2 Metric Analyzed	Mean/Median Concentration ppb	Upper Percentile Concentrations ppb
Barraza-Villarreal et al. (2008), Escamilla-Nuñez et al. (2008)	Mexico City, Mexico	June 2003–June 2005	8-h max NO_2	37.4	Max: 77.6
Mann et al. (2010)	Fresno/Clovis, CA	Winter–Summer, 2000–2005	24-h avg NO_2	Median: 18.6	75th: 24.7 Max: 52.4
Zora et al. (2013)	El Paso, TX	Mar–June 2010	96-h avg NO_2	School 1: 9.3 School 2: 3.4	Max: 16.2 Max: 7.5
Jalaludin et al. (2004)	Western and Southwestern Sydney, Australia	Feb–Dec 1994	15-h avg NO_2 (6 a.m.–9 p.m.)	15.0	Max: 47.0
Spira-Cohen et al. (2011)	Bronx, NY	Spring 2002, Spring/Fall 2004, Spring 2005	6-h avg NO_2 (9 a.m.–3 p.m.)	NR	NR
Sarnat et al. (2012)	El Paso, TX and Ciudad Suarez, Mexico	Jan–Mar 2008	96-h avg NO_2	El Paso schools: 4.5, 14.2; central sites: 14.0, 18.5, 20.5 Ciudad Juarez schools: 18.7, 27.2; central site: none	NR
Holguin et al. (2007)	Ciudad Juarez, Mexico	2001–2002	1-week avg NO_2	18.2	NR
Gillespie-Bennett et al. (2011)	Bluff, Dunedin, Christchurch, Porirua, Hutt Valley, New Zealand	Sept 2006	4-week avg NO_2	3.9	NR
Gent et al. (2009)	New Haven County, CT	Aug 2000–Feb 2004	NO_2—avg time NR	NR	NR
Delfino et al. (2003)	Los Angeles, CA (Huntington Park area)	Nov 1999–Jan 2000	1-h max NO_2 8-h max NO_2	7.2 5.9	90th: 9.0; max: 14 90th: 7.9; max: 11
Delfino et al. (2002)	Alpine, CA	Mar–Apr 1996	1-h max NO_2 8-h max NO_2	24 15	Max: 53 Max: 34
O'Connor et al. (2008)	Boston, MA Bronx, NY Chicago, IL Dallas, TX New York, NY Seattle, WA Tucson, AZ	Aug 1998–July 2001	24-h avg NO_2	NR	NR
Mortimer et al. (2002)	Bronx & East Harlem, NY Chicago, IL Cleveland, OH	June–Aug 1993	4-h avg NO_2 (6 a.m.–10 a.m.)	NR	NR

Table 5-11 (Continued): Mean and upper percentile concentrations of nitrogen dioxide (NO_2) in epidemiologic studies of respiratory symptoms in populations with asthma.

Study[a]	Location	Study Period	NO_2 Metric Analyzed	Mean/Median Concentration ppb	Upper Percentile Concentrations ppb
	Detroit, MI St. Louis, MO Washington, DC				
Just et al. (2002)	Paris, France	Apr–June 1996	24-h avg NO_2	28.6[b]	Max: 59.0[b]
Ostro et al. (2001)	Central Los Angeles, CA	Aug–Oct 1993	24-h avg NO_2	Los Angeles: 79.5 Pasadena: 68.1	Max: 220 Max: 170
Boezen et al. (1998)	Amsterdam Meppel, the Netherlands	Winter 1993–1994	24-h avg NO_2	24.5[b] 14.2[b]	Max: 40.4[b] Max: 28.9[b]
Forsberg et al. (1998)	Landskrona, Sweden	Jan–Mar, yr NR	24-h avg NO_2	16.2[b]	Max: 38.1[b]
von Klot et al. (2002)	Erfurt, Germany	Sept 1996–Nov 1997	24-h avg NO_2	24.5[b]	Max: 63.3[b]
Maestrelli et al. (2011)	Padua, Italy	1999–2003	24-h avg NO_2	Across seasons and yr: 20.9–37.0[b]	75th: 23.0−42.5[b]
Wiwatanadate and Liwsrisakun (2011)	Chiang Mai, Thailand	Aug 2005–June 2006	24-h avg NO_2	17.2	90th: 26.5 Max: 37.4
Hiltermann et al. (1998)	Bilthoven, the Netherlands	July–Oct 1995	24-h avg NO_2	11.2[b]	Max: 22.5[b]
Laurent et al. (2009)	Strasbourg, France	2004, all yr	24-h avg NO_2 Dispersion model	18.6[b]	NR
Carlsen et al. (2012)	Reykjavik, Iceland	Mar 2006–Dec 2009	24-h avg NO_2 1-h max	11.7[b] 27.4[b]	Max: 52.9[b] Max: 64.4[b]
Kim et al. (2012)	Seoul and Kyunggi Province, South Korea	2005–2009	24-h avg NO_2	Asthma exacerbation 34.3 spring, 26.6 summer, 30.6 fall, 38.8 winter	75th: asthma exacerbation: 41.3 spring, 35.3 summer, 42.0 fall, 46.8 winter
				No asthma exacerbation: 32.7 spring, 26.0 summer, 30.6 fall, 37.7 winter	No asthma exacerbation: 41.4 spring, 35.2 summer, 41.6 fall, 48.9 winter
Karakatsani et al. (2012)	Amsterdam, the Netherlands Athens, Greece Birmingham, U.K. Helsinki, Finland	Oct 2002–Mar 2004	24-h avg NO_2	20.4[b] 21.2[b] 18.3[b] 12.1[b]	Max: 51.8[b] Max: 59.0[b] Max: 44.2[b] Max: 41.4[b]
Feo Brito et al. (2007)	Ciudad Real Puertollano, Spain	May–June 2000–2001	24-h avg NO_2	17.4[b] 29.5[b]	Max: 35.6[b] Max: 100.5[b]

NR = not reported, NO_2 = nitrogen dioxide.
[a]Studies presented in order of first appearance in the text of this section.
[b]Concentrations converted from µg/m^3 to ppb using the conversion factor of 0.532 assuming standard temperature (25°C) and pressure (1 atm).

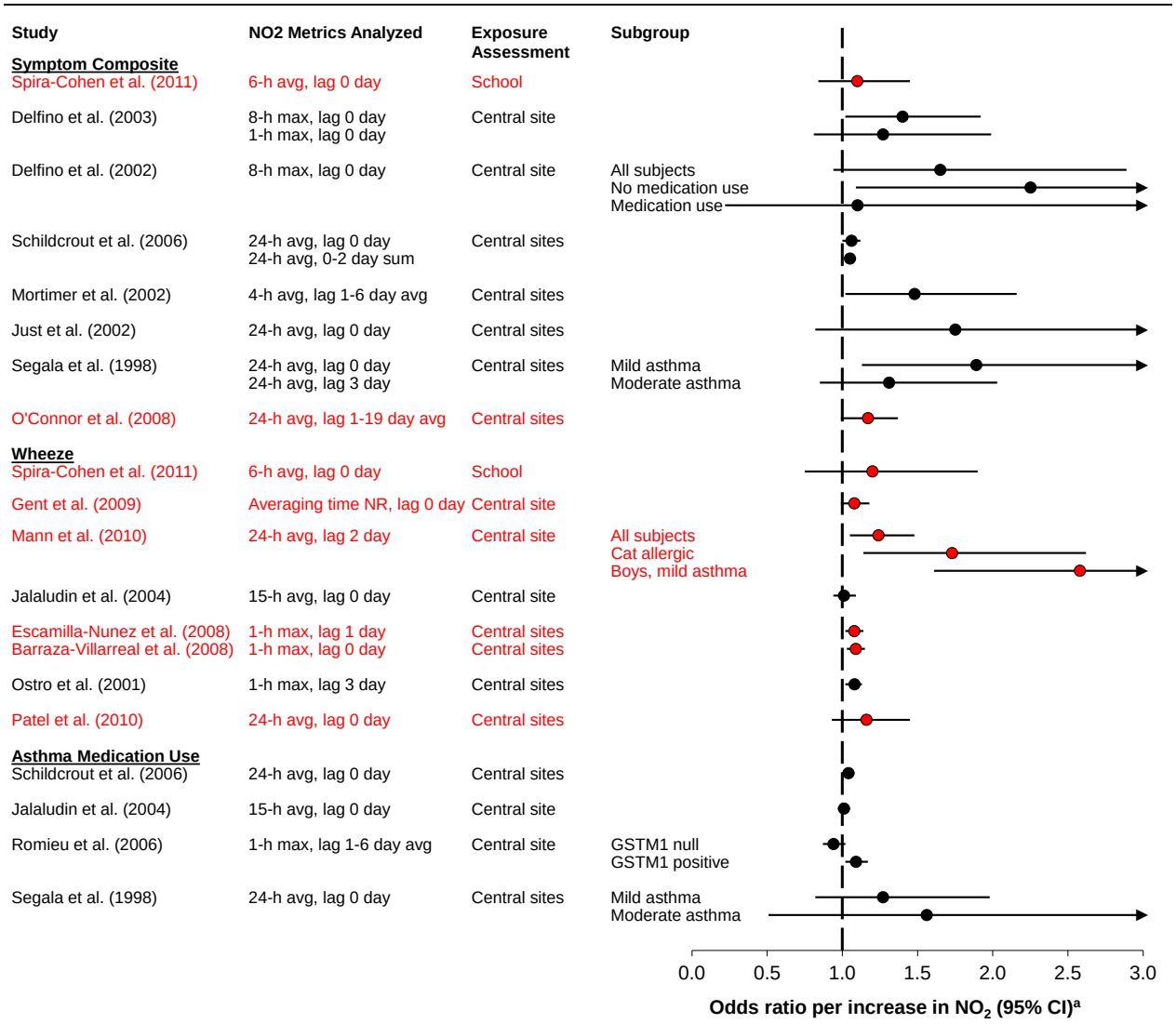
Children with Asthma

Several recent studies add to the evidence for increases in respiratory symptoms in children with asthma associated with short-term increases in ambient NO_2. Across previous and recent studies, there is heterogeneity in the magnitude and precision (width of 95% CIs) of the association. However, the results collectively indicate a pattern of elevated risk of respiratory symptoms across the various symptoms and lags of NO_2 exposure examined (Figure 5-4 and Table 5-12). The consistency of findings also is supported by a meta-analysis of 24 mostly European studies and some U.S. studies, including several reviewed in the 2008 ISA for Oxides of Nitrogen. In the meta-analysis, there was some evidence of publication bias with exclusion of the multicounty European PEACE studies, but with adjustment for publication bias, an increase in 24-h avg NO_2 was associated with increased risk of asthma symptoms (Weinmayr et al., 2010). Across individual studies reviewed in this ISA, the most consistent results were for total respiratory or asthma symptoms, wheeze, and cough. Increases in ambient NO_2 concentrations were not consistently associated with increases in rescue inhaler or beta-agonist use in children with asthma (Patel et al., 2010; Romieu et al., 2006; Schildcrout et al., 2006; Segala et al., 1998).

Study populations were recruited from schools, asthma or allergy clinics, or doctors' offices. Asthma was assessed by parental report of physician-diagnosed asthma or clinical examination. Neither of these methodological issues appeared to affect whether an association was found. Many studies reported follow-up participation rates of 77−92%, and none reported selective drop-out among a particular group within the study population. In a-priori-determined comparisons of children with and without asthma, one study found stronger associations in children with asthma (Patel et al., 2010); another found stronger associations in children without asthma, 72% of whom had atopy (Barraza-Villarreal et al., 2008; Escamilla-Nuñez et al., 2008).

Many asthma study populations had high prevalence of atopy (47−89%), and larger NO_2-associated increases in symptoms were found in children with asthma who also had allergies (Zora et al., 2013; Mann et al., 2010). These results were based on 16 to 47% of the study populations but are coherent with experimental evidence for NO_2-induced allergic responses in adults with asthma and animal models of allergic disease (Section 5.2.2.5). Study populations also varied in asthma severity; some studies examined mostly children with mild, intermittent asthma and others examined children with persistent asthma. Comparisons by asthma severity indicated larger NO_2-related increases in respiratory symptoms among children with mild, intermittent asthma than severe or moderate asthma (Mann et al., 2010; Segala et al., 1998), but these results also were based on small numbers of subjects. Jalaludin et al. (2004) found that elevated risk

was limited to children with more severe asthma (asthma plus airway hyperresponsiveness). But, results were based on a 3-pollutant model that can produce unreliable results because of potential multicollinearity.



Note: Results from more informative studies in terms of the exposure assessment method and potential confounding considered are presented first within an outcome group. Red = recent studies, black = previous studies. Study details and quantitative results are reported in Table 5-12. The figure presents a subset of results included in Table 5-12 for which quantitative results were available for NO_2 examined as a linear variable and for specific outcomes examined by multiple studies.
[a]Effect estimates are standardized to a 20-ppb, 25-ppb, and 30-ppb increase for 24-h avg, 8-h max, and 1-h max NO_2, respectively. Effect estimates for other averaging times (4-h avg to 15-h avg) are not standardized and presented as reported in their respective studies (Section 5.1.2.3).

Figure 5-4 Associations of ambient nitrogen dioxide (NO_2) concentrations with respiratory symptoms and asthma medication use in children with asthma.

Table 5-12 Epidemiologic studies of respiratory symptoms and asthma medication use in children with asthma.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Odds Ratio (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Studies with small spatial scale exposure assessment and/or examination of copollutant confounding				
†Zora et al. (2013) El Paso, TX n = 36, mean age 9.3 (SD: 1.5) yr, 47% with atopy Repeated measures. Asthma control questionnaire given weekly at school for 13 weeks. Questionnaire ascertains symptoms, activity limitations, asthma medication use. Recruitment from schools via school nurses. Parent report of physician-diagnosed asthma. No information on participation rate. Linear mixed effects model adjusted for random subject effect and humidity, temperature, school.	NO_2-school outdoor 24-h avg 1 school 91 m from major road, 1 school in residential area.	0−4 avg	Change in asthma control score (higher score indicates poorer control): Allergy, n = 17 0.56 (−0.10, 1.22) No allergy, n = 19 −0.29 (−1.07, 0.49)	No copollutant models analyzed for subgroups. BC, benzene, toluene, also associated with poorer asthma control. Correlations with NO_2 weak to high. Spearman r = 0.29 to 0.56 for BC, 0.37 to 0.71 for benzene, 0.16 to 0.71 for toluene.
†Sarnat et al. (2012) El Paso, TX and Ciudad Juarez, Mexico n = 29 per city, ages, 6−12 yr, asthma and current symptoms Repeated measures. Daily symptom diaries. Recruitment from schools representing a gradient of traffic, subjects from non-smoking homes. No information on participation rate. Self report of physician-diagnosed asthma. GLM with subject as random effect and adjustment for school, temperature, relative humidity. Adjustment for medication use, cold symptoms did not alter results.	NO_2-school outdoor 24-h avg In each city, 1 school 91 m from major road, 1 in residential area.	0−4 avg	No quantitative results reported; associations reported to be consistent with the null.	No copollutant model.
†Holguin et al. (2007) Ciudad Juarez, Mexico n = 95, ages 6−12 yr, 78% mild, intermittent asthma, 58% with atopy Repeated measures. Daily symptom diaries given by parents for 4 mo, checked biweekly. 87% participation. Parent report of physician-diagnosed asthma. Linear and nonlinear mixed effects model with random effect for subject and school adjusted for sex, body mass index, day of week, season, maternal and paternal education, passive smoking exposure.	NO_2-school outdoor 24-h avg Schools located 239−692 m from homes.	0−6 avg	No quantitative results reported. No air pollutants reported to be associated with respiratory symptoms.	No copollutant model. Road density at home and school reported not to be associated with respiratory symptoms.

Table 5-12 (Continued): Epidemiologic studies of respiratory symptoms and asthma medication use in children with asthma.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Odds Ratio (95% CI) Single-Pollutant Model[a]	Copollutant Examination
†Spira-Cohen et al. (2011); Spira-Cohen (2013) Bronx, NY n = 40, ages 10−12 yr, 100% nonwhite, 44% with asthma ED visit or hospital admission in previous 12 mo Repeated measures. Daily symptom diaries for 1 mo, checked daily. 454 observations. Recruitment from schools by referrals from school nurses. Parental report of physician-diagnosed asthma. 89% time indoors. No information on participation rate. Mixed effects model with subject as random effect adjusted for temperature, height, sex. Adjustment for school (indicator of season) did not alter results.	NO_2-school outdoor 6-h avg (9 a.m.−3 p.m.) Schools 53−737 m from highways with varying traffic counts. Most children walk to school.	0	Total symptoms: 1.10 (0.84, 1.45) Wheeze: 1.20 (0.75, 1.9) OR per 60-ppb increase NO_2 (5th to 95th percentile)	Personal EC associated with symptoms with NO_2 adjustment. No quantitative data reported.
†Gillespie-Bennett et al. (2011) Bluff, Dunedin, Christchurch, Porirua, Hutt Valley, New Zealand n = 358, ages 6−13 yr Cross-sectional. Daily symptom diaries for 112 days. Recruitment from a home heating intervention. 77% participation. Mixed effects model with log-transformed NO_2 and random effect for subject. Adjustment for age, sex, region, ethnicity, intervention, income, temperature did not alter results.	NO_2-home outdoor 24-h avg 1 measure per subject.	4-week avg	Lower respiratory symptom: 1.09 (0.78, 1.51) Reliever inhaler: 1.47 (0.96, 2.26) OR per log increase NO_2	No copollutant model. No other pollutants examined.
	NO_2-home indoor 24-h avg Up to 4 measures per subject.		Lower respiratory symptom: 1.14 (1.12, 1.16) Reliever inhaler: 1.14 (1.11, 1.17)	
†Gent et al. (2009) New Haven county, CT n = 149, ages 4−12 yr Repeated measures. Daily symptom diaries reported monthly. Recruitment from larger cohort, pediatric asthma clinic, and school. Parent report of physician diagnosed asthma. No information on participation rate. GEE adjusted for season, day of week, date, motor vehicle factor obtained by source apportionment.	NO_2-central site Avg time not reported 1 site 0.9−30 km of homes (mean 10.2 km).	0	NR	Wheeze with source apportionment factor of EC, zinc, lead, copper, selenium: 1.08 (0.99, 1.18). Factor results not altered by NO_2 adjustment. Moderate correlation with NO_2. Pearson r = 0.49.

Table 5-12 (Continued): Epidemiologic studies of respiratory symptoms and asthma medication use in children with asthma.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Odds Ratio (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Delfino et al. (2003) Los Angeles, CA (Huntington Park region) n = 16, ages 10−16 yr, 100% Hispanic, 27% on anti-inflammatory medication Repeated measures. Daily symptom diaries for 3 months, collected weekly. Recruitment from schools of non-smoking children from non-smoking homes. Self or parental report of physician diagnosed asthma. 92% follow-up participation. GEE with autoregressive parameter and adjusted for respiratory infections. Excluded potential confounding by weekend, maximum temperature.	NO_2-central site 4.8 km of home & school		Asthma symptoms not interfering with daily activity	Copollutant model results in figure. ORs for NO_2 not altered by xylene or toluene adjustment. Smaller but positive ORs for NO_2, wider 95% CI with adjustment for benzene, ethylbenzene, acetylaldehyde, formaldehyde. Moderate to high correlations with 8-h max NO_2. Spearman r = 0.57 (benzene) to 0.72 (xylene). No interactions between NO_2 and VOCs. ORs for VOCs attenuated with NO_2 adjustment. No copollutant model with $PM_{2.5}$, EC, OC. r = 0.38−0.62. No association with CO.
	8-h max	0	1.27 (1.05, 1.54)	
	1-h max		1.18 (0.96, 1.43)	
			Asthma symptoms interfering with daily activity	
	8-h max	0	1.40 (1.02, 1.92)	
	1-h max		1.27 (0.81, 1.99)	
			ORs per 1.4 ppb increase in 8-h max and 2.0 ppb increase in 1-h max NO_2 (interquartile ranges).	
Delfino et al. (2002) Alpine, CA and adjacent areas n = 22, ages 9−19 yr, 36% with mild persistent or more severe asthma, 77% with atopy Repeated measures. 92% follow-up. Daily symptom diaries for 61 days, collected weekly or biweekly. 1,248 observations (94% of expected). Recruitment from schools. Asthma diagnosis based on referrals from health maintenance organization and newspaper advertisements. Subjects were nonsmokers from nonsmoking homes. GEE with autoregressive lag 1 correlation matrix with no covariates. Adjustment for day of week, linear trend, temperature, humidity did not alter results. Adjustment for respiratory infection increased pollutant ORs.	NO_2-central site 1-h max	0	Symptoms interfering with daily activity: All subjects: 1.35 (0.82, 2.20) No anti-inflammatory medication, n = 12 1.80 (0.89, 3.64) On anti-inflammatory medication, n = 10 0.91 (0.21, 3.97)	Positive interaction for 8-h max NO_2 with 1-h max PM_{10} (p < 0.01) and 1-h max O_3 (p = 0.12). Fungi and pollen allergen associated with symptoms. No NO_2-allergen interactions. No quantitative results for NO_2-allergen copollutant models, but ORs reported to decrease. Moderate correlations with NO_2. Pearson r = 0.29 for fungi, 0.27 for pollen, 0.55 for PM_{10}.
	8-h max 1 site 1−4.7 km from subjects' homes.		All subjects: 1.65 (0.94, 2.89) No anti-inflammatory medication, n = 12 2.25 (1.09, 4.63) On anti-inflammatory medication, n = 10 1.10 (0.22, 5.46)	

Table 5-12 (Continued): Epidemiologic studies of respiratory symptoms and asthma medication use in children with asthma.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Odds Ratio (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Schildcrout et al. (2006) Albuquerque, NM; Baltimore, MD; Boston, MA; Denver, CO; San Diego, CA; St. Louis, MO; Toronto, ON, Canada (CAMP cohort) n = 990, ages, 5−12 yr, mild to moderate asthma Repeated measures. Daily symptom diaries for 21−201 days. No information on participation rate. GEE for individual cities combined for study-wide estimates. City-specific models adjusted for day of week, ethnicity, annual family income, response to methacholine, maximum temperature, humidity, temperature×humidity, calendar date. Pollutant analyzed as daily deviation from subject mean.	NO_2-central site 24-h avg Average of multiple sites within 80 km of ZIP code.	0 0−2 sum	Asthma symptoms: 1.06 (1.00, 1.12) Rescue Inhaler use: 1.04 (1.00, 1.08) Asthma symptoms: 1.05 (1.01, 1.09)	Joint effect models NO_2+CO: 1.07 (1.0, 1.14) NO_2+SO_2: 1.06 (0.98, 1.15) NO_2+PM_{10}:1.06 (0.99, 1.13) Moderate to high correlations with NO_2. r = 0.23 to 0.58 for SO_2, 0.26 to 0.64 for PM_{10}, 0.63 to 0.92 for CO.
†Mann et al. (2010) Fresno, Clovis, CA n = 280, ages 6−11 yr, 47% mild persistent asthma, 25% moderate to severe asthma, 63% with atopy Repeated measures. Daily symptom diaries for 14 days every 3 mo. Recruitment from schools, advertisements, physician's offices, local media. Imputed wheeze values for 7.6% days. Participation from 89% of original cohort. Group examined representative of original cohort. GEE adjusted for fitted daily mean wheeze, home ownership, race, sex, asthma severity, panel group, 6-mo cohort, 1-h minimum temperature. Adjustment for medication use did not alter results.	NO_2-central site 24-h avg 1 site within 20 km of homes.	2	Wheeze: All subjects: 1.24 (1.05, 1.48) Fungi allergic, n = 85 1.61 (1.24, 2.08) Cat allergic, n = 49 1.73 (1.14, 2.62) Boys, intermittent asthma, n = 47 2.58 (1.61, 4.13)	With $PM_{10-2.5}$, all subjects: 1.14 (0.95, 1.37). $PM_{10-2.5}$ association not altered by NO_2 adjustment. Weak correlation with NO_2. r = 0.12.
Mortimer et al. (2002) Bronx and East Harlem, NY; Chicago, IL; Cleveland, OH; Detroit, MI; St. Louis, MO; Washington, DC (NCICAS cohort) n = 846, ages 4−9 yr Repeated measures. Daily symptom data collected for 2-week periods every 3 mo. Recruitment from ED visits and clinics. Parent report of physician-diagnosed asthma and symptoms in previous 12 mo or asthma symptoms for >6 weeks, or family history of asthma. Participation from 55% full cohort. Sample representative of full cohort except for greater asthma medication use. Mixed effects model adjusted for city, follow-up period, day of study, 24-h rainfall, 12-h avg temperature.	NO_2-central site 4-h avg (6 a.m.−10 a.m.) Average of all city monitors.	Lag 1−6 avg	Morning symptoms: 1.48 (1.02, 2.16) OR per 20 ppb increase in NO_2 (interquartile range).	With O_3 (summer): 1.40 (0.93, 2.09) Weak correlation with NO_2. r = 0.27. O_3 effect estimate also slightly attenuated. SO_2 and PM_{10} also associated with symptoms. Correlations with NO_2 not reported.

Table 5-12 (Continued): Epidemiologic studies of respiratory symptoms and asthma medication use in children with asthma.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Odds Ratio (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Studies with central site exposure assessment and no examination of copollutant confounding				
Jalaludin et al. (2004) Sydney, Australia n = 125, mean age 9.6 yr, 45 with wheeze, asthma, and airway hyperresponsiveness, 60 with wheeze and asthma, 20 with wheeze Repeated measures. Daily symptom diary mailed in monthly for 11 mo. Recruitment from schools. Parent-report of physician-diagnosed asthma. 84% follow-up participation. GEE adjusted for time trend, temperature, humidity, number of hours spent outdoors, total pollen and alternaria, season.	NO_2-central site 15-h avg (6 a.m.−9 p.m.) Site within 2 km of schools.	0	Wheeze: 1.01 (0.94, 1.09) Wet cough: 1.05 (1.00, 1.10) Beta agonist use: 1.01 (0.97, 1.05) OR per 8.2 ppb increase in NO_2 (interquartile range)	NO_2 associations found in children with asthma/airway hyperresponsiveness but examined only in multipollutant model with O_3 and PM_{10}. Negative or weak correlations with NO_2. r = −0.31 for O_3, 0.26 for PM_{10}.
†Escamilla-Nuñez et al. (2008) Mexico City, Mexico n = 147, ages 6−14 yr, 43% with persistent asthma, 89% atopy Repeated measures. Symptom data collected every 15 days for mean 22 weeks. Children with asthma recruited from pediatric clinic. Asthma severity assessed by pediatric allergist. No information on participation rate. Linear mixed effects model with random effect for subject and adjusted for asthma severity, atopy, lag 1 minimum temperature, time, sex. Adjustment for outdoor activities, smoking exposure, season did not alter results.	NO_2-central site 1-h max Site within 5 km of school or home.	1	Cough: 1.07 (1.02, 1.12) Wheeze: 1.08 (1.02, 1.14)	No copollutant model. $PM_{2.5}$ and O_3 also associated with symptoms. No statistically significant interaction between NO_2 and $PM_{2.5}$ or O_3. Quantitative results not reported.
†Barraza-Villarreal et al. (2008) Mexico City, Mexico n = 126, ages 6−14 yr, 44% persistent asthma, 89% with atopy Part of same cohort as above. No information on participation rate. Linear mixed effects model with random effect for subject and adjusted for sex, body mass index, lag 1 minimum temperature, ICS use, time. Adjustment for outdoor activities, smoking exposure, anti-allergy medication use, season did not alter results.	NO_2-central site 1-h max Site within 5 km of school or home. Low correlation for central site vs. school: Spearman r = 0.21	0	Wheeze: 1.09 (1.03, 1.15) Cough: 1.09 (1.04, 1.14)	No copollutant model. $PM_{2.5}$ and O_3 also associated with symptoms. Moderate correlations with NO_2. Pearson r = 0.61 for $PM_{2.5}$, 0.28 for O_3.
Romieu et al. (2006) Mexico City, Mexico n = 151, mean age 9 yr, mild or moderate asthma	NO_2-central site 1-h max Site within 5 km of home.	1−6 avg	Cough by genotype: GSTM1 null 1.09 (1.00, 1.19) GSTM1 positive 1.19 (1.11, 1.27) GSTP1 Ile/Ile or Ile/Val 1.19 (1.11, 1.27)	No copollutant model. Associations with O_3 found with different variants than NO_2. Moderate correlation with NO_2. Pearson r = 0.57 for O_3 and PM_{10}.

Table 5-12 (Continued): Epidemiologic studies of respiratory symptoms and asthma medication use in children with asthma.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Odds Ratio (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Repeated measures. Daily symptom diaries 61−92 days per subject, collected weekly. Recruitment from allergy clinic as part of a Vitamin C/E supplementation trial. Diagnosis by clinical examination. 99% follow-up participation. GEE adjusted for supplementation group, minimum temperature, smoking exposure, asthma severity, time.			GSTP1 Val/Val 1.08 (0.99, 1.18) BD use by genotype: GSTM1 null 0.94 (0.87, 1.02) GSTM1 positive 1.09 (1.02, 1.17) GSTP1 Ile/Ile or Ile/Val 1.08 (1.02, 1.14) GSTP1 Val/Val 0.94 (0.85, 1.04)	
Ostro et al. (2001) Central Los Angeles and Pasadena, CA n = 138 (83% LA), ages 8−13 yr, 85% mild or moderate asthma, 100% African American Repeated measures. 90% follow-up. Daily symptom diaries for 13 weeks, mailed in weekly. Excluded subjects returning diaries after 2 weeks. 9,126 observations. Recruitment from hospitals, urgent care clinics, medical practices, asthma camps in Los Angeles and school nurses in Pasadena. GEE adjusted for day of study, age, income, town, lag 1 temperature, lag 1 humidity.	NO_2-central site 1-h max Los Angeles site within 16 km of 90% of subjects' homes. Pasadena site within 8 km of subjects' homes.	3	Onset of shortness of breath: 1.08 (0.99, 1.18) Onset of wheeze: 1.08 (1.02, 1.13) Onset of Cough: 1.07 (1.00, 1.15) No quantitative results for extra medication use but reported not to be associated with NO_2.	No copollutant model. Symptoms associated with $PM_{2.5}$, PM_{10}, fungi. Weak to moderate correlations with NO_2. r = 0.18 for pollen, 0.26−0.48 for fungi, 0.34 for $PM_{2.5}$, 0.63 for PM_{10}.
†Patel et al. (2010) New York City and nearby suburb, NY n = 57, ages 14−20 yr Repeated measures. Daily symptom diaries for 4−6 weeks, collected weekly. Recruitment from schools. Self-report of physician-diagnosed asthma. 75−90% participation across schools. GLMM with random effect for subject and school and adjusted for weekend, daily 8-h max O_3, urban location. Adjustment for season, pollen counts did not alter results.	NO_2-central site 24-h avg 1 site 2.2−9.0 km from schools, 1 site 40 km from schools.	0	Wheeze: 1.16 (0.93, 1.45) Chest tightness: 1.26 (1.00, 1.58)	No copollutant model with BC. BC also associated with symptoms. Across locations, moderate to high correlations with NO_2. Spearman r = 0.56−0.90.
Just et al. (2002) Paris, France n = 82, ages 7−15 yr, asthma attack in previous 12 mo and daily asthma medication use, 90% atopy	NO_2-central site 24-h avg Average of 11 sites	0	Asthma attack: 1.75 (0.82, 3.70) Night cough: 2.11 (1.20, 3.71)	No copollutant model BS associated with cough. High correlation with NO_2. Pearson r = 0.92.

Table 5-12 (Continued): Epidemiologic studies of respiratory symptoms and asthma medication use in children with asthma.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Odds Ratio (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Repeated measures. Daily symptom diaries for 3 mo, collected weekly. Recruitment from hospital outpatients. 82% participation. GEE adjusted for time trend, day of week, pollen, temperature, humidity.				
Segala et al. (1998) Greater Paris area, France n = 43 mild asthma, 41 moderate asthma, 89% atopy, 69% ICS users, ages 7−15 yr Repeated measures. Daily symptom diary for 25 weeks, collected weekly. Recruitment from outpatients of children's hospital. 84% follow-up participation. GEE adjusted for day of week, time trend, temperature, humidity, age, sex.	NO_2-central site 24-h avg Average of 8 sites	0	Incident asthma: Mild asthma, n = 43 1.89 (1.13, 3.15)	No copollutant model. Associations also found with BS, PM_{13}, & SO_2. Moderate correlations with NO_2. Pearson r = 0.61 for BS, 0.55 for PM_{13}, 0.54 for SO_2.
		3	Moderate asthma, n = 41 1.31 (0.85, 2.03)	
		3	Beta agonist use: Mild asthma, n = 43 1.27 (0.82, 1.98)	
		0	Moderate asthma, n = 41 1.56 (0.51, 4.73)	
†O'Connor et al. (2008) Boston, MA; Bronx, NY; Chicago, IL; Dallas, TX; New York, NY; Seattle, WA; Tucson, AZ (ICAS cohort) n = 861, ages 5−12 yr, persistent asthma and atopy, 82% black or Hispanic Repeated measures. Symptom data collected for 2 week period every 2 mo for 2 yr. Recruitment from intervention of physician feedback. 89% of maximum possible diaries obtained. Mixed effects model adjusted for site, mo, site×mo interaction, temperature, intervention group.	NO_2-central site 24-h avg All monitors near home, not near industry. Median distance to site = 2.3 km.	1−19 avg	Wheeze-cough: 1.17 (0.99, 1.37) Slow Play: 1.25 (1.04, 1.51) Missed school in 2 week period: 1.65 (1.18, 2.32)	Only 3-pollutant model analyzed. Associations also found with $PM_{2.5}$ and CO. Moderate correlations with NO_2. r = 0.59 for $PM_{2.5}$, 0.54 for CO.

Note: More informative studies in terms of the exposure assessment method and potential confounding considered are presented first.
BD = bronchodilator; CAMP = Childhood Asthma Management Program; GEE = generalized estimating equations; GLM = generalized linear model; GLMM = generalized linear mixed model; GST = Glutathione-S-transferase; ICAS = Inner City Asthma Study; ICS = inhaled corticosteroid; NCICAS = National Cooperative Inner-city Asthma Study, CI = confidence interval, CO = carbon monoxide, EC = elemental carbon, ED = emergency department, NO_2 = nitrogen dioxide, O_3 = ozone, OC = organic carbon, OR = odds ratio, PM = particulate matter, SD = standard deviation, SO_2 = sulfur dioxide, VOC = volatile organic compound.
[a]Effect estimates are standardized to a 20 ppb for 24-h avg NO_2, 25 ppb for 8-h max, and a 30-ppb increase for 1-h max NO_2. Effect estimates for other averaging times are not standardized but presented as they are reported in their respective studies (Section 5.1.2.3).
†Studies published since the 2008 ISA for Oxides of Nitrogen.

Several studies are noteworthy for NO_2 exposure assessment in subjects' locations or analysis of the influence of other traffic-related pollutants on NO_2 associations. Outdoor school or home NO_2 concentrations are spatially aligned with a location of subjects and may better represent ambient exposures for that location. In a group of 17 children with asthma and allergy in El Paso, TX, a 20-ppb increase in outdoor school 4-day avg NO_2 was associated with a 0.56 (95% CI: −0.17, 1.28)-point poorer asthma control score (composite of symptoms, activity limitation and asthma medication use) (Zora et al., 2013). Among children in Bronx, NY, 6-h avg school-day NO_2 (9 a.m.−3 p.m.) was associated with total symptoms (OR: 1.10 [95% CI: 0.84, 1.45] per 60-ppb NO_2) and wheeze (OR: 1.05 [95% CI: 0.87, 1.39]) but 95% CIs were wide (Spira-Cohen et al., 2011). Other studies did not indicate associations of school or home NO_2 with respiratory symptoms in children with asthma, but it is not clear whether their results represent inconsistency in the evidence base. Studies conducted in El Paso, TX and Ciudad Juarez, Mexico only reported that NO_2 was not associated with respiratory symptoms in children with asthma but did not report quantitative results (Sarnat et al., 2012; Holguin et al., 2007). Outdoor home NO_2 was associated with reliever inhaler use but not respiratory symptoms among children with asthma in multiple New Zealand towns (Gillespie-Bennett et al., 2011). However, daily outcomes were analyzed with a single 4-week sample of NO_2, which cannot represent temporal variability in exposure. Home indoor NO_2, which was represented as up to four measurements per subject, showed stronger associations with both outcomes.

Most studies observed NO_2-related increases in respiratory symptoms with adjustment for temperature, humidity, season, and day of week. A few studies additionally adjusted for asthma medication use, colds, smoking exposure, and allergens (Table 5-12). Some studies with central site exposure assessment are informative for their analysis of copollutant confounding or interactions. Studies with school and central site exposure assessment found symptoms associated with the traffic-related pollutants EC/BC, OC, CO, and VOCs, which showed a wide range of correlations with NO_2 (r = 0.16−0.92) (Table 5-12). In New Haven County, CT, NO_2 was associated with wheeze in children with asthma with adjustment for a source apportionment factor comprising EC, zinc, lead, copper, and selenium (OR: 1.08 [95% CI: 0.99, 1.18] per unspecified increase in lag 0 NO_2) (Gent et al., 2009). In El Paso, TX, neither NO_2 nor BC was associated with asthma control in a copollutant model (Zora et al., 2013). However, these results were based on the whole study population. NO_2 and BC were associated with asthma control only in children with asthma and allergies; thus, the copollutant model results do not clearly inform potential confounding. Among children in Los Angeles, CA, CO was not associated with symptoms, and NO_2-asthma symptom associations were relatively unchanged with adjustment for various VOCs. No NO_2-VOC interaction was found (Delfino et al., 2003). NO_2 concentrations assigned from a central site within 4.8 km of

children's homes may well represent the broad variability in NO_2 in the study area given the high correlations observed among monitors in the area (Section 2.5.3), although it is uncertain whether variability within neighborhoods is adequately represented.

In the multicity Childhood Asthma Management Program (CAMP) study, the joint effect of NO_2 and CO (OR: 1.07 [95% CI: 1.00, 1.14] for a 20-ppb increase in lag 0−2 day sum of 24-h avg NO_2) was similar to the NO_2 (OR: 1.05 [95% CI: 1.01, 1.09]) and CO single-pollutant ORs (Schildcrout et al., 2006). These results indicate a lack of multiplicative interaction between NO_2 and CO but do not inform potential confounding. Although analysis is limited in both the number of studies and array of traffic-related copollutants, there is evidence that NO_2 has an association with respiratory symptoms in children with asthma independent of EC or VOCs, but interactions are not demonstrated. Interactions with NO_2 were not found consistently for PM_{10} and not found at all for SO_2, O_3, or allergens (Schildcrout et al., 2006; Delfino et al., 2002). These copollutants were not examined as potential confounding factors. An NO_2-wheeze association was observe to decrease in magnitude and precision (i.e., wider 95% CI) with adjustment for $PM_{10-2.5}$ (r = 0.12) (Mann et al., 2010), based on exposures assessed from a site up to 20 km of children's homes.

Other studies largely corroborate the aforementioned evidence but do not provide a strong basis for assessing an independent effect of NO_2 exposure on respiratory symptoms in children with asthma because of both central site exposure assessment and no examination of potential confounding by other traffic-related pollutants (Table 5-11). In these multi- and single-city studies, central sites were located 2−16 km from children's homes or schools (Patel et al., 2010; Barraza-Villarreal et al., 2008; Escamilla-Nuñez et al., 2008; Romieu et al., 2006; Jalaludin et al., 2004; Ostro et al., 2001), averaged across city sites (O'Connor et al., 2008; Just et al., 2002; Mortimer et al., 2002), or an unspecified method (Segala et al., 1998). For most locations, information was not reported to assess whether the temporal variation in these metrics represented the variation across the study areas. Low within-city correlations in NO_2 were reported for Mexico City (Barraza-Villarreal et al., 2008; Escamilla-Nuñez et al., 2008), and high correlations for NO_2 were reported for Los Angeles/Pasadena (Ostro et al., 2001) (Section 2.5.3). The recent multicity ICAS found increases in symptoms, slow play, and missed school in association with a 19-day avg of 24-h avg NO_2 (O'Connor et al., 2008), but there is potential for residual temporal confounding for associations with NO_2 exposure on the order of weeks. ICAS could not examine shorter lags because symptom data were collected with a time resolution of 2 weeks.

In addition to NO_2, most studies with central site exposure assessment found associations with the traffic-related copollutants BC, black smoke (BS), $PM_{2.5}$, and CO, which were

moderately to highly correlated with NO_2 (r = 0.34−0.92) (Patel et al., 2010; Barraza-Villarreal et al., 2008; Just et al., 2002; Ostro et al., 2001). A potential confounding effect of these traffic-related pollutants was examined only in multipollutant models, which can produce unreliable results because of potential collinearity (Kim et al., 2012; Escamilla-Nuñez et al., 2008; O'Connor et al., 2008). Copollutant confounding was not examined for PM_{10}, SO_2, or O_3 either. These pollutants were moderately correlated with NO_2 (r = 0.28−0.31) in most studies, although some reported higher correlations (r = 0.54−0.68) (Ostro et al., 2001; Segala et al., 1998).

In addition to the limited findings from copollutant models with traffic-related copollutants, an independent effect of NO_2 exposure is supported by numerous studies that show increases in respiratory symptoms in association with increases in indoor NO_2 averaged over 3 to 7 days or a 4-week avg (Lu et al., 2013; Gillespie-Bennett et al., 2011; Hansel et al., 2008). Previous findings indicated reductions in respiratory symptoms after an intervention to switch to flued gas heaters led to a reduction in indoor classroom NO_2 concentrations (Pilotto et al., 2004). Although potential differences in pollutant mixtures between the indoor and outdoor environments have not been well characterized, a recent study found that correlations between NO_2 and copollutants differed between the indoor and outdoor environments for BC, PM, and SO_2 (Sarnat et al., 2012), suggesting that NO_2 may exist as part of a different pollutant mixture in the indoor and outdoor environments.

Adults with Asthma

Previous and recent evidence indicates associations of ambient NO_2 concentrations with respiratory symptoms (Maestrelli et al., 2011; Wiwatanadate and Liwsrisakun, 2011; von Klot et al., 2002; Boezen et al., 1998; Forsberg et al., 1998) and asthma medication use or sales (Carlsen et al., 2012; Laurent et al., 2009; von Klot et al., 2002; Forsberg et al., 1998; Hiltermann et al., 1998) among adults with asthma or bronchial hyperresponsiveness. Most studies were conducted in Europe and recruited subjects primarily from clinics, doctors' offices, and administrative databases. Subjects represented a mix of asthma severity and prevalence of ICS use and atopy. A few studies did not find associations with symptoms, including Kim et al. (2012), which analyzed only a multipollutant model with SO_2, PM_{10}, O_3, and CO, whose results can be unstable. Null results also were reported in studies with more reliable statistical analysis that were conducted in four European countries (Karakatsani et al., 2012) and one with adults with asthma and allergy (Feo Brito et al., 2007). Results from the latter study contrast those from experimental studies showing NO_2-induced allergic inflammation in humans with asthma and animal models of allergic disease (Section 5.2.2.5). Across studies, respiratory symptoms were associated with lag day 0 NO_2. Medication use or sales were associated more strongly with multiday averages of NO_2 (i.e., lag 3−5 avg, 0−5 avg,

0−6 avg) than with single-day lags (Carlsen et al., 2012; von Klot et al., 2002; Hiltermann et al., 1998), and Carlsen et al. (2012) found a stronger association for beta-agonist sales with 1-h max than 24-h avg NO_2.

Despite the consistency of evidence, there is limited basis for inferring NO_2 effects on respiratory symptoms in adults with asthma. Most studies assigned NO_2 exposure from a single central site monitor located in the community and did not report information to assess whether the NO_2 metrics were representative of the temporal variability across the study areas and of subjects' ambient exposures. With exception of Boezen et al. (1998), studies found associations with the traffic-related pollutants CO, BS, $PM_{2.5}$, and UFP, and few analyzed potential confounding. Confounding is an uncertainty also for the association between beta-agonist sales and block-level NO_2 estimated with a dispersion model (Laurent et al., 2009). Block-level NO_2 was highly correlated with measured concentrations (r = 0.87), but other traffic-related pollutants were not examined. Only von Klot et al. (2002) conducted copollutant modeling and found an association between lag 0−4 day avg NO_2 and beta-agonist use with adjustment for $PM_{2.5}$ or UFP (OR: 1.22 [95% CI: 1.05, 1.43] per 20-pbb increase in NO_2, with adjustment for UFP, Pearson r = 0.66). The NO_2-wheeze association was attenuated with adjustment for UFP (OR: 1.02 [95% CI: 0.86, 1.21]). Copollutant effect estimates were attenuated with NO_2 adjustment. Thus, an independent NO_2 association was found for medication use, but an independent association with wheeze was not discerned for either NO_2 or UFP.

Controlled Human Exposure Studies

Controlled human exposure studies do not provide strong evidence for NO_2-induced increases in respiratory symptoms in adults or adolescents with asthma. The majority of these controlled human exposure studies that assessed respiratory symptoms before, during, or after exposure to NO_2 did not find changes. Unlike studies of airway responsiveness (Section 5.2.2.1), symptom studies did not include a challenge agent with NO_2 exposure. One recent study is available in addition to the studies reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a). Study details are presented in Table 5-13; overall, studies subjected participants to NO_2 exposures of 120−4,000 ppb for 2−5 hours and then conducted an assessment of symptoms 24 hours later.

The majority of studies reported no change in symptoms, as measured by symptom score, in healthy subjects or in adults with asthma (Jörres et al., 1995; Linn et al., 1985b; Kleinman et al., 1983) or in adolescents (Koenig et al., 1987). Vagaggini et al. (1996) reported a small, but statistically significant increase in symptom score during NO_2 exposures in healthy adults, but not those with asthma. Riedl et al. (2012) recently reported an increase in symptom score in adults with asthma during, but not after,

exposure to 350 ppb NO_2 for 2 hours with alternating periods of exercise. The increase in symptom score corresponded to a subject experiencing a mild increase in any two symptoms or moderate elevation of any one symptom. Symptom scores were not different between air and NO_2-exposed subjects when categorically grouped as respiratory, cardiovascular, or miscellaneous; nor were they different when subjects were exposed to allergen after NO_2 exposure Riedl et al. (2012).

Table 5-13 Controlled human exposure studies of respiratory symptoms.

Study	Disease Status; n, Sex; Age (mean ± SD)	Exposure Details (Concentration; Duration)	Time of Symptom Assessment
Jörres et al. (1995)	Asthma; n = 8 M, 4 F; 27 ± 5 yr Healthy; n = 5 M, 3 F; 27 yr (range: 21−33)	1,000 ppb for 3 h; Exercise 10 min on/10 min off at individual's maximum workload	Immediately and 6 and 24 h after exposure.
Kleinman et al. (1983)	Asthma; n = 12 M, 19 F; 31 ± 1 yr	200 ppb for 2 h; Exercise 15 min on/15 min off at $\dot{V}_E$ = ~2 times resting	Before, immediately after, and day after exposure.
Koenig et al. (1987)	Asthma; (1) n = 4 M, 6 F (2) n = 7 M, 3 F; Healthy; (1) n = 3 M, 7 F (2) n = 4 M, 6 F; 14.4 yr (range: 12−19)	(1) 120 ppb NO_2 (2) 180 ppb NO_2; (1−2) Exposures were 30 min at rest with 10 min of moderate exercise	Immediately after, a day after, and a week after exposure.
Linn et al. (1985b)	Asthma; n = 12 M, 11 F; (range: 18−34 yr) Healthy; n = 16 M, 9 F; (range: 20−36 yr)	4,000 ppb for 75 min; Two 15 min periods of exercise at $\dot{V}_E$ = 25 L/min and 50 L/min	Before, during, immediately after, 1 day after and 1 week after exposure.
Riedl et al. (2012)	Asthma Phase 1 (methacholine challenge); n = 10 M, 5 F; 37.3 ± 10.9 yr Phase 2 (cat allergen challenge); n = 6 M, 9 F; 36.1 ± 12.5 yr	350 ppb for 2 h; Exercise 15 min on/15 min off at $\dot{V}_E$ = 15−20 L/min	Before, during, 1−22 h after exposure.
Vagaggini et al. (1996)	Asthma; n = 4 M, 4 F; 29 ± 14 yr Healthy; n = 7 M; 34 ± 5 yr	300 ppb for 1 h; Exercise at $\dot{V}_E$ = 25 L/min	Before and 2 h after exposure.

F = female, M = male, NO_2 = nitrogen dioxide, SD = standard deviation.

5.2.2.4 Hospital Admissions and Emergency Department Visits for Asthma

The evidence for NO_2-related effects on increasing airway responsiveness, decreasing lung function, and increasing respiratory symptoms detailed in the preceding sections are all indicators of asthma exacerbation that may lead people with asthma to seek medical interventions. Thus, the preceding evidence is coherent with associations observed between short-term increases in ambient NO_2 concentrations and hospital admissions and ED visits for asthma. Since the completion of the 2008 ISA for Oxides of Nitrogen, epidemiologic studies have continued to examine the association between short-term exposure to ambient NO_X or NO_2 and respiratory-related hospital admissions and ED visits, but are primarily limited to single-city studies. The sections within this chapter that detail the studies of respiratory-related hospital admissions and ED visits characterize recent studies in the context of the collective body of evidence evaluated in the 2008 ISA for Oxides of Nitrogen. As summarized in Section 5.2.6, the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a) included the first thorough evaluation of respiratory morbidity in the form of respiratory-related hospital admissions and ED visits, including asthma. Previous studies of asthma hospital admissions and ED visits consistently reported positive associations with short-term NO_2 exposures (Figure 5-7, Table 5-16) and observed associations that were generally robust and independent of the effects of ambient particles or gaseous copollutants (e.g., O_3, SO_2, CO, benzene) (U.S. EPA, 2008a). The strongest evidence for associations between short-term NO_2 exposures and asthma were from studies of all ages and children.

Within this section focusing on asthma, as well as the rest of the chapter, studies of respiratory-related hospital admissions and ED visits are evaluated separately because often only a small percentage of respiratory-related ED visits will be admitted to the hospital. Therefore, ED visits may represent potentially less serious, but more common, outcomes. The air quality characteristics of the city, or across all cities, and the exposure assignment approach used in each asthma hospital admission and ED visit study evaluated in this section are presented in Table 5-14. Other recent studies of asthma hospital admissions and ED visits are not the focus of this evaluation because they were conducted in small individual cities, encompass a short study duration, had insufficient sample size, and/or did not examine potential copolluant confounding. The full list of these studies, as well as study specific details, can be found in Supplemental Table S5-3 (U.S. EPA, 2014h).

Table 5-14 Mean and upper percentile concentrations of oxides of nitrogen in studies of asthma hospital admissions and emergency department (ED) visits.

Study	Location Years	Exposure Assignment	Metric	Mean/Median Concentration ppb	Upper Percentile of Concentrations (ppb)	Copollutant Examination
Hospital Admissions						
(Linn et al., 2000)	Los Angeles, CA (1992−1995)	Average of NO_2 concentrations over all monitors.	24-h avg	3.4	NR	Correlations (r): Range across seasons CO: 0.84−0.94 PM_{10}: 0.67−0.88 O_3: −0.23 to 0.35 Copollutant models: none
(Burnett et al., 1999)	Toronto, Canada (1980−1994)	Average of NO_2 concentrations from 4 monitors.	24-h avg	25.2	NR	Correlations (r): $PM_{2.5}$: 0.55 $PM_{10-2.5}$: 0.38 PM_{10}: 0.57 CO: 0.64 SO_2: 0.54 O_3: −0.08 Copollutant models: none
†Samoli et al. (2011)	Athens, Greece (2001−2004)	Average of NO_2 concentrations across 14 monitors.	1-h max	44.4	75th: 53.1	Correlations (r): SO_2: 0.55 Copollutant models: PM_{10}, SO_2, O_3
†Iskandar et al. (2012)	Copenhagen, Denmark (2001−2008)	All hospitals located within 15 km of a central site monitor.	24-h avg	NO_2: 11.3 NO_X: 14.5	75th: NO_2: 14.2 NO_X: 17.7	Correlations (r): NO_X: 0.93 PM_{10}: 0.43 $PM_{2.5}$: 0.33 UFP: 0.51 Copollutant models: NO_X, $PM_{2.5}$, PM_{10}, UFP
†Ko et al. (2007b)	Hong Kong (2000−2005)	Average of NO_2 concentrations across 14 monitors.	24-h avg	28.3	75th: 33.8 Max: 79.5	Correlations (r): SO_2: 0.57 PM_{10}: 0.76 O_3: 0.41 $PM_{2.5}$: 0.77 Copollutant models: O_3

Table 5-14 (Continued): Mean and upper percentile concentrations of oxides of nitrogen in studies of asthma hospital admissions and emergency department (ED) visits.

Study	Location Years	Exposure Assignment	Metric	Mean/Median Concentration ppb	Upper Percentile of Concentrations (ppb)	Copollutant Examination
†Son et al. (2013)	8 South Korean cities (2003−2008)	Average of hourly ambient NO_2 concentrations from monitors in each city.	24-h avg	11.5−36.9	NR	Correlations (r): PM_{10}: 0.5 O_3: −0.1 SO_2: 0.6 CO: 0.7 Copollutant models: none
ED Visits						
Peel et al. (2005)	Atlanta, GA (1993−2000)	Average of NO_2 concentrations from monitors for several monitoring networks.	1-h max	45.9	NR	Correlations (r): $PM_{2.5}$: 0.46 PM_{10}: 0.49 $PM_{10-2.5}$: 0.46 UFP: 0.26 PM2.5 water soluble Metals: 0.32 $PM_{2.5}$ sulfate: 0.17 $PM_{2.5}$ acidity: 0.10 $PM_{2.5}$ OC: 0.63 $PM_{2.5}$ EC: 0.61 Oxygenated HCs: 0.30 O_3: 0.42 CO: 0.68 SO_2: 0.34 Copollutant models: none
Tolbert et al. (2000)	Atlanta, GA (1993−2004)	Average of NO_2 concentrations from monitors for several monitoring networks.	1-h max	81.7	Max: 306	Correlations (r): $PM_{2.5}$: 0.47 PM_{10}: 0.53 $PM_{10-2.5}$: 0.4 $PM_{2.5}$ sulfate: 0.14 $PM_{2.5}$ OC: 0.62 $PM_{2.5}$ EC: 0.64 $PM_{2.5}$ TC: 0.65 $PM_{2.5}$ water soluble Metals: 0.32 Oxygenated HCs: 0.24 O_3: 0.44 CO: 0.70 SO_2: 0.36 Copollutant models: CO, PM_{10}, O_3

Table 5-14 (Continued): Mean and upper percentile concentrations of oxides of nitrogen in studies of asthma hospital admissions and emergency department (ED) visits.

Study	Location Years	Exposure Assignment	Metric	Mean/Median Concentration ppb	Upper Percentile of Concentrations (ppb)	Copollutant Examination
Jaffe et al. (2003)	2 Ohio cities (Cincinnati and Cleveland) (1991−1996)	When more than 1 NO_2 monitor operating in a day, monitor with highest 24-h avg concentration used.	24-h avg	Cincinnati: 50 Cleveland: 48	NR	Correlations (r): Cincinnati PM_{10}: 0.36 O_3: 0.60 SO_2: 0.07 Cleveland PM_{10}: 0.34 SO_2: 0.28 O_3: 0.42 Copollutant models: none
Ito et al. (2007b)	New York, NY (1999−2002)	Average of NO_2 concentrations from 15 monitors.	24-h avg	31.1	NR	Correlations (r): NR Copollutant models: $PM_{2.5}$, O_3, SO_2, CO
ATSDR (2006)	Bronx and Manhattan, N (1999−2000)	NO_2 concentrations from 1 monitor in Bronx and 1 in Manhattan.	24-h avg	Bronx: 36 Manhattan: 31	NR	Correlations (r): Bronx O_3: 0.03 SO_2: 0.47 FRM $PM_{2.5}$: 0.61 Max $PM_{2.5}$: 0.55 Manhattan: NR Copollutant models: O_3, FRM and Max $PM_{2.5}$, SO_2
†Strickland et al. (2010)	Atlanta, GA (1993−2004)	Combined daily NO_2 concentrations across monitors using population weighting.	1-h max	23.3	NR	Correlations (r): NR Copollutant models: O_3
†Sarnat et al. (2013a)	Atlanta, GA (1999−2002)	NO_X concentrations predicted using fused spatially interpolated background pollutant concentrations and local-scale AERMOD output for 186 zip codes.	24-h avg	NO_X: 30.1	75th: 40.1 95th: 94.4 Max: 517.8	Correlation (r) with NO_X: CO: 0.93 O_3: -0.03 $PM_{2.5}$: 0.40 Copollutant models: none
†Villeneuve et al. (2007)	Edmonton, Canada (1992−2002)	Average of NO_2 concentrations across 3 monitoring stations.	24-h avg	50th: 17.5 Summer 50th: 28.5 Winter	75th: 22.0 summer 75th: 35.5 winter	Correlations (r): CO: 0.74 Copollutant models: CO

Table 5-14 (Continued): Mean and upper percentile concentrations of oxides of nitrogen in studies of asthma hospital admissions and emergency department (ED) visits.

Study	Location Years	Exposure Assignment	Metric	Mean/Median Concentration ppb	Upper Percentile of Concentrations (ppb)	Copollutant Examination
†Jalaludin et al. (2008)	Sydney, Australia (1997−2001)	Average of NO_2 concentrations across 14 monitoring stations.	1-h max	23.2	Max: 59.4	Correlations (r): PM_{10}: 0.67 $PM_{2.5}$: 0.68 O_3: 0.21 CO: 0.71 SO_2: 0.52 Copollutant models: PM_{10}, $PM_{2.5}$, O_3, CO, SO_2
†Stieb et al. (2009)	7 Canadian cities (1992−2003)	Average NO_2 concentrations from all monitors in each city. Number of NO_2 monitors in each city ranged from 1−14.	24-h avg	9.3−22.7	75th: 12.3−27.6	Correlations (r) only reported by city and season. Copollutant models: none
†Orazzo et al. (2009)	6 Italian cities (1996−2002)	Average of NO_2 concentrations from all monitors in each city.	24-h avg	21.4−41.2	NR	Correlations (r): NR Copollutant models: none
†Strickland et al. (2011)	Atlanta, GA (1993−2004)	NO_2 concentrations obtained from 3 networks of stationary monitors. Each air pollutant measured by at least 3 monitoring stations. 3 exposure metrics used: (1) 1 downtown monitor was selected to be the central site monitor, (2) all monitors used to calculate unweighted average of pollutant concentrations for all monitors, and (3) population-weighted average concentration.	1-h max	Central monitor: 42.0 Unweighted average: 27.7 Population-weighted average: 22.0	NR	Correlations (r): NR Copollutant models: none

Table 5-14 (Continued): Mean and upper percentile concentrations of oxides of nitrogen in studies of asthma hospital admissions and emergency department (ED) visits.

Study	Location Years	Exposure Assignment	Metric	Mean/Median Concentration ppb	Upper Percentile of Concentrations (ppb)	Copollutant Examination
†Li et al. (2011b)	Detroit, MI (2004−2006)	Average of NO_2 concentrations from 2 monitors in Detroit metropolitan area that measure NO_2.	24-h avg	15.7	75th: 21.2 Max: 55.2	Correlations (r), range across monitors: CO: 0.37−0.40 $PM_{2.5}$: 0.56−0.66 SO_2: 0.42−0.55 Copollutant models: none
†Gass et al. (2014)	Atlanta, GA (1999−2009)	Population-weighted average NO_2 concentrations based on same methods as (Strickland et al., 2010).	24-h avg	NR	NR	Correlations (r): NR Copollutant models: none
†Winquist et al. (2014)	Atlanta, GA (1998−2004)	Population-weighted average of NO_2 concentrations.	1-hr max	Warm (May−Oct): 22.3 Cold (Nov−April): 25.6	75th: Warm: 28.7 Cold: 31.7	Correlations (r): Warm: O_3: 0.54 CO: 0.75 SO_2: 0.44 $PM_{2.5}$: 0.52 EC: 0.68 Sulfate: 0.27 Secondary $PM_{2.5}$: 0.31 Cold: O_3: 0.30 CO: 0.74 SO_2: 0.41 $PM_{2.5}$: 0.49 EC: 0.57 Sulfate: 0.08 Secondary $PM_{2.5}$: 0.12

Table 5-14 (Continued): Mean and upper percentile concentrations of oxides of nitrogen in studies of asthma hospital admissions and emergency department (ED) visits.

Study	Location Years	Exposure Assignment	Metric	Mean/Median Concentration ppb	Upper Percentile of Concentrations (ppb)	Copollutant Examination
Physician Visits						
†Burra et al. (2009)	Toronto, Canada (1992−2001)	Average of NO_2 concentrations from 6 monitors.	1-h max	39.2	95th: 60 Max: 105	Correlations (r): NR Copollutant models: none
†Sinclair et al. (2010)	Atlanta, GA (1998−2002)	NO_2 concentrations collected as part of AIRES at SEARCH Jefferson street site.	1-h max	1998−2000: 49.8 2000−2002: 35.5 1998−2002: 41.7	NR	Correlations (r): NR Copollutant models: none

NR = not reported, AIRES = Aerosol Research Inhalation Epidemiology Study, CO = carbon monoxide, EC = elemental carbon, ED = emergency department, NO_2 = nitrogen dioxide, NO_X = sum of NO and NO_2, O_3 = ozone, OC = organic carbon, PM = particulate matter, SO_2 = sulfur dioxide, UFP = ultrafine particles, SEARCH = Southeastern Aerosol Research and Characterization.
†Studies published since the 2008 ISA for Oxides of Nitrogen.

Hospital Admissions

Generally, studies evaluated in the 2008 ISA for Oxides of Nitrogen that examined the effect of short-term NO_2 exposures on asthma hospital admissions were limited to single cities. It is important to note the results of these studies should be viewed with caution because they tended to include ages <5 years in the study population, which is problematic considering the difficulty in reliably diagnosing asthma within this age range [National Asthma Education and Prevention Program Expert (NAEPP, 2007)]. However, it is unlikely the inclusion of these individuals in a study would introduce a systematic positive bias. In contrast, the majority of studies on asthma ED visits (discussed in the next section) have excluded ages <2 years in analyses to account for this difficulty.

In a time-series study conducted in Athens, Greece, Samoli et al. (2011) evaluated the association of multiple ambient air pollutants and pediatric asthma hospital admissions for ages 0−14 years. In an all-year analysis, the authors reported a positive association with NO_2 (6.4 % [95% CI: −3.8, 17.6]; lag 0 increase for a 30-ppb increase in 1-h max NO_2 concentrations). An examination of additional lags (quantitative results not presented) revealed a similar pattern of associations at lag 2 and a 0−2 days distributed lag. In copollutant analyses, NO_2 risk estimates were robust when O_3 (7.6% [95% CI: −2.7, 19.0]) was included in the model, and were attenuated but remained positive with wide confidence intervals when including PM_{10} in the model (3.1% [95% CI: −7.3, 14.6]). There was evidence of confounding of the NO_2 association when SO_2 was included in the model as demonstrated by an effect estimate and confidence interval for NO_2 reflective of a null association (−4.3% [95% CI: −16.9, 10.2]). Of the two copollutants examined, SO_2 was most highly correlated with NO_2 (r = 0.55).

The association between short-term air pollution exposures and asthma hospital admissions in children (0−18 years of age) was also examined in a study conducted by Iskandar et al. (2012) in Copenhagen, Denmark. In a time-stratified case-crossover analysis using an a priori lag of 0−4 days, the authors reported positive associations for both NO_2 (OR: 1.3 [95% CI: 1.1, 1.6] for a 20-ppb increase in 24-h avg NO_2 concentrations) and NO_X (OR: 1.6 [95% CI: 1.3, 2.1] for a 40-ppb increase in 24-h avg NO_X concentrations), which are larger in magnitude than those observed in Samoli et al. (2011). Within this study NO_X and NO_2 were highly correlated (r = 0.93). Correlations for NO_X and NO_2 with $PM_{2.5}$ and UFPs ranged from, r = 0.28−0.33 and r = 0.45−0.51, respectively. The high correlation between NO_X and NO_2, and the fact NO_2 is part of NO_X, suggests that these pollutants should not be included in the same model due to the inability to clearly examine whether one pollutant has an independent effect compared to

the other. In additional copollutant models, NO_2 and NO_X associations remained relatively unchanged in models with $PM_{2.5}$ and UFP Table 5-15).

Table 5-15 Copollutant model results from Iskandar et al. (2012) for a 20-ppb increase in 24-h avg nitrogen dioxide (NO_2) concentrations and a 40-ppb increase in 24-h avg NO_X (sum of NO and NO_2) concentrations.

Pollutant	Copollutant	Odds Ratio (95% CI)
NO_X	---	1.6 (1.3, 2.1)
	NO_2	1.7 (0.8, 3.5)
	PM_{10}	1.4 (1.1, 1.8)
	$PM_{2.5}$	1.6 (1.2, 2.1)
	UFP	1.6 (1.2, 2.2)
NO_2	---	1.3 (1.1, 1.6)
	NO_X	1.0 (0.6, 1.6)
	PM_{10}	1.3 (1.0, 1.5)
	$PM_{2.5}$	1.4 (1.2, 1.7)
	UFP	1.5 (1.2, 1.8)

CI = confidence interval, NO_2 = nitrogen dioxide, NO_X = sum of NO and NO_2, PM = particulate matter, UFP = ultrafine particles.

Ko et al. (2007b) examined the association between short-term air pollution exposures and asthma hospital admissions for all ages at both single- and multiday lags in Hong Kong. In a time-series analysis the authors reported positive associations at single-day lags that were smaller in magnitude than those observed in Samoli et al. (2011) [e.g., 3.4% (95% CI: 1.9, 5.4%); lag 0 for a 20-ppb increase in 24-h avg NO_2 concentrations]. However, the results of Ko et al. (2007b) are consistent with those of Son et al. (2013) in eight South Korean cities, which found the strongest association at lag 0 between short-term NO_2 exposures and asthma as well as allergic disease hospital admissions, which encompasses asthma (3.6% [95% CI: 0.5, 6.8] and 3.8% [95% CI: 1.0, 6.6], respectively for a 20-ppb increase in 24-h avg NO_2 concentrations). However, unlike Samoli et al. (2011) and Son et al. (2013), Ko et al. (2007b) found the strongest evidence of an association between short-term NO_2 exposures and asthma hospital admissions at multiday lags of 0−3 (10.9% [95% CI: 8.1, 13.8] and 0−4 (10.9% [95% CI: 8.1, 13.4]) days. In a copollutant analysis with O_3, the authors reported evidence of a reduction in NO_2 risk estimates although they remained positive (2.3% [95% CI: −0.8, 5.8]; lag 0−4 days), which is not consistent with the results of the copollutant analysis in Samoli et al. (2011). This attenuation occurred even though NO_2 and O_3 were not well correlated (r = 0.41) in Hong Kong.

Emergency Department Visits

Similar to the asthma hospital admission studies evaluated in the 2008 ISA for Oxides of Nitrogen, the majority of ED visit studies were limited to single-city studies. However, these studies provided additional information with regard to potential seasonal differences in risk estimates, indicating some evidence of larger associations during warmer months.

Strickland et al. (2010) examined the association between NO_2 exposure and pediatric asthma ED visits (ages 5−17 years) in Atlanta, GA, using air quality data over the same years as two studies that focused on total respiratory ED visits, Darrow et al. (2011) and Tolbert et al. (2007) (Section 5.2.6). However, unlike Darrow et al. (2011) and Tolbert et al. (2007), which used a single-site centrally located monitor and the average of multiple monitors to assign exposure, respectively, Strickland et al. (2010) used population weighting to combine daily pollutant concentrations across monitors. In this study, the authors developed a statistical model using hospital-specific time-series data that is essentially equivalent to a time-stratified case-crossover analysis (i.e., using interaction terms between year, month, and day-of-week to mimic the approach of selecting referent days within the same month and year as the case day). Strickland et al. (2010) reported an 8.6% (95% CI: 4.2, 13.3) increase in ED visits for a 30-ppb increase in 1-h max NO_2 concentrations at lag 0−2 days in an all-year analysis. The potential confounding effects of other pollutants on the NO_2-asthma ED visit relationship was only examined in a copollutant model with O_3 and correlations between pollutants were not presented. In the copollutant model, NO_2 risk estimates were found to be relatively unchanged upon the inclusion of O_3 (quantitative results not presented).

The magnitude of the association between short-term NO_2 concentrations and asthma ED visits observed in Strickland et al. (2010) is larger than that observed in Sarnat et al. (2013a) in a study also conducted in Atlanta, GA, which focused on the influence of air exchange rates on air pollution-asthma ED visit associations detailed in Chapter 3 and Chapter 7. Instead of using monitored NO_2 concentrations, the authors used NO_X concentrations estimated by "fus(ing) spatially interpolated background pollutant concentrations and the local-scale air quality model AERMOD output for the 186 ZIP code centroids" in the Atlanta metro area. Also, focusing on a lag of 0−2 days, Sarnat et al. (2013a) reported a 1.3% increase in asthma ED visits (95% CI: 0.0, 2.4) for a 40-ppb increase in 24-h avg NO_X concentrations. The authors did not examine copollutant models, but NO_X was found to be highly correlated with CO (r = 0.93). The magnitude of the association between Strickland et al. (2010) and Sarnat et al. (2013a) differs, which could be a reflection of: (1) exposure measurement error and differences in exposure assessment methods for NO_X compared to NO_2 and (2) the different age ranges included

in both studies, which is supported by the earlier studies focusing on all ages conducted in Atlanta by Tolbert et al. (2000) and Peel et al. (2005) that report associations similar in magnitude to that observed in Sarnat et al. (2013a) (Figure 5-7).

Additional evidence for an association between short-term increases in NO_2 concentrations and asthma ED visits comes from studies conducted in Edmonton, Canada (Villeneuve et al., 2007) and Sydney, Australia (Jalaludin et al., 2008). Villeneuve et al. (2007) reported evidence of positive associations between short-term NO_2 concentrations and asthma ED visits for multiple lag structures (lag 1, lag 0−2, and lag 0−4 days) in the population aged 2 years and older. The authors observed the strongest association for lag 0−4 days (4.5% [95% CI: 0, 7.5] for a 20-ppb increase in 24-h avg NO_2 concentrations). There was no evidence of an association at lag 0. In this study, NO_2 and CO were strongly correlated (r = 0.74), and as a result associations were examined in copollutant models for each age group examined in the study, focusing on the warm season (April−September). In copollutant models with CO, NO_2 associations with asthma ED visits were relatively similar to single-pollutant results except for one age group, 15−44 years, but in all instances NO_2 associations were larger in magnitude than those for CO (quantitative results not provided).

In a study focusing on children 1−14 years old, Jalaludin et al. (2008) examined air pollution associations with asthma ED visits for single day lags up to 3 days as well as the average of 0−1 day lags. Jalaludin et al. (2008) observed a similar magnitude of an association for both lag 0 (7.5% [95% CI: 4.5, 10.5]) and lag 0−1 days (7.8% [95% CI: 4.5, 11.1] for a 30-ppb increase in 1-h max NO_2 concentrations). An examination of the potential confounding effects of other pollutants was assessed in copollutant models with PM_{10}, $PM_{2.5}$, O_3, CO, or SO_2. NO_2 was moderately to weakly correlated with each of these pollutants (r ranging from 0.44−0.56). In copollutant models, the NO_2-asthma ED visit association remained positive, but was slightly attenuated with the magnitude of the association ranging from a 4.2−6.1% increase in asthma ED visits. In addition to analyzing ages 1−14 years, the authors examined whether risks varied among age ranges within this study population (see Chapter 7).

In contrast with the majority of the evidence, short-term increases in NO_2 concentrations were not associated with asthma ED visits in a multicity study conducted in seven Canadian cities (Stieb et al., 2009). Compared to the other asthma ED visit studies evaluated, mean NO_2 concentrations across the cities included in this study were the lowest with all cities having mean 24-h avg concentrations <23 ppb (Table 5-14). Stieb et al. (2009) examined the association between short-term NO_2 exposure and a number of respiratory-related ED visits for all ages. There was no evidence that NO_2 was associated with asthma ED visits at single-day lags of 0 to 2 days (0.0% [95% CI: −2.6, 2.7]; lag 2

for a 20-ppb increase in 24-h avg NO_2 concentrations). Additionally, there was no evidence of associations between respiratory-related ED visits, including asthma, and air pollution averaged over-sub-daily time scales (i.e., 3-h avg of ED visits vs. 3-h avg pollutant concentrations).

Emergency Department Visits for Wheeze

As stated previously [National Asthma Education and Prevention Program Expert (NAEPP, 2007)], asthma is difficult to diagnose in children less than 5 years of age; however, asthma-like symptoms in children within this age range are often presented in the form of transient wheeze. Although studies that examine ED visits for wheeze do not directly inform upon the relationship between short-term NO_2 exposures and asthma, they can add supporting evidence. Also, it should be noted that some studies that examine asthma ED visits, as well as hospital admissions, often include International Classification of Diseases (ICD) codes for wheeze in the definition of asthma (e.g., (Sarnat et al., 2013a)). Orazzo et al. (2009) examined the association between NO_2 and wheeze ED visits in children, (ages 0−2 years) in six Italian cities. Daily counts of wheeze were examined in relation to air pollution using a time-stratified case-crossover approach in which control days were matched on day of week in the same month and year as the case day. PM_{10}, SO_2, CO, and O_3 were also evaluated, but correlations with NO_2 were not reported nor were copollutant analyses conducted. The authors reported positive associations between short-term 24-h avg NO_2 exposures and wheeze ED visits when examining various multiday lags (0−1 through 0−6 days) with risk estimates ranging from 1.1% (95% CI: −1.2, 3.4) for lag 0−1 days to 2.5% (95% CI: −0.9, 6.0) for lag 0−6 days.

Outpatient and Physician Visit Studies

Several recent studies examined the association between ambient NO_2 concentrations and less severe asthma exacerbation, which are often encountered through physician or outpatient (non-hospital, non-ED) visits. Burra et al. (2009) examined asthma physician visits among patients aged 1−17 and 18−64 years focusing on differences by sex and income within age categories in Toronto, Canada. The authors reported evidence of consistently positive associations between short-term increases in NO_2 concentrations and asthma physician visits across the single- and multi-day lags examined (i.e., 0, 0−1, 0−2, 0−3, and 0−4 days). The magnitude of the effect estimates were found to be similar between sexes, income quintiles, and both within and between ages. In a study conducted in Atlanta, GA, Sinclair et al. (2010) examined the association between air pollution and a number of respiratory-related outpatient visits from a managed care organization,

including asthma. The authors separated the analysis into two time periods to compare the air pollutant concentrations and relationships for acute respiratory visits for the 25-month time-period examined in Sinclair and Tolsma (2004) (i.e., August 1998−August 2000) and an additional 28-month time-period of available data from the Atlanta Aerosol Research Inhalation Epidemiology Study (AIRES) (i.e., September 2000−December 2002). Across the two time periods, mean 1-h max NO_2 concentrations were lower in the 28-month versus the 25-month time period, 35.5 versus 49.8 ppb, respectively (Table 5-14). A comparison of the two time periods indicated that risk estimates across outcomes tended to be larger in the earlier 25-month period compared to the later 28-month period, with evidence of consistently positive associations at lags of 0−2 and 3−5 days for asthma, but confidence intervals were relatively large.

Examination of Seasonal Differences

In addition to examining the association between short-term NO_2 concentrations and asthma hospital admissions and ED visits in all-year analyses, some studies also conducted seasonal analyses. Overall, these studies generally provide evidence of larger associations in the warm or summer season compared to cooler months (Figure 5-7). However, it should be noted that these studies did not examine potential copollutant confounding by season, which could further explain the results reported across studies.

In the study of eight South Korean cities, Son et al. (2013) examined potential seasonal differences across respiratory hospital admission outcomes, including asthma and allergic disease. For both outcomes, the association with NO_2 was largest in magnitude during the summer (asthma: 16.2% [95% CI: 5.1, 28.6], lag 0; allergic disease: 15.9 [95% CI: 4.6, 28.5], lag 0 for a 20-ppb increase in 24-h avg NO_2 concentrations) despite the lowest NO_2 concentrations during the summer season (<20 ppb compared to >24 ppb in the other seasons) across the eight cities. However, when using the warm season as the referent in Hong Kong, Ko et al. (2007b) reported evidence of larger effects in the winter (i.e., December to March), suggesting that differences in seasonal associations may vary by geographic location. The difference in seasonal associations by geographic location is further highlighted in a study by Samoli et al. (2011) conducted in Athens, Greece that reported results consistent with Son et al. (2013). Although risk estimates for asthma hospital admissions were relatively consistent across winter, spring, and autumn, ranging from a 13.1 to a 13.8% increase per 20-ppb increase in 24-h avg NO_2, the largest percentage increase was observed for the summer (28.7% [95% CI: −3.4, 71.3]).

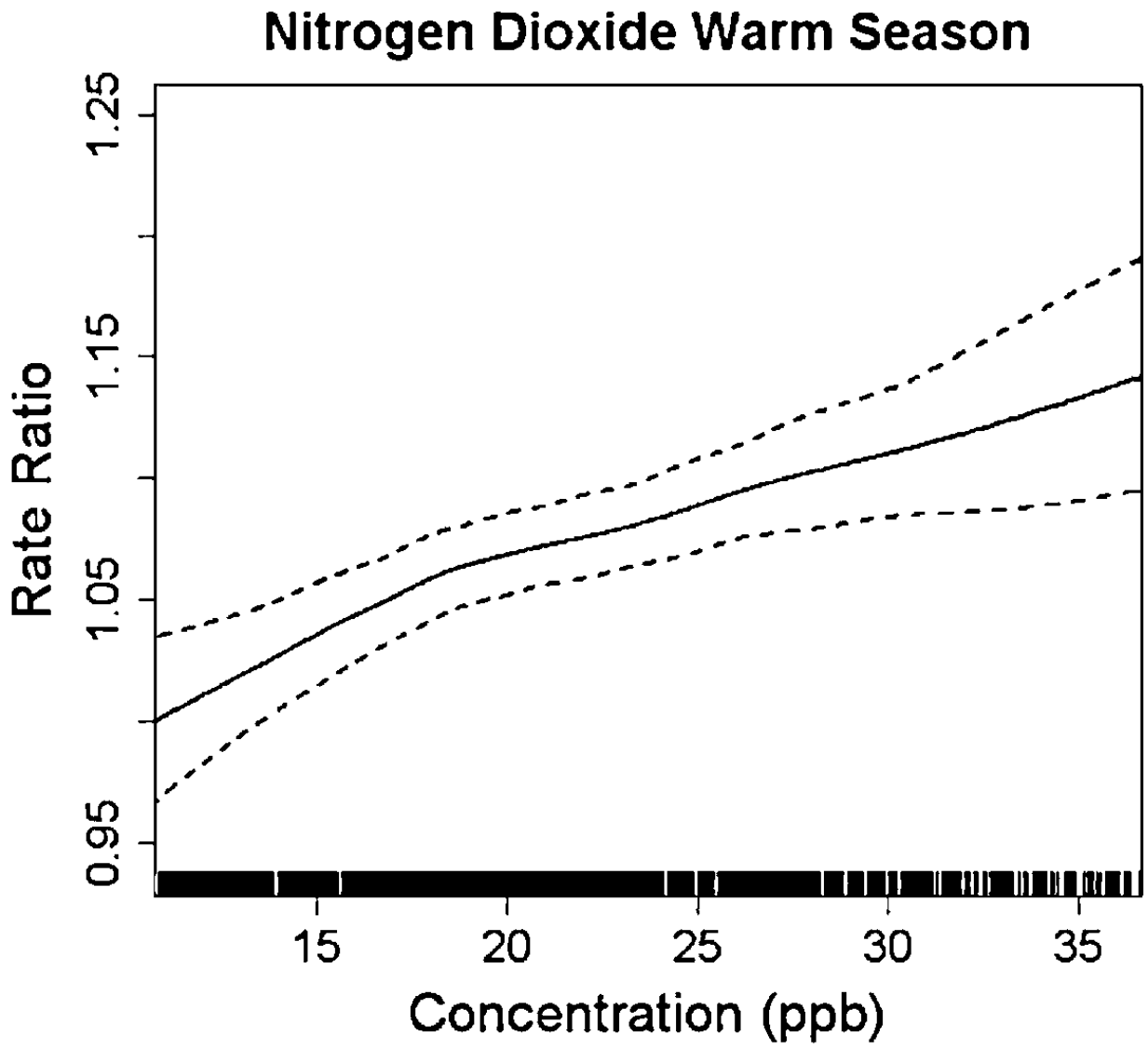
The asthma ED visit studies that conducted seasonal analyses also reported seasonal patterns similar to those observed in the hospital-admission studies. Villeneuve et al. (2007) reported associations to be generally stronger in the warm season (e.g., 21.4%

[95% CI: 13.6, 31.0] at lag 0−4 days for a 20-ppb increase in 24-h avg NO_2 concentrations) than in the cold season (−2.9% [95% CI: −7.3, 1.5]) in Edmonton, Canada. Additionally, Jalaludin et al. (2008) found evidence of larger effects during the warm months (November−April) compared to the cold months (May−October) in Sydney, Australia (Figure 5-7). These results are consistent with Strickland et al. (2010), which reported stronger associations during the warm season (i.e., May−October) (16.0% [95% CI: 9.1, 23.5]; lag 0−2 days) than the cold season (3.8% [95% CI: −1.9, 9.6]; lag 0−2 days) in a study of pediatric asthma ED visits in Atlanta, GA. Additional support for these seasonal differences in associations was presented by Orazzo et al. (2009), who focused on wheeze ED visits in six Italian cities, where associations were slightly larger in the summer compared to the winter, but the confidence intervals were wide and overlapping (quantitative results not provided). In the study of seven Canadian cities, Stieb et al. (2009) also conducted seasonal analyses, but did not present detailed results. However, the authors did state that there was no evidence of consistent associations during the winter months (October−March) between any pollutant and respiratory outcomes, including asthma.

Additional evidence for potential seasonal differences in NO_2-associations with asthma hospital admissions and ED visits comes from the analysis of asthma physician visits by Sinclair et al. (2010). When focusing on asthma in children, the authors reported larger risk estimates in the warm season at all lags for the 25-month period (e.g., warm: 9.6% [95% CI: −7.4, 30.0]; cold: 1.2% [95% CI: −12.4, 16.8] at lag 0−2 days for a 30-ppb increase in 1-h max NO_2 concentrations), with less consistent evidence for seasonal differences in the 28-month period.

Concentration-Response Relationship

To date, few studies have examined the concentration-response (C-R) relationship between NO_2 exposures and respiratory morbidity. In recent studies, Strickland et al. (2010) and Li et al. (2011b) examined the shape of the NO_2-pediatric asthma ED visit relationship using different analytical approaches. Strickland et al. (2010) examined the C-R relationship by conducting quintile and locally weighted scatterplot smoothing (LOESS) C-R analyses. In the quintile analysis, NO_2 associations were positive and stronger at quintiles representing higher concentrations, ranging from 28 ppb to >181 ppb, relative to the first quintile (i.e., NO_2 concentrations <28 ppb). Additionally, the LOESS C-R relationship analysis provides evidence indicating elevated NO_2 associations along the distribution of concentrations from the 5th to 95th percentile (Figure 5-5). Collectively, these analyses do not provide evidence of a threshold.



Source: Reprinted with permission of the American Thoracic Society (Strickland et al., 2010).

Figure 5-5 Locally weighted scatterplot smoothing concentration-response estimates (solid line) and twice-standard error estimates (dashed lines) from generalized additive models for associations between 3-day avg (lag 0−2) nitrogen dioxide (NO_2) concentrations and emergency department visits for pediatric asthma at the 5th to 95th percentile of NO_2 concentrations in the Atlanta, GA area.

In a study conducted in Detroit, MI, Li et al. (2011b) focused on the C-R relationship by examining if there is evidence of a deviation from linearity. Associations were examined in both a time-series and time-stratified case-crossover study design assuming: (1) no

deviation from linearity and (2) a change in linearity at 23 ppb [i.e., the maximum likelihood estimate within the 10th to 95th percentile concentration where a change in linearity may occur (~80th percentile)]. The analysis assumed a deviation in linearity but did not assume zero risk below the inflection point. The focus of the analysis was on identifying whether risk increased above the risk observed in the linear models at NO_2 concentrations above 23 ppb. In the analyses assuming linearity, effect estimates varied across models for a 0−4-day lag (time series: 2.9% [95% CI: −7.9, 15.1]; case-crossover: 9.1% [95% CI: −0.83, 20.2] for a 20-ppb increase in 24-h avg NO_2 concentrations). In the models that assumed a deviation from linearity, the authors did not observe evidence of higher risk in either the time-series or case-crossover analyses at NO_2 concentrations greater than 23 ppb.

Exposure Assignment

Questions often arise in air pollution epidemiologic studies with regard to the method used to assign exposure. Strickland et al. (2011) assessed this question in a study conducted in Atlanta, GA focusing on pediatric asthma ED visits. Using data from the warm season from a previous analysis (Strickland et al., 2010), Strickland et al. (2011) examined the relative influence of different exposure assignment approaches (i.e., central monitor, unweighted average across available monitors, and population-weighted average) on the magnitude and direction of associations between NO_2 and pediatric asthma hospital admission. Strickland et al. (2011) reported that effect estimates per IQR increase in NO_2 were similar across the metrics; however, based on a standardized increment, the magnitude of the association between NO_2 and pediatric asthma ED visits varied (central monitor: 7.9% [95% CI: 4.2, 11.8] < unweighted average: 12.1% [95% CI: 6.7, 17.9] < population-weighted average: 16.2% [95% CI: 9.1, 23.7] for a 30-ppb increase in 1-h max NO_2 concentrations at lag 0−2 days). Although Strickland et al. (2011) represents one study in one location, the results suggest that the different approaches used to assign exposure across the studies evaluated may alter the magnitude, but not direction, of the associations observed.

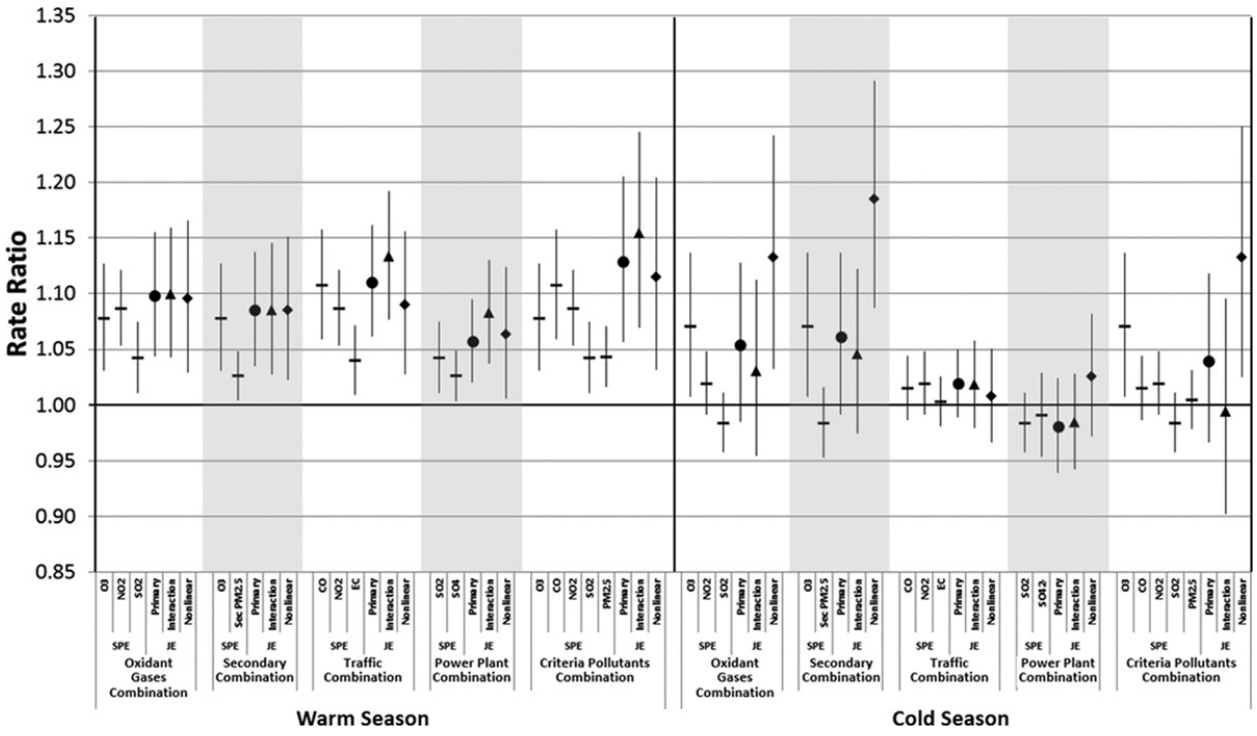
Nitrogen Dioxide within the Multipollutant Mixture

An important question often encountered during the review of any criteria air pollutant, is whether the pollutant has an independent effect on human health. In the case of NO_2, this is questioned because it is often found to be highly correlated with other traffic-related pollutants. However, ambient exposures to criteria air pollutants are in the form of mixtures, which make answering this question difficult and primarily limited to

examining copollutant models. Recent studies conducted by Gass et al. (2014) and Winquist et al. (2014), both of which use pediatric asthma ED visit data from Atlanta, use novel approaches to assess whether specific mixtures are more strongly associated with health effects compared to others. Although the primary objective of these types of studies is not to directly assess the independent effects of a pollutant, they can inform the role of NO_2 in the air pollution mixture.

Gass et al. (2014) used a classification and regression tree (C&RT) approach to examine the association between short-term exposures to unique daily multipollutant mixtures of NO_2, CO, $PM_{2.5}$, and O_3, and pediatric (i.e., ages 2−18 years) asthma ED visits in Atlanta. C&RT is a supervised learning approach that creates various groupings of pollutants based on an outcome variable, which differs from similar techniques, such as principal component analysis, that do not consider the outcome (Gass et al., 2014). For this approach, daily pollutant concentrations were divided into quartiles with the referent group comprised of all days in which each pollutant was in the lowest quartile. The C&RT analysis identified 13 different unique daily pollutant combinations or terminal nodes. Similar to Strickland et al. (2010), Gass et al. (2014) examined the relationship between each combination and pediatric asthma ED visits using a Poisson model in the context of a time-referent case-crossover analysis. Of the 13 unique combinations, 5 of the largest relative risks (RRs) (i.e., RR ranging from 1.05 to 1.08) were observed for combinations where NO_2 concentrations were in the 3rd or 4th quartile. Of note for three of the five combinations with the largest RRs, $PM_{2.5}$ concentrations were also high, with concentrations in the 4th quartile. However, the RR largest in magnitude was observed for a combination where NO_2 concentrations were low (1st and 2nd quartiles) and $PM_{2.5}$ concentrations were high (4th quartile). Overall, these results suggest that high daily concentrations of NO_2 alone and in combination with high daily concentrations of $PM_{2.5}$ can impact respiratory morbidity.

Winquist et al. (2014) took a different approach to examining multipollutant mixtures by focusing on the joint effect (i.e., the combined effect of multiple pollutants) of pollutants often associated with specific air pollution sources. Associations between short-term NO_2 exposures and pediatric asthma ED visits (i.e., ages 5−17) were examined in single-pollutant models and also in a multipollutant context in joint models for pollutant combinations representative of oxidant gases (i.e., O_3, NO_2, SO_2), traffic (i.e., CO, NO_2, EC), and criteria pollutants (i.e., O_3, CO, NO_2, SO_2, $PM_{2.5}$). Using the model detailed in Strickland et al. (2010), the authors reported results for an IQR increase for lag 0−2 days in single-pollutant analyses as well as three types of joint effect models [i.e., no interaction terms (primary), first-order multiplicative interactions between pollutants (interactions), and nonlinear pollutant terms (nonlinear)] (Figure 5-6).



Source: Winquist et al. (2014).
SPE = single-pollutant model estimate; JE = joint model estimate.

Figure 5-6 Rate ratio and 95% confidence intervals for single-pollutant and joint effect models for each pollutant combination in warm and cold season analyses for an interquartile range (IQR) increase in each pollutant at lag 0−2 days. IQR for 1-h max nitrogen dioxide (NO_2) concentrations = 12.87 ppb.

Across pollutant combinations that contained NO_2, in the warm season, joint effect models reported consistent positive associations with pediatric asthma ED visits. For each pollutant combination the association observed was larger in magnitude than any single-pollutant association, including NO_2, but not equivalent to the sum of each individual pollutant association for a specific combination. Furthermore, in the warm season analysis, associations across the different joint effects models were found to be relatively similar. The results during the cold season were inconsistent; however, when focusing on the traffic pollutant combination, results from the joint effects models were relatively similar to the single-pollutant results. The results of Winquist et al. (2014) suggest that NO_2 alone and in combination with other pollutants is associated with asthma ED visits, but also highlight the difficulty in separating out the independent effect of a pollutant that is part of a mixture where multiple pollutants are often highly correlated.

Summary of Asthma Hospital Admissions and Emergency Department Visits

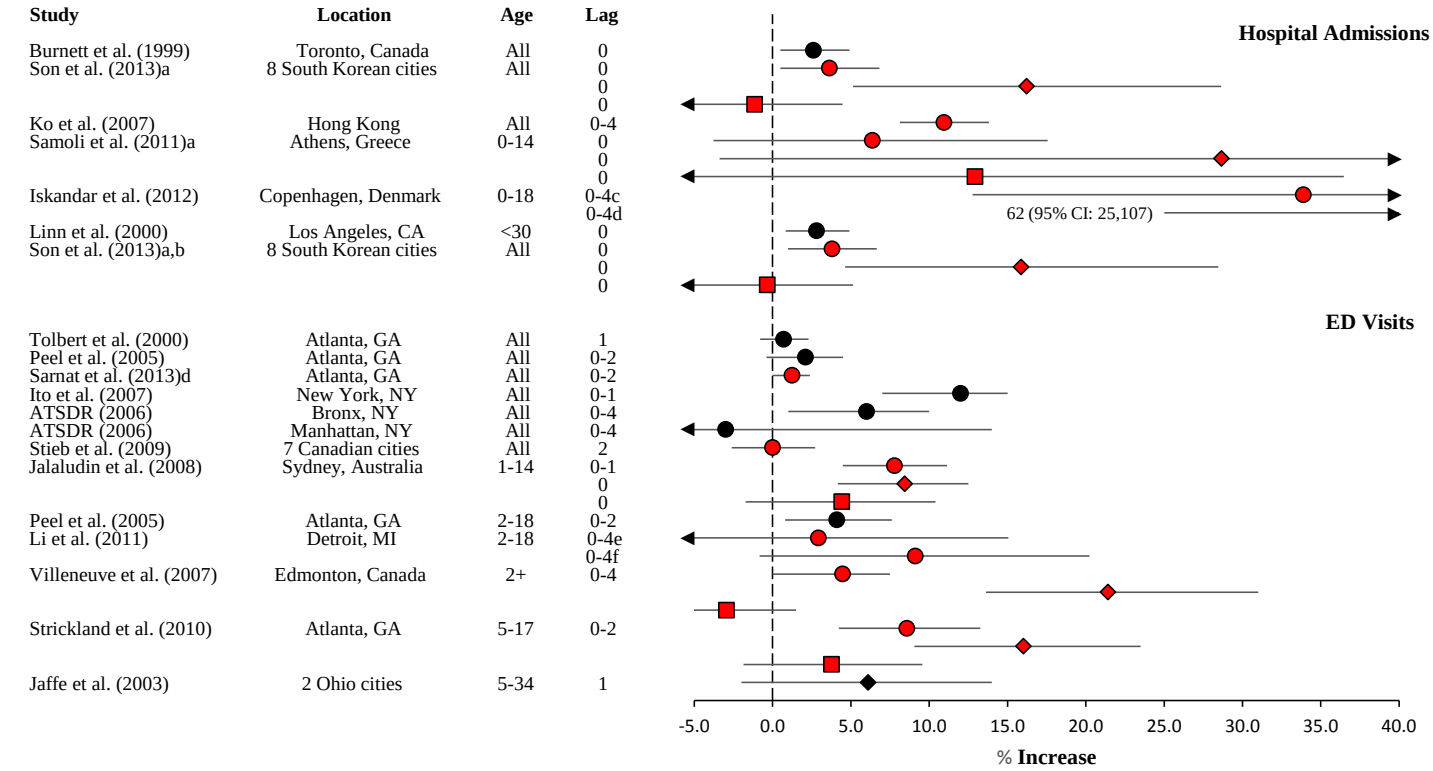
Recent studies that examined the association between short-term NO_2 exposure and asthma hospital admissions and ED visits report relatively consistent positive associations which supports the results of U.S. and Canadian studies evaluated in the 2008 ISA for Oxides of Nitrogen (Figure 5-7, Table 5-16). Across asthma hospital admission and ED visit studies, there was some evidence of a different pattern of associations for each outcome, with more immediate effects (i.e., lag 0) for asthma hospital admissions and evidence of prolonged effects for asthma ED visits, with a number of studies showing effects at multiday lags ranging from 0−2 to 0−4 days. Of the studies that examined potential copollutant confounding, evidence supported that associations between short-term NO_2 exposures and asthma hospital admissions and ED visits remained relatively unchanged in copollutant models (i.e., similar in magnitude or attenuated slightly, but remaining positive). Additionally it is important to note that NO_2 is often found to be highly correlated with other traffic-related pollutants (e.g., $PM_{2.5}$, UFPs, CO); therefore, limiting the ability to determine if short-term NO_2 exposures are independently associated with asthma hospital admissions and ED visits. Recent studies of multipollutant exposures further inform upon the effect of short-term NO_2 exposures on respiratory morbidity, specifically asthma. These studies demonstrate that: high daily concentrations of NO_2 alone and in combination with high daily concentrations of other pollutants, such as $PM_{2.5}$, can impact respiratory morbidity; and associations are observed between asthma ED visits and NO_2 alone and in combination with other traffic-related pollutants, oxidants, and criteria pollutants.

A number of recent studies also examined whether there was evidence that the association between short-term NO_2 exposures and asthma hospital admissions and ED visits was modified by season or some other individual- or population-level factor (Chapter 7). An examination of seasonal differences in NO_2-asthma hospital admission and ED visit associations provide some evidence of NO_2 effects being larger in magnitude in the summer or warm season, and that seasonal associations may vary by geographic location. Studies of individual- and population-level factors, provide evidence of differences in associations by lifestage, with larger NO_2 effects for children and older adults, and more limited evidence for differences by sex, race/ethnicity, and socioeconomic status (SES), specifically insurance status (Chapter 7). Additionally, there is evidence that exposure differences, specifically whether a population lives in housing that has low or high AERs that may influence the association between short-term NO_X exposures and asthma ED visits.

Additionally some recent studies examined various study design issues, including model specification and exposure assignment. An examination of model specification, as

detailed in Section 5.2.6, indicates that the relationship between short-term NO_2 exposures and respiratory-related hospital admissions, including those for asthma and allergic disease, are sensitive to using less than 6 degrees of freedom (df) per year to account for temporal trends, but robust to alternative lags and df, ranging from 3 to 6, for weather covariates (Son et al., 2013). An examination of various exposure assignment approaches including single central site, average of multiple monitors, and population-weighted average, suggests that each approach can influence the magnitude, but not direction, of the NO_2-asthma ED-visit risk estimate (Strickland et al., 2011).

Finally, a few recent studies examined whether the shape of the NO_2-asthma ED visit relationship is linear or provides evidence of a threshold. These studies provide evidence of a linear, no-threshold relationship between short-term NO_2 exposures and asthma ED visits (Li et al. (2011b); Strickland et al. (2010)).



Note: Results are standardized to a 20-ppb increase in 24-h avg NO_2, a 30-ppb increase in 1-h max NO_2, and a 40-ppb increase in 24-h avg NO_X. a = results were presented for four seasons; however the summer and winter estimates represented the largest and smallest estimates across seasons; b = this estimate is for allergic disease, which includes asthma; c = risk estimate for NO_2; d = risk estimate for NO_X; e = time-series results; f = case-crossover results. Black = U.S. and Canadian studies evaluated in the 2008 ISA for Oxides of Nitrogen; red = recent asthma hospital admission and ED visit studies. Circle = all-year; diamond = warm/summer months; square = cool/winter months.

Figure 5-7 Percentage increase in asthma hospital admissions and emergency department (ED) visits from U.S. and Canadian studies evaluated in the 2008 Integrated Science Assessment for Oxides of Nitrogen and recent studies in all-year and seasonal analyses.

Table 5-16 Corresponding risk estimates for studies presented in Figure 5-7.

Study	Location	Age	Avg Time	Season	Lag	% Increase (95% CI)
Hospital Admissions						
Burnett et al. (1999)	Toronto, Canada	All	24-h avg	All	0	2.6 (0.5, 4.9)
†Son et al. (2013)	8 South Korean cities	All	24-h avg	All	0	3.6 (0.5, 6.8)
				Summer		16.2 (5.1, 28.6)
				Winter		−1.1 (−6.5, 4.5)
Ko et al. (2007b)	Hong Kong	All	24-h avg	All	0−4	10.9 (8.1, 13.8)
†Samoli et al. (2011)	Athens, Greece	0−14	1-h max	All	0	6.4 (−3.8, 17.6)
				Summer		28.7 (−3.4, 71.3)
				Winter		12.9 (−6.6, 36.5)
†Iskandar et al. (2012)	Copenhagen, Denmark	0−18	24-h avg	All	0−4	34.0 (13.0, 58.0)[a] 62.0 (25.0, 107)[b]
Linn et al. (2000)	Los Angeles, CA	<30	24-h avg	All	0	2.8 (0.8, 4.9)
†Son et al. (2013)[c,d]	8 South Korean cities	All	24-h avg	All	0	3.8 (1.0, 6.6)
				Summer		15.9 (4.6, 28.4)
				Winter		−0.3 (−5.4, 5.1)
ED Visits						
Tolbert et al. (2000)	Atlanta, GA	All	1-h max	All	1	0.7 (−0.8, 2.3)
Peel et al. (2005)	Atlanta, GA	All	1-h max	All	0−2	2.1 (−0.4, 4.5)
†Sarnat et al. (2013a)	Atlanta, GA	All	24-h avg	All	0−2	1.3 (0.0, 2.4)[b]
Ito et al. (2007b)	New York, NY	All	24-h avg	All	0−1	12.0 (7.0, 15.0)
ATSDR (2006)	Bronx, NY	All	24-h avg	All	0−4	6.0 (1.0, 10.0)
ATSDR (2006)	Manhattan, NY	All	24-h avg	All	0−4	−3.0 (−18.0, 14.0)
†Stieb et al. (2009)	7 Canadian cities	All	24-h avg	All	2	0.0 (−2.6, 2.7)
†Jalaludin et al. (2008)	Sydney, Australia	1−14	1-h max	All	0−1	7.8 (4.5, 11.1)
				Warm	0	8.4 (4.2, 12.5)
				Cold	0	4.4 (−1.7, 10.4)
Peel et al. (2005)	Atlanta, GA	2−18	1-h max	All	0−2	4.1 (0.8, 7.6)
†Li et al. (2011b)	Detroit, MI	2−18	24-h avg	All	0−4[e]	2.9 (−7.9, 15.1)
				All	0−4[f]	9.1 (−0.8, 20.2)
†Villeneuve et al. (2007)	Edmonton, Canada	2+	24-h avg	All	0−4	4.5 (0.0, 7.5)
				Warm		21.4 (13.6, 31.0)
				Cold		4.4 (−1.7, 10.4)
†Strickland et al. (2010)	Atlanta, GA	5−17	1-h max	All	0−2	8.6 (4.2, 13.3)

Table 5-16 (Continued): Corresponding risk estimates for studies presented in Figure 5-7.

Study	Location	Age	Avg Time	Season	Lag	% Increase (95% CI)
				Warm		16.0 (9.1, 23.5)
				Cold		3.8 (−1.9, 9.6)
Jaffe et al. (2003)	2 Ohio cities	5−34	24-h avg	Summer	1	6.1 (−2.0, 14.0)

CI = confidence interval, ED = emergency department.
[a]Risk estimate for NO_2.
[b]Risk estimate for NO_X.
[c]Results were presented for four seasons; the summer and winter estimates represented the largest and smallest estimates for each season.
[d]Estimate for allergic disease, which includes asthma.
[e]Time-series analysis results.
[f]Case-crossover analysis results.
†Studies published since the 2008 ISA for Oxides of Nitrogen.

5.2.2.5 Subclinical Effects Underlying Asthma Exacerbation: Pulmonary Inflammation and Oxidative Stress

The evidence described in the preceding sections for NO_2-related increases in airway responsiveness (Section 5.2.2.1), decreases in lung function and increases in respiratory symptoms in children with asthma (Sections 5.2.2.2 and 5.2.2.3), and asthma hospital admissions and ED visits (Section 5.2.2.4) is coherent and consistent with a sequence of key events by which NO_2 can plausibly lead to asthma exacerbation. Adding to the proposed mode of action is evidence indicating NO_2 exposure-mediated pulmonary inflammation, a key early event in asthma exacerbation that can mediate increases in airway responsiveness (Section 4.3.2.5). The initiation of inflammation by NO_2 exposure is supported by observations of NO_2-induced increases in eicosanoids, which mediate recruitment of neutrophils (Section 4.3.2.3). Further, NO_2-induced increases in reactive oxygen species (ROS) and reactive nitrogen species may impair epithelial barrier function and initiate inflammation (Section 4.3.2.1), as many transcription factors regulating expression of pro-inflammatory cytokines are redox sensitive. Most information on the effects of NO_2 on pulmonary oxidative stress and injury is in healthy people and animal models, and findings are inconsistent at ambient-relevant concentrations (Section 5.2.7.4).

The 2008 ISA for Oxides of Nitrogen described evidence for NO_2-induced increases in pulmonary inflammation in some controlled human exposure studies and animal toxicological studies (U.S. EPA, 2008a). There was coherence with findings from the few available epidemiologic studies in children with asthma, which found associations between short-term increases in ambient NO_2 concentrations and increases in exhaled nitric oxide (eNO). In particular, coherence is found among disciplines for

NO_2-associated increases in allergic inflammation. Recent studies, most of which were epidemiologic, continued to find NO_2-associated increases in pulmonary inflammation and oxidative stress. Biological indicators of pulmonary inflammation and oxidative stress included those measured in exhaled breath; bronchoalveolar, bronchial, and nasal lavage fluid; and sputum. Indicators of systemic inflammation in blood are evaluated in the context of cardiovascular effects in Section 5.3.

Experimental Studies

As described in Section 5.2.2.1, controlled human exposure studies in adults with asthma and allergy demonstrated increases in airway responsiveness in response to NO_2 exposure with or without allergen challenge. These observations are supported by findings in controlled human exposure studies involving adults with asthma and allergy and in a rat model of allergic airway disease that NO_2 exposure with or without an allergen challenge resulted in increased indicators of allergic inflammation. This includes increases in IgE and the influx and/or activation of eosinophils and neutrophils. Results provide evidence that NO_2 exposure can lead to exacerbation of allergic airways disease (discussed below and in Section 4.3.2.6). These results provide support for epidemiologic evidence of NO_2−associated increases in inflammation in children with asthma and allergy.

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a) described several studies that examined inflammatory responses in adults with mild allergic asthma who were exposed to NO_2 followed by a specific allergen challenge (Table 5-17). In a series of studies from the Karolinska Institute in Sweden, adults at rest were exposed to air or 260 ppb NO_2 for 15−30 minutes followed by an antigen (birch or timothy pollen) challenge 4 hours later. Bronchoalveolar lavage (BAL) and bronchial wash fluids were collected 19 hours after allergen challenge. NO_2 exposure for 30 minutes increased polymorphonuclear cells (PMN) in the BAL and bronchial wash fluids and increased ECP in the bronchial wash fluid compared with air exposure (Barck et al., 2002). Reduced cell viability of BAL cells and reduced volume of BAL fluid were also reported. ECP is released by activated eosinophils; it is toxic to respiratory epithelial cells and thought to play a role in the pathogenesis of airway injury in asthma. In a subsequent study, Barck et al. (2005a) exposed adults with mild allergic asthma to air or NO_2 for 15 minutes on Day 1 and twice on Day 2, and for 15 minutes with allergen challenges following all of the exposures. NO_2 exposure induced an increased level of ECP in both sputum and blood and increased myeloperoxidase levels in blood. These results suggest that NO_2 may prime circulating eosinophils and enhance activation of airway eosinophils and neutrophils in response to an inhaled allergen. Nasal responses to nasal allergen challenge were also examined following a 30-minute exposure to NO_2 (Barck et al., 2005b). No enhancement of nasal

allergen responses was observed in adult subjects. As noted in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a), these studies indicate that brief exposures to 260 ppb NO_2 can enhance allergen responsiveness in individuals with asthma.

Table 5-17 Controlled human exposure studies of pulmonary inflammation in populations with asthma.

Study	Disease Status; Age; n, Sex	Exposure Details	Endpoints Examined
Barck et al. (2002)	Adults with mild asthma and allergy to birch or timothy pollen; mean age: 29 yr; n = 6 M, 7 F	Histamine inhalation test to confirm airway hyperresponsiveness. 266 ppb NO_2 for 30 min Inhaled allergen challenge 4 h after pollutant exposure.	Albumin in serum samples. BW and BAL cell parameters-volume recovered, cell viability, total cell counts, macrophage concentrations, % of neutrophils, # eosinophils, # mast cells (performed 19 h after allergen challenge). ECP, MPO, IL-5, IL-8, eotaxin, ICAM-1.
Barck et al. (2005a)	Adults with mild asthma and allergy to birch or timothy pollen; mean age: 32 yr; n = 10 M, 8 F	260 ppb NO_2 Day 1: one 15 min exposure with bronchial challenge 4 h after exposure. Day 2: two 15 min exposures with bronchial challenge 3 h after 2nd exposure.	Total and differential cells counts of induced sputum and venous blood (samples taken on morning of Days 1−3). ECP, MPO in sputum.
Barck et al. (2005b)	Adults with rhinitis and mild asthma; mean age = 31 yr; n = 9 M, 7 F	Seasonal allergy confirmed by positive nasal challenge of allergen. AHR confirmed by histamine test. 260 ppb NO_2 Nasal allergen challenge 4 h after exposure.	Total and differential cell counts and cell viability in NAL (performed before exposure, before allergen challenge, and 1 h, 4 h, and 18 h after challenge). ECP and MPO in NAL fluid and blood.
Ezratty et al. (2014)	Asthma; median 29 yr (range: 20−69 yr); n = 14 M, 5 F;	(1) Filtered air (2) 203 ppb NO_2 ± 1.5% (3) 581 ppb NO_2 ± 3.2% Same design for each exposure, 30 min on Day 1, twice for 30 min of Day 2 separated by 1 h.	Induced sputum at baseline, 6 h, 32 h, and 48 h after the end of the first exposure. Cell counts, ECP. Spirometry for flow volume curve at baseline, and daily before and immediately following exposure and immediately before sputum induction. Symptom questionnaire 0, 15, and 30 min into exposure. FEV1 and PEF by portable spirometer twice during exposure and hourly for 6 h following exposure.

Table 5-17 (Continued): Controlled human exposure studies of pulmonary inflammation in populations with asthma.

Study	Disease Status; Age; n, Sex	Exposure Details	Endpoints Examined
Jörres et al. (1995)	Asthma; 27 ± 5 yr; n = 8 M, 4 F; Healthy; 27 yr (range: 21−33 yr); n = 5 M, 3 F;	1,000 ppb for 3 h; Exercise 10 min on/10 min off at individual's maximum workload.	BAL fluid analysis 1 h after exposure (cell counts, histamine, prostaglandins).
Riedl et al. (2012)	Phase 1: adults with mild asthma; mean age: 37 yr; n = 10 M, 5 F Phase 2: adults with mild asthma and cat allergy; mean age: 36 yr; n = 6 M, 9 F	Inhalation challenge to detect bronchoconstrictive response Phase 1: methacholine; Phase 2: cat allergen). (1) 100 µg/m^3 DEP for 2 h with intermittent exercise (2) 350 ppb NO_2 control for 2 h with intermittent exercise	Total counts and differential cell counts (alveolar macrophages, lymphocytes, PMNs, eosinophils) in induced sputum (taken 22 h after exposure). Induced sputum fluid assay-RANTES, eotaxin, ECP, IgG, IgG4, IgA, IgM, IgE. Cat-specific IL-4, IL-5, IL-8, IL-12, GM-CSF, IFN-γ, TNF-α, tryptase.

Vagaggini et al. (1996)	Asthma;
	29 ± 14 yr;
	n = 4 M, 4 F;
	Healthy; 34 ± 5 yr;
	n = 7 M;
		300 ppb for 1 h;
		Exercise at $\dot{V}_E$ = 25 L/min
			Cell counts in sputum 2-h post-exposure.

Wang et al. (1995a); Wang et al. (1995b)	Adults with seasonal rhinitis; mean age: 26 yr; n = 6 M, 10 F	Nasal provocation with grass pollen allergen to confirm increase in nasal airway resistance. (1) 400 ppb NO_2 for 6 h (2) 400 ppb NO_2 for 6 h + allergen challenge	Nasal lavage for inflammatory mediators fluid-ECP, MCT, MPO, IL-8 (30 min after allergen challenge).
Wang et al. (1999)	Adults with grass allergy; mean age: 32 yr; n = 8 M, 8 F	Nasal airway resistance tests at rest, after saline, and after allergen challenge to confirm reactivity for inclusion in study. (1) 200 µg Fluticasone propionate (FP) + 400 ppb NO_2 for 6 h (2) Matched placebo + 400 ppb NO_2 for 6 h	NAL—total and differential cell counts (30 min after allergen challenge). Immunoassay of NAL fluid-ECP, RANTES.
Witten et al. (2005)	Adults with asthma and house dust mite allergy; mean age: 32 yr; n = 6 M, 9 F	Inhaled allergen challenge to determine predicted allergen PC20. 400 ppb NO_2 for 3 h w/intermittent exercise 2nd inhaled allergen challenge, starting at 4 doubling doses less than APC20 and doubling until 20% decrease in FEV_1.	Total and differential cell counts in induced sputum—macrophages, lymphocytes, neutrophils, and eosinophils (samples taken at 6 and 26 h after allergen challenge).

BW = bronchial wash, ECP = eosinophil cationic protein, F = female, FEV = forced expiratory volume, GM-CSF = granulocyte macrophage-colony stimulating factor, HDM = house dust mite, ICAM-1 = inter-cellular adhesion molecule 1, IL = interleukin, M = male, MPO = myeloperoxidase, NAL = nasal lavage, NO_2 = nitrogen dioxide, PC = provocative concentration, PEF = peak expiratory flow, PMN = polymorphonuclear cells.

Additional studies have been performed using longer NO_2 exposures (Table 5-17). (Wang et al. (1999); Wang et al. (1995a); Wang et al. (1995b)) found that exposure of adults to 400 ppb NO_2 for 6 hours enhanced allergen responsiveness in the nasal mucosa in subjects with allergic rhinitis. Mixed grass pollen was used as the challenge agent and was administered immediately after the NO_2 exposure. Responses included increased numbers of eosinophils and increased levels of myeloperoxidase and ECP in nasal lavage fluid collected 30 minutes after the allergen challenge. Witten et al. (2005) did not observe enhanced airway inflammation with allergen challenge in adults with asthma and allergy to HDM allergen who were exposed to 400 ppb NO_2 for 3 hours with intermittent exercise. HDM allergen was administered immediately after the NO_2 exposure and a decrease in sputum eosinophils was found 6 hours later (Witten et al., 2005). Sputum ECP levels were increased although this change did not reach statistical significance. The authors suggested that their findings may be explained by a decreased transit of eosinophils across the bronchial mucosa occurring concomitantly with NO_2−induced eosinophilic activation. Other investigators have noted that numbers of eosinophils do not always correlate with allergic disease activity (Erjefält et al., 1999). Airway mucosal eosinophilia is a characteristic feature of asthma and rhinitis; eosinophils exert their effects via degranulation or cytolysis resulting in release of ECP and other mediators. However, under conditions favoring eosinophil cytolysis, ECP concentrations may be high and numbers of eosinophils may be low.

As noted in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a), differing findings between the studies in allergic individuals could be due to differences in timing of the allergen challenge, the use of multiple- or single-allergen challenges, the use of BAL fluid versus sputum versus nasal lavage fluid, exercise versus rest during exposure, and differences in subjects. Furthermore, study protocols varied in the timing of biological sample collection post-exposure to NO_2 or allergen.

A recent study of adults with mild allergic asthma also did not find enhanced airway inflammatory responses following exposure to NO_2 (350 ppb NO_2, 2 hours, intermittent exercise) (Table 5-17) (Riedl et al., 2012). Subjects exposed to NO_2 followed by methacholine challenge 1.5 hours later had increased levels of blood IgM and decreased levels of sputum IgG4, interleukin (IL)‑4, eotaxin, RANTES, and fibrinogen measured 22 hours after exposure. Subjects exposed to NO_2 followed by cat allergen 1.5 hours later did not exhibit changes in sputum cell counts measured 22 hours after exposure. While these results are not consistent with NO_2 enhancing airway inflammatory responses, it should be noted that markers of eosinophil activation were not measured.

Several other studies investigated allergic inflammation following NO_2 exposure in the absence of a challenge. Jörres et al. (1995) exposed healthy adults and those with asthma

and allergy to 1,000 ppb NO_2 for 3 hours and performed bronchoscopy 1 hour later. The macroscopic appearance of the bronchial epithelium was altered after exposure in adults with asthma compared to healthy controls; however, no accompanying changes in cell counts in the BAL fluid were observed. Eicosanoid levels were also measured; thromboxane B2 was increased in healthy adults and those with asthma following NO_2 exposure while prostaglandin D2 was increased and 6-keto prostaglandin F1α was decreased after exposure only in adults with asthma. Because eicosanoids are known mediators of inflammation, these results suggest that exposure to NO_2 resulted in activation of cell signaling pathways associated with inflammation. Vagaggini et al. (1996) observed a decrease in eosinophils in sputum collected from adults with asthma following a 1-hour exposure to 300 ppb NO_2, though this decrease was not statistically significant. In contrast, a recent controlled human exposure study reported an increase in eosinophils and ECP following repeated NO_2 exposure in adults with atopic asthma Ezratty et al. (2014). Subjects were exposed to 203 or 581 ppb NO_2 for 30 minutes on one day and twice for 30 minutes on the second day. Compared with baseline, statistically significant increases in the amount of ECP and the number and percentage of eosinophils in sputum were observed after the three exposures to 600, but not 200 ppb NO_2. Furthermore, ECP was highly correlated with eosinophil count in sputum. No increases in either of these parameters were observed 6 hours after the first exposure to 600 ppb NO_2.

Allergic inflammatory responses were also investigated in animal models of allergic airways disease (Table 5-18). These studies involved sensitization and challenge with an antigen followed by exposure to NO_2. In one study in rats, which were sensitized and challenged with HDM allergen, exposure to NO_2 (5,000 ppb, 3 hours) enhanced specific immune responses and increased the numbers of lymphocytes, neutrophils, and eosinophils in the airways (Gilmour et al., 1996). In this study, the most pronounced responses occurred when rats were exposed to NO_2 immediately after sensitization and immediately after challenge with HDM antigen. Rats exposed to NO_2 twice had increased levels of antigen-specific IgG and IgA and increased levels of IgE in BAL fluid 7 days post-exposure to NO_2. In addition, an increase in the ratio of inflammatory cells (i.e., lymphocytes, neutrophils, eosinophils) to alveolar macrophages was observed 7 days post-exposure to NO_2, although the total number of lavagable cells did not change.

In several studies in mice, which were sensitized and challenged with ovalbumin, NO_2 exposure over several hours or days failed to increase allergic inflammatory responses. Exposures to 700 or 5,000 ppb NO_2 for 3 hours on a single day, for 2 hours on 3 consecutive days or for 6 hours on 3 consecutive days either reduced or had no effect on indicators of eosinophil inflammation such as eosinophil counts, eosinophil peroxidase activity, and total cellularity (Poynter et al., 2006; Hubbard et al., 2002;

Proust et al., 2002). Other findings included decreases in IL‑5 levels in the BAL fluid at both 24 and 72 hours after exposure to 5,000 ppb NO_2 and reductions in perivascular and peribronchial cellular infiltrates after exposure to 700 ppb NO_2. Others have noted that the ovalbumin-induced airway inflammation in mice does not involve substantial eosinophil degranulation or cytolysis, which is characteristic of asthma and allergic rhinitis in humans (Malm-Erjefält et al., 2001). This suggests that species-related differences may account for NO_2-induced decreases in eosinophilic inflammation seen in mouse models. Mechanisms underlying the NO_2‑induced decrease in airways eosinophilia are unknown.

In summary, several controlled human exposure studies of adults with asthma and allergy found that exposures to 260 ppb NO_2 for 15‑30 minutes or 400 ppb NO_2 for 6 hours increased inflammatory responses to an allergen challenge. These responses included increases in number and activation of eosinophils and neutrophils. In the absence of an allergen challenge, repeated exposure to 600 ppb NO_2 for 30 minutes also enhanced allergic inflammation in subjects with asthma and allergy. Other studies involving a single exposure to NO_2 (300−350 ppb, 1−2 hours; 1,000 ppb, 3 hours) did not show these responses. Allergic inflammation was also enhanced by a 3-hour exposure to 5,000 ppb NO_2 in a rat model of allergic airways disease, as demonstrated by increases in IgE levels and numbers of eosinophils and neutrophils. These results provide evidence for NO_2-induced exacerbation of allergic airways disease both in the presence and absence of an allergen challenge (Section 4.3.2.6).

Table 5-18 Animal toxicological studies of pulmonary inflammation.

Study	Disease Status; Age; n; Sex	Exposure Details	Endpoints Examined
Gilmour et al. (1996)	Rats (Brown Norway); 6 weeks; F; n = 5/group	Immunization with 100 µg antigen (*D. farina* and *D. pteronyssinuss*) + killed *Bordetella pertussis* in 0.3 mL saline Challenge with 50 µg allergen (2 weeks after immunization), followed by: 5,000 ppb NO_2 for 3 h	Endpoints examined 7 days after exposure: Total and differential cell counts from lung lavage Antigen-specific IgG, IgA, IgE antibodies in serum and lavage fluid Lymphocyte proliferation responsiveness
Proust et al. (2002)	Mice (BALB/c); 6−7 weeks; M; n = 5/group	Immunization with injection of 10 µg OVA (Day 0 and Day 7) Challenge with either 10 µg OVA or saline control (Day 14) Exposure following OVA/saline challenge: (1) 5,000 ppb NO_2 (2) 20,000 ppb NO_2 Challenge to 0.1 M aerosol of methacholine for 20 sec	Endpoints examined 24 h after exposure: BAL fluid total and differential cell counts Eosinophil peroxidase activity Immunoassay of IL-4, IL-5 Anti-OVA IgE and IgG1 in serum Lung histology
Hubbard et al. (2002)	Mice (CB57Bl/6); adult; M/F	Sensitization by weekly injections of 25 µg OVA for 3 weeks Challenge with 20 mg/m^3 OVA aerosol for 1 h for 3 days or 10 days Exposure following OVA aerosol challenge: (1) 700 ppb NO_2 for 2 h (2) 5,000 ppb NO_2 for 2 h	Total and differential cell counts from lung lavage (24 h after exposure) Histology analysis (24 h after exposure)
Poynter et al. (2006)	Mice (C57BL/6)	Sensitization by 20 µg of OVA via i.p. injections on Days 0 and 7 Challenge with OVA aerosol (1% in phosphate buffered saline) for 30 min on Days 14−16 Exposures subsequent to OVA challenge: (1) 5,000 ppb NO_2 for 6 h/day for 1, 3, 5 days (2) 25,000 ppb NO_2 for 6 h/day for 1, 3, 5 days *Select groups given 20-day recovery period Methacholine challenge (0, 3.125, 12.5, 50 mg/mL in aerosol)	Endpoints examined after last day of exposure or after 20 day recovery: BAL fluid-total and differential cell counts; LDH Histopathology analysis mRNA levels of Gob5, Muc5AC, Th2, dendritic cell chemokine CCL20 and eotaxin-1

F = female, IL = interleukin, LDH = lactate dehydrogenase, M = male, mRNA = messenger RNA, NO_2 = nitrogen dioxide, OVA = ovalbumin, Th2 = T-derived lymphocyte helper 2.

Epidemiologic Studies of Populations with Asthma

The observations described in the preceding sections for NO_2-induced increases in allergic inflammation provide support for the epidemiologic associations observed for ambient or personal NO_2 with increases in inflammation in children with asthma and allergy. The limited evidence in adults with asthma is inconclusive. The number of these epidemiologic studies has increased dramatically since the 2008 ISA for Oxides of Nitrogen, and recent studies expand on previous studies with exposure assessment conducted in subjects' locations (e.g., homes, schools) and additional examination of potential confounding by traffic-related copollutants. Ambient NO_2 concentrations, locations, and time periods for epidemiologic studies of pulmonary inflammation and oxidative stress are presented in Table 5-19.

Table 5-19 Mean and upper percentile concentrations of nitrogen dioxide (NO_2) in epidemiologic studies of pulmonary inflammation and oxidative stress in populations with asthma.

Study[a]	Location	Study Period	NO_2 Metric Analyzed	Mean Concentration (ppb)	Upper Percentile Concentrations (ppb)
Liu et al. (2009)	Windsor, ON, Canada	Oct–Dec 2005	24-h avg NO_2	19.8	95th: 29.5
Barraza-Villarreal et al. (2008)	Mexico City, Mexico	June 2003–June 2005	8-h max NO_2	37.4	Max: 77.6
Delfino et al. (2006)	Riverside, CA	Aug–Dec 2003	24-h avg NO_2	Personal: 24.3	Max: 47.6
	Whittier, CA	July–Nov 2004		Personal: 30.9	Max: 106
Delfino et al. (2013)	Riverside, CA		8-h max NO_2	Central site: 39.3	Max: 72.4
	Whittier, CA			Central site: 35.1	Max: 96
	2 sites combined		24-h avg NO_2	Central site: 27.4	Max: 73.8
Martins et al. (2012)	Viseu, Portugal	Jan and June, 2006 and 2007	1-week avg NO_2[b]	Across 4 periods: 4.5, 3.5, 9.8, 8.2[c]	Max across 4 periods: 4.6, 4.0, 10.9, 9.4[c]
Sarnat et al. (2012)	El Paso, TX and Ciudad Juarez, Mexico	Jan–Mar 2008	96-h avg NO_2	El Paso school: 4.5, 14.2, central sites: 14.0, 18.5, 20.5 Ciudad Juarez school: 18.7, 27.2, central site: none	NR
Greenwald et al. (2013)	El Paso, TX	Mar–June 2010	96-h avg NO_2	School A: 6.5 School B: 17.5	NR
Holguin et al. (2007)	Ciudad Juarez, Mexico	2001–2002	1-week avg NO_2	18.2	NR

Table 5-19 (Continued): Mean and upper percentile concentrations of nitrogen dioxide (NO_2) in epidemiologic studies of pulmonary inflammation and oxidative stress in populations with asthma.

Study[a]	Location	Study Period	NO_2 Metric Analyzed	Mean Concentration (ppb)	Upper Percentile Concentrations (ppb)
Flamant-Hulin et al. (2010)	Clermont-Ferrand, France	NR	5-day avg NO_2	Schools <14 ppb: 10.1 Schools >14 ppb: 17.4	Across schools: 75th: 14.0[c] Max: 19.7[c]
Lin et al. (2011)	Beijing, China	June 2007 Sept 2007 Dec 2007 June 2008 Sept 2008	24-h avg NO_2	24.3 30.4 45.3 26.6 25.9	NR NR NR NR NR
Liu et al. (2014a)	Munich and Wesel, Germany	NR	24-h avg NO_2	15.9[c]	95th: 29.7[c]
Berhane et al. (2011)	13 southern California communities	Sept−June 2004−2005	24-h avg NO_2	NR	NR
Romieu et al. (2008)	Mexico City, Mexico	Jan−Oct 2004	8-h max NO_2	35.3	Max: 73.5
Qian et al. (2009a)	Boston, MA; New York City, NY; Philadelphia, PA; Madison, WI; Denver, CO; San Francisco, CA	Feb 1997−Jan 1999	24-h avg NO_2	23.6	75th: 28.8 Max: 48.1
Maestrelli et al. (2011)	Padua, Italy	1999−2003	24-h avg NO_2	Range across seasons and years: 20.9−37.0[c]	Range of 75th: 23.0−42.5[c]

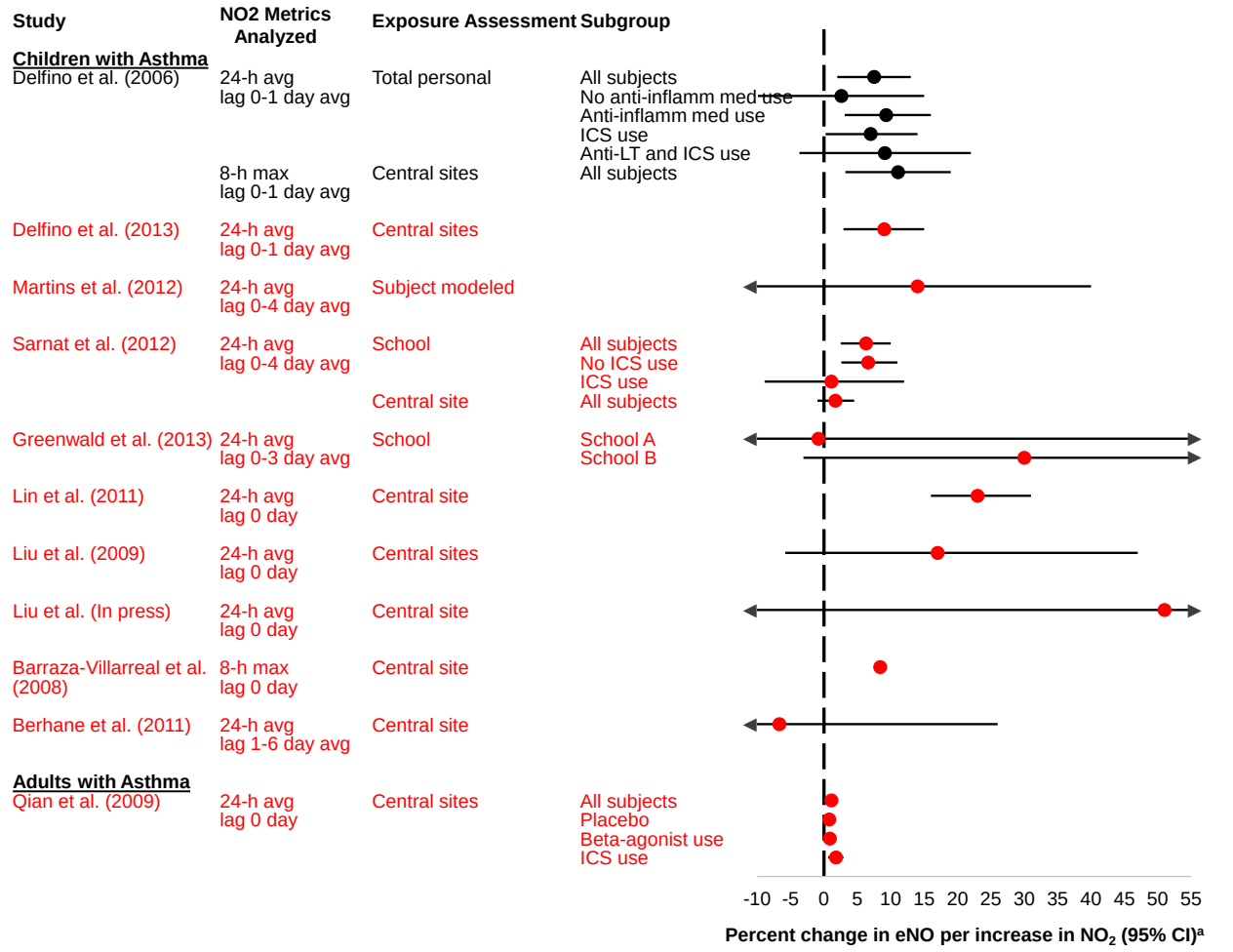
NR = not reported, NO_2 = nitrogen dioxide.
[a]Studies presented in order of first appearance in the text of this section.
[b]Subject-level exposure estimates calculated from outdoor NO_2 at schools and other locations plus time-activity patterns.
[c]Concentrations converted from µg/m^3 to ppb using the conversion factor of 0.532 for NO_2 assuming standard temperature (25°C) and pressure (1 atm).

As in previous studies, the majority of evidence is for eNO. Across studies, eNO was collected with a similar protocol, following the guidelines established by the ATS (2000). eNO assessment methods also accounted for NO in the collection room, although eNO has not been shown to be a reliable indicator of NO exposure (Section 4.2.3). eNO has not been examined in controlled human exposure or animal toxicological studies of NO_2 exposure, but several observations support the epidemiologic findings. NO_2 exposure has been shown to increase some pro-inflammatory cytokines and increase neutrophils and eosinophils (Sections 4.3.2.6), which can activate inducible nitric oxide synthase or produce NO in the lung during an inflammatory response (Barnes and Liew, 1995). Higher eNO has been associated with higher eosinophil counts (Brody et al., 2013). Further, eNO commonly is higher in children and adults with asthma and increases

during acute exacerbation (Soto-Ramos et al., 2013; Carraro et al., 2007; Jones et al., 2001; Kharitonov and Barnes, 2000).

Children with Asthma

Several recent and previous studies found associations between short-term increases in ambient NO_2 concentration and increases in pulmonary inflammation in children with asthma. Children were recruited mostly from schools, supporting the likelihood that study populations were representative of the general population of children with asthma. Asthma was assessed as self- or parental report of physician-diagnosed asthma, but the studies varied in whether they assessed asthma severity or required the presence of current symptoms in subjects. Across studies, associations varied in magnitude and statistical significance; however, the consistent pattern of increasing eNO with increasing short-term NO_2 exposure provides evidence of an association (Figure 5-8 and Table 5-20). Most studies analyzed multiple endpoints, pollutants, lags of exposure, or subgroups; however, with a few exceptions (Liu et al., 2009; Barraza-Villarreal et al., 2008), a pattern of association was found across the multiple comparisons, thus reducing the likelihood of associations found by chance alone or from publication bias.



Note: Results from more informative studies in terms of exposure assessment method and potential confounding considered are presented first. Red = recent studies, black = previous studies. Study details and quantitative results reported in Table 5-20. Table 5-20 presents results for an array of indications of inflammation and oxidative stress for which there was not sufficient numbers to present in a figure. For some studies, eNO results could not be presented in the figure because results were not reported in terms of percentage change eNO.

[a]Effect estimates are standardized to a 20-ppb increase for 24-h avg NO_2 and 30-ppb increase for 1-h max NO_2.

Figure 5-8 Associations of personal or ambient nitrogen dioxide (NO_2) with exhaled nitric oxide (eNO) in populations with asthma.

Table 5-20 Epidemiologic studies of pulmonary inflammation and oxidative stress in children and adults with asthma.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Children with asthma: studies with small spatial scale exposure assessment and/or examination of copollutant confounding				
Delfino et al. (2006) Riverside, Whittier, CA n = 45, ages 9−18 yr, persistent asthma and exacerbation in previous 12 mo Repeated measures. Examined daily for 10 days, 372 observations. Recruitment in schools of non-smokers from non-smoking homes. No information on participation rate. Self-report of physician-diagnosed asthma. Mixed-effects model with random effect for subject with pollutant concentrations centered on subject mean and adjusted for personal measures of relative humidity, measures of personal temperature, follow-up period. Adjustment for city, daily beta agonist use, weekend did not alter results.	NO_2-total personal 24-h avg Compliance assessed with motion detectors. Monitoring checked daily.		eNO:	Copollutant model results in figure only. With $PM_{2.5}$, EC, or OC: NO_2 results robust but increase in 95% CI. Copollutant results robust to NO_2 adjustment. Weak correlations for personal exposures. Spearman r = 0.20−0.31. Stronger correlations for central site pollutants. Pearson r = 0.25−0.70. Central site CO not associated with eNO.
		0	All subjects: 1.2% (−2.0, 4.3)	
		0−1 avg	All subjects: 7.5% (2.0, 13)	
			No anti-inflammatory medication, n = 14 2.6% (−9.9, 15)	
			Anti-inflammatory medication, n = 31 9.3% (3.1, 16)	
			ICS use, n = 19: 7.0% (0.23, 14)	
			Anti-leukotrienes + ICS use, n = 12 9.1% (−3.7, 22)	
	NO_2-central site 8-h max	0	All subjects: 0.81% (−4.5, 6.1)	
		0−1 avg	All subjects: 11% (3.2, 19)	
†Delfino et al. (2013) Riverside, Whittier, CA Same population and methodology as Delfino (2006) above. Analysis also indicated lack of confounding by respiratory infections.	NO_2-central site 24-h avg 1 site Riverside within 12 km of subjects' homes 2 sites Whittier averaged, distance NR		eNO:	For lag 0−1 avg: With oxidative potential of $PM_{2.5}$: 3.8% (−5.1, 14) With in vitro ROS from $PM_{2.5}$: 5.8% (−1.9, 14) Copollutant associations attenuated with NO_2 adjustment. Moderate correlations with NO_2. Spearman r = 0.43 for ROS, 0.49 for oxidative potential.
		0	−0.12% (−3.8, 3.7)	
		1	5.0% (1.2, 9.1)	
		0−1 avg	9.0% (2.9, 15)	

Table 5-20 (Continued): Epidemiologic studies of pulmonary inflammation and oxidative stress in children and adults with asthma.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
†Martins (2013); Martins et al. (2012) Viseu, Portugal n = 51, mean age 7.3 (SD: 1.1) yr, 53% with atopy Repeated measures. 4 measurements over 2 different seasons. Recruitment from urban and suburban schools. ~66% participation rate. Parental report of wheeze in previous 12 mo. GEE adjusted for age, sex, parental smoking, parental education, atopy, time of visit, average temperature, relative humidity. Also included height, weight, older siblings, mold/dampness in home, fireplace in home, pets in home because their inclusion changed the effect estimate for at least 1 pollutant by >10%.	NO_2-subject modeled outdoor 24-h avg Estimated from school outdoor NO_2, 20 city locations, MM5/CHIMERE modeling, and daily activity patterns. 20% time spent at school, 65% at home.	0−4 avg	eNO: 14% (−12, 40) Exhaled breath condensate pH: −2.6% (−3.9, −1.3)	For EBC pH only: With PM_{10}: 0.30 (−3.0, 3.6) With benzene: −1.7 (−3.6, 0.26) With ethylbenzene: −1.6 (−3.7, 0.49) PM_{10} robust to adjustment for NO_2. VOCs attenuated to null. Negative or weakly positive correlations with NO_2. Spearman r = −0.72 to −0.55 for PM_{10}, −0.43 to 0.14 for various VOCs.
†Sarnat et al. (2012) El Paso, TX and Ciudad Suarez, Mexico n = 29 per city, ages 6−1 yr, asthma and current symptoms Repeated measures. Examined weekly for 16 weeks, 697 observations. Recruitment from schools representing a gradient of traffic, subjects from non-smoking homes. No information on participation rate. Self-report of physician-diagnosed asthma. GLM with subject as random effect and adjustment for school, temperature, relative humidity, indoor NO. Adjustment for medication use, cold symptoms did not alter results.	NO_2-school outdoor Each city: one school 91 m from major road, one in residential area. NO_2-school indoor NO_2-central site 1 site in El Paso, TX near major road. All 24-h avg	0−4 avg	eNO: All subjects: 6.3% (2.5, 10) No ICS use, n = 10: 6.6% (2.6, 11) ICS use, n = 19: 1.1% (−8.9, 12) All subjects: 0.53% (0.11, 1.0) All subjects: 1.7% (−1.0, 4.5)	With O_3: 8.8% (4.6, 13) No copollutant model with $PM_{2.5}$ or $PM_{10-2.5}$, which were associated with eNO. No association with BC among all subjects. Weak to moderate correlations with NO_2. Spearman r = −0.39 to 0.32 for $PM_{2.5}$; −0.24 to 0.04 for $PM_{10-2.5}$.
†Greenwald et al. (2013) El Paso, TX n = 38, mean age 10 yr, 76% Mexican-American Repeated measures. Examined weekly for 13 weeks, 436 observations. Recruitment from schools in low- and high-traffic area. No information on participation rate. School record of physician-diagnosed asthma. GLM with subject as random effect and adjusted for school, temperature, relative humidity, indoor NO.	NO_2-school outdoor School A: residential area, School B: 91 m from major road. NO_2-school indoor All 24-h avg	0−3 avg	eNO: School A: −0.86% (−38, 58) School B: 30% (−3.1, 73) School A: −16% (−53, 47) School B: 5.6% (−19, 37%)	No copollutant model. BC, VOCs (central site) associated with eNO. Moderate correlations with NO_2. Pearson r = 0.47−0.62. BTEX associated with eNO. Highly correlated with NO_2. r = 0.77.

Table 5-20 (Continued): Epidemiologic studies of pulmonary inflammation and oxidative stress in children and adults with asthma.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
†Holguin et al. (2007) Ciudad Juarez, Mexico n = 95, ages 6−12 yr, 78% mild asthma, 58% with atopy Repeated measures. Examined biweekly for 4 mo. 87% participation. Self-report of physician-diagnosed asthma. Linear and non-linear mixed effects model with random effect for subject and school adjusted for sex, body mass index, day of week, season, maternal and paternal education, passive smoking exposure.	NO_2-school outdoor 24-h avg Schools located 239−692 m from homes.	0−6 avg	No quantitative results reported for eNO. No association was reported.	No copollutant model. Road density but not $PM_{2.5}$ or EC associated with eNO.
†Zhu (2013); Lin et al. (2011) Beijing, China n = 36, ages 9−12 yr, 22% with asthma Repeated measures before and after Olympics. Examined daily for five 2-week periods. 1,581 observations. Recruitment from school. Selection from 437 (60%) students who responded to initial survey, 95% follow-up participation. GEE adjusted for temperature, relative humidity, body mass index.	NO_2-central site 24-h avg Site 650 m from school.	0 1	eNO: All subjects: 22% (18, 26) Asthma: 23% (16, 31) Asthma: 12% (4.0, 20)	Among all subjects: With BC: 5.6% (0.38, 11) With $PM_{2.5}$: 14% (9.5, 19) No change in BC with NO_2 adjustment. $PM_{2.5}$ reduced but positive. NO_2 highly correlated with BC (r = 0.68), moderately correlated with $PM_{2.5}$ (r = 0.30).
†Flamant-Hulin et al. (2010) Clermont-Ferrand, France n = 34, mean age: 10.7 (SD: 0.7) yr, 44% with atopy Cross-sectional. Recruitment from schools. 69% participation rate. Self- or parental-report of lifetime asthma. For some subjects, eNO measured up to 1 week before pollutants. GEE adjusted for atopy, mother's birth region, parental education, family history of allergy, prenatal and childhood smoking exposure. Did not consider potential confounding by weather.	NO_2-school outdoor 24-h avg NO_2-school indoor 24-h avg	0−4 avg	log eNO comparing ≥14.3 vs. <14.3 ppb NO_2: 0 (−0.14, 0.14) 0 (−0.13, 0.14)	No copollutant model. $PM_{2.5}$, acetylaldehyde associated with eNO.
†Liu (2013); Liu et al. (2009) Windsor, ON, Canada n = 182, ages 9−14 yr Repeated measures. Examined weekly for 4 weeks, same day of week. 672 observations. Recruitment from schools. No information on participation rate. Parental report of physician-diagnosed asthma. Mixed effect model with random effect for subject and adjusted for testing period, temperature, relative humidity, daily medication use.	NO_2-central site 24-h avg Average of 2 sites. 99% subjects live within 10 km of sites.	0 1 0−2 avg 0 1 0−2 avg	eNO: 17% (−5.8, 47) 7.7% (−12, 32) 1.5% (−32, 50) TBARS: 48% (3.9, 111) 22% (−11, 67) 131% (23, 334)	For TBARS only: with $PM_{2.5}$: 31% (−30, 145) with SO_2: 43% (−10, 126) Small decrease in $PM_{2.5}$ estimate with adjustment for NO_2. NO_2 highly correlated with $PM_{2.5}$ (Spearman r = 0.71), weakly with SO_2 (r = 0.18).

Table 5-20 (Continued): Epidemiologic studies of pulmonary inflammation and oxidative stress in children and adults with asthma.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
†Liu et al. (2014a) Munich and Wesel, Germany n = 192, age 10 yr Cross-sectional. Recruitment from GINIplus, LISAplus birth cohort studies. No information reported on participation rate or ascertainment. Parental report of physician-diagnosed asthma. GAM adjusted for cohort, city, sex, parental education, parental history of atopy, indoor gas pollution, current pets, maternal prenatal smoking, smoking exposure at age 10 yr, temperature. Results not altered by adjustment for asthma medication use or annual avg NO_2 estimated from LUR.	NO_2-central site 24-h avg 1 site per city in suburban locations.	0	eNO: Both cities: 51% (−11, 154) Results in figure show association only in Munich, null in Wesel.	With PM_{10}: 23% (−37, 137) among children with asthma. PM_{10} results not altered with NO_2 adjustment. 34% (15, 56) among all 1,985 children. PM_{10} association attenuated with NO_2 adjustment. Moderate correlated with NO_2. Spearman r = 0.59.
Children with asthma: studies with central site exposure assessment and no examination of copollutant confounding				
†Barraza-Villarreal et al. (2008) Mexico City, Mexico n = 119−129, ages 6−14 yr, 54% persistent asthma, 89% atopy Repeated measures. Examined every 15 days for mean 22 weeks. 1,004 observations. Recruited from pediatric clinic. Asthma severity assessed by pediatric allergist. No information on participation rate. Linear mixed effects model with random effect for subject and adjusted for sex, body mass index, lag one minimum temperature, ICS use, time. Adjustment for outdoor activities, smoking exposure, antiallergy medication use, season did not alter results.	NO_2-central site 8-h max Monitors within 5 km of school or home. Low correlation for school vs. central site: Spearman r = 0.21	0	eNO: 8.4% (7.9, 9.0) Interleukin−8: 1.2% (1.1, 1.3) EBC pH: −0.5% (−1.5, 0.50)	No copollutant model. $PM_{2.5}$ and O_3 associated with eNO and IL−8. Moderate or weak correlation with NO_2. Pearson r = 0.61 for $PM_{2.5}$, 0.28 for O_3.
†Romieu et al. (2008) Mexico City, Mexico n = 107, mean age 9.5 yr. 48% persistent asthma, 90% atopy Repeated measures. EBC collected every 2 weeks for 2−16 weeks. 480 observations. Recruitment from allergy clinic. No information on participation rate. 25% EBC samples below detection limit, assigned random value 0−4.1 nmol. Malondialdehyde associated with wheeze and asthma medication use. GEE model adjusted for sex, school shift, temperature, chronological time. Adjustment for outdoor activities, parental smoking did not alter results.	NO_2-central site 8-h max Similar results for 1-h max and 24-h avg. Monitors within 5 km of school or home.	0	Log malondialdehyde: 0.13 (−0.10, 0.35)	No copollutant model. $PM_{2.5}$, distant to closest avenue, 4.5-h traffic count, and O_3 also associated with malondialdehyde. Moderate correlation with NO_2. Pearson r = 0.44 for O_3 and 0.54 for $PM_{2.5}$.

Table 5-20 (Continued): Epidemiologic studies of pulmonary inflammation and oxidative stress in children and adults with asthma.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
†Berhane et al. (2011) 13 Southern CA towns n = 169, ages 6−9 yr Cross-sectional. Recruitment from schools. Parental report of physician-diagnosed asthma and history of respiratory allergy. Linear regression adjusted for community, race/ethnicity, age, sex, asthma, asthma medication use, history of respiratory allergy, eNO collection time, body mass index, smoking exposure, parental education, questionnaire language, season, multiple temperature metrics, eNO collected outdoors.	NO_2-central site 24-h avg Sites in each community. # sites in each community NR.	1−6 avg	eNO: −6.7% (−31 26%)	No copollutant model. $PM_{2.5}$, PM_{10}, O_3 associated with eNO. Moderate or weak correlations with NO_2. Pearson r = 0.47 for $PM_{2.5}$, 0.49 for PM_{10}, 0.15 for O_3.
Adults with Asthma: central site exposure assessment, no examination of potential confounding by traffic-related copollutants				
†Qian et al. (2009a) Boston, MA; New York, NY; Denver, CO; Philadelphia, PA; San Francisco, CA; Madison, WI. n = 119, ages 12−65 yr, persistent asthma, nonsmokers Repeated measures. Examined every 2−4 weeks for 16 weeks. 480 person-days. No information on participation rate. Study population representative of full cohort. Asthma medication trial and a priori comparison of medication regimens. Linear mixed effects model adjusted for age, sex, race/ethnicity, center, season, week, daily average temperature, daily average humidity. Adjustment for viral infections did not alter results.	NO_2-central site 24-h avg Average of all monitors within 51 km of subject ZIP code centroid.	0	eNO: All subjects: 1.1% (0.52, 1.7) Placebo: 0.79% (−0.08, 1.7) Beta-agonist use: 0.86% (0.08, 1.6) ICS use: 1.8% (0.62, 2.9)	With PM_{10}: 0.69% (−0.09, 1.5) With O_3: 0.94% (0.43, 1.5) With SO_2: 1.2% (0.52, 1.9) Copollutant effect estimates attenuated with adjustment for NO_2. Correlations NR.
		0−3	All subjects: 0.94% (0.09, 1.8)	
†Maestrelli et al. (2011) Padua, Italy n = 32, mean age 39.6 (SD: 7.5) yr, 81% persistent asthma Repeated measures. Examined 6 times over 2 yr. Selected from database of beta-agonist users (>6/yr for 3 yr), diagnosis clinically confirmed. 76% follow-up participation. Drop outs did not differ from participants. GEE adjusted for daily average temperature, humidity, atmospheric pressure, asthma medication use, current smoking status.	NO_2-central site 24-h avg 2 sites in city	0	eNO (ppb): All subjects: 3.1 (−14, 21) Nonsmokers, n = 22: 2.9 (−20, 26)	No copollutant model. Personal and central site $PM_{2.5}$ and PM_{10} not associated with eNO. No associations with central site CO. Association found with O_3 and SO_2. Correlations NR.
			EBC pH: All subjects: 0 (−0.19, 0.21) Nonsmokers: −0.09 (−0.24, 0.05)	

Note: More informative studies in terms of the exposure assessment method and potential confounding considered are presented first.

ICS = inhaled corticosteroid, eNO = exhaled nitric oxide, NR = not reported, ROS = reactive oxygen species, GEE = generalized estimating equation, EBC = exhaled breath condensate, GLM = generalized linear mixed effects model, TBARS = thiobarbituric acid reactive substances, GAM = generalized additive models, LUR = land-use regression, CI = confidence interval, CO = carbon monoxide, EC = elemental carbon, IL = interleukin, NO_2 = nitrogen dioxide, O_3 = ozone, OC = organic carbon, PM = particulate matter, SD = standard deviation, VOC = volatile organic compound.

[a]Effect estimates are standardized to a 20 ppb increase for 24-h avg NO_2 and 25 ppb increase for 8-h max NO_2.

†Studies published since the 2008 ISA for Oxides of Nitrogen.

Key evidence was provided by studies with NO_2 exposures assessed for subjects' locations, comparison of various exposure metrics, and/or examination of confounding by traffic-related copollutants. As reported by few studies, participation rates were high (87, 95%, Table 5-20). Selective participation by certain groups was not indicated. These studies examined a limited number of exposure lags but specified them a priori. Across studies, associations were found with multiday averages of NO_2 (i.e., 0−1 avg to 0−6 avg) (Figure 5-8 and Table 5-20), with Delfino et al. (2006) finding a stronger association of eNO with lag 0−1 avg than lag 0 or 1 day NO_2. Strong exposure assessment was characterized as personal monitoring (Delfino et al., 2006); estimation of individual outdoor exposures based on monitoring, modeling, and daily activity patterns (Martins et al., 2012); monitoring at or near schools (Greenwald et al., 2013; Sarnat et al., 2012; Lin et al., 2011; Holguin et al., 2007); or examination of central site ambient concentrations that are temporally correlated with total personal NO_2 measurements (Delfino et al., 2013).

In comparisons with central site NO_2, associations with eNO were similar to personal NO_2 among children with asthma in Riverside and Whittier, CA. A 20-ppb increase in 24-h avg (lag 0−1 day avg) NO_2 was associated with a 7.5% (95% CI: 2.0, 13%) increase in eNO for personal NO_2 exposure (Delfino et al., 2006) and a 9.0% (95% CI: 2.9, 15%) increase for ambient NO_2 averaged between central sites in each community (Delfino et al., 2013). An increase in 8-h max NO_2 assigned from each child's community central site was associated with a similar increase in eNO as 24-h avg personal NO_2 based on the interquartile ranges of NO_2 (1.4% [95% CI: 0.39, 2.3] per 12-ppb increase in 8-h max central site NO_2 and 1.6% [95% CI: 0.43, 2.8] per 17-ppb increase in 24-h avg personal NO_2). Personal and central site NO_2 were moderately correlated (Spearman r = 0.43). Thus, despite the potential for greater exposure measurement error due to within-community variability in ambient NO_2 concentrations and variation in time-activity patterns (Section 3.4.4), daily variation in ambient NO_2 to some extent is represented in daily variation in personal NO_2 exposures of these children that is associated with eNO. Such results provide a rationale for drawing inferences about ambient NO_2 exposure from associations observed with total personal NO_2 exposures.

Among children with wheeze in Portugal, a 20-ppb increase in 1-week avg individual estimates of ambient NO_2 exposure was associated with a 14% (95% CI: −12, 40%) increase in eNO and a −2.6% (95% CI: −3.9, −1.3%) change in exhaled breath condensate (EBC) pH (Martins et al., 2012). School and home indoor NO_2 concentrations were nondetectable, providing support for an association with ambient NO_2. Further, time-weighted averages of microenvironmental NO_2 have shown good agreement with personal NO_2 (Section 3.4.3.1). Children were reported to spend 85% of time at home or

school, underscoring the importance of the individual-level exposure estimation in this study.

Evidence also points to associations of eNO in children with asthma with NO_2 concentrations measured outside schools. Of the studies conducted in communities along the Texas/Mexico border, most found NO_2-associated increases in eNO. In comparisons of NO_2 exposure metrics, eNO was more strongly associated with outdoor school NO_2 than central site NO_2 (Sarnat et al., 2012) or school indoor NO_2 (Greenwald et al., 2013; Sarnat et al., 2012) (Figure 5-8 and Table 5-20). In the Texas/Mexico study, a 20-ppb increase in 96-h avg NO_2 concentration was associated with increases in eNO of 6.3% (95% CI: 2.5, 10.2%) for outdoor school, 0.5% (95% CI: 0.1, 1.0%) for indoor school, and 1.7% (95% CI: −1.0, 4.5%) for central site. NO_2 from the single central site in El Paso was moderately to strongly correlated (Spearman r = 0.63−0.91) with school NO_2 (Sarnat et al., 2012), suggesting that for some schools, the central site measures captured temporal variation in school-based measures. However, the variability in NO_2 found across schools (coefficient of variation = 59%) indicates that the stronger associations with school NO_2 may be attributable to school measurements better representing variability in NO_2 within the area. Misrepresenting variability has been shown to influence exposure measurement error (Section 3.4.5.1). Holguin et al. (2007) did not find an association with eNO in children with asthma in Ciudad Juarez schools. No association was found in a study of children in France (Flamant-Hulin et al., 2010). However, this study had weaker methodology because of its cross-sectional design, comparison of eNO between low and high NO_2 (means 10.1 and 17.4 ppb), and for some subjects, measurement of eNO 1 week before NO_2. NO_2 measured within 650 m of subjects' schools (lag 0 day of 24-h avg) was associated with eNO among children in Beijing, China examined before and after the 2008 Olympics (Lin et al., 2011).

With regard to confounding, most studies with small spatial-scale exposure assessment adjusted for temperature and humidity, with a few additionally adjusting for asthma medication use (Sarnat et al., 2012; Delfino et al., 2006). An array of traffic-related copollutants was examined, and most studies found associations with EC/BC, OC, $PM_{2.5}$, and VOCs. These copollutants showed a wide range of correlations with NO_2 (Pearson or Spearman r = −0.43 to 0.77). There is some evidence for NO_2 effects that are independent from these traffic-related copollutants. NO_2-eNO associations were found with adjustment for personal $PM_{2.5}$, EC, or OC. Personal exposure measures were more weakly correlated with NO_2 (Spearman r = 0.20−0.33) than central site measures (r = 0.20−0.70) (Delfino et al., 2006). For central site NO_2, associations with eNO decreased but remained positive with adjustment for BC or the oxidative potential of ambient $PM_{2.5}$ extracts measured in vitro (Delfino et al., 2013; Lin et al., 2011) (Table 5-20, Figures 5-16 and 5-17). The latter results support an independent association

with NO_2 because oxidative stress is a key event in the mode of action for NO_2, $PM_{2.5}$, and other traffic-related pollutants (Section 5.1.2). The studies conducted in El Paso, TX and Ciudad Juarez, Mexico did not analyze copollutant models with EC/BC or $PM_{2.5}$. However, NO_2 associations were less variable across schools than were $PM_{2.5}$ associations, and in Ciudad Juarez, NO_2 but not EC or $PM_{2.5}$, was associated with eNO (Sarnat et al., 2012; Holguin et al., 2007).

Pulmonary inflammation also was associated with VOCs (Greenwald et al., 2013; Martins et al., 2012). In the El Paso schools, because of the high correlation (Pearson $r = 0.77$) between NO_2 and benzene, toluene, ethylbenzene, xylene (BTEX), an independent association is not discernible for either pollutant. Reporting copollutant-adjusted results only for EBC pH, Martins et al. (2012) found that associations for individual estimates of outdoor NO_2 exposure were similar after adjustment for VOCs, which showed no or negative correlations with NO_2 (range of Spearman correlation coefficient across four visits: $r = -0.42$ to 0.03) (Table 5-20). VOC estimates were attenuated to the null with adjustment for NO_2; thus, NO_2 may have confounded associations for VOCs. Other pollutants, O_3, SO_2, PM_{10}, and $PM_{10-2.5}$, were associated with pulmonary inflammation and oxidative stress but did not show strong positive correlations with NO_2 ($r = -0.72$ to 0.18). NO_2 effect estimates increased with adjustment for O_3 (Sarnat et al., 2012) and became null with PM_{10} (Martins et al., 2012). But, PM_{10} and NO_2 were strongly negatively correlated ($r = -0.55$ to -0.77).

Other studies have weaker implications for inferring an independent effect of NO_2 on pulmonary inflammation and oxidative stress in children with asthma. They all assigned NO_2 exposure as ambient concentrations from one city central site or sites 5 km or 10 km from subjects' homes. While they adjusted for potential confounding by meteorological factors and asthma medication use, most did not examine confounding by traffic-related copollutants. Findings were variable for indicators of inflammation among eNO, IL-8, and exhaled breath condensate pH as well as indicators of oxidative stress related to lipid peroxidation. Some studies found associations with ambient NO_2 (Liu et al., 2014a; Barraza-Villarreal et al., 2008) or inconsistent associations across the lags of exposure or specific endpoints examined (Liu et al., 2009). In others, effect estimates with wide 95% CIs did not support associations (Berhane et al., 2011; Romieu et al., 2008) (Figure 5-8 and Table 5-20). Studies also found associations with traffic proximity and volume and with $PM_{2.5}$, which was moderately to highly correlated with NO_2 ($r = 0.49-0.71$) (Liu et al., 2014a; Berhane et al., 2011; Liu et al., 2009; Romieu et al., 2008);(Barraza-Villarreal et al., 2008). Copollutant modeling was conducted in a study of children in Windsor, Canada, and effect estimates for NO_2 were largely attenuated with adjustment for $PM_{2.5}$ (Table 5-20) (Liu et al., 2009). $PM_{2.5}$ estimates were less altered with adjustment for NO_2; however, the reliability of the copollutant model is questionable because of the high

NO_2-$PM_{2.5}$ correlation (r = 0.71). In the limited analysis of copollutant models with PM_{10} (r = 0.59) or SO_2 (r = 0.18), NO_2 remained associated with pulmonary inflammation or oxidative stress (Liu et al., 2014a; Liu et al., 2009).

Studies of children with asthma did not clearly identify potential factors that could modify ambient NO_2-associated increases in pulmonary inflammation but primarily conducted post hoc analyses. Associations were not found to differ by sex (Sarnat et al., 2012; Liu et al., 2009; Delfino et al., 2006). Larger associations were found in children not using ICS in some (Sarnat et al., 2012; Liu et al., 2009), but not all, (Delfino et al., 2006) studies. Because of the heterogeneity in the definition of ICS use and lack of assessment of ICS compliance, it is not clear whether ICS use represents well-controlled or more severe asthma across populations. Several studies specified comparisons between children with and without asthma a priori. While children with asthma had higher eNO, results indicated no difference in associations with NO_2 between groups (Patel et al., 2013; Lin et al., 2011; Flamant-Hulin et al., 2010; Holguin et al., 2007) or larger associations in children without asthma (Berhane et al., 2011; Barraza-Villarreal et al., 2008).

Adults with Asthma

Recent epidemiologic studies of pulmonary inflammation in adults with asthma, which were not available for the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a), showed contrasting associations with ambient NO_2. Both studies examined adults predominately with persistent asthma, assessed NO_2 exposure from central site monitors, and adjusted for temperature and humidity. In a U.S. multicity (Boston, MA; New York, NY; Philadelphia, PA; San Francisco, CA; Madison, WI) study nested within an asthma medication trial, a 20-ppb increase in lag 0 day of 24-h avg NO_2 (averaged from monitors located within 32 km of subjects' homes) was associated with a 0.26-ppb (95% CI: 0.12, 0.40) increase in eNO (Qian et al., 2009a). A similar increase in eNO was found for lag 0−3 day avg NO_2 but not lags 1, 2 or 3. A larger effect was estimated in the daily ICS group than the placebo or beta-agonist groups only for lag 0 day NO_2. Among children and adults with asthma in Padua, Italy, a large percentage of whom reported ICS use, lag 0 day of 24-h avg ambient NO_2 was not associated with eNO or exhaled breath condensate pH (Maestrelli et al., 2011). The U.S. multicity study did not examine whether the association for ambient NO_2 was independent of other traffic-related pollutants. Copollutant models were examined only for PM_{10}, SO_2, and O_3, in which NO_2 remained associated with eNO (Qian et al., 2009a). Adjustment for NO_2 attenuated the effect estimates for PM_{10}, SO_2, and O_3, indicating that the copollutant associations were confounded by NO_2.

5.2.2.6 Summary of Asthma Exacerbation

Evidence integrated across the array of health outcomes and disciplines strongly supports a relationship between short-term NO_2 exposure and asthma exacerbation. The evidence for allergic inflammation, increased airway responsiveness, and clinical events, such as respiratory symptoms in populations with asthma as well as ED visits and hospital admissions for asthma, is consistent with the sequence of key events within the mode of action for asthma exacerbation (Figure 4-1) and provides biological plausibility for a relationship with NO_2 exposure. Much of this evidence, especially from experimental studies, was described in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a). Recent findings, primarily from epidemiologic studies, continue to indicate NO_2-associated increases in asthma exacerbation. Many recent epidemiologic studies contribute additional exposure assessment in subjects' locations and examination of potential confounding by or interactions with other traffic-related pollutants.

Epidemiologic studies consistently demonstrate associations between increases in ambient NO_2 concentration and increases in asthma hospital admissions and ED visits among subjects of all ages and children (Section 5.2.2.4). The robustness of evidence is demonstrated by associations found in studies conducted in diverse locations in the U.S., Canada, and Asia, including several multicity studies. These observations are coherent with evidence in children and adults with asthma for increases in respiratory symptoms (Section 5.2.2.3), the major reason for seeking medical treatment. NO_2 was associated with the use or sale of asthma medication in adults with asthma but not children with asthma. Individual epidemiologic studies examined multiple outcomes and lags of exposure; however, a pattern of association was consistently observed with NO_2, which does not point to a higher probability of findings due to chance alone.

Although controlled human exposure studies do not provide strong evidence for NO_2 exposure inducing respiratory symptoms in adults with asthma (Section 5.2.2.3), biological plausibility for effects of NO_2 on asthma exacerbation is provided by evidence for NO_2-induced increases in airway responsiveness in adults with asthma, particularly in response to nonspecific challenge agents and exposures in which subjects did not exercise (Section 5.2.2.1). Of all the health outcomes examined in controlled human exposure studies of NO_2, increased airway responsiveness was induced by the lowest NO_2 concentrations, 100 ppb for 1 hour and 200−300 ppb for 30 minutes. Further, a meta-analysis indicates a clinically relevant doubling reduction in provocative dose in adults with asthma in response to NO_2 exposure relative to air exposure. Increased airway responsiveness can lead to poorer asthma control. Thus, the evidence for relatively low NO_2 exposures inducing clinically relevant increases in airway responsiveness in adults with asthma provides key support that ambient exposures to NO_2 can exacerbate asthma.

T-derived lymphocyte helper 2 (Th2)-mediated airway obstruction can lead to both an increase in respiratory symptoms and decrease in lung function. Lung function decrements, as measured by supervised spirometry, were observed in epidemiologic studies in association with ambient or personal NO_2 concentrations (Section 5.2.2.2). Most controlled human exposure studies showed no effect of ambient-relevant NO_2 exposures (200−4,000 ppb for 30 minutes to 6 hours) on lung function but did not include a challenge agent. Information delineating mechanisms underlying NO_2-related lung function decrements is limited. Neural reflexes do not appear to be involved for ambient-relevant exposures; however, there is some evidence for mast cell degranulation mediating changes in lung function (Section 4.3.2.2). Mast cell degranulation leads to histamine release, indicating a role for allergic inflammation in mediating NO_2-induced lung function decrements. Consistent with this mechanistic evidence, NO_2-associated decreases in lung function are found in populations with asthma that have a high prevalence of atopy (53−84%) and groups of children with asthma not using anti-inflammatory ICS (Hernández-Cadena et al., 2009; Liu et al., 2009). In the few NO_2-controlled human exposure studies of adolescents or adults with atopic asthma, NO_2-induced decreases in lung function were found at 1,000 ppb NO_2 (3 hours) but not 120−400 ppb (for 30 minutes to 6 hours) (Jenkins et al., 1999; Jörres et al., 1995; Koenig et al., 1987).

In addition to supporting NO_2-related decreases in lung function in populations with asthma and allergy, evidence for NO_2-induced allergic inflammation demonstrates that NO_2 can affect key events within the mode of action for asthma exacerbation (Section 5.2.2.5). Not all experimental studies of adults with asthma or animal models of allergic disease found effects; however, several found NO_2-induced increases in eosinophil number and activation of eosinophils and/or neutrophils following exposures with and without an allergen challenge. Similar to airway responsiveness, allergic inflammation was enhanced by lower NO_2 exposures than many other health effects examined in experimental studies: 260 ppb NO_2 for 15−30 minutes or 400 ppb NO_2 for 6 hours. In controlled human exposure studies, NO_2 did not consistently increase other indicators of pulmonary inflammation in adults with asthma, including those with atopy, in the absence of allergen challenge. Epidemiologic studies generally did not find NO_2-associated changes in inflammatory cell counts in populations with asthma; however, they did consistently indicate ambient or personal NO_2-associated increases in eNO (Figure 5-8 and Table 5-20). These findings are coherent with experimental evidence for allergic inflammation because increases in eosinophils and neutrophils are linked with NO production during an inflammatory response. The limited studies in adults with asthma produced conflicting results, but the large body of findings in children with asthma shows a consistent pattern of association across the various lags of exposure and outcomes examined. Collectively, the evidence for NO_2-related increases in allergic

inflammation provide biological plausibility for NO_2-associated increases in respiratory symptoms in children with atopy (Barraza-Villarreal et al., 2008; Escamilla-Nuñez et al., 2008). Such associations were not observed in adults with asthma and pollen allergy (Feo Brito et al., 2007).

The evidence in children with asthma is substantiated by several studies with strong exposure assessment characterized by spatially aligning NO_2 concentrations with subjects' location(s). Respiratory symptoms, lung function decrements, and pulmonary inflammation were associated with total and ambient personal NO_2 exposures (Martins et al., 2012; Delfino et al., 2008a; McCreanor et al., 2007) and NO_2 measured outside schools (Greenwald et al., 2013; Zora et al., 2013; Sarnat et al., 2012; Spira-Cohen et al., 2011; Holguin et al., 2007). Given the high variability in NO_2 concentrations, these spatially-aligned measures may better represent temporal variation in subjects' ambient NO_2 exposures than area-wide central site concentrations (Sections 2.5.3 and 3.4.4). Among studies that compared various exposure assessment methods, Delfino et al. (2008a) found similar associations with total personal and central site NO_2, and Sarnat et al. (2012) found stronger associations for school than central site NO_2 concentrations. Observations that daily variation in central site ambient NO_2 was related to variation in total personal NO_2 (r = 0.43) (Delfino et al., 2008a) and that indoor home or school NO_2 concentrations were negligible (Martins et al., 2012) provide additional support for a relationship of asthma exacerbation with ambient NO_2.

A key uncertainty noted in the 2008 ISA for Oxides of Nitrogen was whether NO_2 had an effect independent of other traffic-related pollutants (U.S. EPA, 2008a). Epidemiologic studies of asthma-related respiratory effects found associations with NO_2 as well as with the traffic-related pollutants CO, BC/EC, UFP, $PM_{2.5}$, other PM constituents, and VOCs (Figures 5-16 and 5-17). Among the studies that examined copollutant confounding, most indicate an independent association with NO_2. The predominant method for evaluation was copollutant models, which have well-recognized limitations for distinguishing independent pollutant associations (Section 5.1.2.2). However, several studies with strong exposure assessment provide a sound basis for inferring an independent NO_2 association, when integrated with experimental evidence.

In populations with asthma, total and ambient personal NO_2, NO_2 measured outside or 650 m from children's schools, and NO_2 measured on location of outdoor exposures were associated with respiratory symptoms, decreased lung function, and pulmonary inflammation with adjustment for BC/EC, UFP, OC, or $PM_{2.5}$ (Martins et al., 2012; Lin et al., 2011; Delfino et al., 2008a; McCreanor et al., 2007; Delfino et al., 2006) (Figures 5-16 and 5-17). In a few cases, adjustment for UFP or VOCs attenuated the NO_2 association with one outcome in a study but not another (Martins et al., 2012; McCreanor

et al., 2007), indicating the potential for confounding to differ by outcome. Among children with asthma in El Paso, TX, school NO_2 was not associated with symptoms after adjusting for BC (Zora et al., 2013). However, a copollutant model was not examined in the group also with atopy, to whom the association with NO_2 was limited. Copollutant associations adjusted for NO_2 were robust in some cases (Lin et al., 2011; Delfino et al., 2006) and attenuated in other cases (Martins et al., 2012; Delfino et al., 2008a). Thus, in some studies, NO_2 appeared to confound associations for traffic-related copollutants. The spatial alignment of NO_2 with subjects' location(s) may have reduced differences in exposure measurement error between NO_2 and copollutants, thereby improving the reliability of copollutant model results. A wide range of correlations were reported between NO_2 and traffic-related copollutants (r = −0.42−0.75). Also improving inference from copollutant model results are the low NO_2-copollutant correlations found for personal measurements (r = 0.20−0.30 for EC, OC, $PM_{2.5}$ and −0.42 to 0.08 for benzene and ethylbenzene) (Martins et al., 2012; Delfino et al., 2006).

Consistent with findings for NO_2 exposures in subjects' locations, copollutant models based on central site concentrations indicate ambient NO_2 remains associated with asthma-related hospital admissions, ED visits, symptoms, medication use, and lung function with adjustment for CO, UFP, a source apportionment factor comprising EC and various metals, $PM_{2.5}$, or oxidative potential of $PM_{2.5}$ extracts (Delfino et al., 2013; Iskandar et al., 2012; Dales et al., 2009; Gent et al., 2009; Jalaludin et al., 2008; Villeneuve et al., 2007; Delfino et al., 2003; von Klot et al., 2002). Several traffic-related PM constituents are shown to induce oxidative stress (Section 5.1.2.1). Copollutant adjustment also had effects on central site NO_2 associations that differed by outcomes within studies (Liu et al., 2009; Andersen et al., 2008a; von Klot et al., 2002). Central site NO_2 tended to show moderate correlations with traffic-related copollutants (r = 0.28−0.56, but 0.66 for UFP and 0.71 for $PM_{2.5}$), but differences in spatial distributions may result in differential exposure measurement error for central site concentrations of NO_2 and traffic-related copollutants. Such differences may influence findings from Delfino et al. (2008a), where the association between ambient NO_2 and lung function remained positive but was reduced with adjustment for personal $PM_{2.5}$. Also supporting an independent association for NO_2, some studies found associations with NO_2 but not EC, OC, or $PM_{2.5}$ for school or personal measurements (Sarnat et al., 2012; Delfino et al., 2008a; Holguin et al., 2007) or CO or $PM_{2.5}$ for central site measurements (Lagorio et al., 2006; Delfino et al., 2003).

Epidemiologic evidence also indicates that NO_2 associations with asthma-related effects are independent of other pollutants and temporally varying factors (Tables 5-9, 5-12, 5-20). In most cases, NO_2 associations were found with adjustment for SO_2, PM_{10}, $PM_{10-2.5}$, or O_3 (Liu et al., 2014a; Sarnat et al., 2012; Mann et al., 2010; Patel et al., 2010;

Strickland et al., 2010; Dales et al., 2009; Qian et al., 2009a; Jalaludin et al., 2008; Mortimer et al., 2002). In some copollutant models, associations for both NO_2 and copollutant were attenuated (Martins et al., 2012; Liu et al., 2009; Qian et al., 2009a), and an independent or confounding effect was not distinguished for either NO_2 or copollutant. In exception, Samoli et al. (2011) indicated that the NO_2 association with asthma ED visits was confounded by SO_2 or PM_{10} but not vice versa. Most epidemiologic studies found associations between NO_2 and asthma-related effects with adjustment for potential confounding by temperature, humidity, and season. As examined in fewer studies, NO_2 associations were found with adjustment for day of week, smoking, and asthma medication use.

A few studies of asthma-related respiratory effects examined combined effects of central site NO_2 and traffic-related copollutants. Limited information indicates increases in asthma ED visits when ambient concentrations of NO_2 and $PM_{2.5}$, CO, or EC are high (Gass et al., 2014; Winquist et al., 2014), but does not provide evidence of interactions between NO_2 and VOCs or CO (Schildcrout et al., 2006; Delfino et al., 2003). Limited epidemiologic information indicates joint effects of NO_2, O_3, and SO_2 (Winquist et al., 2014), but interactions are not strongly demonstrated in controlled human exposure studies (Jenkins et al., 1999; Devalia et al., 1994; Hazucha et al., 1994; Adams et al., 1987).

Another line of evidence supporting an independent effect of short-term NO_2 exposure on asthma exacerbation is the coherence of evidence between indoor and outdoor NO_2. Except for Greenwald et al. (2013), indoor home or school NO_2 concentrations were associated with respiratory symptoms and pulmonary inflammation among children with asthma (Lu et al., 2013; Sarnat et al., 2012; Gillespie-Bennett et al., 2011; Hansel et al., 2008). Sarnat et al. (2012) found that correlations between NO_2 and copollutants differed between the indoor and outdoor environments for BC, PM, and SO_2, suggesting that NO_2 may exist as part of different pollutant mixtures in the indoor and outdoor environments.

In summary, epidemiologic studies consistently indicate associations of short-term increases in NO_2 concentrations with asthma-related hospital admissions, ED visits, symptoms, and pulmonary inflammation. A majority of these studies examine central site NO_2 concentrations and do not examine potential confounding by traffic-related copollutants. Therefore, evidence for increased airway responsiveness and allergic inflammation in experimental studies is key in demonstrating the independent effects of NO_2. That these effects are found with exposures to 100−400 ppb NO_2 for 15 minutes to 6 hours substantiates the supposition that ambient-relevant NO_2 exposures can exacerbate asthma. Additional support is provided by associations found with NO_2 exposures assessed in subjects' locations, in copollutant models with another traffic-related

pollutant, and indoor NO_2. Not all respiratory outcomes are associated with NO_2 or show coherence between epidemiologic and controlled human exposure studies, particularly lung function. Potential confounding has not been assessed for all correlated traffic-related pollutants, and reliable methods are not available for simultaneous control for multiple pollutants. However, the evidence integrated across clinical events and key events within the mode of action characterizes biological pathways by which short-term NO_2 exposure may induce asthma exacerbation.

5.2.3 Allergy Exacerbation

The evidence from experimental studies for the effects of short-term NO_2 and allergen co-exposure on increasing allergic inflammation in adults with asthma and animal models of allergic disease (Section 5.2.2.5) not only supports NO_2-related asthma exacerbation but also indicates that NO_2-induced allergy exacerbation may be biologically plausible. Support also is provided by in vitro findings that NO_2 can increase the allergencity of pollen (Cuinica et al., 2014; Sousa et al., 2012). Studies examining clinical indications of allergy exacerbation have become available since the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a). In contrast with asthma exacerbation, short-term NO_2 exposure is not clearly related with clinical indications of allergy exacerbation.

Equivocal epidemiologic evidence in adults with allergies is indicated by associations of ambient NO_2 with physician visits, allergic rhinitis, or nonspecific respiratory symptoms that are either inconsistent across the lags of exposure examined (Villeneuve et al., 2006b), negative, or positive but with wide 95% CIs (Annesi-Maesano et al., 2012; Feo Brito et al., 2007). The latter results are based on a multipollutant model, which can produce unreliable results (Annesi-Maesano et al., 2012) (Table 5-21). Studies only reported that ambient NO_2 exposure was assigned as ambient concentrations from one central site or the average of multiple sites. And, it is uncertain whether the temporal variability in these NO_2 metrics adequately represents the variability in ambient concentrations across the study area or in subjects' ambient exposures. Similarly inconclusive, a recent controlled human exposure study of adults with allergic asthma did not find increases in allergic inflammation after NO_2 exposure and found increases in respiratory symptoms only during exposure (Riedl et al., 2012).

Table 5-21 Epidemiologic studies of allergy exacerbation.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
†Correia-Deur et al. (2012) São Paolo, Brazil, Apr-Jul 2004 n = 36, one test positive (see below); n = 28, three tests positive, ages 9−11 yr Repeated measures. Daily supervised spirometry for 15 school days. Number of observations not reported. Recruitment from school. 86% participation. Allergic sensitization ascertained by skin prick test, blood eosinophils, and serum IgE. GEE with autoregressive correlation matrix adjusted for date, school absence, temperature, humidity.	NO_2-outdoor school 24-h avg, lag 0 day Mean: 69.9 ppb[b] 75th: 84.5 ppb[b] 90th: 102 ppb[b]	% change PEF: Group with 1 positive test −0.87% (−1.7, −0.04) Group with 3 positive tests −0.30% (−1.7, 1.1)	All subjects, lag 0 With CO (r = 0.51) −1.5% (−3.0, 0) With SO_2 (r = 0.60) −1.9% (−3.3, −0.37) With PM_{10} (r = 0.59) −0.75% (−4.4, 3.1) With O_3 (r = 0.40) −1.5% (−3.3, 0.38) Associations for CO & O_3 not altered by NO_2 adjustment. SO_2 & PM_{10} attenuated.
†Barraza-Villarreal et al. (2008) Mexico City, Mexico, Jun 2003−Jun 2005 n = 50, ages 6−14 yr, 72% with atopy Repeated measures. Examined every 15 days for mean 22 weeks. Participation rate not reported. 1,503 observations. Recruitment from friends or schoolmates of subjects with asthma. Clinical assessment of allergy. Supervised spirometry. Linear mixed effects model with random effect for subject and adjusted for sex, BMI, temperature, ICS use, time. Adjustment for outdoor activities, smoking exposure, anti-allergy medication use, season did not alter results.	NO_2-central site 8-h max NO_2 Closest site, within 5 km of homes or schools. R = 0.21 for central site and school NO_2. Mean: 37.4 ppb Max: 77.6 ppb	OR for cough: lag 0−1 day avg 1.28 (1.04, 1.57) % change FEV_1: lag day 1−4 avg −0.64% (−2.1, 0.82)	No copollutant model. $PM_{2.5}$ associated with lung function and cough. Moderate correlation with NO_2. Pearson r = 61.
†Escamilla-Nuñez et al. (2008) Mexico City, Mexico, Jun 2003−Jun 2005 n = 50, ages 6−14 yr, 79% with atopy Part of same cohort as above. Participation rate not reported. Linear mixed effects model with random effect for subject and adjusted for atopy, temperature, time, sex. Adjustment for outdoor activities, smoking exposure, season did not alter results.	NO_2-central site 1-h max NO_2, lag 0−1 day avg Closest site, within 5 km of homes or schools. Mean: 68.6 ppb Upper percentile: NR	OR for cough: 1.23 (1.03, 1.47)	Only multipollutant model with O_3 and $PM_{2.5}$ analyzed. Moderate correlation between $PM_{2.5}$ and NO_2. Pearson r = 0.62.
Villeneuve et al. (2006b) Toronto, Canada, 1995−2000 n = 52,971 physician visits for allergic rhinitis, ages 65 yr or older Time-series analysis. GLM adjusted for temperature, relative humidity, daily number visits for influenza, allergen levels natural spline for time trend.	NO_2-central site 24-h avg, lag 0 day Average of 9 city sites. Mean: 25.4 ppb Max: 71.7 ppb	Quantitative results NR NO_2 associated with physician visits for allergic rhinitis at lag 0 day. Negative or null associations at lag 1 day to 6 days.	No copollutant model. Association also observed for SO_2 but not $PM_{2.5}$ or CO. Correlations with NO_2 NR.

Table 5-21 (Continued): Epidemiologic studies of allergy exacerbation.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
†Feo Brito et al. (2007) Ciudad Real and Puertollano, Spain May−June 2000 or 2001 n = 137, ages NR, mild/moderate asthma and pollen allergy Repeated measures, 90% follow-up participation. Daily symptom diaries. Number of observations not reported. Recruitment from allergy clinics. Clinical assessment of allergy. Poisson regression adjusted only for linear and quadratic terms for season.	NO_2-central site 24-h avg 4 sites in Puertollano, 1 mobile site in Ciudad Real Mean and Max Ciudad Real: 17.4[b], 35.6[b] Puertollano: 29.5[b], 100[b]	% change in symptoms: Ciudad Real, lag day 4 4.75% (−5.75, 16.4) Puertollano, lag day 3 −3.00% (−9.55, 4.03)	No copollutant model. PM_{10}, SO_2, O_3 associated with symptoms only in Puertollano. Moderate correlation with NO_2. r = 0.67, 0.36, 0.36. Pollen associated with symptoms only in Ciudad Real. r with NO_2 = −0.10.
†Annesi-Maesano et al. (2012) Multiple metropolitan locations, France, May−Aug 2004 n = 3,708 with severe allergic rhinitis, ages 6 yr and older, 82% adults Cross-sectional. Recruitment from physicians' offices. No information on participation rate. Clinical assessment of allergy and symptom severity. Multilevel model adjusted for age, date of physician visit, asthma status, postal code. Did not consider confounding by meteorology or SES.	NO_2-central site 24-h avg, lag day 1 Site in postal code of home. Mean: 9.9[b] Max: 38.9[b]	NR	Only multipollutant model analyzed with SO_2, O_3, PM_{10}, pollen. Correlation only reported for pollen, r = −0.12.

Note: More informative studies in terms of the exposure assessment method and potential confounding considered are presented first.

GEE = generalized estimating equations, BMI = body mass index, ICS = inhaled corticosteroid, NR = not reported, OR = odds ratio, CI = confidence interval, CO = carbon monoxide, FEV_1 = forced expiratory volume in 1 second, GLM = generalized linear model, NO_2 = nitrogen dioxide, O_3 = ozone, PEF = peak expiratory flow, PM = particulate matter, SES = socioeconomic status, SO_2 = sulfur dioxide.

[a]Effect estimates are standardized to a 20 ppb for 24-h avg NO_2.

[c]Concentrations converted from μg/m^3 to ppb using the conversion factor of 0.532 assuming standard temperature (25°C) and pressure (1 atm).

†Studies published since the 2008 ISA for Oxides of Nitrogen.

In children with allergies, increases in ambient NO_2 were associated with decreases in lung function (Correia-Deur et al., 2012; Barraza-Villarreal et al., 2008), with an association with cough found in a cohort in Mexico City (Barraza-Villarreal et al., 2008; Escamilla-Nuñez et al., 2008). Strengths of these studies include the clinical assessment of allergy and the supervised measurement of lung function. Although not specific to allergy exacerbation, lung function can decrease during an allergy exacerbation due to airway obstruction caused by Th2 cytokine-mediated inflammation. The studies in children aimed to account for heterogeneity in ambient NO_2 concentrations. In one cohort, exposures were assigned from sites within 5 km of children's home or school, but a Pearson correlation of r = 0.21 between school and central site NO_2 indicates the variability at the central site may not represent the variability in the subjects' locations

(Barraza-Villarreal et al., 2008). Another study examined NO_2 from a central site in the backyard of the subjects' school (Correia-Deur et al., 2012), providing a stronger basis for inference of NO_2 effects. Increases in NO_2 lagged 2 hours, from the same day, and averaged over 3 days were associated with decreases in PEF. However, counter to expectation, associations were observed for the 36 children identified as having atopy with a less stringent definition (one positive test among skin prick test, serum IgE, or blood eosinophils: −0.87% [95% CI: −1.7, −0.04] per 20-ppb increase in lag 0 day NO_2), not the 28 children with atopy defined more stringently (all three tests positive: −0.30% [95% CI: −1.7, 1.1]).

Respiratory effects in populations with allergy were associated with other traffic-related pollutants such as CO and $PM_{2.5}$ as well as with PM_{10} and SO_2, so it is unclear whether the supporting epidemiologic evidence represents an independent effect of NO_2. Only Correia-Deur et al. (2012) examined copollutant models. In analyses combining children with and without allergies, NO_2 remained associated with PEF with adjustment for CO, which also was measured at school (Table 5-21). NO_2 also remained associated with PEF with adjustment for school SO_2 concentrations. However, both NO_2 and PM_{10} (r = 0.60) associations were attenuated when adjusted for each other, and a confounding or independent effect cannot be distinguished for either pollutant. O_3 potentially could confound NO_2 effects in the warm season, and interactions between O_3 and allergens have been reported (U.S. EPA, 2013a). However, NO_2 was associated with PEF after adjustment for O_3 (r = 0.40) (Table 5-21) (Correia-Deur et al., 2012).

In summary, the evidence does not clearly indicate whether NO_2 exposure independently induces allergy exacerbation. While there is evidence for effects on key events in the mode of action, the limited evidence for effects on clinical events related to allergy exacerbation is inconclusive. Further, in the limited analysis of copollutants, there is evidence for an effect of NO_2 on lung function decrements independent of CO or O_3 measured at children's schools but uncertainty regarding confounding by $PM_{2.5}$, PM_{10}, and the array of other traffic-related pollutants, which were not examined (Table 5-1).

5.2.4 Exacerbation of Chronic Obstructive Pulmonary Disease

COPD is characterized by deterioration of lung tissue and airflow limitation. In exacerbation of COPD, episodes of reduced airflow, which can be indicated by decreases in lung function, can lead to symptoms such as cough, sputum production, and shortness of breath. Severe exacerbation can lead to hospital admissions or ED visits. This spectrum of outcomes comprises the majority of investigations of the effects of short-term NO_2 exposure on COPD exacerbation, and as described in the sections that

follow, the consistency of findings from previous and recent studies varies among outcomes. As described at the end of the section (Section 5.2.4.3), limited recent information does not show NO_2-related increases in pulmonary inflammation in adults with COPD. Pulmonary inflammation is a key early event in COPD exacerbation, mediating narrowing of the airways and reducing airflow.

5.2.4.1 Lung Function Changes and Respiratory Symptoms in Adults with Chronic Obstructive Pulmonary Disease

Evidence does not clearly indicate a relationship for NO_2 exposure with changes in lung function or respiratory symptoms in adults with COPD. Evidence is inconsistent in both controlled human exposure and epidemiologic panel studies, many of which examine both respiratory symptoms and lung function. Most of these studies were reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a), and the only recent study, which is epidemiologic, does not support associations for ambient NO_2 concentrations with either respiratory symptoms or lung function decrements in adults with COPD.

Lung Function Changes

Epidemiologic studies recruited adults with COPD from clinics, and the nonrandom selection of the general population may produce study populations less representative of the COPD population. NO_2 exposures were assessed primarily from central site measurements of 24-h avg NO_2, and results are equally inconsistent for NO_2 exposures assigned from one site or averaged from multiple city sites (Table 5-22). Most previous and recent epidemiologic studies in adults with COPD assessed lung function with unsupervised home measurements, and associations with ambient NO_2 concentrations were inconsistent among the various lung function parameters (e.g., FEV_1, PEF) or NO_2 exposure lags (0-, 1-, 2-, or 2- to 7-day avg) examined (Peacock et al., 2011; Silkoff et al., 2005; Higgins et al., 1995). Lagorio et al. (2006) found an association between ambient NO_2 concentrations and FEV_1 (Table 5-22), with similar effects estimated for adults with COPD and asthma.

Table 5-22 Epidemiologic panel studies of adults with chronic obstructive pulmonary disease (COPD).

Study Population Examined and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
†Peacock et al. (2011) London, U.K., Oct 1995−Oct 1997 n = 28−94, ages 40−83 yr Repeated measures. Home PEF. Examined daily for 21−709 days. Recruitment from outpatient clinic. 75% follow-up. GEE adjusted for temperature, season. Lung function measures adjusted for indoor temperature and time spent outdoors.	NO_2-central site 1-h max NO_2, lag 1 day 1 city site Mean: 51.4 ppb 75th: 56 ppb	PEF: 0.17% (0.03, 0.32) PEF >20% below predicted: OR: 1.0 (0.86, 1.2) Symptomatic fall in PEF: OR: 1.1 (0.97, 1.3)	Symptomatic fall in PEF With BS: 1.1 (0.84, 1.3) With PM_{10}: 0.97 (0.81, 1.2) Correlations NR. 95% CI for BS also increased. No change in OR for PM_{10} with NO_2 adjustment.
Silkoff et al. (2005) Denver, CO, Winters 1999−2000 and 2000−2001 n = 34 with COPD, mean age 66 yr, 75% severe COPD Repeated measures. Home PEF. Recruitment from outpatient clinics, research registries, advertisements. 93−96% diaries completed. Mixed effects model with random effect for subjects and adjusted for temperature, relative humidity, barometric pressure.	NO_2-central site 24-h avg, lag 0, 1, 2 days 1 city site Means: 1999−2000: 16 ppb 2000−2001: 29 ppb 75th and Max: 1999−2000: 30, 54 ppb 2000−2001: 36, 54 ppb	No quantitative data. Negative, positive, and null associations with symptoms across NO_2 lags.	No copollutant model. Mixed positive, negative, null associations for $PM_{2.5}$, PM_{10}, O_3.
Desqueyroux et al. (2002) Paris, France, Oct 1995−Mar 1996, Apr−Sept 1996 n = 39, severe COPD, mean age 67 yr Repeated measures. Recruitment from physicians' offices. No information on participation. GEE adjusted for FEV_1, smoking, CO_2 pressure, oxygen treatment, dyspnea, temperature, humidity, season, holiday.	NO_2-central site 24-h avg, lag 1−5 day avg Average of 15 city sites Means for Periods 1 & 2 31.4, 26.1 ppb[b] Max for Periods 1 & 2 68.1, 56.4 ppb[b]	Physician visits for COPD exacerbation OR: 0.76 (0.28, 2.10)	with O_3: 0.47 (0.02, 9.45) O_3 association robust to NO_2 adjustment. Correlations not reported. SO_2 and PM_{10} not associated with COPD.
Lagorio et al. (2006) Rome, Italy, May−June, Nov−Dec 1999 n = 11, ages 40−64 yr, nonsmokers Repeated measures. Supervised spirometry. Examined 2/week for two 1-mo periods. Mean 9, 15 observations per subject. Recruitment from outpatient clinic. Participation rate NR. GEE adjusted for season, temperature, humidity, beta-agonist use.	NO_2-central site 24-h avg, lag 0 day Average of 5 city sites within 2 km of subjects' census tracts. Mean: 37.6 ppb[b] Max: 54.3 ppb[b]	% predicted FEV_1: −2.3 (−3.6, −1.0)	No copollutant model. Lung function associated with $PM_{2.5}$, PM_{10}. Moderate correlation with NO_2. Spearman r = 0.43 for $PM_{2.5}$, 0.45 for PM_{10}.

Table 5-22 (Continued): Epidemiologic panel studies of adults with chronic obstructive pulmonary disease (COPD).

Study Population Examined and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Harre et al. (1997) Christchurch, New Zealand, June−Aug 1994 n = 40, ages 55−83 yr, nonsmokers Repeated measures. Home PEF. Recruitment from doctors' offices, COPD support group, advertisements. 66% participation. Loglinear model adjusted for day of study, temperature, wind speed, CO, PM_{10}, SO_2.	NO_2-central site 24-h avg, lag 1 day # site NR. Concentrations NR	PEF: −0.72% (−1.5, 0.07)	Only multipollutant model analyzed. PM_{10}, CO, SO_2 not associated with PEF.
†Brüske (2014); Bruske et al. (2010) Erfurt, Germany, Oct 2001−May 2002 n = 38, ages 35−78 yr, all male, 53% also with asthma Repeated measures. Examined 2/mo for 6 mo. 381 observations after excluding concurrent fever or infection. Method of recruitment and COPD assessment and participation rate NR. Additive mixed models with random intercept for subject and adjusted for infection/antibiotic use in previous 2 weeks, long-term time trend, temperature, humidity as linear terms or penalized splines. Also evaluated confounding by barometric pressure and corticosteroid use.	NO_2-central site 24-h avg, lag 0 0−23 h before blood collection 1 site 3.5 km from subjects' homes. Mean: 13.5 ppb[b] 75th: 16.6 ppb[b]	PMN: −8.0% (−18, 3.1) Lymphocytes: 8.4% (−5.0, 24)	PMN with UFP: 7.3% (−14, 34). Lymphocytes with UFP: 8.4% (−7.2, 27) CO associated with lymphocytes. NO_2 highly correlated with UFP and CO. Spearman r = 0.66, 0.78. No copollutant model for NO.
	NO-central site Mean: 10.8 ppb[b] 75th: 14.0 ppb[b]	PMN: −0.80% (−10, 9.9) Lymphocytes: 13% (−1.9, 23) NO_2 and NO reported not to be associated with eosinophils. No quantitative results.	

Note: More informative studies in terms of the outcome examined, exposure assessment method and potential confounding considered are presented first.

PEF = peak expiratory flow, GEE = generalized estimating equations, CI = confidence interval, CO = carbon monoxide, CO_2 = carbon dioxide, COPD = chronic obstructive pulmonary disease, FEV_1 = forced expiratory volume in 1 second, NO = nitric oxide, NO_2 = nitrogen dioxide, NR = not reported, O_3 = ozone, OR = odds ratio, PM = particulate matter, PMN = polymorphonuclear cells, SO_2 = sulfur dioxide, UFP = ultrafine particles.

[a]Effect estimates were standardized to a 20-ppb increase in 24-h avg NO_2 or a 30-ppb increase 1-h max NO_2.

[b]Concentrations converted from µg/m^3 to ppb using the conversion factor of 0.532 assuming standard temperature (25°C) and pressure (1 atm).

†Studies published since the 2008 ISA for Oxides of Nitrogen.

In addition to the inconsistent evidence for changes in lung function in adults with COPD, there is uncertainty regarding an independent association of NO_2 from that of copollutants. Studies did not examine a broad range of traffic-related copollutants. Lagorio et al. (2006) found FEV_1 decrements in association with NO_2 but not $PM_{2.5}$, which was moderately correlated with NO_2. Only Peacock et al. (2011) conducted copollutant modeling, and the NO_2-PEF effect estimate was attenuated with adjustment for BS. In contrast, the effect estimate for BS was relatively unchanged with adjustment for NO_2. With respect to PM_{10}, no association was found with FEV_1 (Lagorio et al.,

2006), or the NO_2 association with PEF was attenuated with adjustment for PM_{10} (Peacock et al., 2011).

Similar to the epidemiologic studies, the controlled human exposure studies, which were reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a) and examined older adults diagnosed with COPD, provide mixed results regarding decrements in lung function with NO_2 exposure. As presented in Table 5-23, exposures ranged from 200 to 4,000 ppb NO_2 for 75 minutes to 6 hours, and most studies incorporated exercise in the exposure period to assess lung function during various physiological conditions. Morrow et al. (1992) exposed older adults diagnosed with COPD to 300 ppb NO_2 for 3 hours and reported consistent reductions in FVC that reached statistical significance at the end of exposure, while Vagaggini et al. (1996) reported decreases in FEV_1 in subjects with COPD exposed to 300 ppb NO_2 for 1 hour. In contrast, Linn et al. (1985a) and Gong et al. (2005) reported that exposure to 400−2,000 ppb for 1−2 hours had no effect on lung function in adults with COPD. Furthermore, Gong et al. (2005) did not find NO_2 to enhance respiratory effects of PM exposures.

Respiratory Symptoms

The limitations and uncertainties described above for the evidence base relating NO_2 exposure to lung function changes in adults with COPD largely apply to the evidence base for respiratory symptoms. Epidemiologic panel studies examining adults with COPD, many conducted in Europe, (Table 5-22) either found no association (Peacock et al., 2011; Desqueyroux et al., 2002) between ambient NO_2 and respiratory symptoms or inconsistent associations across the lags of exposure or range of outcomes examined (Silkoff et al., 2005; Harre et al., 1997). Most epidemiologic panel studies recruited subjects from outpatient clinics or doctors' offices. Results were equally inconsistent for symptoms such as cough, wheeze, dyspnea, total symptoms, and medication use (Table 5-22). No pattern of association was found for either 24-h avg or 1-h max NO_2 or for a particular lag day of exposure examined (0, 1, or longer). Most of these studies assigned exposures from a single central site, but associations with symptoms and medication were inconsistent for NO_2 assigned from the closest site (Desqueyroux et al., 2002) or site within 5 km (Harre et al., 1997).

In the studies that found associations with specific symptoms or lags of NO_2, associations also were found with traffic-related pollutants such as $PM_{2.5}$, BS, and CO (Peacock et al., 2011; Silkoff et al., 2005; Harre et al., 1997). Among adults in New Zealand, an increase in 24-h avg NO_2 was associated with an increase in inhaler use in a multipollutant model with CO, PM_{10}, and SO_2 (Harre et al., 1997), which has limited implications because of multicollinearity. A recent study of adults in London, U.K. found that associations

between lag day 1 of 1-h max NO_2 and dyspnea were null with adjustment for BS or PM_{10} (Peacock et al., 2011). Thus, in the few associations found between increases in ambient NO_2 concentration and increases in symptoms or medication among adults with COPD, there is uncertainty as to whether ambient NO_2 has effects independent of other traffic-related pollutants.

The equally inconsistent findings from controlled human exposure studies (Table 5-23) do not address uncertainties in the epidemiologic evidence base. Some studies reported no change in symptoms, as measured by symptom score, in adults with COPD (Gong et al., 2005; Morrow et al., 1992), though some studies reported small, but statistically significant increases in symptom scores during NO_2 exposures of 300−2,000 ppb for 1 hour with exercise (Vagaggini et al., 1996; Linn et al., 1985a).

Table 5-23 Controlled human exposure studies of respiratory symptoms in adults with chronic obstructive pulmonary disease (COPD).

Study	Disease Status; n, Sex; Age (mean ± SD)	Exposure Details (Concentration; Duration)	Time of Outcome Assessment
Gong et al. (2005)	Healthy: n = 2 M, 4 F; 68 ± 11 yr COPD: n = 9 M, 9 F; 72 ± 7 yr	(1) 400 ppb NO_2 for 2 h (2) 200 µg/m^3 CAPs for 2 h (3) 400 ppb NO_2 + 200 µg/m^3 CAPs for 2 h (1−3) Exercise 15 min on/15 min off at $\dot{V}_E$ = ~2 times resting	Pulmonary function tests before and immediately after exposure and 4 h and 22-h post-exposure. Symptoms before, during, and after exposure.
Linn et al. (1985a)	COPD n = 13 M, 9 F (1 never smoker, 13 former smokers, and 8 current smokers); 60.8 ± 6.9 yr	500, 1,000, and 2,000 ppb for 1 h; Exercise 15 min on/15 min off $\dot{V}_E$ = 16 L/min	Pulmonary function tests before, during, and after exposure. Symptoms before, during, immediately after, 1 day after, and 1 week after exposure.
Morrow et al. (1992)	Healthy: n = 10 M, 10 F (13 never smokers, 4 former smokers, 3 current smokers) COPD: n = 13 M, 7 F (14 current smokers, 6 former smokers); 59.9 ± 7.0 yr	300 ppb for 4 h; Three 7-min periods of exercise at $\dot{V}_E$ = ~4 times resting	Pulmonary function tests before, during, and after exposure and 24-h post-exposure. Symptoms before, during, and after exposure and 24-h post-exposure.
Vagaggini et al. (1996)	Healthy: n = 7 M; 34 ± 5 yr COPD: n = 7 M; 58 ± 12 yr	300 ppb for 1 h; Exercise at $\dot{V}_E$ = 25 L/min	Pulmonary function tests before and 2 h after exposure. Symptoms before and 2 h after exposure.

CAPs = concentrated ambient particles, COPD = chronic obstructive pulmonary disease, F = female, M = male, NO_2 = nitrogen dioxide, SD = standard deviation.

5.2.4.2 Hospital Admissions and Emergency Department Visits for Chronic Obstructive Pulmonary Disease

In contrast with the inconsistent evidence for the effects of short-term NO_2 exposure on lung function changes and respiratory symptoms in adults with COPD (Section 5.2.4.1), epidemiologic evidence is consistent for NO_2-related increases in hospital admissions and ED visits for COPD. The few studies of COPD hospital admissions or ED visits evaluated in the 2008 ISA for Oxides of Nitrogen provided initial evidence of a positive association between short-term NO_2 exposures and COPD hospital admissions and ED visits, with more studies focusing on hospital admissions (Figure 5-9 and Table 5-25). However, these studies were more limited in their evaluation of potential confounders

and other factors that may modify the relationships of NO_2 exposure with COPD hospital admissions and ED visits. Consistent with the 2008 ISA for Oxides of Nitrogen, a few recent studies have examined COPD hospital admissions and ED visits and generally add to the initial evidence of a positive association observed in the 2008 ISA for Oxides of Nitrogen. The air quality characteristics of the study cities and the exposure assignment approach used in each study evaluated in this section are presented in Table 5-24. Other recent studies of COPD hospital admissions and ED visits are not the focus of this evaluation, as detailed in Section 5.2.2.4, but the full list of these studies and study details, can be found in Supplemental Table S5-3 (U.S. EPA, 2014h).

Table 5-24 Mean and upper percentile concentrations of nitrogen dioxide (NO_2) in studies of hospital admission and emergency department visits for chronic obstructive pulmonary disease.

Study	Location Years	Exposure Assignment	Metric	Mean Concentration (ppb)	Upper Percentile of Concentrations (ppb)	Copollutant Examination
Hospital Admissions						
†Faustini et al. (2013)	6 Italian cities (2001−2005)	Average of NO_2 concentrations over all monitors within each city. Number of NO_2 monitors in each city ranged from 1−5[a]	24-h avg	24.1−34.6	NR	Correlations (*r*), across cities: PM_{10}: 0.22−0.79 Copollutant models: PM_{10}
Ko et al. (2007a)	Hong Kong (2000−2004)	Average of NO_2 concentrations across 14 monitors.	24-h avg	27.2	75th: 34.0 Max: 83.8	Correlations (*r*): $PM_{2.5}$: 0.44 PM_{10}: 0.40 SO_2: 0.66 O_3: 0.34 Copollutant models: none
†Qiu et al. (2013a)	Hong Kong (1998−2007)	Of 14 monitors, average NO_2 based on data from 10 monitors. 3 monitors sited near roads and 1 monitor on a remote island were excluded.	24-h avg	30.9	NR	Correlations (*r*): NR Copollutant models: PM_{10}
†Wong et al. (2009)	Hong Kong (1996−2002)	Average of NO_2 concentrations across 8 monitors.	24-h av[g]	31.2	75th: 37.0 Max: 89.4	Correlations (*r*): NR Copollutant models: none

Table 5-24 (Continued): Mean and upper percentile concentrations of nitrogen dioxide (NO_2) in studies of hospital admission and emergency department visits for chronic obstructive pulmonary disease.

Study	Location Years	Exposure Assignment	Metric	Mean Concentration (ppb)	Upper Percentile of Concentrations (ppb)	Copollutant Examination
Emergency Department Visits						
†Stieb et al. (2009)	7 Canadian cities (1992−2003)	Average NO_2 concentrations from all monitors in each city. Number of NO_2 monitors in each city ranged from 1−14.	24-h avg	9.3−22.7	75th: 12.3−27.6	Correlations (*r*) only reported by city and season. Copollutant models: none
†Arbex et al. (2009)	São Paulo, Brazil (2001−2003)	Average of NO_2 concentrations across 4 monitors.	1-h max	63.0	75th: 78.6 Max: 204.6	Correlations (*r*): PM_{10}: 0.60 SO_2: 0.63 CO: 0.56 Copollutant models: none

CO = carbon monoxide, NO_2 = nitrogen dioxide, NR = not reported, O_3 = ozone, PM = particulate matter, SO_2 = sulfur dioxide.

[a]Monitoring information obtained from Colais et al. (2012).

†Studies published since the 2008 ISA for Oxides of Nitrogen.

Hospital Admissions

Consistent with the 2008 ISA for Oxides of Nitrogen, relatively few recent studies have focused on the outcome of COPD hospital admissions, but these studies build upon the initial evidence of a positive association (Figure 5-9). Faustini et al. (2013) examined the relationship between short-term air pollution exposures and respiratory-related hospital admissions, including COPD, specifically on the adult population (i.e., individuals 35 years of age and older) in six Italian cities. In a time-series analysis, the authors examined the lag structure of associations through single-day lags as well as cumulative lags using cubic polynomial distributed lags to identify whether the NO_2 effect on respiratory-related hospital admissions was immediate (lag 0, lag 0−1 days), delayed (lag 2−5 days), or prolonged (lag 0−3, 0−5 days). For COPD hospital admissions, the authors observed stronger evidence for immediate (lag 0: 4.6% [95% CI: 0.64, 8.6] for a 20-ppb increase in 24-h avg NO_2 concentrations) NO_2 effects on COPD hospital admissions. Smaller associations were observed when examining prolonged effects, (3.3% for lag 0−3 days and 3.1% for lag 0−5 days). There was no evidence for delayed effects (lag 2−5 days). In a copollutant model with PM_{10} at lag 0, the association between NO_2 and COPD hospital admissions remained relatively unchanged compared to the single-pollutant model results (3.9% [95% CI: −1.7, 9.8]).

In a study conducted in Hong Kong from 2000−2004, Ko et al. (2007a) also examined the lag structure of associations between short-term air pollution exposures and COPD hospital admissions. In analyses of both single-day lags and multiday averages, Ko et al. (2007a) observed the largest magnitude of an association at lags ranging from 0−3 to 0−5 days (10.1% [95% CI: 8.5, 12.2] for a 20-ppb increase in 24-h avg NO_2 concentrations at both 0−3 and 0−5 day lags). These associations are larger in magnitude than those reported by Qiu et al. (2013a) at lag 0−3 (4.7% [95% CI: 3.3, 6.2] for a 20 ppb increase in 24-h avg NO_2 concentrations) for a study also conducted in Hong Kong, but for a longer duration (1998−2007). Although Ko et al. (2007a) reported associations larger in magnitude for multiday averages, the authors also observed a positive association across single-day lags, with lag 0 having one of the stronger associations (3.4% [95% CI: 1.9, 5.0]), which is of similar magnitude to the lag 0 effect observed in Faustini et al. (2013). Ko et al. (2007a) only examined the potential confounding effects of copollutants through the use of three- and four-pollutant models, which are difficult to interpret. In comparisons of the single-pollutant results for NO_2 and the other pollutants examined (O_3, $PM_{2.5}$, and PM_{10}), similar patterns of associations were observed across pollutants. Additionally, Ko et al. (2007a) examined whether there was evidence of seasonal differences in NO_2-COPD hospital admission associations. When using the warm season as the referent, the authors reported evidence of larger associations in the

cold season (i.e., December to March). These results are consistent with the results of Ko et al. (2007b) for asthma (Section 5.2.2.4) and support potential differences in seasonal associations by geographic location.

In addition to examining the association between short-term air pollution exposures and COPD hospital admissions, Qiu et al. (2013a) also examined whether air pollution associations with COPD hospital admissions were modified by the interaction between season and humidity. In models stratifying by both season (warm: May−October; cold: November−April) and humidity (high or humid: ≥80%; low or dry: <80%) the authors found larger NO_2 associations in the cool season and high humidity days (5.6 and 6.3%, respectively) compared to the warm season and low humidity days (3.8 and 4.6%, respectively) for a 20-ppb increase in 24-h avg NO_2 concentrations at lag 0−3 days. When examining the joint effect of season and humidity, Qiu et al. (2013a) found that the magnitude of the association was larger when season and humidity were considered together. Specifically, the largest associations were observed for the combination of warm season and humid days 7.3% [95% CI: 3.7, 11.1]; lag 0−3) and cool season and dry days (9.3% [95% CI: 6.2, 12.5]; lag 0−3). When examining a copollutant model only with PM_{10}, across all combinations of models that examined the effect of season and humidity on NO_2-COPD hospital admissions, the associations were attenuated and in some cases null, specifically for the combination of warm season and dry days and cool season and humid days. These results further highlight the different seasonal patterns in NO_2 associations that have been reported across different geographic areas as well as the potential influence of different weather conditions on NO_2-related health effects.

Emergency Department Visits

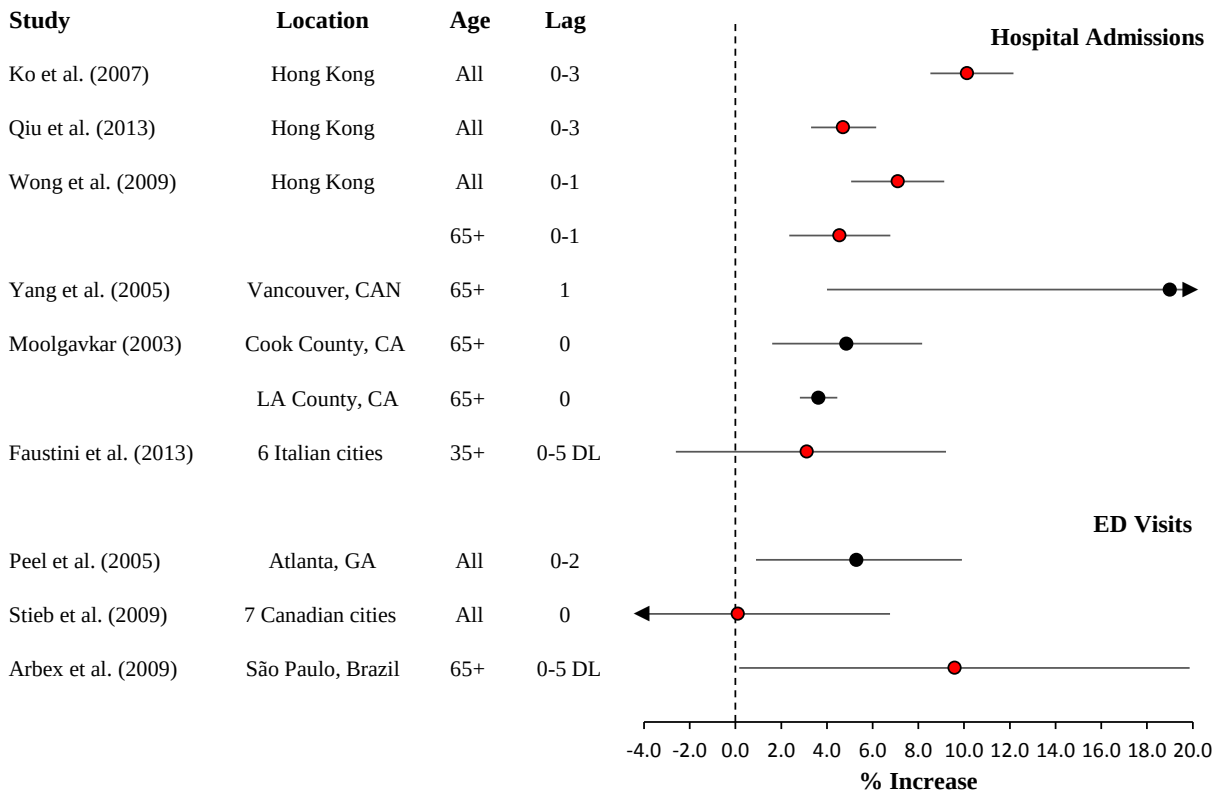
As in the 2008 ISA for Oxides of Nitrogen, relatively few studies have examined the relationship between short-term NO_2 exposures and ED visits, compared to hospital admissions. In the seven Canadian cities discussed previously, consistent with the asthma ED visits results, Stieb et al. (2009) did not find evidence of associations between 24-h avg NO_2 and COPD ED visits at individual lags ranging from 0 (0.1% [95% CI: −6.1, 6.8] for a 20-ppb increase in 24-h ave NO_2) to 2 (−5.2% [95% CI: −12.4, 2.7]) days. Additionally, there was no evidence of consistent associations between any pollutant and COPD ED visits at sub-daily time scales (i.e., 3-h avg of ED visits vs. 3-h avg pollutant concentrations).

Arbex et al. (2009) also examined the association between COPD and several ambient air pollutants, including NO_2, in a single-city study conducted in São Paulo, Brazil, for individuals over age 40 years. Associations between NO_2 exposure and COPD ED visits were examined in both single-day lags (0 to 6 days) and a polynomial distributed lag

model (0−6 days). However, for NO_2, only those results that were statistically significant were presented, that is, for individuals 65 years of age and older for lag 5 (4.3% [95% CI: 0.5, 8.3] for a 20-ppb increase in 24-h avg NO_2 concentrations) and a distributed lag of 0−5 days (9.6% [95% CI: 0.2, 19.9]). The authors did not analyze copollutant models but reported moderate correlations between NO_2 and PM_{10} (r = 0.60), SO_2 (r = 0.63), and CO (r = 0.56).

Summary of Chronic Obstructive Pulmonary Disease Hospital Admissions and Emergency Department Visits

In combination with those studies evaluated in the 2008 ISA for Oxides of Nitrogen, recent studies add to the growing body of literature that has examined the association between short-term NO_2 exposures and COPD hospital admissions and ED visits. Overall, these studies have reported consistent positive associations with evidence of NO_2-COPD hospital admissions and ED visits occurring immediately (lag 0) as well as a few days after exposure (average of lags up to 5 days) (Figure 5-9). However, caution should be used in inferring the independent effects of NO_2 exposure due to the relative sparseness of copollutant model analyses as well as the high correlation often observed between NO_2 and other traffic-related pollutants (e.g., CO, $PM_{2.5}$). Additionally, studies that have focused on COPD hospital admissions and ED visits have not thoroughly examined potential seasonal differences in associations; however, initial evidence suggests that the combination of season and weather conditions, such as humidity, may have a larger effect on NO_2-COPD hospital admission associations than either individually. Additionally, these studies have provided limited information on individual- or population-level factors that could modify the NO_2-hospital-admission or ED visit relationship, or the shape of the C-R relationship.



Note: Black circles = U.S. and Canadian studies evaluated in the 2008 Integrated Science Assessment for Oxides of Nitrogen; Red = recent studies. Effect estimates are standardized to a 20-ppb increase in 24-h avg nitrogen dioxide and 30-ppb increase in 1-h max nitrogen dioxide.

Figure 5-9 Percentage increase in chronic obstructive pulmonary disease hospital admissions and emergency department (ED) visits in relation to nitrogen dioxide concentrations from U.S. and Canadian studies evaluated in the 2008 Integrated Science Assessment for Oxides of Nitrogen and recent studies.

Table 5-25 Corresponding risk estimate for studies presented in Figure 5-9.

Study	Location	Age	Avg Time	Lag	% Increase (95% CI)
Hospital Admissions					
Ko et al. (2007a)	Hong Kong	All	24-h avg	0−3	10.1 (8.5, 12.2)
†Qiu et al. (2013a)[a]	Hong Kong	All	24-h avg	0−3	2.2 (0.8, 4.4)
†Wong et al. (2009)[a]	Hong Kong	All	24-h avg	0−1	7.1 (5.1, 9.1)
		65+	24-h avg	0−1	4.6 (2.4, 6.8)
†Yang et al. (2005)	Vancouver, Canada	65+	24-h avg	1	19.0 (4.0, 37.0)
†Moolgavkar (2003)	Cook County, IL	65+	24-h avg	0	4.9 (1.6, 8.2)
	LA County, CA	65+	24-h avg	0	3.6 (2.8, 4.5)
†Faustini et al. (2013)	6 Italian cities	35+	24-h avg	0−5 DL	3.1 (−2.6, 9.2)
Emergency Department Visits					
Peel et al. (2005)	Atlanta, GA	All	1-h max	0−2	5.3 (0.9, 9.9)
†Stieb et al. (2009)	7 Canadian cities	All	24-h avg	0	0.1 (−6.1, 6.8)
†Arbex et al. (2009)	São Paulo, Brazil	65+	24-h avg	0−5 DL	9.6 (0.2, 19.9)

CI = confidence interval, DL = distributed lag.
†Studies published since the 2008 ISA for Oxides of Nitrogen.

5.2.4.3 Subclinical Effects Underlying Chronic Obstructive Pulmonary Disease—Pulmonary Inflammation

Exacerbation of COPD can be precipitated by increases in airway responsiveness and pulmonary inflammation. While there is some supporting evidence for an effect of NO_2 exposure in initiation of inflammation (Sections 4.3.2.3 and 4.3.2.1), the effects of NO_2 on airway responsiveness and inflammation are not well characterized in adults with COPD. Thus, little information on mode of action is available to support the associations observed between ambient NO_2 concentrations and hospital admissions and ED visits for COPD (Section 5.2.4.1). In a recent epidemiologic study, neither ambient NO_2 nor NO was associated with indicators of inflammation such as increases in the numbers of blood

eosinophils or lymphocytes in adults with COPD consistently among lag days 0, 1, and 2 ambient concentrations (Table 5-22) (Bruske et al., 2010). NO_2 at lag day 0 was associated with an increase in neutrophils only with adjustment for UFP. However, the 95% CI was wide, indicating an imprecise association, and the NO_2-UFP correlation was high (Spearman r = 0.68). UFP and OC were associated with decreases in neutrophils, of which the relation to COPD exacerbation is not clear. Neither in adults with COPD nor healthy adults did Vagaggini et al. (1996) find changes in inflammatory cell counts in sputum following exposure to 300 ppb NO_2 for 1 hour.

5.2.4.4 Summary of Exacerbation of Chronic Obstructive Pulmonary Disease

Evidence for the effects of short-term NO_2 exposure on COPD exacerbation is inconsistent among the various outcomes examined and across scientific disciplines. In epidemiologic studies, short-term increases in ambient NO_2 concentration are consistently associated with increases in hospital admissions and ED visits for COPD (Section 5.2.4.2) but not with increases in respiratory symptoms or decreases in lung function among adults with COPD (Section 5.2.4.1). In limited examination, an epidemiologic and controlled human exposure study do not indicate NO_2-related increases in inflammation in adults with COPD (Section 5.2.4.3). Thus, a mode of action for NO_2 effects on COPD exacerbation is not clear. Epidemiologic studies assigned NO_2 exposure as central site ambient concentrations (average of multiple monitors, nearest site), and many found associations with the traffic-related pollutants CO, BS, UFP, and $PM_{2.5}$. In the one study that examined potential confounding by traffic-related copollutants, the 95% CIs for associations for NO_2 and BS with respiratory symptoms increased, and an independent or confounding effect was not discerned for either pollutant (Peacock et al., 2011). Also, controlled human exposure studies do not consistently find NO_2-induced (200−4,000 ppb for 30 minutes to 6 hours) increases in respiratory symptoms or decreases in lung function in adults with COPD (Section 5.2.4.1). Because of the inconsistent evidence across disciplines for effects on clinical indications of COPD exacerbation and the lack of evidence for effects on underlying mechanisms, there is uncertainty regarding a relationship between short-term NO_2 exposure and COPD exacerbation.

5.2.5 Respiratory Infection

The respiratory tract is protected from exogenous pathogens and particles through various lung host defense mechanisms that include mucociliary clearance, particle transport and

detoxification by alveolar macrophages, and innate and adaptive immunity. The 2008 ISA for Oxides of Nitrogen reported clear evidence from animal toxicological studies for NO_2-induced susceptibility to bacterial or viral infection with some coherence with results from controlled human exposure and epidemiologic studies (U.S. EPA, 2008a). There is some mechanistic support for these observations, with NO_2-induced impairments in alveolar macrophage (AM) function found in some but not all animal toxicological studies. Effects on mucociliary clearance and activity were not in a consistent direction, but the exact mechanism by which mucociliary clearance may impair host defense is not well characterized. Recent contributions to the evidence base are limited to epidemiologic studies. These studies show associations between increases in ambient NO_2 concentrations and increases in hospital admissions and ED visits for respiratory infections but do not consistently show associations with respiratory infections reported or diagnosed in children.

5.2.5.1 Susceptibility to Bacterial or Viral Infection in Experimental Studies

Toxicological Studies

A large body of evidence, provided by studies reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a), demonstrates increased susceptibility of rodents to viral or bacterial infection following short-term NO_2 exposure. These studies used a variety of experimental approaches but in most cases included an infectivity model of exposing animals to NO_2 or filtered air and then combining treatment groups for a brief exposure to an aerosol of a viable agent, such as *Streptococcus zooepidemicus*, *Streptococcus pyogenesi*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. The majority of studies measured mortality over a specified number of days following the challenge, but several studies also examined endpoints such as bacterial counts and clearance (Table 5-26). While there are differences in sensitivity across species to various infectious organisms, host defense mechanisms are shared, and the infectivity model is well accepted as an indicator of impaired or weakened pulmonary defense.

Table 5-26 Animal toxicological studies of susceptibility to infection.

Study	Species (Strain); Age; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Amoruso et al. (1981)	Rat (Sprague-Dawley); F, n = 4/group	1,300, 1,900, and 3,000 ppb NO_2 for 3 h	Analysis of BAL fluid and superoxide production by AMs (PMA stimulation).
Davis et al. (1991)	Mice (C57BL/6N); 8-10 weeks; n = 6/group	5,000 ppb NO_2 for 4 h; *Mycoplasma pulmonis* challenge immediately after exposure.	Bacterial clearance, bactericidal activity.
Dowell et al. (1971)	Dog (beagle); n = 11	3,000 ppb NO_2 for 1 h	Histopathological evaluation and lung surfactant properties.
Ehrlich et al. (1977)	Mice (CF-1); 5-8 weeks; F; n = 5−88/group	0, 1,500, 2,000, 3,500, and 5,000 ppb NO_2 for 3 h; *Streptococcus pyogenes* challenge immediately after exposure.	Mortality
Ehrlich (1980)	(1,2) Mice; 6-8 weeks; n ≥ 88/group (3) Mice, hamsters, and squirrel monkeys	(1) 500 ppb NO_2 continuously for 1 week-1 yr (2) 1,500 ppb NO_2 continuously for 2 h-3 mo (3) 1,500-50,000 ppb NO_2 for 2 h (1-3) *Klebsiella pneumoniae* challenge immediately after exposure.	(1-3) Mortality
Gardner et al. (1979)	Mice (Swiss albino); F; n = 20/group	(1) 500 ppb NO_2 continuously for 7 days-1 yr (2) 1,500 ppb NO_2 continuously for 2 h-21 days (3) 1,500 ppb NO_2 7 h/day for 7 h-11 days (3) 3,500 ppb NO_2 continuously for 30 min-16 days (4) 3,500 ppb NO_2 7 h/day for 7 h-13 days (1-4) *Streptococcus pyogenes* challenge immediately after exposure.	Mortality
Goldstein et al. (1973)	Mice (Swiss albino); M; n = 30/group	(1) *Staphylococcus aureus* challenge immediately before exposure; 0, 1,900, and 3,800 ppb NO_2 for 4 h (2) 0, 1,000, and 2,300 ppb NO_2 for 17 h; *Staphylococcus aureus* challenge immediately after exposure.	(1) Bacterial counts and bactericidal activity 5 h after challenge (i.e., 1 h after exposure). (2) Bacterial counts and bactericidal activity 0 h and 4 h after challenge.
Goldstein et al. (1974)	Mice (Swiss albino); M; n = 30/group	(1) 1,740 ppb NO_2 + 110 ppb O_3 (2) 1,490 ppb NO_2 + 200 ppb O_3 (3) 2,300 ppb NO_2 + 200 ppb O_3 (4) 1,780 ppb NO_2 + 270 ppb O_3 (5) 4,180 ppb NO_2 + 210 ppb O_3(1-5) 17 h; *Staphylococcus aureus* challenge immediately after exposure.	Bacterial counts, bactericidal activity, and bacterial clearance 0 h and 4 h after challenge.

Table 5-26 (Continued): Animal toxicological studies of susceptibility to infection.

Study	Species (Strain); Age; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Goldstein et al. (1977)	Rat (Sprague-Dawley); F	500, 1,000, and 2,400 ppb NO_2 for 1 and 2 h	Agglutination of AMs
Graham et al. (1987)	Mice (CD-1); 4–6 weeks; n = 5–12/group	(1) 4,500 ppb NO_2 for 1, 3.5, and 7 h (2) 1,500 ppb NO_2 continuously with a daily spike of 4,500 ppb for 1, 3.5, and 7 h; (1–2) *Streptococcus zooepidemicus* challenge immediately and 18 h after exposure.	Mortality
Hooftman et al. (1988)	Rats (Wistar); M; n = 10/group	3,000 ppb NO_2 for 6 h/day, 5 days/week up to 21 days	Histopathological evaluation, analysis of BAL fluid, and AM function and morphology.
Illing et al. (1980)	Mice (CD-1); 5–6 weeks; F; n = 16/group	1,000 ppb, 3,000 ppb NO_2, and air for 3 h; With or without continuous exercise; *Streptococcus pyogenes* challenge immediately after exposure.	Mortality after *Streptococcus pyogenes* challenge.
Mochitate et al. (1986)	Rats (Wistar); M; 19–23 weeks; n = 6/group	4,000 ppb NO_2 continuously up to 10 days	BAL fluid cell counts and MA function and morphology.
Parker et al. (1989)	Mice (C57BL/6N and C3H/HeN); 6–10 weeks	0 and 5,000 ppb NO_2 for 4 h; *Mycoplasma pulmonis* challenge immediately after exposure.	Histopathological evaluation, bacterial infection and clearance 4 h up to 7 days post-challenge, BAL fluid cell counts.
Purvis and Ehrlich (1963)	Mice (Swiss Webster & albino); n > 25/group	1,500, 2,500, 3,500, and 5,000 ppb NO_2 for 2 h; *Klebsiella pneumoniae* challenge 0–27 h post-exposure.	Mortality
Robison et al. (1990)	Rats (Sprague-Dawley)	100, 500, and 1,000 ppb NO_2 for 1 h; AMs exposed ex vivo	Viability, LTB4 production, neutrophil chemotaxis, and superoxide production.
Robison and Forman (1993)	Rats (Sprague-Dawley)	100, 2,000, and 5,000 ppb NO_2 for 1–4 h; AMs exposed ex vivo	Arachidonate metabolite production induced by treatment with a calcium ionophore.
Rombout et al. (1986)	Rats (Wistar); M, 6 weeks; n = 3−6/group	500, 1,390, and 2,800 ppb NO_2 for 1, 2, 4, 8, 16, and 28 days	Histopathological evaluation
Rose et al. (1988) Rose et al. (1989)	Mice (CD-1); 4–6 weeks; n > 4/group	(1) 1,000, 2,500, and 5,000 ppb NO_2 for 6 h/day for 2 days; intra-tracheal inoculation with murine *Cytomegalovirus*; 4 additional days (6 h/day) of exposure. (2) re-inoculation 30 days (air) post-primary inoculation.	Infection 5 and 10 days post-inoculation, histopathological evaluation, and analysis of BAL fluid (LDH, albumin, macrophages).

Table 5-26 (Continued): Animal toxicological studies of susceptibility to infection.

Study	Species (Strain); Age; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Schlesinger (1987b)	Rabbits (New Zealand white); M, n = 5/group	300 or 1,000 ppb NO_2 for 2 h/day for 2, 6, and 13 days	Viability and AM activity (mobility, attachment, and phagocytosis).
Sherwood et al. (1981)	Mice (Swiss albino); M; n = 8−24/group	1,000 ppb NO_2 for 24 and 48 h; *Streptococcus* (Group C) challenge immediately after exposure.	Bacterial counts 0-48-h post-challenge, bacterial clearance, histopathological evaluation, mortality.
Suzuki et al. (1986)	Rats (Fischer 344); M, 7 weeks; n = 8/group	4,000 NO_2 ppb for 1, 3, 5, 7, and 10 days	AM activity (phagocytosis and superoxide production), SOD and G-6-PD activity.

F = female, LDH = lactate dehydrogenase, M = male, NO_2 = nitrogen dioxide, O_3 = ozone, SOD = superoxide dismutase.

In a series of studies, (Goldstein et al. (1974); 1973)) examined bactericidal activity and clearance in mice challenged with radiolabeled *Staphylococcus aureus* either immediately before or after NO_2 exposure. The number of bacteria deposited in the lung was not different in NO_2-exposed animals compared to controls; however, concentration-dependent decreases in bactericidal activity were observed in animals exposed to NO_2 for 4 hours after challenge as well as those exposed to NO_2 for 17 hours before challenge. While the 4-hour exposure did not yield statistically significant differences compared to air controls at NO_2 concentrations less than 7,000 ppb, the 17-hour exposure preceding challenge was statistically significant for concentrations greater than 2,300 ppb. Parker et al. (1989) also used radiolabeled bacteria to determine the effects of NO_2 on susceptibility to infection. This study demonstrated that a 4-hour exposure to 5,000 ppb NO_2 was sufficient to reduce bactericidal activity and increase the number of bacteria in the lungs of C3H/HeN and C57BL/6N mice 3 days after challenge compared to control mice, but did not result in an increase in incidence or severity of lung lesions. These results were corroborated in a similar study by Davis et al. (1991).

It is also important to consider differences in response to NO_2 that are specific to the infectious organism as Jakab (1988) has demonstrated. A 4-hour exposure to 5,000 ppb NO_2 resulted in a decrease in bactericidal activity after challenge with *Staphylococcus aureus*; however, bactericidal activity against *Proteus mirabilis* and *Pasteurella pneumotropica* was not impaired with exposure to NO_2 at concentrations less than 20,000 ppb. Additionally, Sherwood et al. (1981) observed an increase in the propensity of virulent group C Streptococci, but not *Staphylococcus aureus* following exposure to 1,000 ppb NO_2 for 24 or 48 hours. In this study, Streptococci infection did not increase the total mortality compared to controls, but NO_2-exposed mice died significantly earlier.

Several other studies reported that NO_2 exposure increased mortality from bacterial infection. Illing et al. (1980) exposed mice to 3,000 ppb NO_2 with continuous exercise for 3 hours while Ehrlich et al. (1977) exposed mice to 2,000 ppb NO_2 for 3 hours; both studies subsequently exposed mice to an aerosol of *Streptococcus pyogenes* and reported increased mortality rates compared to control animals exposed to clean air. Increases in mortality from *Streptococcus pyogenes* infection following NO_2 exposure were also reported by Ehrlich et al. (1979). In this study, the relationship between concentration and time was examined, and these factors yielded very different results as the concentration was more important than time in determining mortality. Results were consistent with other studies, though mortality increased post-challenge following a 7-day exposure to 3,500 ppb NO_2.

Ehrlich (1980) conducted similar studies to investigate the effects of NO_2 on *Klebsiella pneumoniae*-induced mortality. Challenge following exposure to 1,500 ppb NO_2 for more than 8 hours resulted in increased mortality; however, a longer duration of exposure (3 months) was required to increase infection mortality following 500 ppb NO_2 exposure. This study also demonstrated species differences as increases in *K. pneumoniae* infection mortality in mice were observed after a 2-hour exposure to 3,500 ppb NO_2 while hamsters and squirrel monkeys did not experience increases in mortality at NO_2 concentrations less than 35,000 ppb and 50,000 ppb, respectively (Ehrlich, 1980). Conversely, Purvis and Ehrlich (1963) did not observe increases in *K. pneumonia* infection mortality in mice following a 2-hour exposure to NO_2 at concentrations less than 5,000 ppb.

One study examined effects of NO_2 peak exposures superimposed on a lower continuous background level of NO_2 on susceptibility to *Streptococcus zooepidemicus* infection (Graham et al., 1987). Mice were exposed to 4,500 ppb NO_2 for 1, 3.5, and 7 hours or exposed to these spikes with a continuous background exposure to 1,500 ppb NO_2, followed either immediately or 18 hours later with a *Streptococcus zooepidemicus* challenge. Compared to control animals, the 4,500 ppb spikes alone or the spikes superimposed on a background exposure did not result in differences in mortality from infection; however, combined mortality rates (following the 1-hour exposure to 4,500 ppb and the 1-hour exposure to 4,500 ppb with 1,500 ppb background) were significantly increased from immediate challenge after 4,500 ppb NO_2 and were proportional to duration of the 4,500 ppb exposure. In animals challenged 18 hours after NO_2 exposure, increases in mortality were only statistically significant with 3.5- and 7-hour exposures to 4,500 ppb NO_2.

Controlled Human Exposure Studies

Compared with animal toxicological studies, controlled human exposure studies provided less consistent evidence for NO_2-induced infectivity (Table 5-27). Although Pathmanathan et al. (2003) found increased expression of inter-cellular adhesion molecule 1 (ICAM-1), an extracellular receptor for viruses, in airway biopsies following exposure to 2,000 ppb NO_2 for 4 hours per day for 4 days, Frampton et al. (2002) did not find evidence of increased susceptibility to ex vivo viral challenge in bronchial epithelial cells collected from subjects exposed to 600 ppb or 1,500 ppb NO_2 for 3 hours; however, there was an increase in virus-induced cytotoxicity as measured by lactate dehydrogenase (LDH) release. Consistent with Frampton et al. (2002), Goings et al. (1989) reported no increase in infectivity of administered live, attenuated influenza virus in subjects exposed to 1,000, 2,000, or 3,000 ppb NO_2 for 2 hours/day for 3 consecutive days. This study, however, lacked a sham control. Another study (Frampton et al., 1989) reported a trend of decreased inactivation of influenza virus in AMs collected from subjects after a 3-hour exposure to 600 ppb NO_2, although results were not statistically significant.

Table 5-27 Controlled human exposure studies of susceptibility to infection.

Study	n, Sex; Age (mean ± SD)	Exposure Details (Concentration; Duration)	Endpoints Examined
Frampton et al. (1989)	(1) n = 7 M, 2 F; 30 yr (range: 24–37) (2) n = 11 M, 4 F; 25 yr (range: 19–37)	(1) 600 ppb for 3 h, (2) 1,500 ppb for 3 h; (1,2) Exercise 10 min on/20 min off at $\dot{V}_E$ = ~4 times resting	BAL fluid cell viability and differential counts 3.5 h post-exposure, inactivation of influenza virus by BAL cells, IL-1 activity in BAL cells.
Frampton et al. (2002)	(1,2) n = 12 M, 9 F; F = 27.1 ± 4.1 yr M = 26.9 ± 4.5 yr	(1) 600 ppb for 3 h, (2) 1,500 ppb for 3 h; (1,2) Exercise 10 min on/20 min off at $\dot{V}_E$ = 40 L/min	Bronchial and alveolar lavage fluid cell viability and differential counts 3.5 h post-exposure, influenza and RSV challenge in BAL cells, peripheral blood characterization.
Goings et al. (1989)	(1) n = 44 (2) n = 43 (3) n = 65; range: 18–35 yr	(1) 2,000 ppb for 2 h (2) 3,000 ppb for 2 h (3) 1,000 or 2,000 ppb for 2 h	Nasal wash virus isolation and count 4 days after virus administration. Serum and nasal wash antibody response 4 weeks after virus administration.
Pathmanathan et al. (2003)	n = 8 M, 4 F 26 yr (range: 21−32 yr)	2,000 ppb for 4 h/day for 4 days; Exercise 15 min on/15 min off at 75 watts	Biomarkers in bronchial epithelium-exotoxin, GM-CSF, Gro-α, I-CAM 1, IL-5, IL-6, IL-8, IL-10, IL-13, total and active NF-κβ, and TNF-α (fiberoptic bronchoscopy after end of last exposure).

F = female, IL = interleukin, M = male, RSV = respiratory syncytial virus, SD = standard deviation.

5.2.5.2 Respiratory Infections Reported or Diagnosed in Children

In contrast with findings in rodent models, epidemiologic evidence does not clearly indicate a relationship between short-term NO_2 exposure and respiratory infection in children (ages 0−15 years) as reported by self or parents or more objectively ascertained as laboratory-confirmed or physician-diagnosed cases. Some studies found associations (Esposito et al., 2014; Lu et al., 2014; Stern et al., 2013; Ghosh et al., 2012; Just et al., 2002); others did not or found inconsistent associations among the outcomes examined (Altuğ et al., 2014; Stern et al., 2013; Xu et al., 2013) (Table 5-28). Xu et al. (2013) did not provide strong evidence for an association of ambient NO_2 with laboratory-confirmed cases of influenza (RR: 1.01 [95% CI: 0.97, 1.04] for an unreported increment in NO_2); however, study limitations preclude strong inferences from the results. There were a mean of only two influenza cases per day, and potential collinearity in a multipollutant model with PM_{10} and O_3 (Spearman *r* for correlation with NO_2 = 0.62 and −0.42, respectively) limits inference about NO_2 effects.

Results indicating associations between ambient NO_2 or NO_X and respiratory infections also have weak implications (Esposito et al., 2014; Lu et al., 2014; Stern et al., 2013; Ghosh et al., 2012; Just et al., 2002). All of these studies assigned exposure from central site concentrations (one city site or average of multiple sites) (Table 5-28). None reported information on the spatial distribution of subjects around the monitoring site(s) or the within-city variability in NO_2 or NO_X concentrations to ascertain potential exposure measurement error and its impact on effect estimates. A few studies examined more spatially resolved exposure metrics but also have uncertain implications. Ghosh et al. (2012) reported similar results in analyses restricted to homes for which central site NO_X better represented exposure but did not report how these homes were selected. Further, the adequacy of NO_X to serve as an indicator of NO_2 may vary among subjects because of varying NO_2 to NO_X ratios across locations (Section 2.5.3). NO_2 at the central site nearest to schools was associated with pneumonia prevalence (Lu et al., 2014). However, pneumonia was ascertained as "ever having a diagnosis" and may not be temporally matched to exposure assessed for a three-year period. The only study with spatially-aligned measures of exposure did not observe associations between school NO_2 and colds (Altuğ et al., 2014). The importance of the microenvironmental measures is underscored by the variability in traffic volume and road length reported within the study area.

Also limiting strong inferences from results of these studies is uncertainty regarding confounding by other traffic-related pollutants. In addition to NO_2, respiratory infections were associated with BS and $PM_{2.5}$. Other copollutants such as PM_{10}, SO_2, and O_3 also

were associated with respiratory infection (Table 5-28). Studies did not examine other traffic-related PM components, CO, copollutant models, or other methods to assess the independent or mixture effects of NO_2. Where reported, NO_2 was moderately to highly correlated with copollutants (r = 0.92 for BS; 0.6−0.8 for unspecified copollutants) (Ghosh et al., 2012; Just et al., 2002).

Table 5-28 Epidemiologic studies of respiratory infections reported or diagnosed in children.

Study Population Examined and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
†Altuğ et al. (2014) Eskisehir, Turkey, Feb–Mar, 2007 n = 605, ages 9–13 yr Cross-sectional. Self-reported respiratory infections. Recruitment from schools of participants of a larger study. Participation rate not reported. Logistic regression adjusted for sex, age, asthma, parental smoking, coal or wood stove use, parental education, height, weight, daily average temperature.	NO_2-outdoor school 24-h avg, lag 0-6 day avg 1 site at each of 16 schools Means & max (ppb) Suburban: 9.4, 13 Urban: 13, 18 Urban-traffic: 21, 28	Common cold last 7 days: OR: 1.86 (0.41, 8.42) Commom cold at the moment: OR: 4.59 (0.79, 26)	No copollutant model. O_3 associated with colds. Strong inverse correlation with NO_2. Pearson r = −0.80. NO_2 and $PM_{2.5}$ reported to be highly correlated.
†Esposito et al. (2014) Milan, Italy, Jan–Dec 2012 n = 718, ages 2–18 yr, 329 with wheeze or asthma, 389 healthy children Repeated measures. Daily symptom diaries for 12 mo. Diaries checked weekly, clinic visits conducted every 2 mo. Recruited from respiratory disease section (wheeze/asthma) and outpatient surgery (healthy) sections of pediatric clinic. 89% follow-up participation. Followed cohort similar to cohort at baseline. GEE adjusted for age, sex, siblings, parental education, smokers in home, season, day of week, temperature, humidity.	NO_2-central site 1-h max, Lag 0-2 day avg 8 city sites, 7 surrounding area Weighted avg at municipality level Tertiles (T) in ppb 1: <47.3[b] 2: 47.3–60.1[b] 3: >60.1[b]	RR for pneumonia with T1 as reference Children with asthma: T2: 1.20 (0.75, 1.90) T3: 1.56 (1.01, 2.42) Healthy children: T2: 1.45 (0.80, 0.63) T3: 1.02 (0.93, 1.12)	No copollutant model. PM_{10} associated with pneumonia. Correlations NR.
Just et al. (2002) Paris, France, Apr–Jun 1996 n = 82, ages 7-15 yr, children with asthma, 90% atopy Repeated measures. Daily symptom diaries for 3 mo, collected weekly. Recruitment from hospital outpatients. 82% follow-up participation. GEE adjusted for time trend, day of week, pollen, temperature, humidity.	NO_2-central site 24-h avg, lag 0 day Average of 11 sites Mean: 28.6 ppb[b] Max: 59.0 ppb[b]	Respiratory infection: OR: 7.19 (2.53, 20.4)	No copollutant model. BS associated with cough and infection. High correlation with NO_2. Pearson r = 0.92.
†Stern et al. (2013) Bern, Basel, Switzerland, Apr 1999-Feb 2011 n = 366, ages 0-1 yr	NO_2-central site 24-h avg, lag 5-11 day avg 2 sites Rural mean: 8.1 ppb[b]	Incidence respiratory tract infection: RR: 1.20 (0.82, 1.75) Days with respiratory tract infection:	No copollutant model. PM_{10} lag 7 days associated with respiratory infection. Correlation NR.

Table 5-28 (Continued): Epidemiologic studies of respiratory infections reported or diagnosed in children.

Study Population Examined and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Repeated measures. Symptoms reported weekly by telephone for 1 yr. Recruitment from birth cohort. 95% follow-up participation. GAM adjusted for study week, sex, siblings, nursery care, prenatal maternal smoking, postnatal maternal smoking, birth weight, maternal atopy, parental education.	Urban mean: 25.6 ppb[b] Upper percentiles NR	NO_2 < 26 ppb: reference category NO_2 > 26 ppb: 18% (0, 39)	
†Xu et al. (2013) Brisbane, Australia, 2001–2008, winters only n = 2,922 influenza cases, ages 0–14 yr Time-series. Laboratory-confirmed cases of influenza referred by public or private health sector. Only mean 2/day. No information available on subjects' residential location. Poisson regression adjusted for lag 0–9 day avg temperature, lag day 0–9 avg PM_{10}, lag 0–9 day avg O_3, PM_{10}*temperature interaction.	NO_2-central site 24-h avg, lag 0–9 day avg # sites NR Mean: 5.9 ppb[b] 75th: 7.3 ppb[b] Max: 13.3 ppb[b]	Daily influenza counts: RR: 1.01 (0.97, 1.04) increment of NO_2 NR. Results are presented only for a multipollutant model that also includes PM_{10} and O_3.	Only multipollutant model analyzed.
†Ghosh et al. (2012) Teplice and Prachatice, Czech Republic, May 1994–June 2003 n = 1,113 children, ages 0–4.5 yr Repeated measures. Physician-diagnosed infections between ages 0–4.5 yr ascertained from medical records. Recruitment from birth cohort. Participation rate not reported. GEE with exchangeable correlation and adjusted for city, year of birth, day of week, fuel used for heating and cooking, season, 7-day avg temperature. Restricted analyses to children for whom central site may better represent exposure (method not reported).	NO_X-central site 24-h avg, lag 0–2 day avg 2 sites Mean, 75th (µg/m^3) Teplice: 59.2, 73.3 Prachatice: 20.3, 24.4	Acute bronchitis: Birth to age 2 yr: RR: 1.09 (1.01, 1.16) per 34 µg/m^3 NO_X Age 2 yr–4.5 yr: RR: 1.05 (0.94, 1.14) per 32 µg/m^3 NO_X	No copollutant model. Association with $PM_{2.5}$ reported in separate paper. Moderate to high correlations reported with unspecified copollutants. r = 0.6–0.8
†Lu et al. (2014)[b] Changsha, China, Sep 2011–Jan 2012 n = 2706, ages 3–6 yr Cross-sectional. Recruitment from schools. 59% participation. Potential temporal mismatch of exposure (2008–2011) and ever having pneumonia diagnosis. Two-level model. Pneumonia first adjusted for parental atopy, antibiotic use, new furniture in home, coal, wood or gas used in home, painted walls/air conditioning in home, pets in home, visible mold/dampness in home. Adjusted pneumonia prevalence regressed with NO_2. Confounding by meteorological factors not examined.	NO_2-central site 24-h avg Nearest to school, distance NR Concentrations NR 2008–2011: 7 days >63.8 ppb[b] standard before 2012 89 days >42.6 ppb[b] standard 2012	OR for NO_2 > 63.8 ppb 1 day/yr 1.04 (1.02, 1.05)	No copollutant model PM_{10} and SO_2 associated with pneumonia. Correlations NR.

Note: More informative studies in terms of the exposure assessment method and potential confounding considered are presented first.

GEE = generalized estimating equations, GAM = generalized additive model, NR = not reported, RR = relative risk, CI = confidence interval, NO_2 = nitrogen dioxide, NO_X = sum of NO and NO_2, O_3 = ozone, OR = odds ratio, PM = particulate matter, SO_2 = sulfur dioxide.

[a]Effect estimates are standardized to a 20 ppb for 24-h avg NO_2. NO_X effect estimates are presented as reported in the study (Section 5.1.2.3).

[b]Concentrations converted from µg/m^3 to ppb using the conversion factor of 0.532 assuming standard temperature (25°C) and pressure (1 atm).

†Studies published since the 2008 ISA for Oxides of Nitrogen.

5.2.5.3 Hospital Admissions and Emergency Department Visits for Respiratory Infections

To date, relatively few studies have examined the association between short-term NO_2 exposures and hospital admissions and ED visits due to respiratory infections. The 2008 ISA for Oxides of Nitrogen identified studies that evaluated a number of respiratory infection outcomes, such as upper respiratory infections (URIs), pneumonia, bronchitis, allergic rhinitis, and lower respiratory disease. Across these outcomes, studies have generally not provided consistent evidence of an association between NO_2 and hospital admissions and ED visits due to respiratory infections (U.S. EPA, 2008a). Recent studies add to the body of literature evaluated in the 2008 ISA for Oxides of Nitrogen, but compared to other respiratory-related hospital admission and ED visit outcomes the total body of literature remains limited. The air quality characteristics of the city, or across all cities, and the exposure assignment approach used in each respiratory infection-related hospital admission and ED visit study evaluated in this section are presented in Table 5-29. As detailed in Section 5.2.2.4, other recent studies of respiratory infection-related hospital admissions and ED visits are not the focus of this evaluation, and the full list of these studies, as well as study details, can be found in Supplemental Table S5-3 (U.S. EPA, 2014h).

Table 5-29 Mean and upper percentile concentrations of nitrogen dioxide (NO_2) in studies of hospital admissions and emergency department visits for respiratory infection.

Study	Location Years	Type of Visit (ICD9/10)	Exposure Assignment	Metric	Mean Concentration ppb	Upper Percentile Concentrations ppb	Copollutant Examination
Hospital Admissions							
Burnett et al. (1999)	Toronto, Canada (1980−1994)	Respiratory infection (464, 466, 480−7, 494)	Average of NO_2 concentrations across 4 monitors.	24-h avg	25.2	NR	Correlations (*r*): $PM_{2.5}$: 0.55 $PM_{10-2.5}$: 0.38 PM_{10}: 0.57 CO: 0.64 SO_2: 0.54 O_3: −0.08 Copollutant models: none
Lin et al. (2005)	Toronto, Canada (1998−2001)	Respiratory infection (464, 466, 480−487)	Average of NO_2 concentrations across 7 monitors.	24-h avg	25.5	75th: 29.3	Correlations (*r*): $PM_{2.5}$: 0.48 $PM_{10-2.5}$: 0.40 PM_{10}: 0.54 CO: 0.20 SO_2: 0.61 O_3: 0.0 Copollutant models: none
Karr et al. (2006)	Southern Los Angeles County, CA (1995−2000)	Acute bronchiolitis (466.1)	34 NO_2 monitors, exposure assigned based on nearest monitor to residential ZIP code.	1-h max	59	75th: 69 90th: 90	Correlations (*r*): NR Copollutant models: none

Table 5-29 (Continued): Mean and upper percentile concentrations of nitrogen dioxide (NO_2) in studies of hospital admissions and emergency department visits for respiratory infection.

Study	Location Years	Type of Visit (ICD9/10)	Exposure Assignment	Metric	Mean Concentration ppb	Upper Percentile Concentrations ppb	Copollutant Examination
Zanobetti and Schwartz (2006)	Boston, MA (1995−1999)	Pneumonia (480−487)	Average of NO_2 concentrations across 5 monitors.	24-h avg	NR	50th: 23.2	Correlations (*r*): $PM_{2.5}$: 0.55 BC: 0.70 CO: 0.67 O_3: −0.14 PM non-traffic: 0.14 Copollutant models: none
†HEI (2012) †Mehta et al. (2013)	Ho Chi Minh City, Vietnam (2003−2005)	Acute lower respiratory infection (J13−16, 18, 21)	Average of NO_2 concentrations across 9 monitors.	24-h avg	11.7	Max: 29.2	Correlations (*r*): Dry season: PM_{10}: 0.78 O_3: 0.44 SO_2: 0.29 Rainy season: PM_{10}: 0.18 O_3: 0.17 SO_2: 0.01 Copollutant models: SO_2, PM_{10}, O_3
†Ségala et al. (2008)	Paris, France (1997−2001)	Bronchiolitis	Average of NO_2 concentrations from 21 monitors, representative of urban background.	24-h avg	27.0	Max: 90.4	Correlations (*r*): BS: 0.83 PM_{10}: 0.74 SO_2: 0.78 Copollutant models: none
†Faustini et al. (2013)	6 Italian cities (2001−2005)	LRTI (466, 480−487)	Average of NO_2 concentrations over all monitors within each city. Number of NO_2 monitors in each city ranged from 1−5.[a]	24-h avg	24.1−34.6	NR	Correlations (*r*), across cities: PM_{10}: 0.22−0.79 Copollutant models: PM_{10}

Table 5-29 (Continued): Mean and upper percentile concentrations of nitrogen dioxide (NO_2) in studies of hospital admissions and emergency department visits for respiratory infection.

Study	Location Years	Type of Visit (ICD9/10)	Exposure Assignment	Metric	Mean Concentration ppb	Upper Percentile Concentrations ppb	Copollutant Examination
ED Visits							
Peel et al. (2005)	Atlanta, GA (1993−2000)	Upper respiratory infection (460–6,477) Pneumonia (480−486)	Average of NO_2 concentrations from monitors for several monitoring networks.	1-h max	45.9	NR	Correlations (*r*): $PM_{2.5}$: 0.46 PM_{10}: 0.49 $PM_{10-2.5}$: 0.46 UFP: 0.26 $PM_{2.5}$ water-soluble metals: 0.32 $PM_{2.5}$ sulfate: 0.17 $PM_{2.5}$ acidity: 0.10 $PM_{2.5}$ OC: 0.63 $PM_{2.5}$ EC: 0.61 Oxygenated HCs: 0.30 O_3: 0.42 CO: 0.68 SO_2: 0.34 Copollutant models: none
†Stieb et al. (2009)	7 Canadian cities (1992−2003)	Respiratory infection (464, 466, 480−487)	Average NO_2 concentrations from all monitors in each city. Number of NO_2 monitors in each city ranged from 1−14.	24-h avg	9.3−22.7	75th: 12.3−27.6	Correlations (*r*) only reported by city and season. Copollutant models: none
†Ségala et al. (2008)	Paris, France (1997−2001)	Bronchiolitis	Average of NO_2 concentrations across 21 monitors, representative of urban background.	24-h avg	27.0	Max: 90.4	Correlations (*r*): BS: 0.83 PM_{10}: 0.74 SO_2: 0.78 Copollutant models: none

Table 5-29 (Continued): Mean and upper percentile concentrations of nitrogen dioxide (NO_2) in studies of hospital admissions and emergency department visits for respiratory infection.

Study	Location Years	Type of Visit (ICD9/10)	Exposure Assignment	Metric	Mean Concentration ppb	Upper Percentile Concentrations ppb	Copollutant Examination
†Zemek et al. (2010)	Edmonton, Canada (1992−2002)	Otitis media (382.9)	Average of NO_2 concentrations across 3 monitors.	24-h avg	21.9	75th: 27.6	Correlations (*r*): NR Copollutant models: none
Physician Visits							
†Sinclair et al. (2010)	Atlanta, GA (1998−2002)	Upper respiratory infection Lower respiratory infection	NO_2 concentrations collected as part of AIRES at SEARCH Jefferson Street site.	1-h max	1998−2000: 49.8 2000−2002: 35.5 1998−2002: 41.7	NR	Correlations (*r*): NR Copollutant models: none

CO = carbon monoxide, ED = emergency department, HC = hydrocarbons, LRTI = lower respiratory tract infection, NO_2 = nitrogen dioxide, NR = not reported, O_3 = ozone, OC = organic carbon, PM = particulate matter, SO_2 = sulfur dioxide, UFP = ultrafine particles.

[a]Monitoring information obtained from Colais et al. (2012).

†Studies published since the 2008 ISA for Oxides of Nitrogen.

Hospital Admissions

Few recent studies have examined the association between short-term NO_2 exposures and respiratory infection hospital admissions. A time-series study conducted in Ho Chi Minh City, Vietnam (Mehta et al., 2013; HEI, 2012) examined the association between short-term air pollution exposures and pediatric (ages 28 days−5 years) hospital admissions for acute lower respiratory infections (ALRI, including bronchiolitis and pneumonia). In a time-stratified case-crossover analysis focused only on the average of a 1−6 day lag, there was no evidence of an association between NO_2 and ALRI hospital admissions in the all-year analysis (−4.0% [95% CI: −18.0, 12.5] for a 20-ppb increase in 24-h avg NO_2 concentrations).

In an additional study that also examined respiratory infections (i.e., bronchiolitis) in children, Ségala et al. (2008) focused on associations with winter (October−January) air pollution because it is the time of year when respiratory syncytial virus (RSV) activity peaks. It has been hypothesized that air pollution exposures may increase the risk of respiratory infections, including bronchiolitis due to RSV (Ségala et al., 2008). Focusing on children <3 years of age in Paris, France, the authors conducted a bidirectional case-crossover analysis along with a time-series analysis to examine air pollution (i.e., PM_{10}, BS, NO_2, SO_2) associations with bronchiolitis ED visits and hospital admissions. Although the authors specify the bidirectional case-crossover approach was used to "avoid time-trend bias," it must be noted that the bidirectional approach has been shown to bias results (Ségala et al., 2008; Levy et al., 2001). In the case-crossover analysis NO_2 was associated with bronchiolitis hospital admissions (15.9% [95% CI: 7.7, 29.0], lag 0−4 days for a 20-ppb increase in 24-h avg NO_2 concentrations); NO_2 was not examined in the time-series analysis. Although a positive association was observed, the authors did not analyze copollutant models. The lack of copollutant analyses complicates the interpretation of these results because the pollutants were highly correlated, ranging from $r = 0.74−0.83$.

Faustini et al. (2013), in the analysis of air pollution in six Italian cities, also examined associations with lower respiratory tract infection (LRTI) hospital admissions. However, the authors only focused on LRTIs in individuals with COPD over the age of 35. Unlike the analyses focusing on only COPD hospital admissions where the strongest associations were for immediate effects (i.e., lag 0 and 0−1 days) for the population of individuals with COPD that had a hospital admission for a LRTI there was no evidence of an effect at these shorter durations; the largest associations were observed at lag 2−5 days (10.0% [95% CI: −2.7, 24.3]). The authors examined the NO_2 association with LRTI hospital admissions in copollutant models with PM_{10} at lag 0−5 days and, consistent with the

other endpoints examined, reported that although results were attenuated they remained positive (7.8% [95% CI: −6.5, 24.2]).

Emergency Department Visits

Studies that examined the effect of air pollution on ED visits attributed to respiratory infections have focused on similar outcomes to those examined in the studies of hospital admissions. Stieb et al. (2009), in their study of seven Canadian cities, also examined the association between short-term NO_2 concentrations and respiratory infection ED visits. The authors reported positive associations at lags of 1 and 2 days, but the confidence intervals were wide, providing little evidence of an association. However, Ségala et al. (2008) in the study of winter (October−January) air pollution in Paris, France (discussed above) reported evidence of an association between short-term NO_2 concentrations and bronchiolitis ED visits (11.8% [95% CI: 7.7, 20.1]; lag 0−4 day avg for a 20-ppb increase in 24-h avg NO_2 concentrations) in a bi-directional case-crossover analysis. As mentioned previously the interpretation of these results is complicated by the lack of copollutant analyses and the high correlation between pollutants examined (r = 0.74 to 0.83).

In an additional study conducted in Edmonton, Alberta, Canada, Zemek et al. (2010) examined otitis media (i.e., ear infections) ED visits, for ages 1−3 years. Associations were examined for single-day lags of 0 to 4 days in all-year as well as seasonal analyses. The authors observed that NO_2 was associated with increases in ED visits for otitis media in the all-year analysis at lag 2 days (7.9% [95% CI: 1.6, 12.8] for a 20-ppb increase in 24-h avg NO_2 concentrations). When examining whether there was evidence of seasonal patterns in associations, the authors found that the magnitude of the association was larger in the warm months (April−September), 16.1% (95% CI: 3.1, 31.2), compared to the cold months, (October−March), 4.7% (95% CI: 0, 11.2) at lag 2 days for a 20-ppb increase in 24-h avg NO_2 concentrations. Additionally, it is important to note that the pattern of associations for CO were similar to that observed for NO_2, but the authors did not report correlations between pollutants or conduct copollutant analyses.

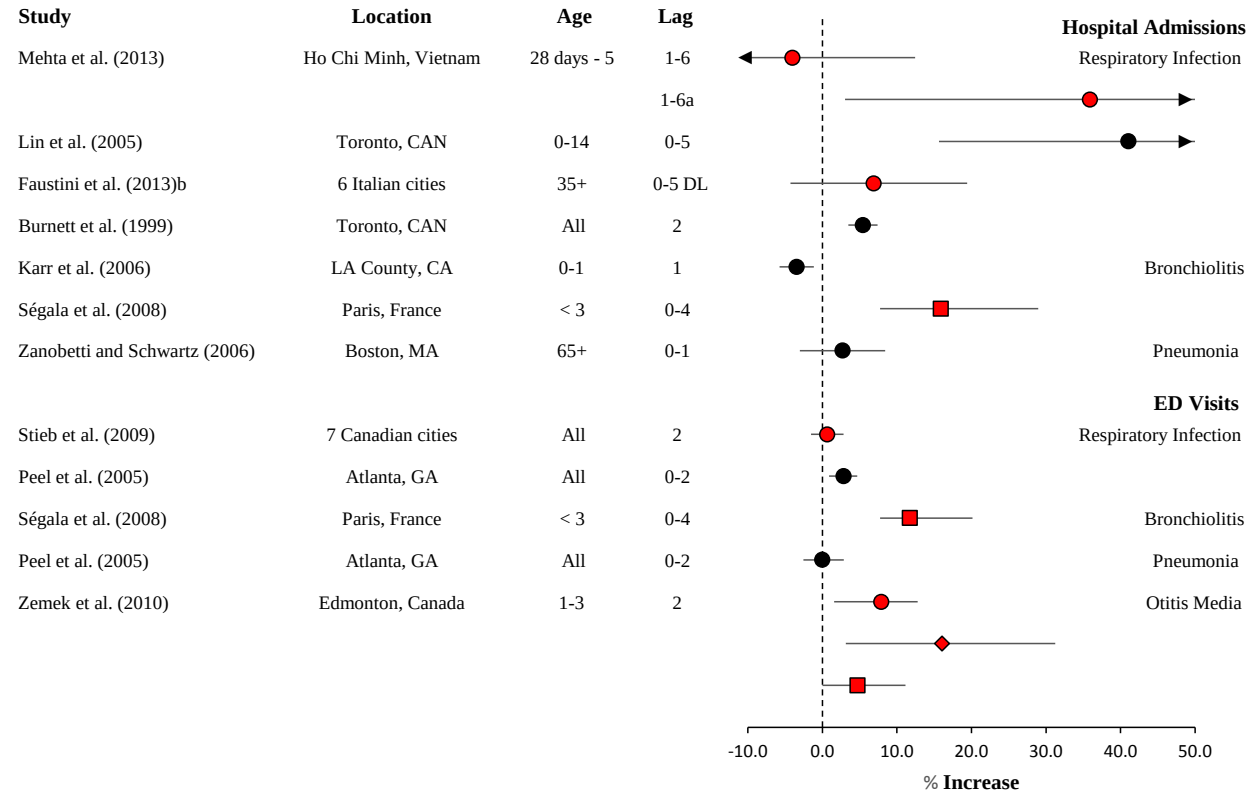
Outpatient and Physician Visits

In addition to examining severe occurrences of a respiratory infection that would require a trip to a hospital, studies have also began to explore whether air pollution may lead to less severe cases, which would be reflective of trips to an outpatient facility. In a study conducted in Atlanta, GA, Sinclair et al. (2010) also examined the association between air pollution and respiratory infection (e.g., upper respiratory infections, lower respiratory infections) outpatient visits from a managed care organization. As detailed previously

(Section 5.2.2.4), the authors separated the analysis into two time periods to compare air pollutant concentrations and relationships for acute respiratory visits for the 25-month time period examined in Sinclair and Tolsma (2004), and an additional 28-month time period of available data from AIRES. Across the two time periods, mean 1-h max NO_2 concentrations were lower in the 28-month versus the 25-month time period, 49.8 ppb versus 35.5 ppb, respectively (Table 5-29). For both outcomes, the daily number of outpatient visit counts varied with LRI being rather small (i.e., 12 per day) compared to that for URI (i.e., 263 per day). A comparison of the two time periods indicated that risk estimates for LRI and URI tended to be larger in the earlier 25-month period compared to the later 28-month period with relatively wide confidence intervals for both outcomes. Additionally, the lag structure of associations varied between each time period. For LRI, the largest magnitude of an association was for both lag 0−2 and 3−5 day avg in the earlier time period, but only lag 3−5 day avg in the latter time period; whereas, for URI the largest associations were for lag 0−2 and 3−5 days for the earlier time period, but a positive association was only observed for lag 6−8 days in the latter time period. The authors also examined potential seasonal differences in associations, but the inconsistent results between the two time periods with respect to the lag structure of associations also complicates the interpretation of seasonal results.

Summary of Respiratory Infection Hospital Admissions and Emergency Department Visits

Recent studies that examined the association between short-term NO_2 exposure and hospital admissions and ED visits due to respiratory infections add to the body of evidence detailed in the 2008 ISA for Oxides of Nitrogen, but studies have not consistently examined similar respiratory infection outcomes (Figure 5-10 and Table 5-30). Of the studies evaluated, the strongest associations are for studies that focused on children, specifically less than 5 years of age. These studies demonstrate associations with respiratory infection, bronchiolitis, and otitis media, specifically during certain times of the year depending on geographic location. The relatively small number of studies that have examined hospital admissions and ED visits due to respiratory infections has resulted in an inadequate assessment of the lag structure of associations and potential copollutant confounding.



Note: a = results are for the dry season (November−April); b = Lower Respiratory Infection in people with COPD. Black = U.S. and Canadian studies evaluated in the 2008 Integrated Science Assessment for Oxides of Nitrogen; red = recent studies. Circle = all-year; diamond = warm/summer months; square = cool/winter months. DL = distributed lag. Effect estimates are standardized to a 20-ppb increase in 24-h avg nitrogen dioxide and 30-ppb increase in 1-h max nitrogen dioxide.

Figure 5-10 Percentage increase in respiratory infection-related hospital admissions and Emergency Department (ED) visits in relation to nitrogen dioxide concentrations from U.S. and Canadian studies evaluated in the 2008 Integrated Science Assessment for Oxides of Nitrogen and recent studies.

Table 5-30 Corresponding risk estimate for studies presented in Figure 5-10.

Study	Location	Age	Avg Time	Lag	Season	% Increase (95% CI)
Hospital Admissions						
Respiratory Infection						
†Mehta et al. (2013)	Ho Chi Minh, Vietnam	28 days−5 yr	24-h avg	1−6	All	−4.0 (−18.0, 12.5)
					Dry[a]	35.9 (3.0, 79.3)
Lin et al. (2005)	Toronto, Canada	0−14	24-h avg	0−5	All	41.1 (15.6, 73.7)
†Faustini et al. (2013)[b]	6 Italian cities	35+	24-h avg	0−5 DL	All	6.9 (−4.3, 19.4)
Burnett et al. (1999)	Toronto, Canada	All	24-h avg	2	All	5.4 (3.5, 7.4)
Bronchiolitis						
Karr et al. (2006)	LA County, CA	0−1	1-h max	1	Winter	−3.5 (−5.8, −1.2)
†Ségala et al. (2008)	Paris, France	<3	24-h avg	0−4	Winter	15.9 (7.7, 29.0)
Pneumonia						
Zanobetti and Schwartz (2006)	Boston, MA	65+	24-h avg	0−1	All	2.7 (−3.0, 8.4)
ED Visits						
Respiratory Infection						
†Stieb et al. (2009)	7 Canadian cities	All	24-h avg	2	All	0.7 (−1.5, 2.8)
Peel et al. (2005)	Atlanta, GA	All	1-h max	0−2	All	2.9 (0.9, 4.7)
Bronchiolitis						
†Ségala et al. (2008)	Paris, France	<3	24-h avg	0−4	Winter	11.8 (7.7, 20.1)
Pneumonia						
Peel et al. (2005)	Atlanta, GA	All	1-h max	0−2	All	0.0 (−2.5, 2.9)
Otitis Media						
†Zemek et al. (2010)	Edmonton, Canada	1−3	24-h avg	2	All	7.9 (1.6, 12.8)
					Summer	16.1 (3.1, 31.2)
					Winter	4.7 (0.0, 11.2)

CI = confidence interval, DL = distributed lag, ED = emergency department.

[a]Dry season was defined as November−April.

[b]LRTI in people with COPD.

†Studies published since the 2008 ISA for Oxides of Nitrogen.

5.2.5.4 Subclinical Effects Underlying Respiratory Infections

Overall, NO_2 exposure shows effects in varying directions on subclinical effects that may characterize key events within the mode of action for respiratory infections (Figure 4-1). Some support for the effects of NO_2 on respiratory infection morbidity and mortality observed in toxicological studies and some epidemiologic studies is provided by toxicological findings for NO_2-induced impairments in alveolar macrophage function. There is uncertainty about the effects of NO_2 on alveolar macrophages and immunoglobulin antibody responses as examined in controlled human exposure and epidemiologic studies, respectively.

Mucociliary and Alveolar Clearance

Airborne substances small enough to be respired may be trapped in the epithelial lining fluid in the conducting airways and physically removed or cleared from the airway by ciliated epithelial cells. Recent animal toxicological studies and studies included in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a) demonstrated that exposure to high concentrations of NO_2, generally above 5,000 ppb, functionally impairs pulmonary clearance and damage the ciliated epithelium of the airway. However, exposures to NO_2 concentrations below 5,000 ppb have varying effects on pulmonary clearance in animal toxicological and controlled human exposure studies. The examination of the effect of NO_2 on pulmonary clearance, which consists of mucociliary and alveolar clearance, is limited to studies that were reviewed in the 2008 ISA.

Studies have been conducted in various animal models and provide evidence that NO_2 exposure can potentially affect mucociliary clearance. Schlesinger (1987b) employed two methods to measure ciliary clearance in rabbits exposed to 310 or 1,030 ppb NO_2 for 2 hours per day for up to 14 days. Mean residence time of radioactive tracer microspheres was not altered 24 hours following 2-, 6-, or 13-day exposures; however, patterns in clearance, measured as the fraction of retained radioactive tracer microspheres, were statistically significantly different from those in controls at both 310 or 1,030 ppb NO_2 over 13 days of exposure. Vollmuth et al. (1986) studied mucociliary clearance in rabbits exposed to 300 or 1,000 ppb NO_2 for 2 hours while Ferin and Leach (1975) exposed rats to 1,000 ppb NO_2 in conjunction with 900 ppb NO for 7 hours per day, 5 days per week for 11 or 22 days; both studies reported accelerated clearance of particles. A study published by Ohashi et al. (1994) found different results, showing that guinea pigs exposed to 3,000 or 9,000 ppb NO_2 for 6 hours/day, 6 days/week for 2 weeks had concentration-dependent reductions in ciliary activity. This study, however, reported

ciliary beat (measured by light refraction) in nasal tissues excised after animals were exposed. This method could have affected the outcome and be less representative of changes that occur from human ambient exposure.

In a controlled human exposure study of healthy adults, Helleday et al. (1995) used fiberoptic bronchoscopy to measure ciliary activity and found a decrease 45 minutes after a brief exposure to 1,500 or 3,500 ppb NO_2. In contrast, increases in ciliary activity were reported 24 hours after a 4-hour exposure to 3,500 ppb NO_2. It is important to note that baseline measurements for each subject in this study were used as control values, and therefore, the study lacked air controls and subject blinding.

Function and Morphology of Alveolar Macrophages

Toxicological Studies

Previous studies reported NO_2 exposure to induce slight morphological differences and increases in AM numbers in BAL fluid (Hooftman et al., 1988; Mochitate et al., 1986; Rombout et al., 1986; Goldstein et al., 1977; Dowell et al., 1971) and diminished superoxide radical production (indicating reduced respiratory burst) at exposures as low as 500 ppb (see Table 5-31 for study details). Robison et al. (1990) and Robison et al. (1993) exposed rat AMs to 100−20,000 ppb for 1 hour ex vivo and found a concentration-dependent decrease in superoxide production, ranging from 81−55% of control levels after phorbol mynstate acetate (PMA) stimulation. Similarly, Sprague Dawley male rats exposed to 500 ppb NO_2 for 8 hours/day for 0.5, 1, 5, or 10 days had superoxide levels 63−75% of those in air-exposed animals after PMA stimulation (Robison et al., 1993). Suzuki et al. (1986) reported comparable observations in AMs isolated from Fisher 344 rats exposed to 4,000 ppb NO_2 for 3, 5, or 10 days. Conversely, PMA-stimulated AMs isolated from Sprague Dawley female rats exposed to NO_2 below 6,100 ppb showed no change in superoxide production compared to controls (Amoruso et al., 1981). Overall, NO_2 exposure appears to decrease the ability of AMs to produce superoxide anion, although inconsistencies are present across studies that could be the result of strain or sex differences in response to NO_2.

Studies also found variable effects of ambient-relevant NO_2 exposures on phagocytic capacity of AMs. Rose et al. (1989) exposed CD-1 mice to 1,000 and 5,000 ppb NO_2 for 6 hours/day for 2 days and reported diminished phagocytosis of colloidal gold particles at both concentrations of NO_2. In contrast, NO_2 exposure increased uptake of murine *Cytomegalovirus*. Studies report both no change and decreased phagocytosis of latex microspheres. Hooftman et al. (1988) exposed rats to 4,000, 10,000, or 25,000 ppb NO_2 for 6 hour/day, 5 days/week and found no changes in phagocytosis of latex microspheres below 10,000 ppb at 1, 2, or 3 weeks. Schlesinger (1987b), however, found decreased

phagocytosis of latex microsphere by AMs isolated from rabbits 24 hours after a 2 or 6-day exposure at 300 or 1,000 ppb [2 hours/day; all animals were co-exposed to 0.5 mg/m^3 sulfuric acid (H_2SO_4)]. Suzuki et al. (1986) also reported decreased phagocytic capacity of AMs isolated from rats exposed to 4,000 ppb NO_2 for 7 days.

Table 5-31 Animal toxicological studies of subclinical lung host defense effects.

Study	Species (Strain); Age; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Amoruso et al. (1981)	Rat (Sprague-Dawley); F, n = 4/group	1,300, 1,900, and 6,100 ppb NO_2 for 3 h	Analysis of BAL fluid and superoxide production by AMs (PMA stimulation).
Dowell et al. (1971)	Dog (beagle); n = 11	3,000 ppb NO_2 for 1 h	Histopathological evaluation and lung surfactant properties.
Ferin and Leach (1975)	Rats (Long-Evans), n = 5−10/group	1,000−24,000 ppb NO_2 for 11 or 22 days (7 h/day, 5 days/week)	Retained TiO_2 particles at 8, 25, and 130 days post-exposure.
Goldstein et al. (1977)	Rat (Sprague-Dawley); F	500, 1,000, and 2,400 ppb NO_2 for 1 and 2 h	Agglutination of AMs.
Hooftman et al. (1988)	Rats (Wistar); M; n = 10/group	4,000, 10,000, 25,000 ppb NO_2 for 6 h/day, 5 days/week for 7−21 days	Histopathological evaluation, analysis of BAL fluid, and AM function and morphology.
Mochitate et al. (1986)	Rats (Wistar); M; 19−23 weeks; n = 6/group	4,000 ppb NO_2 continuously up to 10 days	BAL fluid cell counts and AM function and morphology.
Ohashi et al. (1994)	Guinea pigs (Hartley); n = 10/group	3,000 or 9,000 ppb for 6 h/day, 6 days/week for 2 weeks	Ciliary beat in excised nasal tissue 24 h after exposure.
Robison et al. (1990)	Rats (Sprague-Dawley)	100, 500, and 1,000 ppb NO_2 for 1 h; AMs exposed ex vivo	Viability, LTB4 production, neutrophil chemotaxis, superoxide production.
Robison et al. (1993)	Rat (Sprague Dawley); n > 4/group	500 ppb NO_2 for 8 h/day for 0.5, 1, 5, or 10 days	BAL fluid cell counts and arachidonate metabolite levels, AM arachidonate metabolism, respiratory burst activity, and glutathione content.
Rombout et al. (1986)	Rats (Wistar); F, 6 weeks; n = 3−6/group	500, 1,390, and 2,800 ppb NO_2 for 1, 2, 4, 8, 16, and 28 days	Histopathological evaluation.

Table 5-31 (Continued): Animal toxicological studies of subclinical lung host defense effects.

Study	Species (Strain); Age; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Rose et al. (1988) Rose et al. (1989)	Mice (CD-1); 4−6 weeks; n > 4/group	(1) 1,000, 2,500, and 5,000 ppb NO_2 for 6 h/day for 2 days; intratracheal inoculation with murine *Cytomegalovirus*; 4 additional days (6 h/day) of exposure. (2) re-inoculation 30 days (air) post-primary inoculation.	Infection 5 and 10 days post-inoculation, histopathological evaluation, and analysis of BAL fluid (LDH, albumin, macrophages).
Schlesinger (1987b)	Rabbits (New Zealand white); M, n = 5/group	310 or 1,030 ppb NO_2 for 2 h/day for 2, 6, and 13 days	Viability and AM activity (mobility, attachment, and phagocytosis).
Suzuki et al. (1986)	Rats (Fischer 344); M, 7 weeks; n = 8/group	4,000 NO_2 ppb for 1, 3, 5, 7, and 10 days	AM activity (phagocytosis and superoxide production), SOD and glucose-6-phosphate dehydrogenase activity.
Vollmuth et al. (1986)	Rabbit (New Zealand white); M; n = 5/group	300, 1,000, or 3,000 ppb for 2 h	Retained tracer particles for 14 days following exposure.

LDH = lactate dehydrogenase, M = male, NO_2 = nitrogen dioxide, PMA = phorbol mynstate acetate, SOD = superoxide dismutase.

Controlled Human Exposure

Similar to animal toxicological studies, controlled human exposure studies did not consistently demonstrate that ambient-relevant NO_2 concentrations can alter AM characteristics (see Table 5-32 for study details). Devlin et al. (1999) exposed healthy subjects to 2,000 ppb NO_2 for 4 hours with intermittent exercise and found that AMs isolated from the BAL fluid had decreased phagocytic activity and superoxide production in ex vivo experiments. Conversely, no change in ex vivo macrophage morphology or function was reported after subjects were exposed to 2,000 ppb NO_2 for 6 hours with intermittent exercise (Azadniv et al., 1998). In vitro exposure of human AMs for 3 hours at 5,000 ppb NO_2 did not result in statistically significant changes in cell viability or neutrophil chemotactic factor (IL-8) or IL-1 release, markers of macrophage activity (Pinkston et al., 1988).

Table 5-32 Controlled human exposure studies of subclinical lung host defense effects.

Study	n, Sex; Age (mean ± SD)	Exposure Details (Concentration; Duration)	Endpoints Examined
Azadniv et al. (1998)	n = 11 M, 4 F; Early phase: 28.1 ± 3.5 yr Late phase: 27.4 ± 4.2 yr	2,000 ppb for 6 h; Exercise for approximately 10 of every 30 min at $\dot{V}_E$ = 40 L/min	Alveolar macrophage function 1 h (early phase) and 18 h (late phase) after exposure.
Devlin et al. (1999)	n = 11 M; range: 18−35 yr	2,000 ppb for 4 h; Exercise for 15 min on/15 min off at $\dot{V}_E$ = 50 L/min	BAL fluid macrophage superoxide production and phagocytosis.
Goings et al. (1989)	(1) n = 44 (2) n = 43 (3) n = 65; range: 18−35 yr	(1) 2,000 ppb for 2 h (2) 3,000 ppb for 2 h (3) 1,000 or 2,000 ppb for 2 h	Nasal wash virus isolation and count 4 days after virus administration. Serum and nasal wash antibody response 4 weeks after virus administration.
Helleday et al. (1995)	n = 14 M, 10 F; 27 yrs (range: 23−30 yr)	(1) 1,500 ppb for 45 min (2) 3,500 ppb for 45 min (3) 3,500 ppb for 4 h Baseline obtained 2 weeks prior (each subject served as own control).	Fiberoptic bronchoscopy to record mucociliary activity frequency. (1) and (2) 45 min following exposure. (3) 24 h following exposure.
Pinkston et al. (1988)	Human AMs isolated from 14 M and 1 F; 29 ± 3.9 yr	5,000 ppb for 3 h (ex vivo)	Cell viability and release of neutrophil chemotactic factor and IL-1.

F = female, IL = interleukin, M = male, SD = standard deviation.

Immunoglobulin Antibody Response

Immunoglobulin M antibodies increase in response to infections, and a recent epidemiologic study of adults infected with human immunodeficiency virus and hospitalized for pneumocystis pneumonia found a 34% (95% CI: 6.5, −60) diminished antibody response to pneumocystis proteins per 20-ppb increase in 24-h avg ambient NO_2 concentrations (lag 0−2 day avg) (Blount et al., 2013). Potential confounding by other traffic-related copollutants or factors such as meteorology, sex, and SES was not examined. Further, because subjects were distributed at varying distances of the single central site monitor, which was located within 1 km of major roads, the impact of exposure measurement error on the results is uncertain. Thus, the results do not strongly inform the understanding of the effects of NO_2 on respiratory infections.

5.2.5.5 Summary of Respiratory Infection

Animal toxicological studies provide clear evidence for short-term NO_2 exposure impairing host defense by demonstrating increased mortality from bacterial or viral infection following exposures of experimental animals to 1,500 to 4,500 ppb NO_2 for 1 to 8 hours. Several studies also demonstrated decreased bactericidal activity following exposures of 1,000 to 5,000 ppb for 1 to 17 hours. Compared with animal toxicological studies, controlled human exposure studies provide less consistent evidence for NO_2-induced infectivity assessed as viral titers or inactivation of influenza virus. In humans, NO_2 exposures spanned 600−3,000 ppb for 3 hours for a single or 3-day exposure (Table 5-27). The evidence from animal toxicological studies provides biological plausibility for the associations observed in epidemiologic studies between increases in ambient NO_2 concentrations (5- to 7-day avg) and increases in respiratory infections as ascertained by hospital admissions, ED visits, and parental reports. Studies varied in the specific respiratory infection examined (e.g., bronchiolitis, ear infection, any respiratory infection), and many studies observed null or imprecise associations with wide 95% CIs (Figure 5-10). Epidemiologic associations were observed in children and in study populations with respiratory disease (i.e., children with asthma, adults with COPD). Whereas the association between NO_2 and LTRI in adults with COPD was robust to adjustment for PM_{10} (Faustini et al., 2013), most epidemiologic studies did not examine copollutant models, and respiratory infections also were associated with highly correlated copollutants such as BS, PM_{10}, and SO_2 (r = 0.74 to 0.92).

Also providing some biological plausibility for NO_2-induced impaired host defense, some studies characterized potential mechanisms underlying susceptibility to infection. Although results vary across studies, some animal toxicological studies found NO_2 exposure to decrease the ability of AMs to produce superoxide anion and decrease phagocytic activity. Such observations were made with NO_2 exposures of 300 to 5,000 ppb (Table 5-31). There was heterogeneity across studies in animal species, strain, and sex that may or may not have contributed to inconsistencies observed in response to NO_2. Results for the effects of NO_2 exposure on pulmonary clearance were more variable, with a majority of studies reporting increased pulmonary clearance after ambient-relevant NO_2 exposure.

5.2.6 Aggregated Respiratory Conditions

In addition to individual respiratory conditions, epidemiologic studies examined respiratory effects as an aggregate of multiple respiratory conditions (e.g., asthma, COPD, respiratory infections). The studies from the 2008 ISA for Oxides of Nitrogen

(U.S. EPA, 2008a) and recent studies consistently show associations between short-term increases in ambient NO_2 concentration and increases in aggregated respiratory conditions. This evidence is based primarily on hospital admissions and ED visits for all respiratory diseases, which are the focus of the discussion in this section. Other outcomes include lung function in adults with asthma or COPD and sales of medication for asthma and COPD combined or cough and mucus combined. As described in preceding sections, evidence for the effects of short-term NO_2 exposure varies among specific respiratory outcome groups. Thus, it is not clear whether the evidence for aggregated respiratory conditions reflects associations with each respiratory condition equally or a particular condition(s).

5.2.6.1 Respiratory Symptoms, Lung Function, and Medication Use

Outcomes such as lung function decrements in adults with asthma and/or COPD (Rice et al., 2013; Higgins et al., 2000) and increases in the sale of medication for asthma and COPD combined or for cough and mucus combined (Pitard et al., 2004; Zeghnoun et al., 1999) were associated with ambient NO_2 (24-h avg, lagged 0 to 7 days or 0−1 day avg) in a small group of epidemiologic studies, with exception of the Higgins et al. (1995) study. However, uncertainties in these studies result in weak inference of the independent effects of NO_2. Associations with medication sales were modeled with GAM in S-plus (Pitard et al., 2004), which can produce biased results (U.S. EPA, 2006). In the Framingham cohort study, lung function was associated with $PM_{2.5}$ (r = 0.63) (Rice et al., 2013), and a copollutant model was not analyzed. The other lung function studies did not report what potential confounding factors were examined (Higgins et al., 2000; 1995). Another uncertainty is potential exposure measurement error produced by the use of central site ambient concentrations to represent ambient exposure. In the Framingham study, sites in the Boston, MA area were averaged (Rice et al., 2013). With one to two observations per subject collected over 3−9 years, the analysis relied on both temporal and spatial contrasts in exposure. With individuals distributed across a 40 km area, and variability in ambient NO_2 observed across a range of 3 to 10 km in Boston (Section 2.5.2), is not clear how well the average area concentration represents ambient exposure among study subjects.

5.2.6.2 Hospital Admissions and Emergency Department Visits for All Respiratory Diseases

Epidemiologic studies examining the association between short-term NO_2 exposures and respiratory-related hospital admissions or ED visits were not available until after the completion of the 1993 AQCD for Oxides of Nitrogen. As a result, the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a) contained the first thorough evaluation of respiratory morbidity in the form of respiratory-related hospital admissions and ED visits. The majority of the studies evaluated consisted of single-city, time-series studies that examined all respiratory hospital admissions or ED visits with additional cause-specific studies, as discussed in previous sections. Studies of all respiratory hospital admissions and ED visits consistently reported positive associations with short-term NO_2 exposures (Figure 5-13 and Table 5-34). These associations were generally found to be robust and independent of the effects of ambient particles or gaseous copollutants (U.S. EPA, 2008a). The evidence supporting NO_2-associated increases in all respiratory disease hospital admission and ED visits contributed heavily to the 2008 ISA for Oxides of Nitrogen conclusion that "there is a likely causal relationship between short-term exposure to NO_2 and effects on the respiratory system" (U.S. EPA, 2008a). The air quality characteristics of the cities and the exposure assignment approach used in each study evaluated in this section are presented in Table 5-33. As detailed in Section 5.2.2.4, other recent studies of all respiratory disease hospital admissions and ED visits are not the focus of this evaluation, and the full list of these studies, as well as study details, can be found in Supplemental Table S5-3 (U.S. EPA, 2014h).

Table 5-33 Mean and upper percentile concentrations of nitrogen dioxide in studies of hospital admissions and emergency department visits for aggregrated respiratory conditions.

Study	Location (Years)	Exposure Assignment	Metric	Mean Concentration ppb	Upper Percentile Concentrations ppb	Copollutant Examination
Yang et al. (2003)	Vancouver, Canada (1986−1998)	Average of NO_2 concentrations from 30 monitors.	24-h avg	18.7	NR	Correlations (*r*): O_3: −0.32 Copollutant models: O_3
Fung et al. (2006)	Vancouver, Canada (1995−1999)	Average of NO_2 concentrations over all monitors.	24-h avg	16.8	Max: 33.9	Correlations (*r*): CO: 0.74 CoH; 0.72 O_3: −0.32 SO_2: 0.57 PM_{10}: 0.54 $PM_{2.5}$: 0.36 $PM_{10-2.5}$: 0.52 Copollutant model: none
Burnett et al. (2001)	Toronto, Canada (1980−1994)	Average of NO_2 concentrations from 4 monitors.	1-h max	44.1	146	Correlations (*r*): O_3: 0.52 Copollutant model: O_3
†Cakmak et al. (2006)	10 Canadian cities (1993−2000)	Average of NO_2 concentrations over all monitors within each city.	24-h avg	21.4	Max: 44−134	Correlations (*r*): NR Copollutant models: none
†Wong et al. (2009)	Hong Kong (1996−2002)	Average of NO_2 concentrations across 8 monitors.	24-h avg	31.2	75th: 37.0 Max: 89.4	Correlations (*r*): NR Copollutant models: none
†Dales et al. (2006)	11 Canadian cities (1986−2000)	Average of NO_2 concentrations over all monitors within each city.	24-h avg	21.8	95th: 21−43	Correlations (*r*), across cities: PM_{10}: −0.26 to 0.69 O_3: −0.55 to 0.05SO_2: 0.20−0.67 CO: 0.13−0.76 Copollutant models: none

Table 5-33 (Continued): Mean and upper percentile concentrations of nitrogen dioxide in studies of hospital admissions and emergency department visits for aggregated respiratory conditions.

Study	Location (Years)	Exposure Assignment	Metric	Mean Concentration ppb	Upper Percentile Concentrations ppb	Copollutant Examination
†Son et al. (2013)	8 South Korean cities (2003−2008)	Hourly ambient NO_2 concentrations from monitors in each city.	24-h avg	11.5−36.9	NR	Correlations (*r*): PM_{10}: 0.5 O_3: −0.1SO_2: 0.6 CO: 0.7 Copollutant models: none
†Atkinson et al. (2012)	Meta-analysis (Asia) (Years NR)	NR	24-h avg	NR	NR	Correlations (*r*): NR Copollutant models: none
†Faustini et al. (2013)	6 Italian cities (2001−2005)	Average of NO_2 concentrations over all monitors within each city. Number of NO_2 monitors in each city ranged from 1−5.[a]	24-h avg	24.1−34.6	NR	Correlations (*r*), across cities: PM_{10}: 0.22−0.79 Copollutant models: PM_{10}
Peel et al. (2005)	Atlanta, GA (1993−2000)	Average of NO_2 concentrations from monitors for several monitoring networks.	1-h max	45.9	NR	Correlations (*r*): $PM_{2.5}$: 0.46 PM_{10}: 0.49 $PM_{10-2.5}$: 0.46 UFP: 0.26 $PM_{2.5}$ Water Soluble Metals: 0.32 $PM_{2.5}$ Sulfate: 0.17 $PM_{2.5}$ Acidity: 0.10 $PM_{2.5}$ OC: 0.63 $PM_{2.5}$ EC: 0.61 Oxygenated HCs: 0.30 O_3: 0.42 CO: 0.68 SO_2: 0.34 Copollutant models: none

Table 5-33 (Continued): Mean and upper percentile concentrations of nitrogen dioxide in studies of hospital admissions and emergency department visits for aggregated respiratory conditions.

Study	Location (Years)	Exposure Assignment	Metric	Mean Concentration ppb	Upper Percentile Concentrations ppb	Copollutant Examination
Tolbert et al. (2007)	Atlanta, GA (1993−2004)	Average of NO_2 concentrations from monitors for several monitoring networks.	1-h max	81.7	306	Correlations (*r*): $PM_{2.5}$: 0.47 PM_{10}: 0.53 $PM_{10-2.5}$: 0.4 $PM_{2.5}$ Sulfate: 0.14 $PM_{2.5}$ OC: 0.62 $PM_{2.5}$ EC: 0.64 $PM_{2.5}$ TC: 0.65 $PM_{2.5}$ Water Soluble Metals: 0.32 Oxygenated HCs: 0.24 O_3: 0.44 CO: 0.70 SO_2: 0.36 Copollutant models: CO, PM_{10}, O_3
†Darrow et al. (2011)	Atlanta, GA (1993−2004)	Epidemiologic analysis used NO_2 concentrations from 1 centrally located monitor. Assessment of spatial heterogeneity relied upon all EPA AQS and ARIES monitors.	1-h max 24-h avg Commute (7 a.m.−10 a.m., 4 p.m.−7 p.m.) Daytime (8 a.m.−7 p.m.) Nighttime (12 a.m.−6 a.m.)	1-h max: 43 24-h avg: 22 Commute: 21 Daytime: 17 Nighttime: 25	75th: 1-h max: 53 24-h avg: 28 Commute: 27 Daytime: 22 Nighttime: 35 Max: 1-h max: 181 24-h avg: 74 Commute: 97 Daytime: 82 Night-time: 97	Correlations (*r*), for averaging times specified in current NAAQS: CO, 1-h: 0.61 O_3, 8-h: 0.34 $PM_{2.5}$, 24-h: 0.42 Copollutant models: none

CO = carbon monoxide, CoH = coefficient of haze, EC = elemental carbon, ED = emergency department, HC = hydrocarbon, NAAQS = National Ambient Air Quality Standards, NO_2 = nitrogen dioxide, NR = not reported, O_3 = ozone, OC = organic carbon, PM = particulate matter, SO_2 = sulfur dioxide, TC = total carbon, UFP = ultrafine particles.

[a]Monitoring information obtained from Colais et al. (2012).

†Studies published since the 2008 ISA for Oxides of Nitrogen

Hospital Admissions

Multicity studies conducted in Canada (Cakmak et al., 2006; Dales et al., 2006), Italy (Faustini et al., 2013) and Korea (Son et al., 2013), as well as a single-city study conducted in Hong Kong (Wong et al., 2009) examined the association between short-term NO_2 concentrations and hospital admissions for all respiratory diseases, each focusing on a different age range (Figure 5-13 and Table 5-34). Additional supporting evidence for an association between short-term NO_2 exposures and respiratory hospital admissions comes from a meta-analysis of studies conducted in Asian cities (Atkinson et al., 2012).

Cakmak et al. (2006) focused on all ages in 10 Canadian cities with the primary objective of the study being to examine the potential modification of the effect of ambient air pollution on daily respiratory hospital admissions by education and income using a time-series analysis conducted at the city level (the effect modification analysis is discussed in Chapter 7). The authors calculated a pooled estimate across cities for each pollutant using a random effects model by first selecting the lag day with the strongest association from the city-specific models. For NO_2, the mean lag day across cities that provided the strongest association and for which the pooled effect estimate was calculated was 1.4 days. At this lag, Cakmak et al. (2006) reported a 2.3% increase (95% CI: 0.2, 4.5%) in respiratory hospital admissions for a 20-ppb increase in 24-h avg NO_2 concentrations. This result is consistent with a study conducted in Hong Kong that examined whether influenza modifies the relationship between air pollution exposure and hospital admissions (Wong et al., 2009). Wong et al. (2009) observed a 3.2% (95% CI: 1.9, 4.5) increase in all respiratory disease hospital admissions for all ages at lag 0−1 days for a 20-ppb increase in 24-h avg NO_2 concentrations, with an association slightly smaller in magnitude for acute respiratory disease (2.1% [95% CI: −0.1, 4.3]), which comprises approximately 39% of all respiratory disease hospital admissions in Hong Kong. Cakmak et al. (2006) also examined the potential confounding by other pollutants but only through the use of a multipollutant model (i.e., two or more additional pollutants included in the model). These models are difficult to interpret due to the multicollinearity between pollutants and are not evaluated in this ISA.

In an additional multicity study conducted in 11 Canadian cities, Dales et al. (2006) focused on NO_2-associated respiratory hospital admissions in neonatal infants (ages 0−27 days). The investigators used a statistical analysis approach similar to Cakmak et al. (2006) (i.e., time-series analysis to examine city-specific associations, and then a random effects model to pool estimates across cities). Dales et al. (2006) observed that the mean lag day across cities that provided the strongest association for NO_2 was 1 day, which

corresponded to 6.5% (95% CI: 3.5, 9.6%) increase in neonatal respiratory hospital admissions for a 20-ppb increase in 24-h avg NO_2 concentrations. Similar to Cakmak et al. (2006), Dales et al. (2006) only examined the potential confounding effects of other pollutants on the NO_2-respiratory hospital admission association through the use of multipollutant models, which are not informative due to multicollinearity between pollutants.

The results of Cakmak et al. (2006) and Wong et al. (2009), which focus on all ages, are further supported by Son et al. (2013), a study that examined the association between short-term exposures to air pollution and respiratory-related hospital admissions in eight South Korean cities. It is important to note that South Korea has unique demographic characteristics with some indicators more in line with other more developed countries (e.g., life expectancy, percentage of population living in urban areas), but because it represents a rapidly developing Asian country, it is likely to have different air pollution, social, and health patterns than less industrialized Asian nations or Western nations that developed earlier (Son et al., 2013). In a time-series analysis using a two-stage Bayesian hierarchical model, Son et al. (2013) examined both single-day lags and cumulative lags up to 3 days (i.e., lag 0−3). The authors only presented NO_2 results for the strongest lag and observed a 3.6% increase (95% CI: 1.0, 6.1) in respiratory disease hospital admissions at lag 0 for a 20-ppb increase in 24-h avg NO_2 concentrations. These results are consistent with those of a meta-analysis of studies conducted in Asian cities by Atkinson et al. (2012), which in a random effects model based on five estimates, reported a 3.5% increase (95% CI: 0.6, 6.5) in respiratory hospital admissions for a 20-ppb increase in 24-h avg NO_2 concentrations.

Son et al. (2013)did not conduct copollutant analyses; however, similar patterns of associations were observed across pollutants that were moderately [PM_{10} (r = 0.5); SO_2 (r = 0.6)] to highly correlated [CO (r = 0.7)] with NO_2. Son et al. (2013) also examined potential seasonal differences in all respiratory disease hospital-admission associations. The authors reported that the association with NO_2 was largest in magnitude during the summer (8.3% [95% CI: 2.8, 14.3], lag 0). However, across the eight cities, NO_2 concentrations were lowest during the summer season (<20 ppb compared to >24 ppb in the other seasons), which complicates the interpretation of these results.

Faustini et al. (2013) focused on examining the relationship between short-term air pollution exposures and respiratory hospital admissions, specifically on the adult population (i.e., individuals 35 years of age and older) in six Italian cities. In a time-series analysis the authors examined the lag structure of associations through single-day lags as well as cumulative lags, using cubic polynomial distributed lags, in an attempt to identify whether the NO_2 effect on respiratory-related hospital admissions was immediate (lag 0,

lag 0−1 days), delayed (lag 2−5 days), or prolonged (lag 0−3, 0−5 days). The authors reported that NO_2 was most strongly associated with all respiratory hospital admissions at lag 0−5 days (4.6% [95% CI: 0.87, 8.3] for a 20-ppb increase in 24-h avg NO_2 concentrations), which differs from Cakmak et al. (2006) and Dales et al. (2006) where the strongest effects were observed at lags less than 2 days. However, Faustini et al. (2013) did observe positive associations, although smaller in magnitude (ranging from 2.5−2.9%) at the shorter lags (i.e., lag 0 and 0−1 days). Faustini et al. (2013) only examined potential copollutant confounding of NO_2 associations in models with PM_{10}, and reported that the NO_2 association with respiratory hospital admissions at lag 0−5 days was attenuated slightly, but remained positive (3.3% [95% CI: −1.1, 7.8]).

Emergency Department Visits

Studies of ED visits for aggregated respiratory conditions that were evaluated in the 2008 ISA for Oxides of Nitrogen were few in number and focused almost exclusively on study populations consisting of all ages, and U.S. studies were limited to Atlanta, GA. Building on the previous studies conducted in Atlanta, GA (Tolbert et al., 2007; Peel et al., 2005), Darrow et al. (2011) also examined associations between short-term air pollution exposures and all respiratory ED visits. To examine the association between the various NO_2 exposure metrics and respiratory ED visits, the authors conceptually used a time-stratified case-crossover framework in which control days were selected as those days within the same calendar month and maximum temperature as the case day. However, instead of conducting a traditional case-crossover analysis, the authors used a Poisson model with indicator variables for each of the strata (i.e., parameters of the control days). Darrow et al. (2011) only reported results for a 1 day lag in NO_2 concentrations. For a 30-ppb increase in 1-h max NO_2 concentrations the authors reported a 1.4% increase (95% CI: 0.8, 2.1) in all respiratory ED visits. These results are slightly smaller than those reported by Peel et al. (2005) and Tolbert et al. (2007), but this could be attributed to the fact that the latter two studies used a multiday average of NO_2 concentrations (i.e., lag 0−2 days) instead of the single-day lag used in Darrow et al. (2011).

Model Specification—Sensitivity Analyses

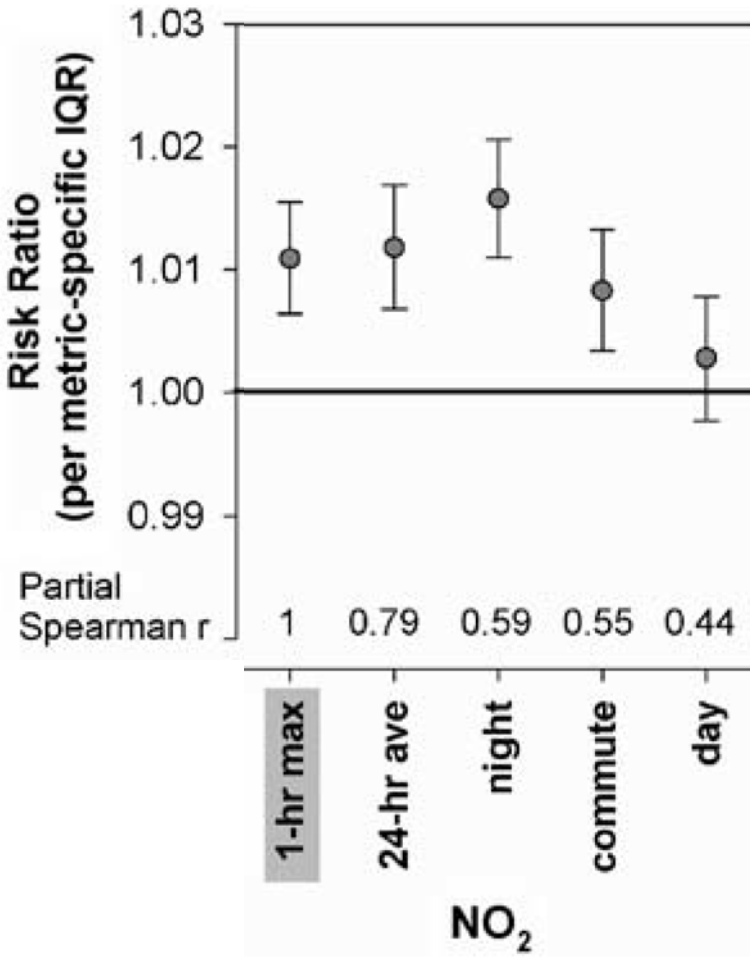
A question that often arises in the examination of associations between air pollution and a health effect is whether the statistical model employed adequately controls for the potential confounding effects of temporal trends and meteorological conditions. Son et al. (2013), in the study of eight South Korean cities, conducted a sensitivity analysis to identify whether risk estimates changed depending on the df used to control for temporal trends and meteorology covariates (i.e., temperature, humidity, and barometric pressure).

Similar to the other respiratory-related hospital admission outcomes examined, the authors reported that the association between short-term NO_2 exposures and all respiratory disease hospital admissions was sensitive to using less than 6 df per year to control for temporal trends, but was stable when using 6−10 df per year. Additionally, when varying the number of df used for the meteorology covariates from 3 to 6 df as well as the lag structure (i.e., lag 0 and lag 0−3 days), the NO_2 association remained robust (i.e., relatively unchanged).

Exposure Assignment

In addition to model specification, the method used to assign exposure in epidemiologic studies has been suggested to influence the magnitude and direction of air pollution-health effects associations. As discussed in Section 5.2.2.4, Strickland et al. (2011) examined exposure assignment in the case of asthma ED visits in Atlanta, GA and found that different exposure assignment approaches could influence the magnitude, but not direction of associations. Darrow et al. (2011) also used data from Atlanta, GA to examine the influence of alternative exposure metrics on the association between short-term NO_2 concentrations and all respiratory ED visits along with the spatial variability of each exposure metric.

To examine whether all respiratory ED visits associations differed depending on the exposure metric used, Darrow et al. (2011) used five different exposure metrics: (1) 1-h max; (2) 24-h avg; (3) commuting period (7:00 a.m. to 10:00 a.m. and 4:00 p.m. to 7:00 p.m.); (4) daytime avg (8:00 a.m. to 7:00 p.m.); and (5) nighttime avg (12:00 a.m. to 6:00 a.m.). The authors reported relatively consistent results (using an a priori lag of 1 day) across exposure metrics with the largest estimate found for the night-time avg and the smallest for the daytime metrics (Figure 5-11). The larger risk estimate for the nighttime metric could be a reflection of NO_2 during this exposure duration being a better surrogate for NO_2 concentrations on the previous day (Darrow et al., 2011). The correlation between NO_2 metrics was not as high compared to that for other pollutants examined in the study (i.e., $r < 0.80$ between 1-h max and all other metrics), but was relatively high for the 24-h avg metric ($r = 0.79$), which is the other metric for NO_2 often used in epidemiologic studies.



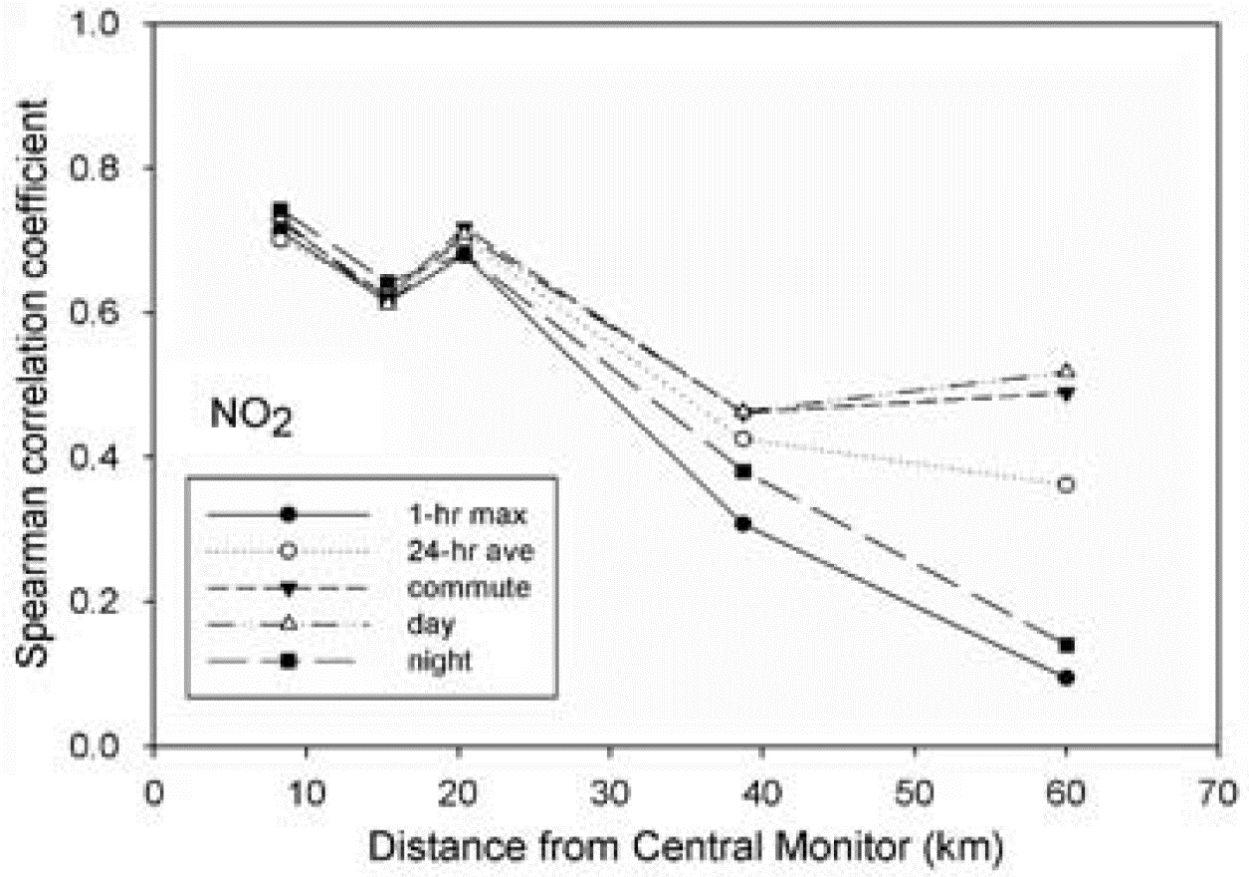
Note: Partial Spearman correlation coefficient between a priori metrics (shaded in gray) and other pollutant metrics shown above the *x*-axis.

Source: Reprinted with permission of Nature Publishing Group (Darrow et al., 2011).

Figure 5-11 Risk ratio and 95% confidence intervals for associations between various lag 1 day nitrogen dioxide (NO_2) metrics and respiratory emergency department visits.

In the analysis of the spatial correlation of exposure metrics for NO_2, Darrow et al. (2011) found that unlike O_3 and $PM_{2.5}$, which were spatially homogenous, there was evidence that correlations for NO_2 metrics decreased dramatically as distance from the central monitor increased (Figure 5-12). This was especially true for the 1-h max and nighttime metrics ($r < 0.20$) at 60 km. The 24-h avg metric was also reduced ($r = \sim 0.40$), but not as dramatically as the 1-h max. Although reduced at greater distances, moderate correlations ($r = \sim 0.50$) were reported with the central monitor for the daytime and

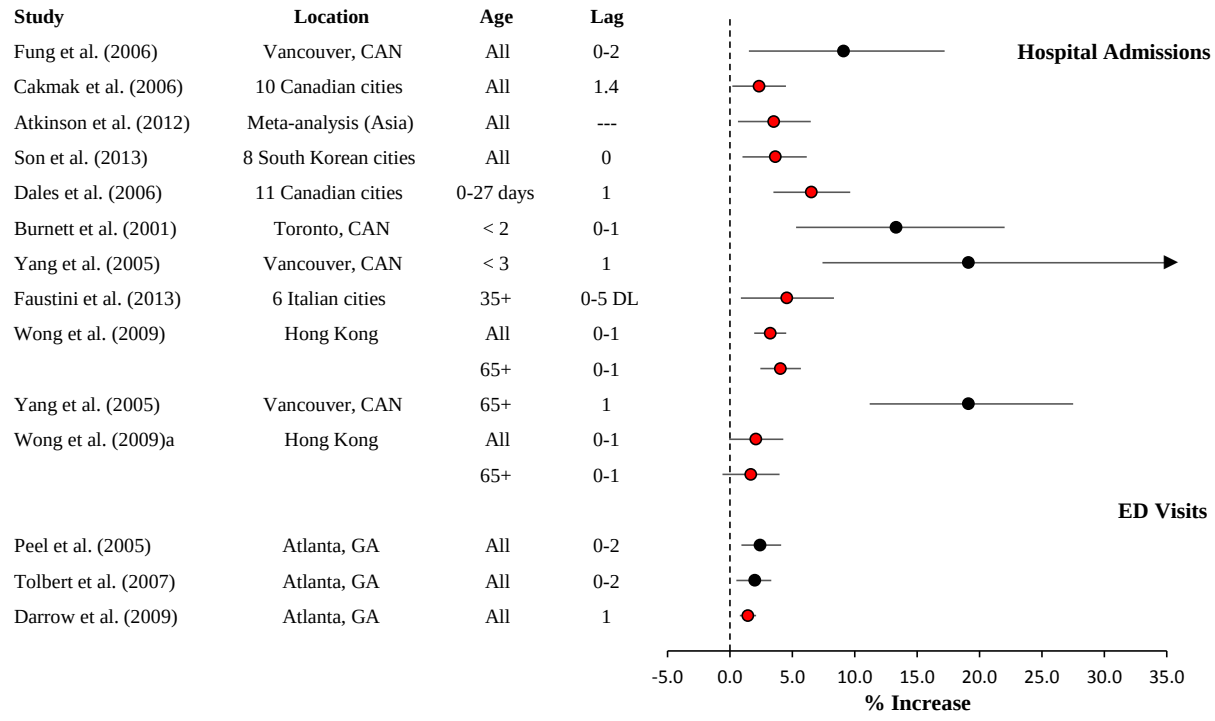
commute time metrics. Overall, these results suggest evidence of potential exposure misclassification for NO_2 with increasing distance from the central monitor across exposure metrics.



Source: Reprinted with permission of Nature Publishing Group Darrow et al. (2011).

Figure 5-12 Spatial correlations for nitrogen dioxide (NO_2) metrics in the Atlanta, GA area.

As detailed within this section, hospital admission and ED visit studies of all respiratory diseases consistently report positive associations with short-term increases in ambient NO_2 concentrations. As presented in Figure 5-13 and Table 5-34, associations are consistently observed in studies evaluated in the 2008 ISA for Oxides of Nitrogen as well as recent studies.



Note: Black circles = U.S. and Canadian studies evaluated in the 2008 Integrated Science Assessment for Oxides of Nitrogen; Red = recent studies. a = This estimate is for acute respiratory diseases, which comprise approximately 39% of all respiratory disease hospital admissions in Hong Kong. DL = distributed lag. Effect estimates are standardized to a 20-ppb increase in 24-h NO_2 or 30-ppb increase in 1-h max NO_2.

Figure 5-13 Percentage increase in all respiratory disease hospital admissions and emergency department (ED) visits in relation to nitrogen dioxide concentrations from U.S. and Canadian studies evaluated in the 2008 Integrated Science Assessment for Oxides of Nitrogen and recent studies.

Table 5-34 Corresponding risk estimate for studies presented in Figure 5-13.

Study	Location	Age	Avg Time	Lag	% Increase (95% CI)
Hospital Admissions					
Fung et al. (2006)	Vancouver, Canada	All	24-h avg	0−2	9.1 (1.5, 17.2)
†Cakmak et al. (2006)	10 Canadian cities	All	24-h avg	1.4	2.3 (0.2, 4.5)
†Atkinson et al. (2012)	Meta-analysis (Asia)	All	24-h avg	---	3.5 (0.6, 6.5)
†Son et al. (2013)	8 South Korean cities	All	24-h avg	0	3.6 (1.0, 6.1)
†Dales et al. (2006)	11 Canadian cities	0−27 days	24-h avg	1	6.5 (3.5, 9.6)
Burnett et al. (2001)	Toronto, Canada	<2	1-h max	0−1	13.3 (5.3, 22.0)
Yang et al. (2003)	Vancouver, Canada	<3	24-h avg	1	19.1 (7.4, 36.3)
†Faustini et al. (2013)	6 Italian cities	35+	24-h avg	0−5 DL	4.6 (0.9, 8.3)
†Wong et al. (2009)[a]	Hong Kong	All	24-h avg	0−1	3.2 (1.9, 4.5)
		65+	24-h avg	0−1	4.0 (2.4, 5.7)
Yang et al. (2003)	Vancouver, Canada	65+	24-h avg	1	19.1 (11.2, 27.5)
Wong et al. (2009)	Hong Kong	All	24-h avg	0−1	2.1 (−0.1, 4.3)
		65+	24-h avg	0−1	1.7 (−0.6, 4.0)
Emergency Department Visits					
Peel et al. (2005)	Atlanta, GA	All	1-h max	0−2	2.4 (0.9, 4.1)
Tolbert et al. (2007)	Atlanta, GA	All	1-h max	0−2	2.0 (0.5, 3.3)
†Darrow et al. (2011)	Atlanta, GA	All	1-h max	1	1.4 (0.8, 2.1)

CI = confidence interval, DL = distributed lag.

[a]This estimate is for acute respiratory diseases, which comprise approximately 39% of all respiratory disease hospital admissions in Hong Kong.

†Studies published since the 2008 ISA for Oxides of Nitrogen.

5.2.6.1 Summary of Aggregated Respiratory Conditions

Previous and recent epidemiologic studies consistently indicate associations between short-term increases in ambient NO_2 concentrations and increases in respiratory effects aggregated across specific conditions such as asthma, COPD, and respiratory infections. A majority of the available evidence is for hospital admissions for all respiratory diseases combined (Figure 5-13 and Table 5-34), with a few additional studies of ED visits for all respiratory diseases, lung function in adults with asthma or COPD, or medication sales for unspecified respiratory effects. Associations of NO_2 with respiratory disease hospital

admissions and ED visits are observed to be larger for children and older adults; limited evidence points to differences in risk by sex and SES.

With respect to the lag structure of associations across studies, evidence indicates that the strongest associations for all respiratory hospital admissions and ED visits are within the first few days after NO_2 exposure, specifically lags of 0 to 2 days. An examination of model specification indicated the NO_2-respiratory hospital admission relationship was robust to alternative lags and df for weather covariates (Son et al., 2013). Thus, varying approaches to modeling weather does not appear to be a source of confounding. NO_2 effect estimates were sensitive to using less than 6 df per year to account for temporal trends, but most studies did not model temporal trends with fewer df. The limited analysis of potential seasonal differences in associations, suggests that NO_2 associations with all respiratory disease hospital admissions are stronger during the summer (Son et al., 2013). In a study of all respiratory disease ED visits, similar associations were observed for 1-h max and 24-h avg NO_2 (Darrow et al., 2011).

The epidemiologic evidence for associations of NO_2 with aggregated respiratory effects is based on exposure assessment from central site monitors. In two study locations, Boston, MA (Section 2.5.2) and Atlanta, GA (Darrow et al., 2011), between-monitor correlation in ambient NO_2 concentration decreased with increasing distance. Thus, it is unclear the extent to which temporal variation in central site NO_2 concentrations represent variation in exposure among subjects. Also, studies of aggregated respiratory effects did not thoroughly examine potential confounding by traffic-related copollutants, which in many studies, showed moderate to high (r = 0.61−0.76, Table 5-33) correlations with NO_2. Limited evidence indicates that NO_2 associations with all respiratory hospital admissions and ED visits persisted in copollutant models with CO or $PM_{2.5}$ (Tolbert et al., 2007) (Figures 5-16 and 5-17). However, potential differential exposure measurement error resulting from central site exposure assessment limits inference from the copollutant model results. Further, given the variable nature of evidence for the effects of short-term NO_2 exposure among specific respiratory conditions (Sections 5.2.2, 5.2.4, 5.2.5), it is not clear whether the evidence for aggregated respiratory conditions reflects associations with each respiratory condition equally or a particular condition(s).

5.2.7 Respiratory Effects in Healthy Populations

Similar to populations with asthma and COPD, an array of respiratory outcomes has been examined in relation to short-term exposure to NO_2 in healthy populations. The 2008 ISA for Oxides of Nitrogen did not draw inferences specifically about respiratory effects of NO_2 exposure in healthy populations (U.S. EPA, 2008a) but described epidemiologic

associations of short-term increases in ambient NO_2 concentration with increases in respiratory symptoms and decreases in lung function in children. Evidence from experimental studies varied across outcomes, indicating no effects on respiratory symptoms or lung function in healthy adults. However, NO_2 exposure did affect underlying key events, inducing increases in airway responsiveness and PMNs in healthy adults generally at 1,000 ppb NO_2 exposure or higher. Recent evidence, which is from epidemiologic studies, continues to indicate NO_2-related respiratory effects in healthy populations, most consistently seen as increases in pulmonary inflammation.

5.2.7.1 Airway Responsiveness in Healthy Individuals

The 2008 ISA for Oxides of Nitrogen reported that increases in nonspecific airway responsiveness were observed in the range of 1,500 to 2,000 ppb NO_2 for 3-hour exposures in healthy adults (U.S. EPA, 2008a). Studies of airway responsiveness in healthy individuals were generally conducted using volunteers ages 18 to 35+ years. (Mohsenin, 1988) found that a 1-hour resting exposure to 2,000 ppb NO_2 increased responsiveness to methacholine. A mild increase in responsiveness to carbachol was observed following a 3-hour exposure to 1,500 ppb NO_2 with moderate intermittent exercise ($\dot{V}_E$ = 40 L/min; 10 of 30 minutes) (Frampton et al., 1991). Kulle and Clements (1988) also showed a tendency for greater FEV_1 decrements from methacholine challenge following 2-hour resting exposures to 2,000 and 3,000 ppb NO_2. Resting exposures to 100 ppb NO_2 for 1 hour have not affected carbachol or methacholine responsiveness in healthy subjects (Ahmed et al., 1983a; Hazucha et al., 1983). Two meta-analyses of the available literature confirm statistically significant effects of NO_2 exposures above 1,000 ppb, but not below, on airway responsiveness in healthy individuals (Kjaergaard and Rasmussen, 1996; Folinsbee, 1992). More recent studies of airway responsiveness in healthy individuals following NO_2 exposure are not available.

5.2.7.2 Lung Function Changes in Healthy Populations

Compared with evidence for airway responsiveness, the 2008 ISA for Oxides of Nitrogen reported weak evidence for the effects of NO_2 exposure on changes in lung function in the absence of a challenge agent in controlled human exposure and epidemiologic studies of healthy adults (U.S. EPA, 2008a). A small body of epidemiologic studies of children in the general population indicated associations between increases in ambient NO_2 concentration and decrements in lung function measured by supervised spirometry. Several recent studies, which are epidemiologic, contribute inconsistent evidence for ambient NO_2-associated lung function decrements in children in the general population.

Epidemiologic Studies of Children in the General Population

As in other populations, ambient NO_2 concentrations are more consistently associated with lung function decrements in children in the general population as measured by supervised spirometry than by home PEF. However, many studies of supervised spirometry did not find associations with ambient NO_2 concentrations. Locations, time periods, and ambient concentrations of oxides of nitrogen for these studies are presented in Table 5-35. The studies recruited children from schools, supporting the likelihood that study populations were representative of the population of children in the study areas.

Table 5-35 Mean and upper percentile oxides of nitrogen concentrations in epidemiologic studies of lung function in the general population.

Study[a]	Location	Study Period	Exposure Metric Analyzed	Mean/Median Concentration ppb	Upper Percentile Concentrations ppb
Altuğ et al. (2014)	Eskisehir, Turkey	Feb–Mar, 2007	24-h avg NO_2	Suburban: 9.4, Urban: 13.0 Traffic: 21.2	Max: 13.1 Max: 17.7 Max: 28.2
Castro et al. (2009)	Rio de Janeiro, Brazil	May, June, Sept, Oct 2004	24-h avg NO_2	49.2[b]	Max: 115[b]
Moshammer et al. (2006)	Linz, Austria	School yr, 2000–2001	8-h avg NO_2 (12–8 a.m.)	NR	NR
			24-h avg NO_2	9.3[b]	75th: 11.4[b]
Scarlett et al. (1996)	Surrey, U.K.	June–July 1994	1-h max NO_2	34.9	Max: 82
Linn et al. (1996)	Upland, Rubidoux, Torrance, CA	School yr, 1992–1994	24-h avg NO_2	33	Max: 96
Oftedal et al. (2008)	Oslo, Norway	Nov 2001–Dec 2002	24-h avg NO_2	14.4[b]	Max: 59.2[b]
Padhi and Padhy (2008)	West Bengal, India	June 2006–July 2007	24-h avg NO_2 indoor	Biomass fuel: 71.7, LPG: 27.5	75th: Biomass fuel: 90, LPG: 44
			24-h avg NO indoor	Biomass fuel: 46.7, LPG: 37.7	75th: Biomass fuel: 55, LPG: 30
Eenhuizen et al. (2013)	3 study areas, the Netherlands	Oct 2000–Nov 2001	24-h avg NO_2	16.0[b]	75th: 23.2[b] Max: 47.9[b]
Chang et al. (2012)	Taipei, Taiwan	Dec 1996–May 1997	6 day avg NO_2	31.8	75th: 41.7
Bagheri Lankarani et al. (2010)	Tehran, Iran	NR	24-h avg NO_2 24-h NO 24-avg NO_X	75.5, 17.6[b] 51.6, 40.4[b] 72.9, 38.8[b]	Max: 119, 25.5[b] Max: 85.1, 110[b] Max: 122, 94.7[b]

Table 5-35 (Continued): Mean and upper percentile oxides of nitrogen concentrations in epidemiologic studies of lung function in the general population.

Study[a]	Location	Study Period	Exposure Metric Analyzed	Mean/Median Concentration ppb	Upper Percentile Concentrations ppb
Steerenberg et al. (2001)	Utrecht, the Netherlands	Feb–Mar 1998	24-h avg NO_2 24-h avg NO	28.2[b] 30.2[b]	Max: 44.7[b] Max: 168[b]
	Bilthoven, the Netherlands		24-h avg NO_2 24-h avg NO	25.5[b] 7.4[b]	Max: 49.5[b] Max: 85.6[b]
Peacock et al. (2003)	Rochester upon Medway, U.K.	Nov 1996–Feb 1997	24-h avg NO_2 1-h max NO_2	17.4, 17.1, 19.2 28.5, 28.1, 31.8	Max: 39, 39, 43 Max: 67, 71, 98
Correia-Deur et al. (2012)	São Paolo, Brazil	Apr–Jul 2004	24-h avg	Mean: 69.9[b]	75th: 84.5[b] 90th: 102[b]
Van Der Zee et al. (2000) van der Zee et al. (1999)	Rotterdam, Nunspeet, Bodegraven/Reeuwij, Amsterdam, Meppel, the Netherlands	Three winters 1992–1993	24-h avg NO_2	27.1, 17.6[b]	Max: 50, 44.2[b]
		1993–1994		25.5, 13.3[b]	Max: 40.4, 28.7[b]
		1994–1995		25.0, 11.7[b]	Max: 43.6, 30.3[b]
Ranzi et al. (2004)	Emiglia-Romagna, Italy	Feb–May 1999	24-h avg NO_2	Urban: 37.0[b] Rural: 18.51[b]	NR NR
Ward et al. (2000)	West Midlands, U.K.	Jan–Mar 1997 May–July 1997	24-h avg NO_2	NR	NR
Roemer et al. (1998)	Sweden, Germany, Finland, Hungary, Norway, Italy, Greece, Czech Republic, the Netherlands	Winter 1993–1994	24-h avg NO_2	Across locations: 6.7–39.8[b]	NR
Timonen and Pekkanen (1997)	Kuopio, Finland	Feb–Apr 1994	24-h avg NO_2	Urban: 14.9[b] Suburban: 7.4[b]	Max: 41.5[b] Max: 27.1[b]
Schindler et al. (2001)	Aarau, Basel, Davos, Geneva, Lugano, Montana, Payerne, Wald, Switzerland	NR	24-h avg NO_2	19.5[b]	Max: 69.3[b]
Steinvil et al. (2009)	Tel Aviv, Israel	Sept 2002–Nov 2007	24-h avg NO_2	19.3	75th: 25.3 Max: 59.9
Cakmak et al. (2011a)	14 Canadian cities	Mar 2006–Mar 2007	24-h avg NO_2	12.6	95th: 29.4
Lepeule et al. (2014)	Boston, MA area	1999–2009	24-h avg NO_2	20.2[b]	95th: 23.9[b]
Son et al. (2010)	Ulsan, Korea	2003–2007	24-h avg NO_2	21.4	75th: 26.1 Max: 44.8
Agarwal et al. (2012)	Patiala, Punjab area, India	Aug–Jan, 2007–2009	1-mo avg NO_2	For 2008 Aug–Sep: 8.4[b] Oct–Nov: 21.9[b] Dec–Jan: 17.4[b]	NR
Weichenthal et al. (2011)	Ottawa, ON, Canada	NR	1-h avg NO_2	High traffic: 4.8 Low traffic: 4.6	Max: 11 Max: 10

Table 5-35 (Continued): Mean and upper percentile oxides of nitrogen concentrations in epidemiologic studies of lung function in the general population.

Study[a]	Location	Study Period	Exposure Metric Analyzed	Mean/Median Concentration ppb	Upper Percentile Concentrations ppb
Thaller et al. (2008)	Galveston, TX	Summers 2002, 2003, 2004	24-h avg NO_2 1-h max NO_2	1.2 3.2	Max: 7.1 Max: 27.7
Strak et al. (2012)	Bilthoven, the Netherlands	Mar−Oct 2009	5-h avg NO_X 5-h avg NO_2	36 20	Max: 96 Max: 34
Dales et al. (2013)	Sault Ste. Marie, ON, Canada	May−Aug 2010	10-h avg NO_2 (8 a.m.−6 p.m.)	Near steel plant: 7.1 Distant site: 4.5	NR

NR = not reported, NO = nitric oxide, NO_2 = nitrogen dioxide, NO_X = sum of NO and NO_2, LPG = liquefied petroleum gas.
[a]Studies presented in order of first appearance in the text of this section.
[b]Concentrations converted from µg/m^3 to ppb multiplying by 0.532 assuming standard temperature (25°C) and pressure (1 atm).

The most informative studies are those examining NO_2 concentrations outdoor schools or at a central site adjacent to schools, which may represent a component of the subjects' ambient exposures. These metrics were inconsistently associated with lung function in children (Altuğ et al., 2014; Castro et al., 2009; Moshammer et al., 2006; Scarlett et al., 1996). The inconsistent evidence does not appear to be related to the health status of the study population. NO_2 was not associated with lung function in children without respiratory symptoms (Altuğ et al., 2014), but results were inconsistent in groups of children with prevalence of asthma or wheeze of 5 or 9% (Castro et al., 2009; Scarlett et al., 1996). Associations were found with same-day NO_2 and NO_2 averaged over 3 to 8 days but were inconsistent for lag day 1. Linn et al. (1996) found that a 20-ppb increase in lag 0 of central site NO_2 was associated with a −5.2 mL (95% CI: −13, 2.3) change in evening FEV_1 among children in three southern California communities. These results have relatively strong inference about an association with ambient NO_2 exposure because daily average personal and ambient NO_2 were reported to be well correlated (r = 0.63).

In the studies examining ambient NO_2 metrics representing subjects' school or total personal exposure, there is uncertainty regarding copollutant confounding. Associations were found with CO, PM_1, and $PM_{2.5}$ (Castro et al., 2009; Moshammer et al., 2006; Linn et al., 1996); other traffic-related pollutants were not examined. The lack of examination of other traffic-related pollutants particularly weakens the implications of Oftedal et al. (2008), who observed high correlations among NO_2, $PM_{2.5}$ and PM_{10} estimated by a dispersion model (r = 0.83−0.95). Potential confounding also is an uncertainty in a recent study in India that found decreases in lung function in association with indoor NO_2, NO, and CO from cooking fuel but did not specify model covariates (Padhi and Padhy, 2008). Linn et al. (1996) did not provide quantitative results and indicated only that NO_2 effect

estimates lost statistical significance with adjustment for $PM_{2.5}$, which was weakly correlated with NO_2 (r = 0.25). Among children in Austria, with pollutants measured at a site adjacent to the school, NO_2 effect estimates were unchanged with adjustment for moderately correlated $PM_{2.5}$ (r = 0.54). (Moshammer et al., 2006). A 25-ppb increase in lag 1 of 8-h avg NO_2 (12−8 a.m.) was associated with a −4.1% change (95% CI: −6.4, −1.7) in FEV_1 in the single-pollutant model and a −4.7% change (95% CI: −7.3, −2.0) with adjustment for $PM_{2.5}$. $PM_{2.5}$ effect estimates were attenuated or became positive with adjustment for NO_2. While these results provide evidence for an independent association with NO_2, other model covariates were not specified, and potential confounding by other factors such as weather cannot be assessed.

Among studies of supervised spirometry, evidence was inconsistent for associations with NO_2 and NO ascertained from central sites (Eenhuizen et al., 2013; Chang et al., 2012; Bagheri Lankarani et al., 2010; Oftedal et al., 2008; Steerenberg et al., 2001). Results were inconsistent for PEF as well as FEV_1, and no association was found with a measure of airway resistance. Controlled human exposure studies, conducted in healthy adults, do not consistently indicate effects on ambient-relevant NO_2 exposures on FEV_1 (see below) or airway resistance (Section 4.3.2.2). In addition to the inconsistent findings, there is uncertainty as to whether the NO_2 concentrations from an average of area central sites or one central site represent the variability in NO_2 concentrations across the study area or subjects' ambient exposure, particularly in the many cross-sectional studies that make up the evidence base. Inconsistencies also were found between studies that measured NO_2 at sites located 2 km from children's schools (Chang et al., 2012; Steerenberg et al., 2001). Repeated measures and cross-sectional studies found associations with adjustment for time-varying factors such as weather as well as between-subject factors such as height, weight, smoking exposure, and SES. However, copollutant confounding was not examined, and lung function also was associated with the traffic-related pollutants CO and BS (Chang et al., 2012; Steerenberg et al., 2001) as well as PM_{10}, SO_2, and O_3.

A fairly large body of studies, conducted in various European countries, does not strongly support NO_2-associated decrements in PEF in children. These studies were similar to studies of supervised lung function in that they examined populations that included children with respiratory symptoms, asthma, or atopy and measured NO_2 concentrations at central sites and schools. Outdoor school NO_2 concentrations were associated with an increase in PEF in children with 25% wheeze prevalence (Peacock et al., 2003). Associations with central site NO_2 tended to be positive (Roemer et al., 1998; Timonen and Pekkanen, 1997) or null (Ranzi et al., 2004; Ward et al., 2000; van der Zee et al., 1999). A recent study found an NO_2-associated decrease in PEF among children that was independent of CO (Correia-Deur et al., 2012) (Table 5-36). Both NO_2 and CO were averaged across multiple city sites.

Table 5-36 Epidemiologic studies of lung function in children and adults in the general population.

Study Population Examined and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Lag day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Children in the General Population				
Linn et al. (1996) Upland, Rubidoux, Torrance, CA n = 269, 4th−5th grades Repeated measures. Supervised spirometry. Examined 1 week/season for 2 yr. Recruitment from schools. 75−90% follow-up participation across communities. Repeated measures ANOVA adjusted for year, day, temperature, rain. Time spent outdoors = 101−136 min across seasons and communities.	NO_2-central site 24-h avg # sites NR, no site in Torrance *r* = 0.63 correlation with personal NO_2	0	p.m. FEV_1: −5.2 (−13, 2.3) mL p.m. FVC: −3.6 (−12, 4.6) mL Diurnal change FEV_1: −7.8 (−14, −1.5) Diurnal change FVC: −2.2 (−9.6, 4.9)	No quantitative results. NO_2 association reported to lose statistical significance with $PM_{2.5}$ adjustment. Associations found with $PM_{2.5}$, weak for O_3. Weak correlation with $PM_{2.5}$. *r* = 0.25.
†Castro et al. (2009) Rio de Janeiro, Brazil n = 118, ages 6−15 yr, 18.4% with asthma Repeated measures. Supervised PEF. Recruitment from school. Examined daily for 6 weeks. 9−122 observations/subject. No information on participation rate. Mixed effects model with random effect for subject and adjusted for weight, height, sex, age, asthma, smoking exposure, time trend, temperature, relative humidity.	NO_2-school outdoor 24-h avg School was within 2 km of homes.	1 1−2 avg 1−3 avg	PEF, L/min: 0.04 (−0.58, 0.65) −0.60 (−1.3, 0.14) −0.83 (−1.7, 0.02)	No copollutant model. Associations also found with PM_{10}. Associations with CO, SO_2 had wide 95% CIs.
Scarlett et al. (1996) Surrey, U.K. n = 154, ages 7−11 yr, 9% with wheeze Repeated measures. Supervised spirometry. Examined daily for 6 weeks. Recruitment from school. No information on participation rate. Lung function adjusted for machine, operator, day of week. Individual subject regressions adjusted for temperature, humidity, pollen. Pooled estimates obtained using weighting method.	NO_2-school outdoor 1-h max		$FEV_{0.75}$: 0.30% (−0.29, 0.89) FVC: 5.5% (−5.1, 17%)	No copollutant model. Association found with PM_{10}. No to moderate correlations with NO_2. *r* = 0.07 for PM_{10}, 0.50 for 8-h max O_3.

Table 5-36 (Continued): Epidemiologic studies of lung function in children and adults in the general population.

Study Population Examined and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Lag day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Moshammer et al. (2006) Linz, Austria n = 163, ages 7−10 yr Repeated measures. Supervised spirometry. Examined every 2 weeks for school yr. Recruitment from schools. No information on participation rate. GEE model, covariates not specified.	NO_2-central site 8-h avg (12 a.m.−8 a.m.) Site adjacent to school	0	FEV_1: −4.1% (−6.4, −1.7) FVC: −2.7% (−5.1, −0.33)	With $PM_{2.5}$: −4.7% (−7.3, −2.0) $PM_{2.5}$ results attenuated or become positive. Associations also found for PM_1, PM_{10}. Moderate correlations with NO_2. r = 0.53 for PM_1, 0.54 for $PM_{2.5}$, 0.62 for PM_{10}.
†Altuğ et al. (2014) Eskisehir, Turkey, Feb−Mar, 2007 n = NR, ages 9−13 yr, no upper respiratory symptoms Cross-sectional. Supervised spirometry. Recruitment from schools of participants of a larger study. No information on participation rate. Logistic regression adjusted for sex, age, asthma, parental smoking, coal or wood stove use, parental education, height, weight, daily average temperature.	NO_2-outdoor school 24-h avg 1 site at each of 16 schools	0−6 avg	FEV_1: 0% (−14, 17) FVC: 3.8% (−7.3, 16)	No copollutant model. O_3 associated with PEF only. Strong inverse correlation with NO_2. Pearson r = −0.80. NO_2 and $PM_{2.5}$ reported to be highly correlated.
†Oftedal et al. (2008) Oslo, Norway N = 2,170, ages 9−10 yr, 5.5% with asthma Cross-sectional. Supervised spirometry. Recruitment from a birth cohort. 67% participation, 60% follow-up. Examined subjects had more “Westernized” parents. Linear regression adjusted for age, sex, height, BMI, current asthma, early life maternal smoking, parental ethnicity, education, smoking, and atopy, lag 1−3 temperature, neighborhood variables (% married, % with income < median, etc.), long-term NO_2.	NO_2-dispersion model NO_2-central site 24-h avg 1 city site	1−3 avg 1−7 avg 1−30 avg	Quantitative results not reported. Association observed with lag 1−3 day avg and 1−7 day avg. Larger effect estimated for lag 1−30 day avg. Central site no association.	No copollutant model. No association reported for $PM_{2.5}$. Correlations among pollutants = 0.83−0.95. Short-term association attenuated with adjustment for early or lifetime NO_2. r = 0.46−0.77 among NO_2 metrics.

Table 5-36 (Continued): Epidemiologic studies of lung function in children and adults in the general population.

Study Population Examined and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Lag day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Steerenberg et al. (2001) Utrecht, Bilthoven, the Netherlands n = 126, ages 8−13 yr, 28% respiratory disease, 20% allergy Repeated measures. Supervised PEF. Examined 1/week for 7−8 weeks. Recruitment from urban and suburban schools. 65% participation. Mixed effects model adjusted for sex, age, # cigarettes smoked in home, presence of a cold, history of respiratory symptoms and allergy. No consideration for potential confounding by meteorological factors.	NO_2-central site 15-h avg (8 a.m.−11 p.m.) 24-h avg Site within 2 km of schools	0 0−2 avg	PEF mL/min: Urban: −17 (−35,0) Suburban: 7, p >0.05 Urban: 0, p > .05 Suburban: 6, p > 0.05	No copollutant model. BS associated with PEF. Correlation with NO_2 and NO NR. Association also found with PM_{10}.
	NO-central site 15-h avg (8 a.m.−11 p.m.) 24-h avg	15-h avg 0−2 avg	Urban: 1, p > 0.05 Suburban: 0, p > 0.05 Urban: −6 (−12, 0) Suburban: 6, p > 0.05	
†Chang et al. (2012) Taipei, Taiwan n = 2,919, ages 12−16 yr Cross-sectional. Supervised spirometry. Recruitment from schools. No information on participation rate. Regression model adjusted for residence in district, age, sex, height, weight, temperature, rainfall.	NO_2-central site 4-h avg (8 a.m.−12 p.m.) 10-h avg (8 a.m.−6 p.m.) Average of 5 city sites within 2 km of schools	0 1 2	FEV_1 in mL: −25 (−57, 7.5) −41 (−70, −11) −2.5 (−50, 45)	No copollutant model. Associations also found with SO_2, CO, O_3, PM_{10}.
†Eenhuizen et al. (2013) 3 study areas, the Netherlands n = 880, age 8 yr Cross-sectional. Recruitment from intervention study of mattress allergy covers. Valid data on 49% subjects, who had higher parental education, less likely to have pets. Linear regression adjusted for sex, age, height, weight, prenatal smoke exposure, smoking in home, gas stove, parental allergy, dampness in home, parental education, season, temperature, humidity.	NO_2-central site 1 site	0 1	Interrupter resistance in kPA*s/L: 0 (−0.04, 0.04) −0.02 (−0.06, 0.03) Positive effect estimate indicates increase in resistance	No associations with PM_{10} or BS. Moderate correlations with NO_2. Pearson r = 0.47 for PM_{10}, 0.60 for BS.
†Bagheri Lankarani et al. (2010) Tehran, Iran n = 562, elementary school age Repeated measures. Examined daily for 6 weeks. No information on participation rate. 158 case-days. Case crossover with control dates as 2 weeks before and after case date. Conditional logistic regression adjusted for daily temperature, lag 0−6 day avg PM_{10}.	NO-central site 24-h avg 2 city sites	0−6 avg	PEF <50% predicted: OR: 18 (1, 326)	No copollutant model. PM_{10} associated with decreased odds of large PEF decrement.

Table 5-36 (Continued): Epidemiologic studies of lung function in children and adults in the general population.

Study Population Examined and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Lag day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
†Padhi and Padhy (2008) West Bengal, India n = 755 from biomass fuel homes, 372 from liquified petroleum gas homes, ages 5−10 yr Cross-sectional. Supervised spirometry. Recruitment method and participation not reported. Multiple regression adjusted for unspecified covariates.	NO_2-indoor home 24-h avg	NR	Biomass fuel homes Lung function units NR FEV_1: −1.05 (−1.75, −0.35) FVC: −1.09 (−1.58, −0.61)	No copollutant model. CO also associated with lung function. Correlation with NO_2 NR.
			Liquified gas petroleum homes FEV_1: −5.41 (−8.33, −2.50) FVC: −5.17 (−9.17, −1.17)	SPM, SO_2, O_3 also associated with lung function.
†Correia-Deur et al. (2012) São Paolo, Brazil n = 31, ages 9−11 yr, no allergic sensitization Repeated measures. Daily supervised spirometry for 15 school days. Number of observations not reported. Recruitment from school. 86% participation. Allergic sensitization ascertained by skin prick test, blood eosinophils, and serum IgE. GEE with autoregressive correlation matrix adjusted for date, school absence, temperature, humidity.	NO_2-outdoor school 24-h avg	0	PEF: −1.0% (−1.7, −0.35)	Lag 0, all subjects with CO: −1.5% (−3.0, 0) Moderate correlation with NO_2. r = 0.51. CO association persists with NO_2 adjustment. with SO_2: −1.9% (−3.3, −0.4) with PM_{10}: −0.8% (−4.4, 3.1) with O_3: −1.5% (−3.3, 0.38) Moderate correlations with NO_2. r= 0.59, 0.60, 0.40. O_3 association persists with NO_2 adjustment. SO_2 & PM_{10} attenuated.
Peacock et al. (2003) Rochester upon Medway, U.K. N = 177, ages 7−13 yr, 25% with wheeze	NO_2-outdoor school 24-h avg	0−4 avg	PEF: −0.20 (−3.0, 2.6) OR for PEF > 20%: 2.3 (1.0, 5.4)	No copollutant model. $PM_{2.5}$ also associated with PEF decrement >20%. Correlation NR.
Repeated measures. Home PEF. Examined daily for 13 weeks. 14−63 observations/subject. Recruitment from rural and urban schools. No information on participation rate. Individual subject regressions adjusted for day of week, date, temperature. Pooled estimates obtained using weighting method.	1-h max		PEF: 1.2 (−1.5, 3.9) OR for PEF > 20%: 1.3 (0.5, 3.4)	

Table 5-36 (Continued): Epidemiologic studies of lung function in children and adults in the general population.

Study Population Examined and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Lag day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
van der Zee et al. (1999) Rotterdam, Bodegraven/Reeuwijk, Amsterdam, Meppel, Nunspeet, the Netherlands n = 633, ages 7−11 yr, 63% with symptoms, 26 and 38% with asthma Repeated measures. Home PEF. Examined daily for 3 mo. Recruitment from school and mail. 47% responded to initial survey, 80% follow-up participation. Logistic regression adjusted for minimum temperature, day of week, time trend, influenza.	NO_2-central site 24-h avg 1 site per community	0 0−4 avg	ORs Urban: 0.96 (0.79, 1.2) Suburban: 0.77 (0.54, 1.1) Urban: 1.1 (0.93, 1.3) Suburban: 0.99 (0.72, 1.4)	Associations found for PM_{10}, BS, SO_4, and SO_2 in urban area. Correlations NR.
Roemer et al. (1998) Germany, Finland, the Netherlands, Czech Republic, Norway, Italy, Greece, Hungary, Sweden—26 locations n = 2,010, ages 6−12 yr, atopy prevalence: 7−81% Repeated measures. Home PEF. Examined daily for 2 mo. 85% of enrolled included in analysis. Regression model adjusted for minimum temperature, school day, time trend. Individual panel results combined in a meta-analysis.	NO_2-central site 24-h avg	0 0−6 avg	PEF, L/min: 0.15 (−0.19, 0.49) 0.23 (−1.2, 1.6)	Association found with PM_{10} and BS, but not consistently across lags.
Ranzi et al. (2004) Emiglia-Romagna, Italy n = 118, ages 6−11 yr, 77% with asthma, 67% with atopy Repeated measures. Home PEF. Examined daily for 12 weeks. 98.4% follow-up participation. Recruited from schools. GLM adjusted for sex, medication use, symptoms, temperature, humidity	NO_2-central site 24-h avg # sites NR	0	No quantitative data. Figure shows no association in group with and without atopy.	$PM_{2.5}$ associated with PEF in urban group.
Ward et al. (2000) West Midlands, U.K. n = 147, age 9 yr, 24% with symptoms, 31% with atopy Repeated measures. Home PEF. Examined daily for two 8-week periods. Recruitment from schools. Individual subject regressions adjusted for time trend, day of week, meteorological variables, pollen count. Individual regressions pooled with weighting method.	NO_2-central site 24-h avg 2 sites	0, 1, 2, 3, 0−4 avg	No quantitative data. Figure shows no association across lags, except at lag day 0 in symptomatic group.	No copollutant model. Associations with $PM_{2.5}$ equally inconsistent.

Table 5-36 (Continued): Epidemiologic studies of lung function in children and adults in the general population.

Study Population Examined and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Lag day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Timonen and Pekkanen (1997) Kuopio, Finland n = 169, ages 7−12 yr, children with cough Repeated measures. Home PEF. Examined daily for 3 mo. Recruitment from schools. 86% participation. Linear mixed model adjusted for time trend, weekend, minimum temperature, relative humidity.	NO_2-central site 24-h avg # sites NR 26% missing data were modeled, r = 0.58	0	FEV_1: Urban: 11 (−14, 35) Suburban: −6.5 (−40, 27)	Associations found for SO_2 in urban group. Weak correlations with NO_2. r= 0.22.
		1−4 avg	PEF: Urban: 13 (−24, 50) Suburban: −22 (−87, 43)	
Adults in the General Population				
†Strak et al. (2012) Utrecht area, the Netherlands n = 31, adults ages 19−26 yr, all healthy, non-smoking Repeated measures. Supervised spirometry. Examined 3−7 times. 107 observations. Recruitment from university. No information on participation rate. Well-defined outdoor exposures at various traffic/non-traffic sites. Heart rate maintained during intermittent exercise. Higher probability of associations found by chance alone. Mixed effects model adjusted for temperature, relative humidity, season, high/low pollen, respiratory infection.	NO_2-personal outdoor 5-h avg Measured next to subjects during outdoor exposures.		FVC post-exposure:	FVC with PNC: NO_2: −3.0% (−7.2, 1.4) NO_X: −0.11% (−2.6, 2.5) Moderate to high correlation with NO_2. Spearman r = 0.56, 0.75. PNC association attenuated with adjustment for NO_2 or NO_X.
		0-h	−4.3% (−7.4, −1.0)	
		2-h	−3.5% (−6.5, −0.43)	
		18-h	−4.5% (−7.4, −1.4)	
	NO_X-personal outdoor 5-h avg			
		0-h	−1.6% (−2.6, −0.51)	
		2-h	−2.0% (−4.9, −0.16)	
		18-h	−2.5% (−5.4, −0.69)	
†Dales et al. (2013) Sault Ste. Marie, Ontario, Canada n = 59, adults mean (SD) age 24.2 (5.8) yr, all healthy Repeated measures. Supervised spirometry. Examined 10 times. Total observations NR. Recruitment from university. No information on participation rate. Well-defined outdoor exposures near steel plant and university campus 4.5 km away. Exposures occurred at rest except for 30-min exercise to increase heart rate to 60% predicted maximum. Mixed effect model with autoregressive correlation matrix and adjusted for site, day of week, mean temperature, humidity.	NO_2-on site of outdoor exposure 10-h avg (8 a.m.−6 p.m.)	0-h Post-exposure	% predicted FEV_1: −10.9 (−13.3, −8.6) % predicted FVC: −9.2 (−14.5, −3.9)	No copollutant model. Associations found with UFP and $PM_{2.5}$. Correlations NR. All pollutants higher at steel plant than at university campus. Associations also found with SO_2, O_3.

Table 5-36 (Continued): Epidemiologic studies of lung function in children and adults in the general population.

Study Population Examined and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Lag day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
†Weichenthal et al. (2011) Ottawa, Canada n = 42, adults ages 19−58 yr, from non-smoking homes, 95% white, 62% with allergies, 33% with asthma Repeated measures. Supervised spirometry. Most examined 3 times. 118 observations. 1-h outdoor exposures during cycling in low and high traffic areas. Recruitment from public advertisements. No information on participation rate. Differential exposure measurement error for personal PM and VOCs and central site NO_2. Mixed effects models with random subject effect adjusted for temperature during cycling, average heart rate. Adjustment for relative humidity, day of week did not affect results.	NO_2-central site 1-h avg 1 site	1-h 4-h Post-exposure	FEV_1 in L: 0.54 (−0.15, 1.2) L 0.40 (−0.12, 0.92) L	No copollutant model. Lung function not associated with O_3 or VOCs, UFP, BC, $PM_{2.5}$.
†Thaller et al. (2008) Galveston, TX n = 142, lifeguards at work, ages 16−27 yr, 13% with asthma, 22% with allergies Repeated measures. Supervised spirometry. Recruitment from worksite. 1,140 observations. Self-report of physician-diagnosed asthma. 81% follow up participation. GLM, covariates not specified.	NO_2 & NO_X-central site 24-h avg, 1-h max 1 site 4−12 km from beaches	0	No quantitative data. NO_2 and NO_X reported not to be significantly associated with lung function.	No copollutant model.
Schindler et al. (2001) Aarau, Basel, Davos, Geneva, Lugano, Montana, Payerne, Wald, Switzerland n = 3,912, ages 18−60 yr, non-smokers Cross-sectional. Supervised spirometry. Recruitment from registry and SALPADIA cohort. Sample representative of full cohort. Regression model adjusted for sex, age, height, weight, day of week, temperature, relative humidity. Adjustment for asthma medication or wheeze did not alter results.	NO_2-central site 24-h avg 1 site per city	0 0−3 avg	FEV_1: −2.5% (−4.5, −0.48) −2.9% (−5.9, 0.21)	with TSP: −1.2% (−3.8, 1.6)
Van Der Zee et al. (2000) Rotterdam, Bodegraven/Reeuwijk, Amsterdam, Meppel, Nunspeet, the Netherlands	NO_2-central site 24-h avg	0	OR for PEF decrease >10% Urban: 0.85 (0.59, 1.2) Suburban: 0.72 (0.50, 1.05)	No copollutant model. PEF associated with PM_{10} and SO_4 in urban group.
n = 274, ages 50−70 yr, no symptoms in previous 12 mo Repeated measures. Home PEF. Examined daily for 3 mo. Recruitment from mailings. 81% enrolled included in final analysis. Logistic regression adjusted for minimum temperature, day of week, time trend, influenza.	1 site per community	0−4 avg	Urban: 0.46 (0.20, 1.08) Suburban: 0.56 (0.27, 1.16)	Wide range of correlations with NO_2. Spearman r = 0.16−0.72 for PM_{10}, 0.25−0.65 for BS.

Table 5-36 (Continued): Epidemiologic studies of lung function in children and adults in the general population.

Study Population Examined and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Lag day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
†Cakmak et al. (2011a) 15 cities, Canada n = 5,011, ages 6−79 yr, mean age 39 yr Cross-sectional. Supervised spirometry. Recruitment by random sampling of households. No information on participation rate. GLMM adjusted for age, sex, income, education, smoking, random effect for site. Adjustment for temperature and relative humidity did not alter results.	NO_2-central site 24-h avg # sites NR	0	% predicted FEV_1: −1.6 (−2.9, −0.35)	No copollutant model. O_3 and $PM_{2.5}$ also associated with lung function. Correlations NR.
†Lepeule et al. (2014) Boston, MA area n = 776, all male, mean (SD) age at baseline 72.3 (6.8) yr, Normative Aging Study Longitudinal. Supervised spirometry. Examined 1−4 times over 10 yr. No information on recruitment over follow-up participation. Linear mixed effects model adjusted for age, log-height, race, education, standardized weight, smoking status, pack-years smoking, chronic lung condition, methacholine responsiveness, medication use season, day of week, visit number, temperature, humidity. Adjusting for cardiovascular disease did not alter results.	NO_2-central site 24-h avg Average of 5 sites in Boston area. Median 21.4 km from subjects' homes.	0 0−2 avg 0−27 avg	FEV_1: −0.18% (−1.89, 1.57) −1.62% (−3.89, 0.70) −13.0% (−17.9, 7.75) Low IL-6 gene methylation −11.6% (−17.5, −5.32) High IL-6 gene methylation −13.0% (−18.7, −6.95)	No copollutant model. Associations found with BC, CO, $PM_{2.5}$. Moderate correlation with NO_2. Spearman r = 0.59, 0.52, 0.62, respectively. BC and $PM_{2.5}$ measured at one Boston site. Association also found with O_3. r = −0.31.
†Steinvil et al. (2009) Tel Aviv, Israel n = 2,380, mean age 43 (SD:11) yr, healthy non-smokers Cross-sectional. Supervised spirometry. Recruitment from ongoing survey of individuals attending health center. No information on participation rate. Linear regression adjusted for sex, age, height, BMI, exercise intensity, education, temperature, relative humidity, season, year.	NO_2-central site 24-h avg 3 sites within 11 km of homes	0 5 0−6 avg	FEV_1: −16 (−64, 33) mL −55 (−103, −6.3) mL −97 (−181, −13) mL	w/CO (lag 5): −19 (−88, 50) w/SO_2 (lag 5): −7.8 (−72, 56) SO_2 and CO results persist with adjustment for NO_2. High correlations with NO_2. Pearson r = 0.75 for CO, 0.70 for SO_2.

Table 5-36 (Continued): Epidemiologic studies of lung function in children and adults in the general population.

Study Population Examined and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Lag day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
†Son et al. (2010) Ulsan, Korea n = 2,102, mean age 45 (SD: 17) yr, mean % predicted FEV_1: 83% Cross-sectional. Supervised spirometry. Recruitment during meeting of residents. No information on participation. Regression model adjusted for age, sex, BMI. Did not consider potential confounding by weather, season, or time trend. High correlation among exposure assessment methods. r = 0.84−0.96.	NO_2-central site 13 site average	0−2 avg	% predicted FVC: −7.9 (−10, −5.6)	Associations found with PM_{10}, O_3, SO_2, CO. NO_2 effect estimate slightly reduced with adjustment for O_3. No copollutant model with PM_{10} or SO_2.
	Nearest site		−6.9 (−8.8, −5.0)	
	Inverse distance weighting		−6.9 (−9.1, −4.7)	
	Kriging All 24-h avg		−7.4 (−9.8, −5.1)	
†Agarwal et al. (2012) 5 locations with agricultural burning around Patiala City, Punjab, India. n = 50, ages 13−53 yr, 80% adults, no respiratory conditions Repeated measures. Supervised spirometry. Examined 2 times/mo for 6 mo in each of 3 years. Total observations NR. No information on recruitment method. 40% follow-up participation. Linear regression. Did not report whether covariates were included.	NO_2-central site 24-h avg 1 site per location	1-mo avg	FEV_1: −8.9%, p = 0.054 FVC: −7.5%, p = 0.064	No copollutant model. Association found with $PM_{2.5}$. Correlation with NO_2 NR. Association also found with PM_{10} and SO_2.

Note: Studies are organized by population examined, and more informative studies in terms of exposure assessment method and potential confounding considered are presented first.

GLM = Generalized linear model, BMI = body mass index, ICS = inhaled corticosteroid, SES = socioeconomic status, GEE = generalized estimating equations, CI = confidence interval, CO = carbon monoxide, FEV_1 = forced expiratory volume in 1 second, FVC = forced vital capacity, GLMM = generalized linear mixed model, kPa = kilopascal, IL = interleukin, NO = nitric oxide, NO_2 = nitrogen dioxide, NO_X = sum of NO and NO_2, NR = not reported, O_3 = ozone, OR = odds ratio, PEF = peak expiratory flow, PM = particulate matter, PNC = particle number concentration, SD = standard deviation, SO_2 = sulfur dioxide, TSP = total suspended particles, UFP = ultrafine particles, VOC = volatile organic compound..

[a]Effect estimates were standardized to a 20-ppb increase in 24-h avg NO_2 and a 30-ppb increase 1-h max NO_2. Effect estimates for other averaging times (1-h avg to 15-h avg) are not standardized but presented as they are reported in their respective studies (Section 5.1.2.3).

†Studies published since the 2008 ISA for Oxides of Nitrogen.

Adults in the General Population

In studies reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a), increases in ambient NO_2 concentration were associated with decrements in lung function in adults in the general population as measured by supervised spirometry (Schindler et al., 2001) but not home peak flow (Van Der Zee et al., 2000). Recent studies conducted supervised spirometry, and while the results were inconsistent overall, the studies with stronger exposure assessment and/or examination of copollutant confounding indicated ambient NO_2-associated decreases in lung function in healthy adults. Overall, studies examined a wide range of ages (i.e., 18−79 years) and a mix of healthy populations and those including adults with asthma or allergies, but these factors did not appear to influence the results. Van Der Zee et al. (2000) found no association in adults with or without respiratory symptoms. Locations, time periods, and ambient concentrations of oxides of nitrogen for these studies are presented in Table 5-36.

While many studies found lung function decrements in adults in the general population in association with higher ambient NO_2 concentrations, they do not strongly inform the independent effects of NO_2 exposure (Lepeule et al., 2014; Cakmak et al., 2011a; Son et al., 2010; Steinvil et al., 2009). A major uncertainty is potential confounding. Son et al. (2010) did not examine confounding by meteorological or other time-varying factors. Studies found associations with the traffic-related pollutants CO, BC, and $PM_{2.5}$, which were moderately to highly correlated with NO_2 (r = 0.56 to 0.75) (Lepeule et al., 2014; Agarwal et al., 2012; Cakmak et al., 2011a; Son et al., 2010; Steinvil et al., 2009). Copollutant models were not analyzed, except in Steinvil et al. (2009), where the NO_2 effect estimate was attenuated with CO adjustment, and NO_2 results were mixed among the various lags examined. Lung function also was associated with PM_{10}, total suspended particles (TSP), SO_2, and O_3, and in copollutant models, NO_2 associations remained with adjustment for TSP or O_3 (Son et al., 2010; Schindler et al., 2001) but not for highly correlated SO_2 (r = 0.70) (Steinvil et al., 2009). Another uncertainty pertains to central site ambient NO_2, particularly whether NO_2 concentrations measured at the nearest site, one city site, averaged across multiple sites, or spatially interpolated by inverse distance weighting or kriging were equally representative of ambient exposure among subjects distributed within a city or across multiple communities. Differences in exposure measurement error between subjects may influence results, particularly in cross-sectional studies (Cakmak et al., 2011a; Son et al., 2010; Steinvil et al., 2009; Schindler et al., 2001) and a longitudinal study collecting one to four measures of lung function over 10 years (Lepeule et al., 2014).

Ambient concentrations may better represent exposures in situations when people are outdoors. In adults cycling in various traffic and nontraffic locations or lifeguards

working outdoors, lung function before and after repeated outdoor exposures were not associated with NO_2 assessed from a central site (Weichenthal et al., 2011; Thaller et al., 2008). However, in healthy adults, lung function decrements were associated with NO_2 measured on site of outdoor activity in locations that varied in traffic (Strak et al., 2012) or distance from a steel plant (Dales et al., 2013). Lung function decreased immediately after and 2 to 18 hours after the outdoor exposure period (Dales et al., 2013; Strak et al., 2012). These studies have stronger inference than the aforementioned central site studies because NO_2 measurements are aligned with subjects in both time and space. Both outdoor exposure studies found associations with the moderately to highly correlated traffic-related copollutants $PM_{2.5}$ absorbance, EC, metal components of $PM_{2.5}$, UFP/particle number concentration (PNC), and/or $PM_{2.5}$ (r = 0.67−0.87). Only Strak et al. (2012) examined copollutant models and found that NO_2 associations persisted with adjustment for PNC, EC, $PM_{2.5}$, and other $PM_{2.5}$ components. A 25-ppb increase in 5-h avg NO_2 was associated with a −4.3% (95% CI: −7.4, −1.0%) change in FVC and a −3.0% (95% CI: −7.2, 1.4%) change with adjustment for PNC. The NO_X association was attenuated with adjustment for PNC. Effect estimates for EC, absorbance, and PNC were attenuated with adjustment for NO_2, indicating that NO_2 may have confounded associations for copollutants.

Controlled Human Exposure Studies

Similar to the epidemiologic studies, controlled human exposure studies generally did not report effects of NO_2 on lung function in healthy adults. Overall, exposures ranged from 200 to 4,000 ppb NO_2 for 75 minutes to 6 hours, and most studies incorporated exercise in the exposure period to assess lung function during various physiological conditions (Table 5-37).

Huang et al. (2012b) examined the health effects of NO_2 exposure alone and in combination with exposure to concentrated ambient particles (CAPs) in healthy adults and did not report any effects on pulmonary function during, immediately after, or 18 hours after exposure to 500 ppb NO_2 for 2 hours with intermittent exercise. These results are consistent with previously published studies in healthy adults. For example, Hackney et al. (1978) demonstrated that exposure to 1,000 ppb for 2 h/day for 2 consecutive days did not induce pulmonary function changes with the exception of a 1.5% drop in FVC after exposure on the second day. Similarly, Frampton et al. (1989) reported no differences in lung function before, during, or after exercise or after exposure to 600 or 1,500 ppb NO_2 for 3 hours or a 3-hour base of 50 ppb NO_2 with intermittent peaks of 2,000 ppb. These results were replicated in studies in healthy adults at similar concentrations (Frampton et al., 2002; Devlin et al., 1999). Rasmussen et al. (1992) reported that healthy subjects exposed to 2,300 ppb NO_2 for 5 hours had slight

improvements, though not statistically significant, in FVC and FEV_1 during and after NO_2 exposure compared to air. Blomberg et al. (1999) reported a decrease in FEV_1 and FVC after the first exposure to 2,000 ppb NO_2 for 4 hours. This response was attenuated after a second, third, and fourth exposure.

Other studies examined co-exposures to NO_2 and O_3, which often are weakly correlated in the ambient air (Figure 3-6). Lung function was not affected by a 2-hour exposure to 600 ppb NO_2, but statistically significant reductions in FEV_1 and forced expiratory flow were observed after a subsequent O_3 exposure (Hazucha et al., 1994). Exposure of aerobically trained young men and women to 600 ppb NO_2 or 600 ppb NO_2 + 300 ppb O_3 for 1 hour resulted in an increase in airway resistance with co-exposure, although the increase in resistance with co-exposure was far less than with O_3 exposure alone, and NO_2 exposure alone did affect lung function (Adams et al., 1987).

Table 5-37 Controlled human exposure studies of lung function and respiratory symptoms in healthy adults.

Study	Disease Status[a]; n; Sex; Age (mean ± SD)	Exposure Details (Concentration; Duration)	Endpoints Examined
Adams et al. (1987)	(1−3) n = 20 M, 20 F; F = 21.4 ± 1.5 yr M = 22.7 ± 3.3	(1) 600 ppb NO_2 for 1 h, (2) 300 ppb O_3 for 1 h, (3) 600 ppb NO_2 and 300 ppb O_3 for 1 h; (1−3) Exercise during entire exposure at $\dot{V}_E$ = 75 L/min (M) and $\dot{V}_E$ = 50 L/min (F)	Pulmonary function before and after exposure. Symptoms following exposure.
Blomberg et al. (1999)	n = 8 M, 4 F; 26 yr (range: 21−32 yr)	2,000 ppb, 4 h/day for 4 days; Exercise 15 min on/15 min off at workload of 75 watts	Pulmonary function before and after exposure.
Devlin et al. (1999)	n = 11 M (range: 18−35 yr)	2,000 ppb for 4 h; Exercise for 15 min on/15 min off at $\dot{V}_E$ = 50 L/min	Aerosol bolus dispersion (deposition, FEV_1 and Sraw).
Frampton et al. (1989)	(1) n = 7 M, 2 F; 30 yr (range: 24−37 yr) (2) n = 11 M, 4 F; 25 yr (range: 19−37 yr)	(1) 600 ppb for 3 h, (2) 1,500 ppb for 3 h; (1,2) Exercise 10 min on/20 min off at $\dot{V}_E$ = ~4 times resting	Pulmonary function tests before, during, and after exposure.
Frampton et al. (1991)	(1) n = 7 M, 2 F; 29.9 ± 4.2 yr (2) n = 12 M, 3 F; 25.3 ± 4.6 yr (3) n = 11 M, 4 F; 23.5 ± 2.7 yr	(1) 600 ppb for 3 h, (2) 1,500 ppb for 3 h, (3) 50 ppb for 3 h + 2,000 ppb peak for 15 min/h; (1−3) Exercise 10 min on/20 min off at $\dot{V}_E$ = ~4 times resting	Pulmonary function tests before, during, and after exposure, airway reactivity 30 min post-exposure. Symptoms following exposure.

Table 5-37 (Continued): Controlled human exposure studies of lung function and respiratory symptoms in healthy adults.

Study	Disease Status[a]; n; Sex; Age (mean ± SD)	Exposure Details (Concentration; Duration)	Endpoints Examined
Frampton et al. (2002)	(1,2) n = 12 M, 9 F; F = 27.1 ± 4.1 yr M = 26.9 ± 4.5 yr	(1) 600 ppb for 3 h, (2) 1,500 ppb for 3 h; (1,2) Exercise 10 min on/20 min off at $\dot{V}_E$ = 40 L/min	Pulmonary function tests before and after exposure.
Gong et al. (2005)	Healthy; n = 2 M, 4 F; 68 ± 11 yr COPD; n = 9 M, 9 F; 72 ± 7 yr	(1) 400 ppb NO_2 for 2 h (2) 200 µg/m^3 CAPs for 2 h (3) 400 ppb NO_2 + 200 µg/m^3 CAPs for 2 h (1−3) Exercise 15 min on/15 min off at $\dot{V}_E$ = ~2 times resting	Pulmonary function tests before and immediately after exposure and 4 h and 22-h post-exposure. Symptoms before, during, and after exposure.
Hackney et al. (1978)	n = 16 M; 26.9 ± 5.0 yr	1,000 ppb, 2 h/day for 2 days; Exercise 15 min on/15 min off at $\dot{V}_E$ = 2 times resting	Pulmonary function tests before and after each exposure. Symptoms after each exposure.
Hazucha et al. (1994)	n = 21 F; 22.9 ± 3.6 yr	(1) 600 ppb NO_2 for 2 h, air for 3 h, 300 ppb O_3 for 2 h, (2) air for 5 h, 300 ppb O_3 for 2 h; (1,2) Exercise for 15 min on/15 min off at $\dot{V}_E$ = 35 L/min	Pulmonary function tests before, during, and after exposure; airway reactivity after exposure. Times for symptoms assessment not reported.
Huang et al. (2012b)	(1) n = 11 M, 3 F (2) n = 6 M, 7 F (3) n = 7 M, 6 F; 24.6 ± 4.3 yr	(1) 500 ppb NO_2 for 2 h, (2) 500 ppb NO_2 + 73.4 ± 9.9 µg/m^3 CAPs for 2 h, (3) 89.5 ± 10.7 µg/m^3 for 2 h; (1−3) Exercise 15 min on/15 min off at $\dot{V}_E$ = 25 L/min	Pulmonary function tests before, immediately after, and 18 h after exposure.
Koenig et al. (1987)	Healthy; (1) n = 3 M, 7 F (2) n = 4 M, 6 F Asthma; (1) n = 4 M, 6 F (2) n = 7 M, 3 F 14.4 yr (range: 12−19 yr)	(1) 120 ppb NO_2, (2) 180 ppb NO_2; (1−2) Exposures were 30 min at rest with 10 min of moderate exercise	Pulmonary function tests before, during, and after exposure.
Jörres et al. (1995)	Healthy; n = 5 M, 3 F; 27 yr (range: 21−33) Asthma; n = 8 M, 4 F; 27 ± 5 yr	1,000 ppb for 3 h; Exercise 10 min on/10 min off at individual's maximum workload	Symptoms immediately and 6 and 24 h after exposure.

Table 5-37 (Continued): Controlled human exposure studies of lung function and respiratory symptoms in healthy adults.

Study	Disease Status[a]; n; Sex; Age (mean ± SD)	Exposure Details (Concentration; Duration)	Endpoints Examined
Linn et al. (1985b)	Healthy; n = 16 M, 9 F (range: 20−36 yr) Asthma; n = 12 M, 11 F (range: 18−34 yr)	4,000 ppb for 75 min; Two 15-min periods of exercise at $\dot{V}_E$ = 25 L/min and 50 L/min	Airway resistance before, during, and after exposure. Symptoms before, during, and after exposure and 24-h post-exposure.
Morrow et al. (1992)	Healthy; n = 10 M, 10 F (13 never smokers, 4 former smokers, 3 current smokers) COPD; n = 13 M, 7 F (14 current smokers, 6 former smokers); 59.9 ± 7.0 yr	300 ppb for 4 h; Three 7-min periods of exercise at $\dot{V}_E$ = ~4 times resting	Pulmonary function tests before, during, and after exposure and 24-h post-exposure.
Rasmussen et al. (1992).	n = 10 M, 4 F; 34.4 yr (range: 22−66 yr)	2,300 ppb for 5 h	Pulmonary function tests before, 2 times during, and 3 times after exposure. Symptoms before, during, and after exposure.
Vagaggini et al. (1996)	Healthy: n = 7 M; 34 ± 5 yr Asthma: n = 4 M, 4 F; 29 ± 14 yr COPD: n = 7 M; 58 ± 12 yr	300 ppb for 1 h; Exercise at $\dot{V}_E$ = 25 L/min	Pulmonary function tests before and 2 h after exposure.
Witten et al. (2005)	n = 15; 32 ± 8.6 yr	400 ppb for 3 h; Exercise 30 min on/15 min off at $\dot{V}_E$ = 25 L/min; Inhalation challenge with house dust mite antigen after NO_2 exposure.	Symptoms before and after exposure and 6 h after allergen challenge.

CAPS = concentrated ambient particles, COPD = chronic obstructive pulmonary disease, F = female, FEV_1 = forced expiratory volume in 1 second, M = male, NO_2 = nitrogen dioxide, O_3 = ozone, SD = standard deviation.

[a]Subjects were healthy individuals unless described otherwise.

5.2.7.3 Respiratory Symptoms in Healthy Populations

Epidemiologic Studies of Children in the General Population

Respiratory symptoms in relation to short-term NO_2 exposure have not been examined in epidemiologic studies of healthy adults; however, associations are indicated in

school-aged children in the general population (Tables 5-38 and 5-39). NO_2-associated increases in respiratory symptoms also were found in infants (Stern et al., 2013; Andersen et al., 2008a; Peel et al., 2007) (Table 5-39). These results have weaker implications because symptoms such as wheeze are common in infancy and may not clearly distinguish children who do and do not develop respiratory conditions like asthma later in life (Cano Garcinuno et al., 2013). Further, Peel et al. (2007) examined apnea in infants on home cardiorespiratory monitors, a group unrepresentative of the general population. Another uncertainty is whether the temporal variation in ambient NO_2 concentrations from one central site in the area adequately represents variation in ambient NO_2 exposure in infants, particularly those on cardiorespiratory monitors, who may not spend much time outdoors away from home.

Table 5-38 Mean and upper percentile concentrations of nitrogen dioxide (NO_2) in epidemiologic studies of respiratory symptoms in children in the general population.

Study[a]	Location	Study Period	NO_2 Metric Analyzed	Mean/Median Concentration ppb	Upper Percentile Concentrations ppb
Stern et al. (2013)	Bern, Basel, Switzerland	Apr 1999–Feb 2011	24-h avg NO_2	Rural: 8.1[b] Urban: 25.6[b]	NR NR
Andersen et al. (2008a)	Copenhagen, Denmark	Dec 1998–Dec 2004	24-h avg NO_2	11.8	75th: 14.6
Peel et al. (2011)	Atlanta, GA	Aug 1998–Dec 2002	1-h max NO_2	41.7	90th: 65.6 Max: 109.2
Rodriguez et al. (2007)	Perth, Australia	June 1996–July 1998	1-h max NO_2 24-h avg NO_2	18 7	Max: 48 Max: 24
Ward et al. (2002)	Birmingham, Sandwell, U.K.	Jan–Mar 1997 May–July 1997	24-h avg NO_2	18 13.3	Max: 35 Max: 29
Patel et al. (2010)	New York City and nearby suburb, NY	2003–2005, mo NR	24-h avg NO_2	NR	NR
Wendt et al. (2014)	Harris County, TX (Houston area)	2005–2007	1-h max NO_2	39.26	75th: 48.00 Max: 108
Schwartz et al. (1994)	Watertown, MA; Steubenville, OH; Topeka, KS; St. Louis, MO; Portage, WI; Kingston-Harriman, TN	Apr–Aug, 1984–1988	24-h avg NO_2	13.3	75th: 24.1 Max: 44.2
Moon et al. (2009)	Seoul, Incheon, Busan, Jeju, Korea	Apr–May 2003	24-h avg NO_2	NR	NR

NO_2 = nitrogen dioxide, NR = not reported.
[a]Studies presented in order of first appearance in the text of this section.
[b]Concentrations converted from µg/m^3 to ppb using the conversion factor of 0.532 assuming standard temperature (25°C) and pressure (1 atm).

In school-aged children, not all results were statistically significant, but a pattern of elevated odds ratios indicates consistency in association between short-term NO_2 exposure and respiratory symptoms (Table 5-39). Evidence is stronger for cough than wheeze, which is identified more with asthma. Children were recruited primarily from schools but also from a birth cohort, suggesting study populations were representative of the general populations. A wide range of participation rates was reported (Table 5-39), but no study reported issues with differential participation by a particular group. The health status of study populations was not always specified, and it is not clear whether the NO_2-associated increases in respiratory symptoms reflect associations among all children or those with a respiratory disease. For example, associations were reported in populations with parental history of asthma (Rodriguez et al., 2007) or with 27% asthma prevalence (Ward et al., 2002). Findings for symptoms are uncertain in healthy children. NO_2 was not associated with respiratory symptoms in children without asthma (Patel et al., 2010) but was associated with new diagnosis of asthma in children (Wendt et al., 2014) (Table 5-39). Findings that an increase in a 5-day avg of ambient NO_2 concentrations may induce respiratory symptoms that precipitate an asthma diagnosis have uncertain implications. Asthma diagnosis was ascertained from Medicaid claims as a record of an outpatient or inpatient visit for asthma or dispensing events of asthma medication during a three-year period (Wendt et al., 2014). Among children older than age 3 years, what is defined as a diagnosis instead could represent an exacerbation of previously diagnosed asthma. Among children younger than age 3 years, the reliability of an asthma diagnosis is uncertain. The uncertainty of basing a new asthma diagnosis on a three-year review of medical records is underscored by observations that NO_2 associations were stronger in children older than age 4 years than in children ages 1−4 years (Table 5-39).

Despite the associations found with respiratory symptoms in children, there is uncertainty regarding the extent to which the results reflect an independent relationship with NO_2. Ambient NO_2 exposures were assigned from one central site per city or the average across multiple sites per city. In the study of asthma diagnosis, effect estimates were similar for 1-h max NO_2 assigned to subjects as the average NO_2 across 17 sites covering the 4,400-km^2 area of Harris County, TX and the nearest site within 9.7 km of the zip code centroid (Wendt et al., 2014). The two NO_2 exposure metrics differed in mean concentration, 39.3 ppb versus 27.7 ppb, but the temporal variability of each metric was not reported to assess whether large differences in temporal variability in NO_2 occurred across the study area.

Studies of respiratory symptoms in children also did not clearly identify NO_2 associations that are independent of other traffic-related pollutants. Symptoms also were related to CO, BS, UFP, and $PM_{2.5}$, which tended to be highly correlated with NO_2 (r = 0.61−0.75)

(Wendt et al., 2014; Moon et al., 2009; Andersen et al., 2008a; Rodriguez et al., 2007; Ward et al., 2002). Analysis of potential confounding was limited, and potentially differential exposure measurement error for central site NO_2 and copollutants limits inference from the copollutant model results. In Harris County, TX, 1-h max NO_2 remained associated with diagnosis of asthma in a copollutant model with 24-h avg $PM_{2.5}$ (Table 5-39) (Wendt et al., 2014). NO_2 was weakly correlated with $PM_{2.5}$ (r = 0.21), and while the variability in ambient $PM_{2.5}$ concentrations was not reported, NO_2 concentrations were reported to vary across the county. In infants, ORs for both NO_2 and UFP decreased with mutual adjustment (Table 5-39); thus, an independent or confounding effect was not discerned for either pollutant (Andersen et al., 2008a). Copollutant models were examined in the U.S. Six Cities study for PM_{10} and SO_2. ORs for cough decreased with PM_{10} or SO_2 adjustment to 1.37 (95% CI: 0.98, 2.12) and 1.42 (95% CI: 0.90, 2.22 for a 20-ppb increase in NO_2, respectively (Schwartz et al., 1994). The width of 95% CIs is inflated when presented for a 20-ppb increase in NO_2, which is double the 10-ppb interquartile range for the study areas. The OR for PM_{10} was robust to NO_2 adjustment. Thus, PM_{10} may partly confound NO_2 associations. However, the positive ORs for NO_2 suggest an independent association for NO_2 as well.

Table 5-39 Epidemiologic studies of respiratory symptoms in children in the general population.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Odds Ratio (95% CI) Single-Pollutant Model[a]	Copollutant Examination
†Wendt et al. (2014) Harris County, TX (Houston area) n = 18,264 cases in incident asthma, ages 1−17 yr Case-crossover. Incident asthma cases ascertained for 2004−2007 from Medicaid database. Medicaid enrollment required only for 13 mo. Date of diagnosis defined as earliest date of asthma diagnosis on inpatient or outpatient record or earliest of four asthma medication dispensing events in a year. Uncertainty as to whether outcome represents incident asthma. Conditional logistic regression adjusted for temperature, humidity, mold spores, tree pollen, grass pollen, weed pollen.	NO_2-central site 1-h max Average of 17 sites in 4,400 km^2 area Mean: 39.3 ppb	0−5 avg	Asthma diagnosis May−Oct All ages: 1.22 (1.09, 1.36) 1−4 yr: 1.05 (1.00, 1.09) 15−17 yr: 1.57 (1.06, 2.32) Nov−Apr All ages: 1.03 (0.93, 1.14)	All ages, NO_2 average over 17 county sites, May−Oct. With 24-h avg $PM_{2.5}$: 1.20 (1.06, 1.36) With 8-h max O_3: 1.11 (0.90, 1.37) Low or moderate correlations with NO_2. r = 0.21 for $PM_{2.5}$, 0.49 for O_3. O_3 means similar for county average and site within 9 km. $PM_{2.5}$ and O_3 attenuated with adjustment for NO_2.
	Site within 9.7 km of zip code centroid Mean: 27.6 ppb		No quantitative results. Slightly higher OR than that for 17 site avg	
Schwartz et al. (1994) Watertown, MA; Kingston-Harriman, TN; St. Louis, MO; Steubenville, OH; Portage, WI; Topeka, KS n = 1,844, grades 2−5 Repeated measures. Daily symptom diaries for 5 mo, collected every 2 weeks. Recruitment from schools. No information on participation rate. Logistic regression adjusted for lag day 1 temperature, day of week, city.	NO_2-central site 24-h avg 1 site per community	0 0−3 avg 1	Cough: 1.21 (0.92, 1.59) 1.61 (1.08, 2.40) Lower respiratory symptoms: 1.44 (0.96, 2.16)	For cough: With PM_{10}: 1.37 (0.98, 2.12) With O_3: 1.61 (1.08, 2.41) With SO_2: 1.42 (0.90, 2.22) PM_{10} and O_3 robust to adjustment for NO_2. SO_2 reduced. Moderate correlations with NO_2. R = 0.36 for PM_{10}, 0.35 for $PM_{2.5}$, 0.51 for SO_2, —0.28 for O_3.
†Moon et al. (2009) Seoul, Incheon, Busan, Jeju, Korea n = 696, ages NR Repeated measures. Daily symptom diaries for 2 mo. Recruitment from schools. 69% participation rate. GEE adjusted for temperature, relative humidity.	NO_2-central site 24-h avg # sites NR	0	Lower respiratory symptoms: All subjects 1.02 (1.00, 1.05) Seoul 1.08 (0.99, 1.18) Incheon 1.08 (0.99, 1.18) Busan 1.04 (0.96,1.12) Jeju 0.97 (0.89, 1.06)	No copollutant model. Association also found with CO. Correlation NR.

Table 5-39 (Continued): Epidemiologic studies of respiratory symptoms in children in the general population.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Odds Ratio (95% CI) Single-Pollutant Model[a]	Copollutant Examination
†Patel et al. (2010) New York City and nearby suburb, NY n = 192 children without asthma, ages 14−20 yr Repeated measures. Daily symptom diaries for 4−6 weeks, collected weekly. Recruitment from schools. Self-report of physician-diagnosed asthma. 75−90% participation across schools. GLMM with random effect for subject and school and adjusted for weekend, 8-h max O_3, urban location. Adjustment for season, pollen counts did not alter results.	NO_2-central site 24-h avg 1 site 2.2−9.0 km from schools, 1 site 40 km from schools	0	Wheeze: 0.88 (0.75, 1.03) Chest tightness 0.96 (0.75, 1.23)	No copollutant model with BC. BC also associated with symptoms. Across locations, moderately to highly correlated with NO_2. Spearman r = 0.56−0.90 for BC.
Ward et al. (2002) Birmingham, Sandwell, U.K. n = 162, age 9 yr, 27% with asthma, 31% with atopy Repeated measures. Daily symptom diaries for two 8-week periods, collected weekly. Recruitment from schools. 61% participation rate. Logistic regression adjusted for time trend, temperature, school day.	NO_2-central site 24-h avg Multiple sites	0	Cough: Winter: 0.78 (0.57, 1.09) Summer: 1.14 (1.01, 1.27)	No copollutant model. $PM_{2.5}$ associated with cough.
Rodriguez et al. (2007) Perth, Australia n = 263, ages 0−5 yr, 1 parent with asthma or other atopic disease Repeated measures. Daily symptom diary from birth to age 5 yr. Recruitment from birth cohort. >80% follow-up participation until yr 4 and 5. GEE adjusted for temperature, humidity.	NO_2-central site 24h max 10 site average	0	Wheeze (unit NR): 1.00 (0.99, 1.01) Cough: 1.01 (1.00, 1.02)	No copollutant model. Associations also found for $PM_{2.5}$, BS at lag 0.
	24-h avg		Wheeze: 1.01 (0.98, 1.04) Cough: 1.03 (1.00, 1.06)	
†Andersen et al. (2008a) Copenhagen, Denmark n = 205, ages 0−3 yr, all with maternal asthma Repeated measures. Daily symptom diaries from birth to 3 yrs, collected every 6 mo. Recruitment from birth cohort. 95% follow-up participation. Mean 805 observations/subject. GEE adjusted for age, sex, smoking exposure, paternal asthma, temperature, calendar season.	NO_2-central site 24-h avg 1 site within 15 km of homes	0	Wheeze: Age 0−1 yr 3.13 (1.27, 7.77) Age 2−3 yr 1.71 (0.94, 3.10)	For age 0−1 yr: With UFP: 1.19 (0.14, 75) with PM_{10}: 2.46 (0.72, 8.4) UFP & PM_{10} associations attenuated with adjustment for NO_2. UFP & CO highly correlated with NO_2. Spearman r = 0.67, 0.75. Moderate correlation for PM_{10}. r = 0.43.
	NO_X-central site 24-h avg		Age 0−1 yr 3.26 (1.14, 9.26) Age 2−3 yr 1.80 (0.87, 3.72)	

Table 5-39 (Continued): Epidemiologic studies of respiratory symptoms in children in the general population.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Odds Ratio (95% CI) Single-Pollutant Model[a]	Copollutant Examination
†Stern et al. (2013) Bern, Base, Switzerland n = 366, ages 0−1 yr Repeated measures. Symptoms reported weekly by telephone for 1 yr. Recruitment from birth cohort. 95% follow-up participation. GAM adjusted for study week, sex, siblings, nursery care, maternal atopy, birth weight, prenatal & post-natal maternal smoking, parental education.	NO_2-central site 1-week avg 2 site, urban and rural	5	Daytime respiratory symptom composite: 1.20 (1.04, 1.39)	No copollutant model. PM_{10} lag 7 associated with respiratory symptoms. Correlation NR.
†Peel et al. (2011) Atlanta, GA area n = 4,277, mean age 46 days, 84% premature births Repeated measures. Followed for mean of 42 days. 111,000 person-days. Recruitment from referral center for home cardiorespiratory monitoring of infants. Limited generalizability. Apnea events collected electronically. No information on participation rate. GEE adjusted for long-term trends, age.	NO_2-central site 1-h max 1 site	0−1 avg	Apnea: 1.02 (0.96, 1.08)	With O_3: 1.00 (0.96, 1.05) O_3 association robust to NO_2 adjustment. Moderate correlation with NO_2. Spearman r = 0.45. No association with PM_{10}, coarse PM.

Note: Studies are organized by population examined, and more informative studies in terms of the exposure assessment method and potential confounding considered are presented first.

GEE = generalized estimating equations, GLMM = Generalized linear mixed model, GLM = generalized linear model, NR = not reported, GAM = generalized additive model, CI = confidence interval, CO = carbon monoxide, NO_2 = nitrogen dioxide, NO_X = sum of NO and NO_2, O_3 = ozone, OR = odds ratio, PM = particulate matter, SO_2 = sulfur dioxide, UFP = ultrafine particles.

[a]Effect estimates are standardized to a 20 ppb for 24-h avg NO_2, 25 ppb for 8-h max, a 30-ppb increase for 1-h max NO_2, and 40-ppb increase in 24-h avg NO_X.

†Studies published since the 2008 ISA for Oxides of Nitrogen.

Controlled Human Exposure Studies

Controlled human exposure studies do not provide strong evidence for NO_2-induced increases in respiratory symptoms in healthy adults. Most of these studies were reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a). Study details are presented in Table 5-37; overall, studies involved NO_2 exposures of 200−2,300 ppb for 2−5 hours and assessment of symptoms 24 hours later. The majority of studies in healthy subjects reported no change in symptoms, as measured by symptom score (Gong et al., 2005; Witten et al., 2005; Frampton et al., 2002; Jörres et al., 1995; Morrow et al., 1992; Rasmussen et al., 1992; Linn et al., 1985b), although a few studies reported statistically nonsignificant increases in symptom score following NO_2 exposure (Frampton et al., 2002; Hackney et al., 1978). NO_2 exposure (600 ppb for 1−2 hour) also did not affect respiratory symptoms with simultaneous or sequential O_3 (200−300 ppb for 1−2 hours) co-exposures (Hazucha et al., 1994; Adams et al., 1987).

5.2.7.4 Subclinical Respiratory Effects in Healthy Individuals: Pulmonary Inflammation, Injury, and Oxidative Stress

Pulmonary inflammation, injury, and oxidative stress are mediators of respiratory symptoms and decreases in lung function (Section 4.3.5). Consistent with the evidence described in the preceding sections, epidemiologic studies show ambient NO_2-related increases in pulmonary inflammation and oxidative stress in children and adults in the general population. A few analyses of copollutant models indicate associations for NO_2 persist with adjustment for another traffic-related pollutant. Experimental studies report evidence for increased pulmonary inflammation as PMN increases. Effects on other indicators depended upon exposure concentration, duration, and frequency and were observed more consistently at higher than ambient-relevant concentrations. Limited evidence from experimental studies indicates development of an allergic phenotype with repeated NO_2 exposures.

Epidemiologic Studies

Together, the few epidemiologic studies from the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a) and most recent studies found associations between increases in ambient oxides of nitrogen and increases in pulmonary inflammation or oxidative stress among children and adults in the general population and healthy populations. Locations, time periods, and ambient concentrations of oxides of nitrogen for these studies are

presented in Table 5-40. In this group of studies are several with exposure assessment methods that aim to account for the high variability in ambient oxides of nitrogen.

Table 5-40 Mean and upper percentile concentrations of oxides of nitrogen in epidemiologic studies of pulmonary inflammation and oxidative stress in the general population.

Study[a]	Location	Study Period	Exposure Metric Analyzed	Mean Concentration ppb	Upper Percentile Concentrations (ppb)
Flamant-Hulin et al. (2010)	Clermont-Ferrand, France	NR	5-day avg NO_2	Schools <14.0: 10.1 Schools >14.0: 17.4	Across schools: 75th: 14.0[b] Max: 19.7[b]
Lin et al. (2011)	Beijing, China	Jun 2007 Sept 2007 Dec 2007 Jun 2008 Sept 2008	24-h avg NO_2	24.3 30.4 45.3 26.6 25.9	NR NR NR NR NR
Liu et al. (2014a)	Munich and Wesel, Germany	NR	24-h avg NO_2	15.9[c]	95th: 29.7[b]
Berhane et al. (2011)	13 southern California communities	Sept–June 2004–2005	24-h avg NO_2	NR	NR
Patel et al. (2013)	New York City, NY	May–June 2005	24-h avg NO_2	Median: 23.3	NR
Altuğ et al. (2014)	Eskisehir, Turkey	Feb–Mar 2007	24-h avg NO_2	Suburban: 9.4[b] Urban: 13.0[b] Urban-traffic: 21.2[b]	Max: 13.1[b] Max: 17.7[b] Max: 28.2[b]
Holguin et al. (2007)	Ciudad Juarez, Mexico	2001–2002	1-week avg NO_2	18.2	NR
Steerenberg et al. (2001)	Utrecht, Bilthoven, the Netherlands	Feb–Mar 1998	24-h avg NO_2 24-h avg NO 24-h avg NO_2 24-h avg NO	28.2[b] 30.2[b] 25.5[b] 7.4[b]	Max: 44.7[b] Max: 168[b] Max: 49.5[b] Max: 85.6[b]
Chen et al. (2012a)	New Taipei City, Taiwan	Oct–June 2007; June–Nov 2009	24-h avg NO_2	21.7	NR
Salam et al. (2012)	13 southern California communities	2004–2007, school year	24-h avg NO_2	19.0	Max: 39.4
Steerenberg et al. (2003)	the Netherlands, city NR	May–June; year not reported	24-h avg NO_2 24-h avg NO	17.3[b] 6.3[b]	Max: 28.3[b] Max: 34.5[b]
Steenhof et al. (2013) Strak et al. (2012)	the Netherlands, city NR	Mar–Oct 2009	5-h avg NO_2 5-h avg NO_X	36 20	Max: 96 Max: 34

Table 5-40 (Continued): Mean and upper percentile concentrations of oxides of nitrogen in epidemiologic studies of pulmonary inflammation and oxidative stress in the general population.

Study[a]	Location	Study Period	Exposure Metric Analyzed	Mean Concentration ppb	Upper Percentile Concentrations (ppb)
Adamkiewicz et al. (2004)	Steubenville, OH	Sept−Dec 2000	1-h avg NO_2 24-h avg NO_2 1-h avg NO 24-h avg NO	9.2 10.9 15 11.2	75th: 12.8, Max: 32.9 75th: 14.6, Max: 23.8 75th: 16.1, Max: 215 75th: 14.2, Max: 70.7
Weichenthal et al. (2011)	Ottawa, ON, Canada	NR	1-h avg NO_2	High traffic: 4.8 Low traffic: 4.6	Max: 11 Max: 10
Chimenti et al. (2009)	Palermo, Sicily, Italy	Nov Feb July; year NR	7-day avg NO_2	31.7[b] 27.1[b] 33.9[b]	NR NR NR
Madsen et al. (2008)	Oslo, Norway	Jan−June 2000	24-h avg NO_2 7-day avg NO_2	NR NR	NR NR

NO = nitric oxide. NO_2 = nitrogen dioxide, NO_X = sum of NO and NO_2, NR = not reported.
[a]Studies presented in order of first appearance in the text of this section.
[b]Concentrations converted from µg/m^3 to ppb using the conversion factor of 0.532 for NO_2 and 0.815 for NO assuming standard temperature (25°C) and pressure (1 atm).

Children in the General Population

Ambient NO_2 was associated with pulmonary inflammation and oxidative stress in populations of children, in which the prevalence of asthma ranged from 7.5 to 59% and prevalence of allergy ranged from 20 to 56% (Patel et al., 2013; Berhane et al., 2011; Lin et al., 2011; Steerenberg et al., 2001). Except for Altuğ et al. (2014), studies demonstrated associations in groups without asthma or allergy, with no consistent difference in magnitude of association between children with and without respiratory disease [(Liu et al., 2014a; Berhane et al., 2011; Lin et al., 2011); Figure 5-14 and Table 5-41]. These findings suggest associations between NO_2 exposure and pulmonary inflammation in healthy children.

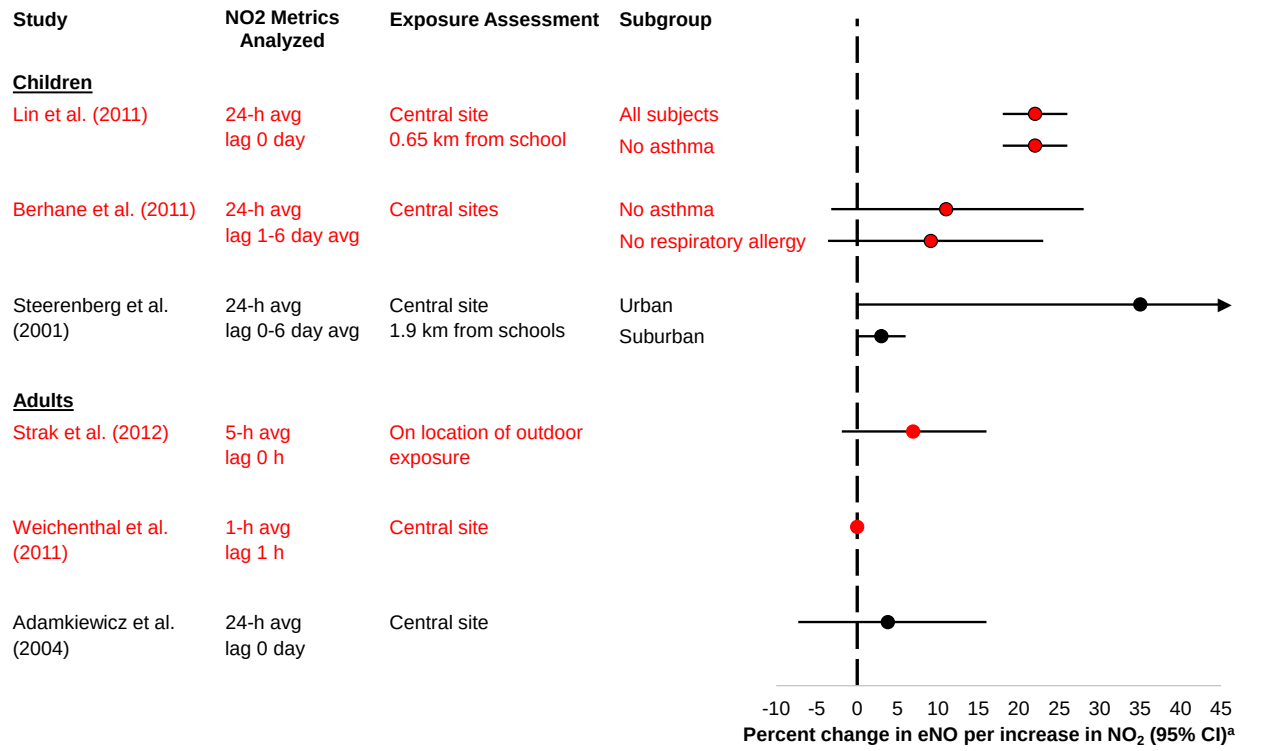
Among children, associations for NO_2 varied among the various indicators of oxidative stress and inflammation. As examined in one study each, associations were not observed with PMNs, eosinophils, exhaled breath condensate pH, or methylation of inducible nitric oxide synthase (iNOS) (Patel et al., 2013; Chen et al., 2012a; Salam et al., 2012; Steerenberg et al., 2001). But several study results pointed to associations with eNO (Berhane et al., 2011; Lin et al., 2011; Steerenberg et al., 2001) (Figure 5-14 and Table 5-41). Most of these studies assigned exposure from one central site per community located between 1 and 14 km of subjects' homes. Further, associations were found with CO, BC, BS, and $PM_{2.5}$, and copollutant models were not analyzed. Moderate to strong correlations were reported for NO_2 with $PM_{2.5}$ and BC (r = 0.47−0.80) (Patel et

al., 2013; Berhane et al., 2011). Thus, the extent to which the results for central site NO_2 reflect an independent association with NO_2 is uncertain.

Other studies examined copollutant confounding and/or ambient NO_2 measurements spatially aligned with a location of subjects, which may better represent ambient microenvironmental exposure. Outdoor school NO_2, averaged over 5 or 7 days, was not associated with pulmonary inflammation in children without respiratory disease (Altuğ et al., 2014; Flamant-Hulin et al., 2010; Holguin et al., 2007). However, Holguin et al. (2007) did not report quantitative results to assess whether there was suggestion of association. And, the cross-sectional comparison of high versus low NO_2 in Flamant-Hulin et al. (2010) lacks the sensitivity to discern incremental changes in eNO that may occur with incremental changes in NO_2 exposure. Also, for some subjects, eNO was measured days before NO_2 was measured.

The study with the strongest inference about NO_2-related increases in pulmonary inflammation in healthy children was conducted in Beijing, China before and after the 2008 Olympics (Lin et al., 2011). Although results were based on 28 children without asthma, a large number of measurements was collected per child. NO_2 and copollutants were measured at a site 0.65 km from schools, improving the spatial alignment of pollutants with subjects over the aforementioned central site studies. A 20-ppb increase in lag 0 day of 24-h avg NO_2 was associated with a 22% (95% CI: 18, 26) increase in eNO. This effect estimate was attenuated two to fourfold with adjustment for BC or $PM_{2.5}$ but remained positive (e.g., 5.6% [95% CI: 0.38, 11] with adjustment for BC). Adjustment for NO_2 attenuated the association of eNO with $PM_{2.5}$ but not BC. These results indicated that the NO_2 association was partly confounded, by BC in particular. But, they also provide evidence for an independent association for NO_2 in this population of children without asthma.

Although (Lin et al., 2011) found that eNO increased in relation to ambient NO_2 measured at subjects' school and independently of the traffic-related pollutant BC or $PM_{2.5}$, other studies had weaker inference. Outdoor school NO_2 was not associated with eNO in children without respiratory disease, but these studies either did not report quantitative results or had other methodological limitations. eNO was consistently associated with NO_2 measured at central sites and also with traffic-related copollutants such as CO, BC, BS, and $PM_{2.5}$. Thus, overall, there is uncertainty in the epidemiologic evidence regarding an independent association of NO_2 exposure with pulmonary inflammation and/or oxidative stress in healthy children.



Note: Results are presented first for children then adults. Within each of these groups, results from more informative studies in terms of the exposure assessment method and potential confounding considered are presented first. Red = recent studies, Black = previous studies. Study details and quantitative results reported in Table 5-41.

[a]Effect estimates are standardized to a 20-ppb increase for 24-h avg NO_2. Effect estimates for 5-h or 1-h avg NO_2 are not standardized but are presented as reported in their respective studies (Section 5.1.2.3).

Figure 5-14 Associations between ambient nitrogen dioxide (NO_2) concentrations and exhaled nitric oxide (eNO) among children and adults in the general population.

Table 5-41 Epidemiologic studies of pulmonary inflammation, injury, and oxidative stress in children and adults in the general population.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Children in the general population: studies with small spatial scale exposure assessment and/or examination of copollutant confounding				
†Zhu (2013); Lin et al. (2011) Beijing, China n = 36, ages 9−12 yr, 8 with asthma, 28 without asthma. Repeated measures before and after Olympics. Examined daily for five 2-week periods. 1,581 observations. Recruitment from school. 60% responded to initial survey, 95% follow-up participation. GEE adjusted for temperature, relative humidity, body mass index.	NO_2-central site 24-h avg Site 650 meters from schools	0 1	eNO: All subjects: 22% (18, 26) No asthma: 22% (18, 26) No asthma: 9.5% (5.8, 13)	w/BC: 5.6% (0.38, 11) w/$PM_{2.5}$: 14% (9.5, 19) BC robust to adjustment for NO_2, $PM_{2.5}$ reduced but positive. Moderate correlations with NO_2. Spearman r = 0.30 for $PM_{2.5}$, 0.68 for BC.
†Flamant-Hulin et al. (2010) Clermont-Ferrand, France n = 70 without asthma, mean age: 10.7 (SD: 0.7) yr, 75% no atopy Cross-sectional. Recruitment from schools. 69% participation. Self or parental report of no asthma. For some subjects, eNO measured up to 1 week before pollutants. GEE adjusted for atopy, mother's birth region, parental education, family history of allergy, smoking exposure. Did not consider confounding by meteorology.	NO_2-school outdoor 24-h avg	0−4 avg	log eNO comparing ≥14.3 vs. <14.3 ppb NO_2 −0.09 (−0.22, −0.04)	No copollutant model. Acetylaldehyde and $PM_{2.5}$ associated with eNO. Correlations with NO_2 not reported.
	NO_2-school indoor 24-h avg	0−4 avg	log eNO comparing ≥16.3 vs. <16.3 ppb NO_2 −0.16 (−0.11, −0.20)	
Children in the general population: studies with central site exposure assessment and no examination of copollutant confounding				
†Chen et al. (2012a) New Taipei City, Taiwan n = 100, mean age 10.6 (SD: 2.5) yr, 33% asthma, 33% atopy Repeated measures. Examined 3−4 times/mo for 10 mo. 824 observations. Recruited from schools. A priori recruitment of children with and without asthma or atopy. Participants similar to nonparticipants. Mixed effects model adjusted for school, age, sex, body mass index, upper respiratory infection, asthma/allergic rhinitis attack, asthma medication use, temperature, humidity, day of week, sampling time, parental education, smoking exposure at home.	NO_2-central site 24-h avg 1 site 2.5 km from schools, most homes 1 km of schools	0 1 2 3	No quantitative data. NO_2 reported not to affect eosinophils, PMNs, monocytes, IL-8.	No copollutant model. Associations found for $PM_{2.5}$, O_3 but not CO. Moderate to no correlation with NO_2. Pearson r = 0.61 for $PM_{2.5}$, −0.01 for O_3.

Table 5-41 (Continued): Epidemiologic studies of pulmonary inflammation, injury, and oxidative stress in children and adults in the general population.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Steerenberg et al. (2001) Utrecht (Urban, near busy roadway) and Bilthoven (Suburban), the Netherlands n = 126, ages 8−13 yr, 28% respiratory disease, 20% allergy Repeated measures. Examined 1/week for 7−8 weeks. Recruitment from urban and suburban schools. 65% participation. Non-standardized eNO collection. Mixed effects model adjusted for sex, age, # cigarettes smoked in home, presence of a cold, history of respiratory symptoms, allergy. No consideration for potential confounding by meteorological factors.	NO_2-central site	0−6 avg	eNO: Urban: 35% (0, 70)[b] Suburban: 3.0%, $p > 0.05$ IL-8 (units NR) Urban OR: 1.08, $p > 0.05$ Suburban OR: 1.03, $p>0.05$	No copollutant model. PM_{10} and BS also associated with eNO, IL-8, uric acid, urea.
	NO-central site All 24-h avg Site within 1.9 km of schools	0−6 avg	eNO: Urban: 6.6% (0, 13)[b] Suburban: 7.3% (0, 15)[b] IL-8 (units NR) Urban OR: 1.05, $p > 0.05$ Suburban OR: 0.95, $p>0.05$	
†Patel et al. (2013) New York City, NY n = 36, ages 14−19 yr, 94% nonwhite, 50% with asthma Repeated measures. EBC collected 2/week for 4 weeks. 217 observations. Recruitment from schools. 89−90% participation rate. A priori recruitment of children with and without asthma or atopy. Self-report of physician-diagnosed asthma and symptoms in previous 12 mo. Mixed effects model with random effects for subject and adjusted for school, daily average temperature, 8-h max O_3. Adjustment for day of week and humidity did not alter results.	NO_2-central site 24-h avg Site 14 km from schools	0 0−3 avg	EBC 8-isoprostane: 1.7 (0.63, 2.7) log units 3.1 (1.3, 4.9) log units	No copollutant model. BC also associated with EBC pH and 8-isoprostane. School BC moderately to highly correlated with NO_2. Pearson r = 0.62, 0.80.
		0 0−3 avg	EBC pH: −0.05 (−0.79, 0.68) −0.11 (−1.2, 1.0)	
†Berhane et al. (2011) Anaheim, Glendora, Long Beach, Mira Loma, Riverside, San Dimas, Santa Barbara, Upland, CA, Children's Health Study n = 169, ages 6−9 yr Cross-sectional. Recruitment from schools. Parental report of physician-diagnosed asthma and history of respiratory allergy. Two different methods used for eNO measurement. No information on participation rate. Linear regression adjusted for community, age, sex, race/ethnicity, asthma, asthma medication use, history of respiratory allergy, eNO collection time, body mass index percentile, smoking exposure, parental education, questionnaire language, season, multiple temperature metrics, eNO collected outdoors.	NO_2-central site 24-h avg Sites in each community. # sites in each community NR	1−6 avg	eNO: No asthma 11% (−3.2, 28) No respiratory allergy: 9.1% (−3.6, 23)	No copollutant model. $PM_{2.5}$, PM_{10}, O_3 associated with eNO. Moderate or weak correlations with NO_2. Pearson r for warm and cold season = 0.47, 0.65 for $PM_{2.5}$, 0.49, 0.55 for PM_{10}, 0.15, −0.4 for O_3.

Table 5-41 (Continued): Epidemiologic studies of pulmonary inflammation, injury, and oxidative stress in children and adults in the general population.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
†Salam et al. (2012) Same cohort as above n = 940, ages 6−11 yr, 14% asthma, 56% respiratory allergy Cross-sectional. Recruitment from schools. Subjects representative of full cohort. Linear regression model adjusted for age, sex, ethnicity, asthma, respiratory allergy, parental education, smoking exposure, community, month of eNO collection. No consideration for confounding by meteorology.	NO_2-central site 24-h avg Sites in each community. # sites in each community NR	1−7 avg	iNOS promoter methylation: 0.40% (−1.0, 1.8) iNOS methylation not strong predictor of eNO.	No copollutant model. $PM_{2.5}$ associated with higher iNOS promoter methylation. Moderate correlation with NO_2. Spearman r = 0.36.
Adults in the general population: studies with small spatial scale exposure assessment and/or examination of copollutant confounding				
†Strak (2013); Strak et al. (2012) †Steenhof et al. (2013) Utrecht area, the Netherlands n = 31, adults ages 19−26 yr, all healthy, nonsmoking Repeated measures. Examined 3−7 times. 107 observations. Recruitment from university. Well-defined outdoor exposures at various sites: underground train station, two traffic sites, farm, and urban background site. Heart rate maintained during intermittent exercise. Multiple comparisons could results in higher probability of associations found by chance alone. No information on participation rate. Mixed effects model adjusted for temperature, relative humidity, season, high/low pollen, respiratory infection.	NO_2 and NO_X-on site of outdoor activity 5-h avg	0-h post-exposure	eNO: NO_2: 6.9% (−1.9, 16) NO_X: 4.7% (−1.8, 11) Per 10.54 ppb increase in NO_2 and 28.05 ppb increase in NO_X	w/PNC: −7.4% (−19, 3.9) for NO_2 −5.8% (−14, 2.4) for NO_X w/EC: 4.1% (−6.0, 14) for NO_2 2.0% (−7.3, 11) for NO_X PNC association persists NO_2/NO_X adjustment. EC & Abs attenuated. For IL-6: w/PNC: 95% (0, 190) w/OC: 67% (−10, 144) Copollutant results robust. Moderate to high correlations with NO_2 & NO_X. Spearman r = 0.56, 0.75 for PNC, 0.74, 0.87 for Abs, 0.67, 0.87 for EC.
		2-h	NO_2 IL-6: 66% (−10, 142) NAL protein: 60% (0, 121)[b]	
Adults in the general population: studies with central site exposure assessment and no examination of copollutant confounding				
†Chimenti et al. (2009) Palermo, Sicily, Italy n = 9, male adults mean age 40 (SD: 3.8) yr, all healthy, nonsmoking Repeated measures. Examined during 3 outdoor races. No information on participation rate. Statistical analyses limited to correlation analyses. No consideration for potential confounding factors or repeated measures.	NO_2-central site Averaging time NR 10 sites		No correlations with plasma PMN or eosinophils. No results reported for CC16.	No copollutant model. Associations found with O_3 and $PM_{2.5}$.

Table 5-41 (Continued): Epidemiologic studies of pulmonary inflammation, injury, and oxidative stress in children and adults in the general population.

Study Population Examined and Methodological Details	NO_2 Metrics Analyzed	Lag Day	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
†Weichenthal et al. (2011) Ottawa, Canada n = 42, adults ages 19−58 yr, from nonsmoking homes, 95% white, 62% with allergies, 33% with asthma Repeated measures. Most examined 3 times. 118 observations. 1-h outdoor exposures during cycling in low and high traffic areas. Recruitment from public advertisements. No information on participation rate. Mixed effects models with random subject effect adjusted for temperature during cycling, average heart rate. Adjustment for relative humidity, day of week did not affect results.	NO_2-central site 1-h avg 1 site	1-h 4-h post-exposure	eNO: −0.01% (−0.08, 0.06) −0.04% (−0.09, 0.01) Per 4-ppb increase in NO_2	No copollutant model. $PM_{2.5}$ associated with eNO. Moderate correlation with NO_2. Spearman r = 0.31 for low traffic site, 0.45 for high traffic site. Potential differential exposure error for personal PM species and VOCs vs. central site NO_2.
†Madsen et al. (2008) Oslo, Norway n = 1,004, male adults ages 67−77 yr, 10% with respiratory disease Cross-sectional. Recruitment from a larger cohort to represent a range of home outdoor NO_2. No information on participation rate. GLM adjusted for age, respiratory disease, alcohol consumption, smoking status, # cigarettes/day, smoking exposure, education, hour of exam, body mass index, temperature.	NO_2-central site NO_2-dispersion model No information on model validation.	0−7 avg	CC16: 30% (7.8, 57) 3.8% (−7.3, 16)	No copollutant model. $PM_{2.5}$ (central site and home) associated with CC16. Moderate correlation with NO_2. Spearman r for home = 0.59.
Adamkiewicz et al. (2004) Steubenville, OH n = 29, adults ages 53-90 yr, nonsmoking, 28% with asthma, 24% with COPD Repeated measures. Examined weekly for 12 weeks. 138−244 total observations. No information on participation rate. GLM with subject-specific intercept and adjusted for time of day, day of week, study week, temperature, pressure, relative humidity. Several NO_2 measurements missing.	NO_2-central site 24-h avg NO-central site 24-h avg 1 site	0 0	eNO: 3.8% (−7.3, 16%) 30% (7.8, 57%)	No copollutant model for NO_2. NO w/$PM_{2.5}$: 9.2% (−1.7, 20) $PM_{2.5}$ result robust. Correlations NR. Ambient NO robust to adjustment for indoor NO.

Note: Studies are organized by population examined, and more informative studies in terms of the exposure assessment method and potential confounding considered are presented first.
GLM = generalized linear mixed effects model, GEE = generalized estimating equation, EBC = exhaled breath condensate, eNO = exhaled nitric oxide, IL = interleukin, NR = not reported, PMNs = polymorphonuclear leukocytes, iNOS = inducible nitric oxide synthase, NAL = nasal lavage, CC16 = club cell protein, Abs = absorbance coefficient, CI = confidence interval, CO = carbon monoxide, COPD = chronic obstructive pulmonary disease, EC = elemental carbon, eNO = exhaled nitric oxide, NO = nitric oxide. NO_2 = nitrogen dioxide, NO_X = sum of NO and NO_2, O_3 = ozone, OC = organic carbon, OR = odds ratio, PM = particulate matter, PNC = particle number concentration, SD = standard deviation, VOC = volatile organic compound.
[a]Effect estimates are standardized to a 20 ppb for 24-h avg NO_2 or NO and 25 ppb for 8-h max NO_2. Effect estimates for other averaging times (1-h avg to 15-h avg) are not standardized but presented as they are reported in their respective studies (Section 5.1.2.3).
[b]95% CI estimated for p = 0.05 based on reported p-value < 0.05.
†Recent studies published since the 2008 ISA for Oxides of Nitrogen.

Adults in the General Population

Among a few studies reviewed in the 2008 ISA for Oxides of Nitrogen that were conducted in older adults (U.S. EPA, 2008a) and recent studies conducted in older adults and adults performing outdoor exercise, several results point to increases in pulmonary inflammation in association with increases in ambient NO_2 concentrations. Pulmonary inflammation was indicated as increases in eNO, nasal lavage IL-6, and indicators of pulmonary injury and lung permeability such as Club cell protein (CC16) and nasal lavage protein levels (Table 5-41). Copollutant-adjusted associations were found with 24-h avg NO (Adamkiewicz et al., 2004) and with 5-h avg NO_2 for some outcomes (Steenhof et al., 2013; Strak et al., 2012). The epidemiologic findings have some support from controlled human exposure and toxicological studies (described in sections that follow), although the evidence for pulmonary injury is inconsistent.

In populations of mostly healthy adults performing outdoor exercise for <1 to 5 hours, increases in pulmonary inflammation were associated with NO_2 measured at the locations of outdoor locations but not at central sites. Compared with studies that do not account for time-activity patterns, examination of subjects during time spent outdoors may better reflect effects related to ambient exposures, particularly for pollutants measured in subjects' outdoor locations. In these studies, subjects had 3−5 separate outdoor exposure periods. In some studies, exposures occurred in locations that represented a gradient of traffic volume. Among adults running or cycling outdoors for 35−90 minutes, eNO and inflammatory cell counts (as measured by PMNs and eosinophils) were not associated with NO_2 measured at central sites (Weichenthal et al., 2011; Chimenti et al., 2009) (Figure 5-14 and Table 5-41). However, increases in eNO and nasal lavage IL-6 and protein were found in healthy adults in association with 5-h avg NO_X and NO_2 measured on the site of outdoor exposures (Steenhof et al., 2013; Strak et al., 2012), which account for variability in exposure better than central site measurements. Increases in eNO and nasal lavage IL-6 and protein were found immediately after and 2 hours after exposures ended but not the morning after, indicating a transient increase in pulmonary inflammation. The multiple analyses conducted across pollutants, including several $PM_{2.5}$ components, increases the potential for associations to be found by chance alone (Strak et al., 2012), but there was good consistency in results.

Among healthy adults, eNO also was associated with EC, Absorbance coefficient (Abs), and PNC (Strak et al., 2012); IL-6 also was associated with $PM_{2.5}$ and OC (Steenhof et al., 2013). In copollutant models, associations of eNO with NO_X and NO_2 were attenuated with adjustment for EC or Abs and became negative with adjustment for PNC (Strak et al., 2012). The PNC effect estimate was robust to adjustment for NO_X or NO_2. NO_X and NO_2 were highly correlated with PNC and EC (e.g., r = 0.75 for NO_X and PNC

and 0.71 for NO_2 and EC). However, NO_2 remained associated with nasal lavage IL-6 and protein after adjustment for PNC or other copollutants (e.g., 67% [95% CI: −10, 144] increase in IL-6 per 30-ppb increase in 5-h avg NO_2 and 95% [95% CI: 0, 190] with adjustment for PNC). Thus, in this study of well-defined outdoor exposures, there was evidence of confounding of NO_2-eNO associations by PNC but independent associations of NO_2 with IL-6 and nasal lavage protein as well as lung function (Figures 5-16 and 5-17).

Increases in pulmonary inflammation were associated with 24-h avg NO or NO_2 measured at central monitoring sites among older adults (ages: 53-90 years) (Madsen et al., 2008; Adamkiewicz et al., 2004). Multiday averages of NO_2 (e.g., lag 0−4 day avg, 0−7 day avg) were associated with CC16 (Madsen et al., 2008). However, there is uncertainty regarding independent associations of NO_2 as Madsen et al. (2008) found an association with central site not home NO_2, and each study found associations with other pollutants. There was evidence of an independent association between lag 0 of 24-h avg NO and eNO among older adults in Steubenville, OH (Adamkiewicz et al., 2004). In a copollutant model, the NO effect estimate decreased, and the 24-h avg $PM_{2.5}$ effect estimate increased. However, the NO effect estimate remained positive.

Controlled Human Exposure Studies

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a) cited several studies addressing the effects of NO_2 exposure on markers of pulmonary inflammation (i.e., differential cell counts, cytokines, eicosanoids), injury (i.e., LDH and protein concentrations), and oxidative stress (i.e., antioxidant molecules and enzymes). The study protocol typically used in these studies includes a single- or multi-day exposure to NO_2 (50−5,000 ppb) followed 1 to 24 hours later by collection of bronchial wash or BAL fluid (Table 5-42). The consistency and biological significance of effects across studies is difficult to evaluate given the variety of exposures and timing of when effects were measured, but there is evidence for pulmonary inflammation that is most consistently demonstrated by increases in PMNs.

Table 5-42 Controlled human exposure studies of pulmonary inflammation, injury, and oxidative stress in healthy adults.

Study	Disease Status[a]; n, Sex; Age (Mean ± SD)	Exposure Details (Concentration; Duration)	Endpoints Examined
Azadniv et al. (1998)	n = 11 M, 4 F; Early phase: 28.1 ± 3.5 yr Late phase: 27.4 ± 4.2 yr	2,000 ppb for 6 h; Exercise for approximately 10 of every 30 min at $\dot{V}_E$ = 40 L/min	BAL fluid analysis 1 h and 18 h after exposure. Protein concentration, differential cell counts.
Blomberg et al. (1999)	n = 8 M, 4 F; 26 yr (range: 21−32 yr)	2,000 ppb, 4 h/day for 4 days; Exercise 15 min on/15 min off at workload of 75 watts	Cell counts from bronchial biopsies, BW, and BAL fluid 1.5-h post-exposure; protein concentration, IL-8, MPO, hyaluronic acid, glutathione, ascorbic acid, and uric acid in BAL fluid and BW 1.5-h post-exposure, blood parameters.
Devlin et al. (1999)	n = 10 M; range: 18−35 yr	2,000 ppb for 4 h; Exercise for 15 min on/15 min off at $\dot{V}_E$ = 50 L/min	Bronchial and alveolar lavage fluid contents 16-h post-exposure. LDH activity, tissue plasminogen factor activity, IL-6 activity, IL-8 activity, PGE2 levels, total protein, ascorbate, urate, and glutathione.
Frampton et al. (1989)	(1) n = 7 M, 2 F; 30 yr (range: 24−37 yr) (2) n = 11 M, 4 F; 25 yr (range: 19−37 yr)	(1) 600 ppb for 3 h, (2) 50 ppb for 3 h + 2,000 ppb peak for 15 min/h; (1,2) Exercise 10 min on/20 min off at $\dot{V}_E$ = ~4 times resting	BAL fluid cell viability and differential counts 3.5-h post-exposure, IL-1 activity in BAL fluid cells.
Frampton et al. (2002)	(1,2) n = 12 M, 9 F; F = 27.1 ± 4.1 yr M = 26.9 ± 4.5 yr	(1) 600 ppb for 3 h, (2) 1,500 ppb for 3 h; (1,2) Exercise 10 min on/20 min off at $\dot{V}_E$ = 40 L/min	Bronchial and alveolar lavage fluid cell viability and differential counts 3.5-h post-exposure, peripheral blood characterization.
Helleday et al. (1994)	n = 8 nonsmokers; median: 26 yr (range: 24−35 yr), n = 8 smokers, median: 29 yr (range: 28−32 yr)	3,500 ppb for 20 min; Exercise last 15 min at 75 watts	Bronchial wash and BAL fluid analysis. Protein concentration, differential cell counts.
Huang et al. (2012b)	(1) n = 11 M, 3 F (2) n = 6 M, 7 F (3) n = 7 M, 6 F; 24.6 ± 4.3 yr	(1) 500 ppb NO_2 for 2 h, (2) 500 ppb NO_2 + 73.4 ± 9.9 µg/m^3 CAPs for 2 h, (3) 89.5 ± 10.7 µg/m^3 CAPS for 2 h; (1−3) Exercise 15 min on/15 min off at $\dot{V}_E$ = 25 L/min	Cell counts and concentrations of IL-6, IL-8, α1-antitrypsin, and LDH in BAL fluid 18-h post-exposure.

Table 5-42 (Continued): Controlled human exposure studies of pulmonary inflammation, injury, and oxidative stress in healthy adults.

Study	Disease Status[a]; n, Sex; Age (Mean ± SD)	Exposure Details (Concentration; Duration)	Endpoints Examined
Jörres et al. (1995)	Healthy; n = 5 M, 3 F; 27 yr (range: 21−33 yr) Asthma; n = 8 M, 4 F; 27 ± 5 yr	1,000 ppb for 3 h; Exercise 10 min on/10 min off at individual's maximum workload	BAL fluid analysis 1 h after exposure (cell counts, histamine, eicosanoids).
Kelly et al. (1996)	n = 44; median: 24 yr (range: 19−45 yr)	2,000 ppb for 4 h; Exercise 15 min on/15 min off at 75 watts	Antioxidant concentrations and malondialdehyde in BAL fluid and bronchial wash at 1.5, 6, or 24-h post-exposure.
Mohsenin (1991)	n = 10 M, 9 F; (range: 21−33 yr)	4,000 ppb for 3 h; Prior to exposure, 4 week course of daily placebo or vitamin C and vitamin E.	BAL fluid immediately after exposure (α1-protease inhibitor, elastase inhibitory capacity, TBARS, conjugated dienes, and phospholipid phosphorus in lipid extraction, albumin).
Pathmanathan et al. (2003)	n = 8 M, 4 F 26 yr (range: 21−32 yr)	2,000 ppb for 4 h/day for 4 days; Exercise 15 min on/15 min off at 75 watts	Biomarkers in bronchial epithelium-exotoxin, GM-CSF, Gro-α, I-CAM 1, IL-5, IL-6, IL-8, IL-10, IL-13, total and active NF-κβ, and TNF-α (fiberoptic bronchoscopy after end of last exposure).
Solomon et al. (2000)	n = 11 M, 4 F; 29.3 ± 4.8 yr	2,000 ppb for 4 h/day for 3 days; Exercise 30 min on/30 min off at $\dot{V}_E$ = 25 L/min	Bronchial wash and BAL fluid analysis immediately after exposure. Differential cell counts, LDH, peripheral blood parameters.
Vagaggini et al. (1996)	Healthy; n = 7 M; 34 ± 5 yr Asthma; n = 4 M, 4 F; 29 ± 14 yr COPD; n = 7 M; 58 ± 12 yr	300 ppb for 1 h; Exercise at $\dot{V}_E$ = 25 L/min	Cell counts in sputum 2-h post-exposure.

BAL = bronchoalveolar lavage, BW = bronchial wash, CAPS = concentrated ambient particles, COPD = chronic obstructive pulmonary disease, F = female, GM-CSF = granulocyte macrophage-colony stimulating factor, IL = interleukin, LDH = lactate dehydrogenase, M = male, NO = nitric oxide. NO_2 = nitrogen dioxide, SD = standard deviation, TBARS - thiobarbituric acid reactive substances, TNF-α = tumor necrosis factor alpha.

[a]Subjects were healthy individuals unless described otherwise.

Several studies reported increases in PMNs and inflammatory mediators following NO_2 exposure. In a study by Frampton et al. (2002), adults exposed to 1,500 ppb NO_2 had increased PMNs in BAL fluid though PMNs were not statistically significantly increased after exposure to 600 ppb, consistent with results from an earlier study (Frampton et al., 1989). No change in BAL fluid protein concentration was reported, but lymphocytes were decreased in peripheral blood and increased in BAL fluid after 600 ppb NO_2. Consistent with Frampton et al. (2002), several studies reported an increase in PMNs in BAL fluid or bronchial wash from adults exposed to 2,000 ppb NO_2 under varying exposure durations and patterns (Solomon et al., 2000; Blomberg et al., 1999; Devlin et al., 1999; Azadniv et al., 1998). Other cell populations, LDH, and protein concentration generally were not altered following NO_2 exposure in these studies. Albumin levels in the bronchial wash fluid were increased following 4 consecutive days of 4-hour exposure to 2,000 ppb NO_2 (Blomberg et al., 1999). In an additional study, Helleday et al. (1994) found that bronchial PMNs were increased in nonsmoking adults while alveolar PMNs were increased in smoking adults 24 hours after a brief exposure to 3,500 ppb NO_2. With respect to cytokine profiles, Devlin et al. (1999) reported increased IL-6 and IL-8 in BAL fluid from adults 16 hours following exposure to 2,000 ppb. However, 2,000 ppb NO_2 for 4 hours repeated over 4 consecutive days did not increase expression of IL-6 and IL-8 in biopsies of the bronchial epithelium obtained from healthy human subjects who were exercising at a light rate but did increase the ICAM-1 in the bronchial epithelium (Pathmanathan et al., 2003). Additionally, Jörres et al. (1995) reported increases in thromboxane B_2 after a 3 hour exposure to 1,000 ppb NO_2, but there were no changes in other eicosanoids. Based on this group of studies, NO_2 exposure can induce pulmonary inflammation in healthy human adults, although evidence does not demonstrate NO_2-induced pulmonary injury.

Studies have also measured effects of NO_2 exposure on antioxidant capacity, but results across studies are mixed. Blomberg et al. (1999) found no changes in glutathione, ascorbic acid, or uric acid levels 1.5 hours after 4 consecutive days of exposure to 2,000 ppb NO_2 for 4 hours. Kelly et al. (1996) examined the kinetics of antioxidant response in the respiratory tract after a single exposure to 2,000 ppb NO_2 and found reduced levels of uric acid and ascorbic acid in bronchial wash and BAL fluid 1.5 hours post-exposure. The levels of these antioxidants were increased or at baseline levels at 6 and 24 hours after exposure. Glutathione was increased at 1.5 and 6 hours in the bronchial wash, but no changes in glutathione were found in the BAL fluid or for reduced glutathione and malondialdehyde at any time after exposure. Additionally, Mohsenin (1991) found increased lipid peroxidation in BAL fluid following a 3-hour exposure to 4,000 ppb NO_2. Supplementation with ascorbate and a-tocopherol decreased the NO_2-induced lipid peroxidation (Mohsenin, 1991), as well as NO_2-induced increases in airway responsiveness (Mohsenin, 1987b) (Section 5.2.2.1). Results from this study

suggest that NO_2 exposure may induce oxidative stress, and antioxidant status may modulate the effects of inhaled NO_2 (Section 4.3.2.1).

Toxicological Studies

Animal toxicological studies reported limited evidence of pulmonary inflammation, injury, and oxidative stress with ambient-relevant, short-term exposures to NO_2 but more consistently indicate effects with long-term exposure (Section 6.2.7.2). Few studies have been published since the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a), and they similarly report inconsistent results. Study details are presented in Table 5-43.

Table 5-43 Animal toxicological studies of pulmonary inflammation, injury, and oxidative stress.

Study	Species (Strain); Age; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Barth et al. (1995)	Rat (Sprague Dawley); M, n = 7/group	5,000, 10,000, and 20,000 ppb NO_2 for 3 or 25 days	Histological evaluation, morphometry, parenchymal and vascular damage, pulmonary arterial thickness, average medial thickness.
Barth and Müller (1999)	Rat (Sprague Dawley); M, n = 5/group	5,000, 10,000, and 20,000 ppb NO_2 for 3 or 25 days	Club cell morphology, cellular proliferation, epithelial proliferation.
de Burbure et al. (2007)	Rat (Wistar); 8 weeks; M; n = 8/group	(1) 1,000 ppb NO_2 for 6 h/day, 5 days/week for 4 weeks; (2) 10,000 ppb NO_2 for 6 h/day, 5 days/week for 4 weeks; (3) 5,000 ppb NO_2 for 6 h/day for 5 days; (1−3) Animals had selenium-deficient or selenium-supplemented diets.	BAL fluid lipid peroxidation, antioxidant enzyme levels, protein concentration, cell counts, oxidant production, selenium levels, and peripheral blood parameters.
Gregory et al. (1983)	Rat (Fischer 344); 14−16 weeks; n = 4−6/group	(1) 1,000 and 5,000 ppb NO_2 for 7 h/day for 5 days/week for up to 15 weeks; (2) 1,000 ppb NO_2 for 0.5 h, 5,000 ppb NO_2 for 1.5 h; (3) 1,000 ppb NO_2 for 3 h, 5,000 ppb NO_2 for 1.5 h; (4) 1,000 ppb NO_2 for 0.5 h for 5 days/week for up to 15 weeks	Histopathological evaluation, BAL fluid and lung homogenate biochemical analysis (protein concentration, LDH, glucose-6-phosphate dehydrogenase, alkaline phosphatase, glutathione reductase, and glutathione peroxidase).

Table 5-43 (Continued): Animal toxicological studies of pulmonary inflammation, injury, and oxidative stress.

Study	Species (Strain); Age; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Hatch et al. (1986)	Guinea pig (Hartley); young adult; n = >3/group	4,800 ppb NO_2 for 3 h in deficient and normal animals; 4,500 ppb NO_2 for 16 h; Animals had Vitamin C deficient or normal diets	BAL fluid protein and antioxidant concentrations, 16 h after the 3 h exposure and within 2 h after the 16 h exposure.
Ichinose et al. (1988)	Mice (ICR), hamster (Golden), rat (Wistar), guinea pig (Hartley); 10 weeks; M; n = NR	400 ppb NO_2, 400 ppb O_3, and 400 ppb NO_2 + 400 ppb O_3 for 24 h/day for 2 weeks	Lipid peroxidation, antioxidant protective enzymes, total proteins, TBA reactants, non-protein sulfhydryls in lung homogenates, immediately after exposure.
Last and Warren (1987)	Rat (Sprague Dawley); M; n = > 4/group	5,000 ppb NO_2, 1.0 mg/m^3 NaCl or H_2SO_4, 5,000 ppb NO_2 + 1.0 mg/m^3 NaCl, 5,000 ppb NO_2 + 1.0 mg/m^3 H_2SO_4 for 23.5 h/day for 1, 3, or 7 days	Collagen synthesis, BAL fluid protein content and lavagable enzyme activities, immediately after exposure.
Müller et al. (1994)	Rat (Sprague Dawley); M; n = 4	800, 5,000, and 10,000 ppb NO_2 for 1 and 3 days	BAL fluid cell counts and protein concentration, phospholipid component, SP-A, morphological changes.
Mustafa et al. (1984)	Mice (Swiss Webster); 8 weeks; M; n = 6/group	(1) 4,800 ppb NO_2; (2) 4,500 ppb O_3; (2) 4,800 ppb NO_2 + 4,500 ppb O_3; (1−3) for 8 h/day for 7 days	Physical and biochemical lung parameters (lung weight, DNA, protein contents, oxygen consumption, sulfhydryl metabolism, NADPH generating enzyme activities), immediately after exposure.
Ohashi et al. (1994)	Guinea pig (Hartley); F; n = 10/group	3,000 and 9,000 ppb NO_2 for 6 h/day, 6 times/week for 2 weeks	Pathology of mucosal samples: accumulation of eosinophils, epithelial injury, mucociliary dysfunction (taken 24 h after end of exposure period).
Pagani et al. (1994)	Rat (CD Cobs); M; n = 5/group	5,000 and 10,000 ppb NO_2 for 24 h and 7 days	Analysis of BAL fluid and superoxide anion production by AMs.
Poynter et al. (2006)	Mice (C57BL/6); n = 5/group	5,000 and 25,000 ppb NO_2 for 6 h/day for 1, 3, or 5 days	Analysis of BAL fluid and histopathological evaluation immediately or 20 days post-exposure.
Robison and Forman (1993)	Rat (Sprague Dawley); M; n = 3/group	100, 1,000, 5,000, and 20,000 ppb NO_2 for 1, 2, and 4 h ex vivo	Enzymatic production of arachidonate metabolites in AMs, cyclooxygenase products.
Robison et al. (1993)	Rat (Sprague Dawley); n > 4/group	500 ppb NO_2 for 8 h/day for 0.5, 1, 5, or 10 days	BAL fluid cell counts and arachidonate metabolite levels, AM arachidonate metabolism, respiratory burst activity, and glutathione content.

Table 5-43 (Continued): Animal toxicological studies of pulmonary inflammation, injury, and oxidative stress.

Study	Species (Strain); Age; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Rose et al. (1989)	Mice (CD-1); 4−6 weeks; n > 4/group	(1) 1,000, 2,500, and 5,000 ppb NO_2 for 6 h/day for 2 days; intra-tracheal inoculation with murine Cytomegalovirus; 4 additional days (6 h/day) of exposure (2) re-inoculation 30 days (air) post-primary inoculation	Infection 5 and 10 days post-inoculation, histopathological evaluation, and analysis of BAL fluid (LDH, albumin, macrophages).
Sherwin et al. (1972)	Guinea pig; M; n = 4/group	2,000 ppb NO_2 continuously for 7, 14, or 21 days	Histopathological evaluation, cellular damage by LDH staining.
Sherwin and Carlson (1973)	Guinea pig; M; n = 9/group	400 ppb NO_2 continuously for 1 week	Protein concentration in BAL fluid.
Schlesinger (1987a)	Rabbit (New Zealand white); M; n = 5/group	0.5 mg/m^3 H_2SO_4 + 300 ppb NO_2, 0.5 mg/m^3 H_2SO_4 + 1,000 ppb NO_2 for 2 h/day for 2, 6, or 13 days	Cell counts in BAL fluid, AM function 24 h after exposure.
Schlesinger et al. (1990)	Rabbit (New Zealand white); M; n = 3/group	(1) 1,000, 3,000, or 10,000 ppb NO_2 for 2 h; (2) 3,000 ppb NO_2 + 300 ppb O_3 for 2 h; (3) 100, 300, or 1,000 ppb O_3 for 2 h	Eicosanoids in BAL fluid, immediately and 24 h after exposure.

H_2SO_4 = sulfuric acid, F = female, LDH = lactate dehydrogenase, M = male, NADPH = reduced nicotinamide adenine dinucleotide phosphate, NO = nitric oxide. NO_2 = nitrogen dioxide, NR = not reported, O_3 = ozone.

Pulmonary Inflammation

Animal studies examined similar endpoints to those in controlled human exposure studies (discussed above) to assess pulmonary inflammation after NO_2 exposure, but effects of NO_2 are inconsistent between humans and laboratory animals. While studies in humans demonstrated increases in BAL fluid PMNs after NO_2 exposure, several studies found no statistically significant changes in BAL fluid inflammatory cells and mediators in rodents exposed to 5,000 ppb NO_2 for up to 7 days (Poynter et al., 2006; Müller et al., 1994; Pagani et al., 1994; Mustafa et al., 1984). Schlesinger (1987a), however, did report an increase in PMNs in BAL fluid from rabbits exposed to 1,000 ppb NO_2 for 2, 6, and 13 days, though all exposures included H_2SO_4.

A series of studies also investigated changes in arachidonic acid metabolism in response to NO_2 exposure. Robison and Forman (1993) exposed rats or rat AMs ex vivo to NO_2 at concentrations as low as 100 ppb and found that in vivo exposure led to statistically significant decreases in eicosanoid levels in as little as 4 hours, while ex vivo exposure of AMs led to statistically significant increases in cyclooxygenase and lipoxygenase activity and slight, but increases in eicosanoids that were statistically nonsignificant. Schlesinger

et al. (1990) studied similar endpoints in rabbits exposed to NO_2 for 2 hours and found an increase in thromboxane B_2 in BAL fluid at 1,000 ppb, but not at 3,000 ppb. Results from NO_2 and O_3 co-exposure suggested that eicosanoid response is more sensitive to O_3 exposure than NO_2.

A study in guinea pigs provides evidence for the development of pro-allergic responses since increases in eosinophils were found in the nasal epithelium and submucosa following a two-week exposure to 3,000 ppb NO_2 (Ohashi et al., 1994) (Table 5-43). The observed increase in numbers of airway eosinophils in this study and expression of Th2 cytokines in Pathmanathan et al. (2003) suggest that inhaled NO_2 may promote Th2 skewing and allergic sensitization (Section 4.3.2.6.3).

Pulmonary Injury

In addition to NO_2-induced changes in inflammatory cells and mediators, toxicological studies have also assessed pulmonary injury at the morphologic and molecular level. For example, Müller et al. (1994) did not find evidence of changes in surfactant or lipid content in BAL fluid at concentrations below 10,000 ppb; however histopathological assessments in lung tissues from this study suggested morphologic changes in the respiratory airways including thickened interstitium and inflammatory cell accumulation. Barth et al. (1995) expanded upon these structural observations and reported pulmonary injury at 10,000 ppb that includes diffuse alveolar damage, epithelial degeneration and necrosis, proteinaceous oedema, inflammatory cell influx, and compensatory proliferation and differentiation. Few morphologic studies have incorporated ambient-relevant NO_2 exposures; however, Barth et al. (1995) reported that slight interstitial edema was present following a 5,000 ppb exposure for 3 days, although this edema was not present after a 25-day exposure. In another study, Barth and Müller (1999) also found slight modifications to the bronchiolar epithelium after 3 days of exposure, though the bronchi appeared normal. The proliferative index of club cells increased in the bronchioles and bronchi relative to air controls following a 3-day exposure to 5,000 ppb, but the number of club cells was only increased in the bronchioles; no changes were observed following a 25-day exposure. Additionally, Last and Warren (1987) found increased collagen synthesis, a feature of fibrosis, in lung homogenates obtained from rats exposed to 5,000 ppb NO_2, which was enhanced with concurrent exposure to H_2SO_4 or NaCl. Overall, short term exposure to NO_2 appears to induce minor morphologic changes in the respiratory tract, although long-term exposure studies (Section 6.2.6) report more profound impacts of exposure.

In addition to pulmonary injury observed at the morphologic level, molecular markers of injury have been described in some studies. Continuous exposure to 400 ppb NO_2 for 1 week resulted in increased BAL fluid protein in guinea pigs on a Vitamin C-deficient

diet (Sherwin and Carlson, 1973), while a 2,000 ppb exposure for 1−3 weeks increased LDH levels in alveolar lung sections (Sherwin et al., 1972). Hatch et al. (1986) also reported increased BAL fluid protein levels in NO_2 exposed Vitamin C-deficient guinea pigs. Gregory et al. (1983) exposed rats to 1,000 and 5,000 ppb NO_2 for up to 15 weeks and found early increases (1.7−2.7 weeks) in LDH in BAL fluid. Rose et al. (1989) did not find any changes in LDH in BAL fluid following a 6-day exposure to 5,000 ppb, though slight increases in albumin were reported, suggesting mild pulmonary injury. In contrast to these studies, a number of studies have shown that NO_2 exposure below 5,000 ppb does not result in an increase in BAL fluid protein and LDH levels in many animal species (Robison et al., 1993; Robison and Forman, 1993; Schlesinger et al., 1990; Last and Warren, 1987).

Oxidative Stress and Antioxidant Status

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a) did not discuss the toxicological evidence relating to effects of NO_2 on antioxidants or oxidative stress, but in the few previous studies, oxidative stress was not consistently induced by ambient-relevant NO_2 concentrations. For example, Ichinose et al. (1988) exposed rats and guinea pigs to 400 ppb NO_2 for 2 weeks and found that levels of lipid peroxides and antioxidants (nonprotein sulfhydryls, Vitamin C, and Vitamin E) were not affected in lung homogenates. Furthermore, there was no change in activity levels of in antioxidant enzymes including glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glutathione S-transferase (GST), glutathione peroxidase (GPx), glutathione reductase, and superoxide dismutase (SOD) after NO_2 exposure; however, combined exposure with O_3 did demonstrate synergistic effects on antioxidant systems.

Studies also report variable effects of NO_2 on glutathione and oxidized glutathione levels in the BAL fluid and peripheral blood. Pagani et al. (1994) found that rats exposed to 5,000 ppb NO_2 for 24 hours had increased total and oxidized glutathione in peripheral blood, though the increase in oxidized glutathione alone was not statistically significant. Conversely, statistically significant increases in oxidized glutathione were reported in the BAL fluid, whereas total glutathione was slightly diminished. de Burbure et al. (2007) reported decreased GPx in plasma immediately and 48 hours after exposure to 1,000 ppb NO_2 for 28 days, whereas GPx increased in the BAL fluid. GST and SOD also increased in BAL fluid after exposure, although SOD returned to control levels by 48 hours post-exposure. Rats exposed to 5,000 ppb NO_2 for 5 days also had reduced levels of GPx in plasma and increased levels of GPx and GST in BAL fluid. SOD also increased in BAL fluid, but only 48 hours post-exposure. Oxidized lipids were transiently increased immediately after exposure in BAL fluid and were not affected in the subacute exposure.

Other studies have reported effects of NO_2 on antioxidant levels or enzyme activity, but those exposures were above ambient-relevant concentrations of NO_2.

Other studies have reported that Vitamin C or E deficiency enhances the effects of NO_2 in the lung, which is plausible given that both vitamins have antioxidant activity in the airways and neutralize reactive oxygen species. Guinea pigs with a Vitamin C-deficient diet had increased BAL fluid protein and lipids following exposure to 1,000 ppb NO_2 for 72 hours or 4,800 ppb for 3 hours relative to air controls or guinea pigs with a normal diet (Hatch et al., 1986; Selgrade et al., 1981). Additionally, exposure to 5,000 ppb for 72 hours resulted in 50% mortality in Vitamin C-deficient guinea pigs. Similarly, rats with diets deficient in Vitamin E had increases in lipid peroxidation and protein content in lung homogenates following a 7-day exposure to 3,000 ppb NO_2 (Elsayed and Mustafa, 1982; Sevanian et al., 1982). Additional support for an influence of Vitamin E is provided by observations that NO_2-induced increases in BAL fluid protein or decreases in glutathione peroxidase activity were attenuated in animals fed Vitamin E-supplemented diets, relative to animals not supplemented with Vitamin E (Guth and Mavis, 1986; Ayaz and Csallany, 1978). These studies demonstrate that antioxidants, particularly Vitamin C and E, can modify the effects of NO_2 on pulmonary injury and oxidative stress in animals.

Development of a pro-allergic phenotype

A few experimental studies indicate that repeated exposures to NO_2 may promote Th2 skewing, which may have implications for allergic sensitization and development of Th2-related conditions such as asthma (Section 4.3.2.6). In guinea pigs, two-week exposure to 3,000 ppb NO_2 led to an increase in eosinophils in the nasal epithelium and submucosa (Ohashi et al., 1994) (Table 5-43). In healthy adults, 2,000 ppb NO_2 exposure for 6 hours on 4 consecutive days increased expression of the Th2 cytokines IL-5, IL-10, and IL-13 in the bronchial epithelium (Pathmanathan et al., 2003). IL-5 promotes eosinophila, and IL-13 promotes airway hyperresponsiveness and mucus production.

5.2.7.5 Summary of Respiratory Effects in Healthy Individuals

The 2008 ISA for Oxides of Nitrogen did not make a specific assessment about the respiratory effects of short-term exposure to oxides of nitrogen in healthy populations (U.S. EPA, 2008a). However, previous and recent epidemiologic evidence indicates ambient NO_2-associated increases in respiratory symptoms and pulmonary inflammation in children in the general population. Neither epidemiologic nor experimental studies clearly support NO_2-related effects on lung function in healthy populations. Experimental

studies do not clearly demonstrate effects on respiratory symptoms in healthy adults either. Although experimental findings are variable across the exposure concentrations and specific outcomes examined, they provide some support for the effects of NO_2 on events that may mediate the occurrence of respiratory symptoms.

Epidemiologic studies show a consistent pattern of elevated odds ratios for associations between short-term increases in ambient NO_2 and cough in school-aged children (Table 5-39). Epidemiologic studies did not examine respiratory symptoms in healthy adults. A majority of evidence was for 24-h avg NO_2, and no clear difference was found between lag day 0 and multiday (2- to 5-day) average concentrations. Most studies recruited children from schools, increasing the likelihood that study populations were representative of the general population. No study reported issues with differential participation by a particular group. Despite the consistency of findings, there is uncertainty as to whether these results support an independent effect of NO_2 on symptoms in healthy children. Associations were reported in study populations with 27% asthma prevalence (Ward et al., 2002) or parental asthma (Rodriguez et al., 2007) but not children without asthma (Patel et al., 2010). Other studies, including a large U.S. multicity study (Schwartz et al., 1994), did not report the health status of study populations. All studies assigned NO_2 exposure from central sites. Symptoms also were associated with CO, BS, and $PM_{2.5}$, PM_{10}, and SO_2, and while there is limited evidence indicating associations for NO_2 with adjustment for PM_{10} or SO_2 (Schwartz et al., 1994), confounding by traffic-related copollutants was not examined. An association found between indoor NO_2 at ice arenas and respiratory symptoms in hockey players (Salonen et al., 2008) provides limited coherence for the findings for outdoor NO_2.

Although evidence for NO_2-associated decreases in lung function is inconsistent overall, associations were observed in healthy adults in studies characterized as having strong exposure assessment with NO_2 and copollutants measured on site of outdoor exposures near busy roads or a steel plant (Dales et al., 2013; Strak et al., 2012). These studies found lung function decrements associated with 5-hour or 10-hour outdoor NO_2 exposures that persisted 0 to 18 hours after exposure. Strak et al. (2012) further indicated NO_2 associations in an array of copollutant models each with another traffic-related pollutant: EC, $PM_{2.5}$ metal components, PNC, or $PM_{2.5}$. That some of the copollutants effect estimates were attenuated with adjustment for NO_2 indicates that NO_2 may have confounded copollutant associations. These findings provide evidence for the independent effects of NO_2 exposure on lung function in healthy individuals, but such informative epidemiologic studies are few in number.

Controlled human exposure studies do not indicate an effect of NO_2 exposures of 200−4,000 ppb (30 minutes to 6 hours) on respiratory symptoms or lung function in

healthy adults (Sections 5.2.7.2 and 5.2.7.3) but provide some support for the epidemiologic findings by demonstrating effects on some key events that can lead to respiratory symptoms (Section 4.3.5). Controlled human exposure studies show increases in airway responsiveness in healthy adults with 3-hour NO_2 exposures above 1,000 ppb but no change at lower concentrations (Section 5.2.7.1). Epidemiologic studies indicate increases in pulmonary inflammation in association with NO_2 measured near children's school or on location of outdoor exposures with adjustment for $PM_{2.5}$, BC, OC, or PNC (Steenhof et al., 2013; Lin et al., 2011). In Steenhof et al. (2013), NO_2 appeared to confound copollutant associations. Experimental studies provide some support for the independent effects of NO_2 exposure. Although pulmonary inflammation and oxidative stress were not always affected in controlled human exposure or toxicological studies with a wide range of NO_2 exposures and durations (800−5,000 ppb for 6 hours to 2 weeks) (Section 5.2.7.4), controlled human exposure studies of healthy adults showed NO_2-induced (1,500−3,500 ppb) increases in PMNs.

Overall, epidemiologic studies find associations of short-term increases in ambient NO_2 concentrations with increases in respiratory symptoms and pulmonary inflammation in children, and to a limited extent, in healthy adults. Evidence specifically attributing the associations to healthy children or to NO_2 independently of other traffic-related pollutants is limited. Experimental studies do not indicate NO_2 effects on respiratory symptoms or lung function in healthy adults. There is experimental evidence for an effect of NO_2 on key events underlying respiratory symptoms, including increases in airway responsiveness and pulmonary inflammation, but variable findings among exposure concentrations and/or specific endpoints limit the extent of support to the epidemiologic evidence for respiratory symptoms and respiratory effects overall in healthy populations.

5.2.8 Respiratory Mortality

Studies evaluated in the 2008 ISA for Oxides of Nitrogen that examined the association between short-term NO_2 exposure and cause-specific mortality consistently found positive associations with respiratory mortality, with some evidence indicating that the magnitude of the effect was larger compared to total and cardiovascular mortality. Recent multicity studies conducted in Asia (Wong et al., 2008), China (Meng et al., 2013; Chen et al., 2012b), and Italy (Faustini et al., 2013; Chiusolo et al., 2011), as well as a meta-analysis of studies conducted in Asian cities Atkinson et al. (2012) add to the initial body of evidence indicating larger respiratory mortality effects (Section 5.4.3, Figure 5-23). However, an additional multicity study conducted in Italy (Bellini et al., 2007), which is an extension of Biggeri et al. (2005), observed relatively consistent risk estimates across mortality outcomes, which differs from the results of the original

analysis and complicates interpretation of whether there is differential risk among mortality outcomes.

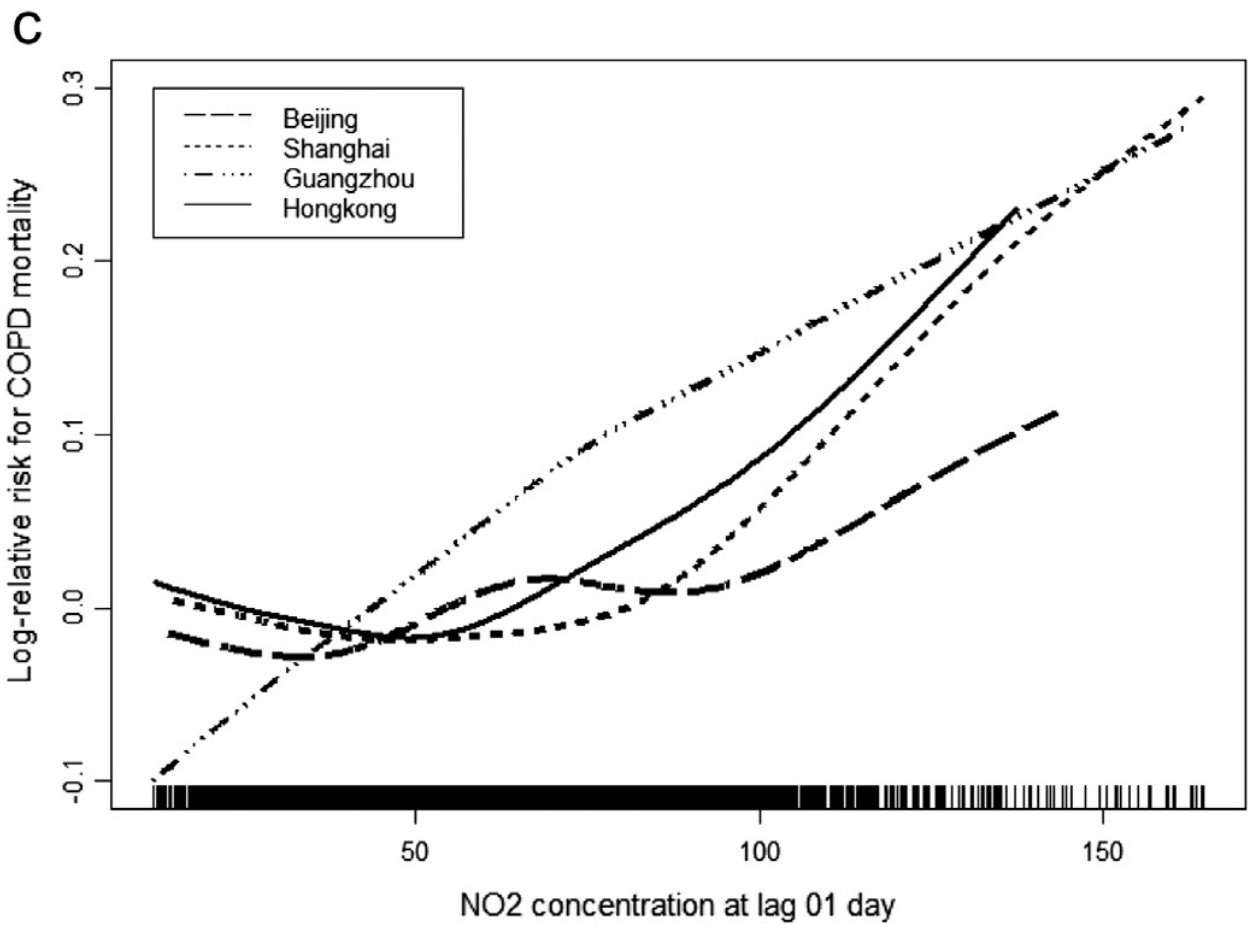
The initial observation of consistent positive NO_2 associations with respiratory mortality was examined in a few studies that conducted copollutant analyses. It is important to note that, similar to interpreting NO_2 associations with total mortality (Section 5.4.4), it is difficult to examine whether NO_2 is independently associated with respiratory mortality because NO_2 is often highly correlated with other traffic-related pollutants. Chen et al. (2012b) in the 17 Chinese cities study [China Air Pollution and Health Effects Study (CAPES)] found that NO_2 risk estimates for respiratory mortality were slightly attenuated, but remained positive in copollutant models with PM_{10} and SO_2 (9.8% [95% CI: 5.5, 14.2]; with PM_{10}: 6.7% [95% CI: 2.9, 10.7]; with SO_2: 7.0% [95% CI: 3.2, 11.0]; for a 20-ppb increase in 24-h avg NO_2 concentrations at lag 0−1 days). These results are consistent with those of Meng et al. (2013) for COPD mortality in a study of four Chinese cities (i.e., 7.1% [95% CI: 5.4, 8.9]; lag 0−1 for a 20-ppb increase in 24-h avg NO_2 concentrations; with PM_{10}: 6.0% [95% CI: 3.2, 8.8]; and with SO_2: 6.9% [95% CI: 4.2, 9.5]). Chiusolo et al. (2011) also found evidence that associations between short-term NO_2 exposure and respiratory mortality remained robust in copollutant models in a study of 10 Italian cities. In both an all-year analysis of NO_2 with PM_{10} (NO_2: 13.7% [95% CI: 2.9, 25.8]; NO_2 with PM_{10}: 13.4% [95% CI: 2.9, 24.9]; for a 20-ppb increase in NO_2 concentrations at lag 1−5 days), and a warm season (April−September) analysis of NO_2 with O_3 (NO_2: 41.3% [95% CI: 16.2, 71.7]; NO_2 with O_3: 43.4% [95% CI: 14.6, 79.5]; for a 20-ppb increase in NO_2 concentrations at lag 1−5 days) NO_2 associations with respiratory mortality were relatively unchanged. However, when focusing on a subset of respiratory mortality, specifically those deaths occurring out-of-hospital, in six Italian cities, Faustini et al. (2013) reported evidence of an attenuation of the NO_2-repiratory mortality association in copollutant models with PM_{10} (NO_2: 24.5% [95% CI: 7.4, 44.2]; lag 0−5 for a 20-ppb increase in 24-h avg NO_2 concentrations; NO_2 with PM_{10}: 11.8% [95% CI: −7.5, 35.0]). Overall, the limited number of studies that have examined the potential confounding effects of copollutants on the NO_2-respiratory mortality relationship generally indicate that associations remain relatively unchanged, but it is remains difficult to disentangle the independent effects of NO_2.

Of the studies evaluated, only the studies conducted in Italy examined potential seasonal differences in the NO_2-respiratory mortality relationship (Chiusolo et al., 2011; Bellini et al., 2007). In a study of 15 Italian cities, Bellini et al. (2007) found that risk estimates for respiratory mortality were dramatically increased in the summer from 1.4 to 9.1% for a 20-ppb increase in 24-h avg NO_2 concentrations at lag 0−1 days, respectively, with no evidence of an association in the winter. These results were further confirmed in a study of 10 Italian cities (Chiusolo et al., 2011), which also observed an increase in risk

estimates for respiratory mortality in the warm season (i.e., April−September) compared to all-year analyses. Chiusolo et al. (2011) did not conduct analyses only for the winter season . Although the respiratory mortality results are consistent with those observed in the total mortality analyses conducted by Bellini et al. (2007) and Chiusolo et al. (2011), as discussed in Section 5.4, studies conducted in Asian cities observed much different seasonal patterns, and it remains unclear whether the seasonal patterns observed for total mortality would be similar to those observed for respiratory mortality in these cities.

An uncertainty that often arises when examining the relationship between short-term air pollution exposures and cause-specific mortality is whether the lag structure of associations and the C-R relationship provide results that are consistent with what is observed for total mortality. Chiusolo et al. (2011) in a study of 10 Italian cities found the strongest evidence for an effect of NO_2 on respiratory mortality at longer lags, with the largest association at lag 2−5 days, which is indicative of a delayed effect (Figure 5-24). These results are supported by the study of (Faustini et al., 2013) in six Italian cities, which found the strongest evidence of an NO_2-association with out-of-hospital respiratory mortality at lags 2−5 and 0−5 days. Evidence of an immediate effect at lag 0−1 day avg was also observed, but the magnitude of the association was smaller compared to lags 2−5 and 0−5 days. However, Chen et al. (2012b) in the CAPES study reported the largest effect at single-day lags of 0 and 1 and the average of lag 0−1 days providing support for an immediate effect of NO_2 on respiratory mortality (Figure 5-25). When examining longer lags Chen et al. (2012b) reported that the magnitude of the association was similar, albeit slightly smaller, for a 0−4 day lag, suggesting a potential prolonged effect. Meng et al. (2013) in a study of COPD mortality in four Chinese cities, of which all are found within the CAPES study cities, reported slightly different results than the CAPES study respiratory mortality results. When examining single-day lags from 0 to 7 days, the authors reported the largest association for lag day 0. However, larger associations were observed in multiday lag analyses with a similar magnitude of an association observed for lags 0−1 and 0−7 days, and the largest magnitude of an association overall for lag 0−4 days.

To date, analyses detailing the C-R relationship between air pollution and cause-specific mortality have been limited. In the analysis of four Chinese cities, Meng et al. (2013) also examined the air pollution and COPD mortality C-R relationship in each individual city. To examine the assumption of linearity the authors fit both a linear and spline model to the city-specific NO_2-COPD mortality relationship. Meng et al. (2013) then computed the deviance between the two models to determine if there was any evidence of non-linearity. An examination of the deviance did not indicate that the spline model improved the overall fit of the NO_2-COPD mortality relationship across the cities examined (Figure 5-15).



Source: Reprinted with permission of Elsevier Meng et al. (2013).

Figure 5-15 City-specific concentration-response curves of nitrogen dioxide and daily chronic obstructive pulmonary disease (COPD) mortality in four Chinese cities.

5.2.9 Summary and Causal Determination

Evidence indicates that there is a causal relationship between short-term NO_2 exposure and respiratory effects based on the coherence among multiple lines of evidence and biological plausibility for effects on asthma exacerbation. There is some support for NO_2-related exacerbation of respiratory allergy and COPD, respiratory infection, respiratory mortality, and respiratory effects in healthy populations. However, because of inconsistency among lines of evidence and consequent uncertainty about the effects of NO_2 exposure, evidence for these other non-asthma respiratory effects does not strongly inform the determination of a causal relationship.

The determination of a causal relationship represents a change from the "sufficient to infer a likely causal relationship" concluded in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a). Consistent with previous findings, recent epidemiologic results indicate associations between ambient NO_2 concentrations and asthma-related respiratory effects. Biological plausibility continues to be provided by the NO_2-induced increases in airway responsiveness and allergic inflammation demonstrated in experimental studies. The 2008 ISA cited uncertainty as to whether NO_2 has an effect independent from other traffic-related pollutants, and additional copollutant model results show ambient NO_2-associated increases in asthma-related effects with adjustment for $PM_{2.5}$, BC/EC, UFP, OC, metals, VOCs, or CO. Thus, much of the evidence for NO_2-related respiratory effects was available in the 2008 ISA. However, the 2008 ISA emphasized epidemiologic findings and did not assess the coherence and biological plausibility for various respiratory conditions separately, which is important given that the weight of evidence varies among respiratory conditions. More than new findings, the evidence integrated across outcomes related to asthma exacerbation, with due consideration of experimental evidence, is sufficient to rule out chance, confounding, and other biases with reasonable confidence and support a change in conclusion from likely to be causal to causal relationship. The evidence for a causal relationship is detailed below using the framework described in the Preamble (Table II). The key evidence as it relates to the causal framework is presented in Table 5-45.

5.2.9.1 Evidence on Asthma Exacerbation

A causal relationship between short-term NO_2 exposure and respiratory effects is strongly supported by evidence for effects across clinical asthma events and pulmonary responses that mediate asthma exacerbation. Epidemiologic studies (Iskandar et al., 2012; Strickland et al., 2010; Jalaludin et al., 2008; Villeneuve et al., 2007) demonstrate associations between increases in ambient NO_2 concentration and increases in asthma hospital admissions and ED visits among subjects of all ages and children (Section 5.2.2.4). Risk estimates ranged from a 4.5 to 34% increase per 20-ppb increase in 24-h avg NO_2 or 30-ppb increase in 1-h max NO_2. These observations are coherent with evidence in children with asthma for increases in respiratory symptoms (Zora et al., 2013; Gent et al., 2009; Delfino et al., 2003; Delfino et al., 2002) (Section 5.2.2.3), the major reason for seeking medical treatment. The recruitment of children from schools supports the likelihood that study populations were representative of the general population of children with asthma. Issues with selective participation by certain groups was not reported. Individual epidemiologic studies examined multiple outcomes and lags of exposure, and not all studies had statistically significant results. However, the pattern

of association observed for NO_2 supports the consistency of evidence and does not indicate a high probability of associations found by chance alone. Consistency also is demonstrated as NO_2-related asthma exacerbation found across diverse locations in the U.S., Canada, and Asia, including several recent multicity studies. Asthma hospital admissions and ED visits were associated with 24-h avg and 1-h max NO_2, whereas respiratory symptoms were associated primarily with 24-h avg NO_2. Most evidence was for multiday lags of NO_2 exposure of 2 to 5 days, but associations also were found with lags of 0 or 1 day. A larger magnitude of association is not clearly indicated for a particular lag of NO_2 exposure. The concentration-response relationship was analyzed for pediatric asthma ED visits in Atlanta, GA and Detroit, MI, and neither a threshold nor deviation from linearity was found in the range of 24-h avg or 1-h max ambient NO_2 concentrations examined (Li et al., 2011b; Strickland et al., 2010).

Epidemiologic evidence for NO_2-related decreases in lung function in populations with asthma is inconsistent as a whole, but associations were found with lung function measured under supervised conditions (Greenwald et al., 2013; Martins et al., 2012; Delfino et al., 2008a; Holguin et al., 2007; McCreanor et al., 2007; Delfino et al., 2003) (Section 5.2.2.2). Many populations had high prevalence of atopy (e.g., 53−84%), which supports the evidence for asthma-related respiratory symptoms, ED visits, and hospital admissions because airway obstruction in response to allergens can lead to lung function decrements and respiratory symptoms. These epidemiologic findings are not clearly supported by findings for respiratory symptoms and lung function in controlled human exposure studies, as most studies found no effect of NO_2 (120−4,000 ppb) in adults with asthma and, in one study, adolescents with asthma. Many studies examined subjects with allergic asthma but did not challenge subjects with an asthma trigger. Only as examined in Riedl et al. (2012), symptoms were not increased after a 3-hour exposure to 350 ppb NO_2 and methacholine challenge.

Although coherence among disciplines is weak for NO_2-related increases in asthma symptoms and decreases in lung function, NO_2-induced increases in airway responsiveness and allergic inflammation in experimental studies provide sufficient biological plausibility for the effects of NO_2 exposure on asthma exacerbation. Increased airway responsiveness can contribute to increases in asthma symptoms such as wheeze. Controlled human exposure studies demonstrated increases in airway responsiveness in adults with asthma at rest and doubling reduction in provocative dose in response to 200 to 300 ppb NO_2 for 30 minutes and 100 ppb for 1 hour [Section 5.2.2.1, (Brown, 2015; Folinsbee, 1992)]. The findings for clinically-relevant airway responsiveness with NO_2 exposures not much higher than peak concentrations near roadways (Section 2.5.3), in particular, support an effect of ambient NO_2 exposures on asthma exacerbation. Further characterizing mechanisms for NO_2-induced airway responsiveness and asthma

symptoms is evidence for NO_2 exposures of 260−400 ppb enhancing allergic inflammation (e.g., eosinophil activation, Th2 cytokines, PMNs) in humans with allergic asthma and a rat model of allergic disease (Ezratty et al., 2014; Barck et al., 2005a; Barck et al., 2002; Wang et al., 1999) (Sections 4.3.2.6 and 5.2.2.5). NO_2-associated increases in pulmonary inflammation also were found in epidemiologic studies of populations with asthma (Section 5.2.2.5). As allergic inflammation promotes bronchoconstriction and airway obstruction, the evidence describes key events in the mode of action for NO_2-associated increases in respiratory symptoms found in populations of children with asthma with prevalence of atopy ranging from 47 to 77%. In experimental studies, ambient-relevant NO_2 exposures increased eicosanoids (involved in PMN recruitment) but did not consistently affect lung injury or pulmonary oxidative stress (Section 5.2.7.4). These inconsistent findings for other key events in the mode of action for asthma exacerbation are not considered to weaken the evidence for a relationship with NO_2 because the effects were studied mostly in healthy humans and animal models.

5.2.9.2 Evidence on Nonasthma Respiratory Effects

Epidemiologic studies demonstrate associations of ambient NO_2 concentrations with hospital admissions and ED visits for all respiratory causes combined (Table 5-45), suggesting that the respiratory effects of short-term NO_2 exposure may extend beyond exacerbation of asthma. However, when other respiratory conditions are evaluated individually, there is uncertainty about relationships with NO_2 because of inconsistency among disciplines and/or inconsistency among outcomes ranging from clinical events to key events in modes of action. Where epidemiologic associations were found, limited examination of potential confounding by traffic-related copollutants results in weak inference about NO_2 effects. Experimental evidence for NO_2-induced increases in airway responsiveness and allergic inflammation supports effects on allergy exacerbation, but epidemiologic evidence is inconsistent (Section 5.2.3). For COPD exacerbation and respiratory infection (Sections 5.2.4 and 5.2.5), evidence from epidemiologic, controlled human exposure, and toxicological studies is inconsistent across outcomes such as hospital admissions, ED visits, symptoms, lung function, and immune cell function; thus, a direct effect of NO_2 exposure is not clearly demonstrated (Table 5-45). Epidemiologic studies consistently found NO_2-associated increases in respiratory mortality (Section 5.2.8), but the spectrum of respiratory effects that can lead to mortality is not entirely clear. Among the leading causes of mortality, COPD, and respiratory infections are the ones related to respiratory causes (Hoyert and Xu, 2012), but these conditions are not clearly related to NO_2 exposure. Epidemiologic evidence also indicates ambient NO_2-associated respiratory effects in healthy populations (Section 5.2.7), as cough and

pulmonary inflammation in children in the general population and healthy adults. However, an independent effect of NO_2 is uncertain because of limited support from experimental studies.

5.2.9.3 Evaluation of Nitrogen Dioxide Exposure Assessment

Most epidemiologic evidence indicating ambient NO_2-related asthma exacerbation is based on exposure assessment from central site concentrations. Substantiating the evidence are several findings for associations with NO_2 concentrations spatially aligned with subjects' location(s), including total and outdoor personal NO_2 as well as NO_2 measured outside schools (Greenwald et al., 2013; Zora et al., 2013; Martins et al., 2012; Sarnat et al., 2012; Spira-Cohen et al., 2011; Delfino et al., 2008a; Holguin et al., 2007; McCreanor et al., 2007). Ambient NO_2 concentrations are highly variable across locations (Sections 2.5.2 and 2.5.3). Thus, compared to area-wide central site concentrations, NO_2 concentrations in subjects' locations may better represent temporal variation in subjects' ambient exposures in those locations. NO_2 concentrations summed across individuals' microenvironments have shown good agreement with total personal NO_2 (Section 3.4.3.1), demonstrating that microenvironmental ambient concentrations are important determinants of exposure. Further supporting asthma exacerbation in relation to ambient NO_2 exposure, for some study areas, central site concentrations were reported to be correlated with total personal NO_2 (Delfino et al., 2008a), outdoor school NO_2 (Sarnat et al., 2012), or NO_2 measured at other central sites in the area (Section 2.5.2). In support of exposure assessment from central sites, larger ambient NO_2-associated increases in respiratory hospital admissions and ED visits were found in the warm season. Personal-ambient NO_2 correlations are higher in the warm than cold season (Section 3.4.4.3), pointing to lower potential NO_2 exposure error.

The studies with microenvironmental exposure assessment provide some, albeit far from conclusive, indication that short-term NO_2 exposures near sources may be related to respiratory effects. In some cases, respiratory effects were observed in association with ambient NO_2 measured across locations with varying traffic intensities or distance to highways (Steenhof et al., 2013; Strak et al., 2012; Spira-Cohen et al., 2011), but NO_2 associations were not compared among locations. Other studies provided stronger support, observing respiratory effects in association with NO_2 at a school in a high but not low traffic area (Greenwald et al., 2013; Sarnat et al., 2012) or larger respiratory effects near a steel plant than in a residential area (Dales et al., 2013). However, none of these studies examined whether the findings were attributable to NO_2 independently of correlated copollutants or differences between locations in population characteristics such as race/ethnicity, body mass index, or asthma medication use. Informing these

uncertainties, McCreanor et al. (2007) observed that adults with asthma had larger decreases in lung function and increases in eosinophil activation after walking along a high traffic road in London, U.K. than after walking in a park. Observations that associations with personal ambient NO_2 persisted with adjustment for personal ambient EC, UFP, or $PM_{2.5}$ provide evidence for an independent relationship between respiratory effects and NO_2 exposures near high traffic roads.

Whether estimated from central sites or subjects' locations, NO_2 exposure metrics largely were integrated over 24 hours or 2-15 hours. The diurnal temporal pattern of exposure (e.g., acute peaks) underlying the associations of respiratory effects with daily average or multiday averages of NO_2 is not well characterized. However, studies conducted in outdoor locations with varying traffic intensities indicate increases in pulmonary inflammation and decreases in lung function in association with 2- or 5-h avg NO_2 exposures that ranged between 5.7 and 153.7 ppb (Strak et al., 2012; McCreanor et al., 2007).

5.2.9.4 Evaluation of Confounding

Also supporting an independent effect of NO_2 exposure on asthma exacerbation are epidemiologic associations found for NO_2 with statistical adjustment for potential confounding factors such as temperature, humidity, season, medication use, and, in particular, copollutants. Based on a common source and moderate to high correlations with NO_2, confounding by other traffic-related pollutants is a major concern (Sections 1.4.3 and 5.1.2.1). Copollutant models were the predominant method used for evaluating copollutant confounding, and most of these studies found that NO_2 associations persisted with adjustment for $PM_{2.5}$, BC/EC, OC, UFP, PNC (Figure 5-16 and Table 5-44), $PM_{2.5}$ metal components, VOCs, or CO (Figure 5-17 and Table 5-44). Copollutant models also indicated that NO_2 associations with asthma and other respiratory effects were independent of $PM_{10-2.5}$, PM_{10}, SO_2, or O_3 [Supplemental Figure S5-1 (U.S. EPA, 2014a)]. O_3 (r = −0.61 to 0.45) and PM_{10} (r = −0.71 to 0.59) showed a wide range of correlations with NO_2, from strongly negative to moderately positive; SO_2 was moderately correlated with NO_2 (r = 0.31−0.56). Inference regarding confounding by traffic-related copollutants is strongest for exposure assessment in subjects' locations. Exposure measurement error due to spatial variability may be similar for NO_2 and copollutants, thereby improving the reliability of copollutant models. These studies reported a wide range of correlations between NO_2 and traffic-related copollutants (r = −0.42 to 0.68). Also strengthening inference from copollutant models, in some studies, personal NO_2 was not strongly positively correlated with personal copollutants (r = 0.20−0.33 for EC, OC, $PM_{2.5}$; −0.42 to 0.08 for ethylbenzene and benzene) (Martins

et al., 2012; Delfino et al., 2006). As examined in multiple populations with asthma, associations of lung function and pulmonary inflammation with total or outdoor personal NO_2 and NO_2 measured within 650 m of a children's school persisted with adjustment for $PM_{2.5}$ or BC/EC (Martins et al., 2012; Lin et al., 2011; Delfino et al., 2008a; McCreanor et al., 2007; Delfino et al., 2006). Results were similar in a study of healthy adults (Steenhof et al., 2013; Strak et al., 2012). In some cases, the 95% CIs for NO_2 associations were exaggerated because the increment used to standardize effect estimates is far larger than the variability in NO_2 concentrations during the study period (Martins et al., 2012; Strak et al., 2012). Most studies examining exposures at subjects' locations did not examine CO, either in single- or co-pollutant models. However, among children in the general population, the association between outdoor school NO_2 and lung function persisted with adjustment for CO (Correia-Deur et al., 2012).

As examined in only one or two studies with exposures assessed in subjects' locations, NO_2 associations persisted with adjustment for OC or metal $PM_{2.5}$ components such as iron and copper (Steenhof et al., 2013; Strak et al., 2012; Delfino et al., 2006). Information on potential confounding by UFP/PNC or VOCs also is limited, and results from copollutant models are more variable. However, rather than clearly demonstrating confounding of NO_2 associations, results show that adjustment for UFP/PNC or benzene attenuated one outcome in a study but not another (Steenhof et al., 2013; Martins et al., 2012; Strak et al., 2012; McCreanor et al., 2007). There also was some evidence that NO_2 exposure confounded associations for traffic-related copollutants. Some associations of personal ambient $PM_{2.5}$, EC/BC, OC, copper, UFP/PNC, or benzene with pulmonary inflammation and lung function were attenuated with adjustment for personal ambient NO_2 (Martins et al., 2012; Strak et al., 2012; McCreanor et al., 2007). Also supporting an independent association for NO_2, some studies found associations with NO_2 but not EC, OC, or $PM_{2.5}$ for school or personal measurements (Sarnat et al., 2012; Delfino et al., 2008a; Holguin et al., 2007).

Copollutant models based on central site concentrations indicate that ambient NO_2 remains associated with asthma- and non-asthma-related respiratory effects with adjustment for $PM_{2.5}$ (Iskandar et al., 2012; Dales et al., 2009; Jalaludin et al., 2008; Villeneuve et al., 2007; von Klot et al., 2002) or as examined in fewer studies, UFP, a source apportionment factor comprising EC and various metals, CO, or VOCs (Delfino et al., 2013; Gent et al., 2009; Tolbert et al., 2007; Delfino et al., 2003). Several traffic-related PM constituents are shown to induce oxidative stress (Table 5-1), and Delfino et al. (2013) found an NO_2 association with adjustment for the oxidative potential of $PM_{2.5}$ extracts. Observations of larger ambient NO_2-associated increases in respiratory-related hospital admissions and ED visits in the warm rather than cold season also support an independent NO_2 association. NO_2-$PM_{2.5}$ correlations are lower in the

warm season (Section 3.4.5.1), pointing to lower potential confounding by $PM_{2.5}$. NO_2 and O_3 are not strongly positively correlated in the warm season. As with NO_2 measured in subjects' locations, some central site NO_2 associations were attenuated with adjustment for a traffic-related copollutant. However, in the same studies, NO_2 associations with other outcomes persisted with $PM_{2.5}$ or UFP adjustment (Dales et al., 2009; Liu et al., 2009; von Klot et al., 2002), and a clear confounding effect was not demonstrated. Similar to NO_2 measured in subjects' locations, central site NO_2 showed a range of correlations with traffic-related pollutants (r = 0.43−0.74). However, because of different spatial distributions (Section 3.3.1.1), exposure measurement error may differ between central site concentrations of NO_2 and traffic-related copollutants, resulting in weaker inference from copollutant models.

Confounding by any particular copollutant was examined to a limited extent, and not all potentially correlated pollutants were examined. Further, inference from copollutant models can be limited (Section 5.1.2.2), and methods to adjust for multiple copollutants simultaneously are not reliable. Thus, residual confounding is likely. However, copollutant model results, particularly for pollutants measured in subjects' locations, integrated with experimental findings, support an effect of ambient NO_2 exposure on asthma exacerbation independent of other traffic-related pollutants.

5.2.9.5 Evaluation of Nitrogen Dioxide-Copollutant Mixture Effects

As a component of an air pollution mixture, NO_2 potentially can induce health effects in combination with other pollutants in the mixture. Controlled human exposure studies, with well-defined NO_2-copollutant co-exposures, do not provide strong evidence that the effects of NO_2 exposure on lung function and airway responsiveness differ when occurring alone or as part of a mixture with $PM_{2.5}$ (Gong et al., 2005), SO_2 (Devalia et al., 1994), or O_3 (simultaneous or sequential exposure) (Jenkins et al., 1999; Hazucha et al., 1994; Adams et al., 1987). Interactions with CO, EC/BC, or UFP have not been examined in controlled human exposure studies. But, limited epidemiologic findings point to increases in asthma-related symptoms and ED visits in association with short-term increases in ambient NO_2 concentration examined alone and jointly with traffic-related pollutants such as $PM_{2.5}$, CO, and EC (Gass et al., 2014; Winquist et al., 2014; Schildcrout et al., 2006) and with O_3 and SO_2 (Winquist et al., 2014). However, there is no clear indication of combined (i.e., synergistic) effects of NO_2 with $PM_{2.5}$, CO, EC, or VOCs larger than effects of individual pollutants alone (Gass et al., 2014; Winquist et al., 2014; Schildcrout et al., 2006; Delfino et al., 2003). Inference from these

epidemiologic findings of joint effects is limited because exposure measurement error in the central site pollutant metrics may obscure interactions between personal exposures.

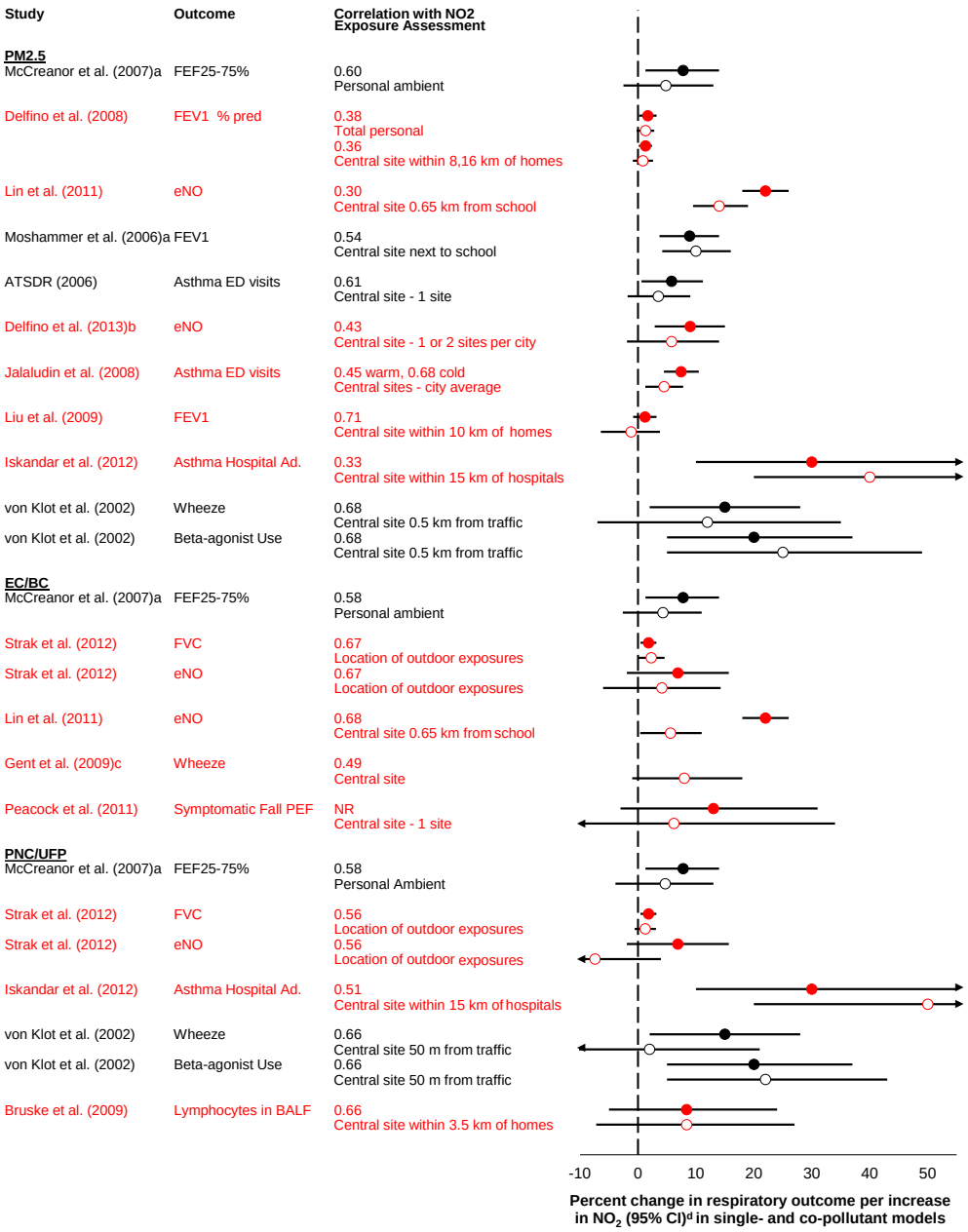
5.2.9.6 Indoor Nitrogen Dioxide-Related Asthma Exacerbation

A causal relationship between NO_2 and respiratory effects also is supported by the coherence of asthma-related effects associated with ambient and indoor NO_2 (Table 5-45). In schools in Ciudad Juarez, Mexico, correlations of NO_2 with BC, $PM_{2.5}$, PM_{10}, $PM_{10-2.5}$, and SO_2 differed indoors and outdoors, suggesting that NO_2 was part of a different pollutant mixture indoors and outdoors. NO_2 also may be part of different pollutant mixtures inside homes and classrooms because gas heaters and not stoves are a major source of classroom NO_2. Cooking has been shown to be a more important determinant of indoor UFP than heating systems (Weichenthal et al., 2007). Thus, associations with indoor classroom NO_2 may be less likely to be confounded by UFP than are associations with indoor home NO_2 or ambient NO_2. Mean concentrations of indoor NO_2, averaged over 3 to 7 days, were in the range of ambient concentrations (Table 5-45), except for a mean of 121 ppb at one school. Indoor NO_2 concentrations, particularly at home, can exhibit acute peaks that deviate from the mean (Table 3-4). As with ambient NO_2, the temporal pattern of NO_2 concentrations underlying associations of respiratory effects with multiday averages of indoor NO_2 is not understood.

5.2.9.7 Conclusion

Multiple lines of evidence support a relationship between short-term NO_2 exposure and asthma exacerbation. Some findings point to effects on allergy, COPD, respiratory infection, respiratory effects in healthy populations, and respiratory mortality, but there is inconsistency among disciplines and outcomes. Increases in asthma hospital admissions, ED visits, as well as respiratory symptoms and pulmonary inflammation in populations with asthma are found in association with 24-h avg and 1-h max NO_2 concentrations, at lags of 0 or 1 day and multiday averages of 2 to 5 days. Across studies finding NO_2-associated effects on asthma, the range of mean ambient concentrations was 11.3−30.9 ppb for 24-h avg NO_2, 75.5 ppb for 2-h avg NO_2, and 23.0−44.4 ppb for 1-h max NO_2. The epidemiologic evidence is substantiated by the consistency of findings among central site ambient NO_2 and NO_2 measured in subjects' location(s), including personal, ambient school, ambient near-road, or indoor concentrations. Further, associations of ambient and personal NO_2 with asthma-related effects persist with adjustment for meteorological factors and/or another traffic-related pollutants such as $PM_{2.5}$, BC/EC, UFP, OC, metals, VOCs, or CO. Inference from copollutant models is

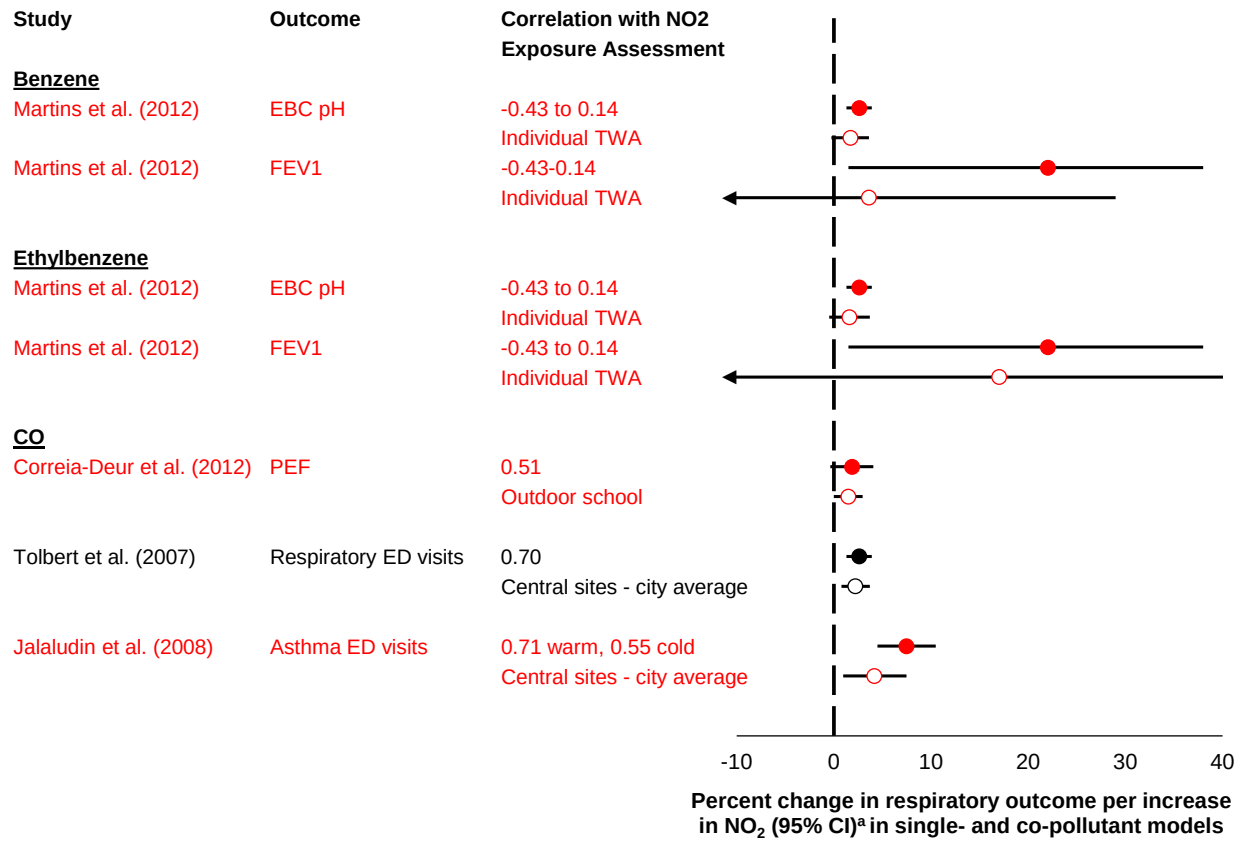
limited as is the breadth of analysis of traffic-related copollutants and copollutant interactions. Thus, the coherence of epidemiologic findings for ambient and indoor NO_2 with effects demonstrated on key events in the mode of action for asthma exacerbation provide strong evidence for an independent effect of NO_2 exposure. Epidemiologic evidence for NO_2-associated asthma exacerbation persisting with adjustment for another traffic-related pollutant and biological plausibility from NO_2-induced increases in airway responsiveness and allergic inflammation in adults with asthma and animal models of asthma are sufficient to conclude that there is a causal relationship between short-term NO_2 exposure and respiratory effects.



Note: EC/BC = elemental/black carbon, NO_2 = nitrogen dioxide, $PM_{2.5}$ = particles with an aerodynamic diameter less than equal to a nominal 2.5 µm, PNC = particle number concentration, UFP = ultrafine particles. Magnitude and precision of effect estimates should not be compared among different outcomes. Results are organized by copollutants analyzed then by exposure assessment method. Percentage change in $FEF_{25-75\%}$, FEV_1, or FVC refers to percentage decrease. Studies in red = recent studies, studies in black = studies reviewed in the 2008 ISA for Oxides of Nitrogen. Effect estimates in closed circles = NO_2 in a single-pollutant model, effect estimates in open circles = NO_2 adjusted for a copollutant. Quantitative results and abbreviations are described in Table 5-44.

[a]To fit results in the figure, effect estimates are multiplied by 10. [b]Copollutant is ROS generated from $PM_{2.5}$ extract. [c]Copollutant is a source apportionment factor comprising EC and various metals. [d]Effect estimates standardized to a 20-ppb increase for 24-h avg NO_2 and a 30-ppb increase for 1-h max NO_2. Effect estimates for 2-h, 5-h, or 15-h avg NO_2 are not standardized but presented as reported in their respective studies (see Section 5.1.2.3).

Figure 5-16 Associations of ambient or personal NO_2 with respiratory effects adjusted for $PM_{2.5}$, EC/BC, or PNC/UFP.



Note: VOC = volatile organic compound, CO = carbon monoxide. Magnitude and precision of effect estimates should not be compared among different outcomes. Results are organized by copollutants analyzed then by exposure assessment method. Percentage change in EBC pH, FEV_1, and PEF refers to percentage decrease. Studies in red = recent studies, studies in black = studies reviewed in the 2008 ISA for Oxides of Nitrogen. Effect estimates in closed circles = NO_2 in a single-pollutant model, effect estimates in open circles = NO_2 adjusted for a copollutants. Quantitative results and abbreviations are described in Table 5-44.

[a]Effect estimates standardized to a 20-ppb increase for 24-avg NO_2 and a 30-ppb increase for 1-h max NO_2.

Figure 5-17 Associations of ambient nitrogen dioxide (NO_2) with respiratory effects adjusted for VOCs or CO.

Table 5-44 Corresponding effect estimates for nitrogen dioxide (NO_2)-associated respiratory effects in single- and co-pollutant models presented in Figures 5-16 and 5-17.

Study	Respiratory Outcome	NO_2 Averaging Time and Lag	Exposure Assessment Method	Correlation with NO_2	% change in outcome(95% CI) per increase in NO_2[a]	
					Single-Pollutant Model	Copollutant model
McCreanor et al. (2007)	$FEF_{25-75\%}$	2-h avg Lag 0 h	Personal ambient	0.60	7.8 (1.3, 14)[b] per 5.3 ppb NO_2	With $PM_{2.5}$: 4.8 (−2.5, 13)[b]
				0.58		With EC: 4.3 (−2.6, 11)[b]
				0.58		With UFP: 4.7 (−3.9, 13)[b]
Delfino et al. (2008a)	FEV_1 % predicted	24-h avg Lag 0−1−day avg	Total personal	0.38	1.7 (0.19, 3.2)	With $PM_{2.5}$: 1.3 (−0.22, 2.8)
			Central site within 8 or 16−km of homes	0.36	1.3 (0.15, 2.4)	With $PM_{2.5}$: 0.86 (−0.89, 2.6)
Zhu (2013)]; Lin et al. (2011)	eNO	24-h avg Lag 0 day	Central site 0.65−km of school	0.30	22 (18, 26)	With $PM_{2.5}$: 14 (9.5, 19)
				0.68		With BC: 5.6 (0.38, 11)
Moshammer et al. (2006)	FEV_1	8-h avg (12−8 a.m.) Lag 0 day	Central site next to school	0.54	8.9 (3.7, 14)[b] per 5.32 ppb NO_2	With $PM_{2.5}$: 10 (4.2, 16)[b]
ATSDR (2006)	Asthma ED visits	24-h avg Lag 0−4 day avg	Central site: 1 site	0.61	5.8 (0.59, 11)	With $PM_{2.5}$: 3.5 (−1.8, 9.0)
Delfino et al. (2013)	eNO	24-h avg Lag 0−1 day avg	Central site: 1 or 2 sites per city	0.43	9.0 (2.9, 15)	With $PM_{2.5}$: 5.8 (−1.9, 14)[c]
Jalaludin et al. (2008)	Asthma ED visits	1-h max Lag 0−1 day avg	Central site: city average	0.45 warm 0.68 cold	7.4 (4.5, 10)	With $PM_{2.5}$: 4.5 (1.3, 7.8)
Liu (2013); Liu et al. (2009)	FEV_1	24-h avg Lag 0−2 day avg	Central site within 10 km of homes	0.71	1.2 (−0.84, 3.2)	With $PM_{2.5}$: −1.2 (−6.4, 3.8)
Iskandar et al. (2012)	Asthma hospital admissions	24-h avg Lag 0−4 day avg	Central site within 15 km of hospital	0.33	30 (10, 60)	With $PM_{2.5}$: 40 (20, 70)
				0.51		With UFP: 50 (20, 80)

Table 5-44 (Continued): Corresponding effect estimates for nitrogen dioxide (NO2)-associated respiratory effects in single- and co-pollutant models presented in Figures 5-16 and 5-17.

Study	Respiratory Outcome	NO_2 Averaging Time and Lag	Exposure Assessment Method	Correlation with NO_2	% change in outcome(95% CI) per increase in NO_2[a]	
					Single-Pollutant Model	Copollutant model
von Klot et al. (2002)	Wheeze	24-h avg Lag 0−4 day avg	Central site 50 m from traffic	0.68	15 (2.0, 28)	With $PM_{2.5}$: 12 (−7.0, 35)
				0.66		With UFP: 2.0 (−14, 21)
	Beta agonist use			0.68	20 (5.0, 37)	With $PM_{2.5}$: 25 (5.0, 49)
				0.66		With UFP: 22 (5.0, 43)
Strak (2013); Strak et al. (2012)	FVC	5-h avg Lag 0 h	Location of outdoor exposures	0.67	1.8 (0.44, 3.2) per 10.54 ppb NO_2	With EC: 2.3 (0, 4.6)
				0.56		With PNC: 1.3 (−0.58, 3.1)
	eNO			0.67	6.9 (−1.9, 16) per 10.54 ppb NO_2	With EC: 4.1 (−6.0, 14)
				0.56		With PNC: −7.4 (−19, 3.9)
Gent et al. (2009)	Wheeze	NR Lag 0 day	Central site	0.49	NR	With source apportionment factor of EC, zinc, copper, lead: 8.0 (−1.0, 18)
Peacock et al. (2011)	Symptomatic fall in PEF	1-h max Lag 1 day	Central site: 1 site	NR	13 (−3.0, 31)	With BS: 6.2 (−17, 34)
Brüske (2014); Bruske et al. (2010)	Lymphocytes in BAL fluid	24-h avg Lag 0−23 h	Central site within 3.5 km of homes	0.66	8.4 (−5.0, 24)	With UFP: 8.4 (−7.2, 27)

Table 5-44 (Continued): Corresponding effect estimates for nitrogen dioxide (NO2)-associated respiratory effects in single- and co-pollutant models presented in Figures 5-16 and 5-17.

Study	Respiratory Outcome	NO_2 Averaging Time and Lag	Exposure Assessment Method	Correlation with NO_2	% change in outcome(95% CI) per increase in NO_2[a]	
					Single-Pollutant Model	Copollutant model
Martins (2013); Martins et al. (2012)	EBC pH	24-h avg Lag 0−4 day avg	Individual TWA based on outdoor monitoring, modeling, time-activity data	−0.43 to 0.14 across time periods	2.6 (1.3, 3.9)	With benzene: 1.7 (−0.26, 3.6)
						With ethylbenzene: 1.6 (−0.49, 3.7)
	FEV_1				22 (1.5, 38)	With benzene: 3.6 (−31, 29)
						With ethylbenzene: 17 (−17, 41)
Correia-Deur et al. (2012)	PEF	24-h avg Lag 0 day	Outdoor school	0.51	1.9 (−0.38, 4.1)	With CO: 1.5 (0, 3.0)
Tolbert (2009); Tolbert et al. (2007)	Respiratory ED visits	1-h max Lag 0−2 day avg	Central sites: city average	0.70	2.6 (1.3, 3.9)	With CO: 2.2 (0.78, 3.7)
Jalaludin et al. (2008)	Asthma ED visits	1-h max Lag 0−1 day avg	Central sites: city average	0.71 warm 0.55 cold	7.4 (4.5, 10)	With CO: 4.2 (0.78, 3.7)

$FEF_{25-75\%}$ = Forced expiratory flow between 25 and 75% of forced vital capacity, FEV_1 = forced expiratory flow in 1 second, eNO = exhaled breath condensate, ED = emergency department, FVC = forced vital capacity, PEF = peak expiratory flow, NR = not reported, EBC = exhaled breath condensate, TWA = time-weighted average.

[a]single- and copollutant model results are standardized to a 20-ppb increase in 24-h avg NO_2 and 30-ppb increase in 1-h max NO_2. Results based on other averaging times are not standardized but presented as reported in their respective studies (Section 5.1.2.3). Percentage change in $FEF_{25-75\%}$, FEV_1, FVC, PEF, and EBC pH refers to percentage decrease.

[b]To fit results in Figure 5-16, results are multiplied by 10.

[c]Copollutant specifically is reactive oxygen species generated from ambient $PM_{2.5}$ extracts.

Table 5-45 Summary of evidence for a causal relationship between short-term nitrogen dioxide (NO_2) exposure and respiratory effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Asthma Exacerbation			
Consistent epidemiologic evidence from multiple, high-quality studies at relevant NO_2 concentrations	Increases in asthma hospital admissions, ED visits in diverse populations in association with 24-h avg and 1-h max NO_2, lags 0 and 3 to 5-day avg among all ages and children.	Strickland et al. (2010), Villeneuve et al. (2007), Jalaludin et al. (2008), Ito et al. (2007a), Iskandar et al. (2012), ATSDR (2006) Section 5.2.2.4, Figure 5-7	Overall study mean 24-h avg: 11.3−31.3 ppb Overall study mean 1-h max: 23−44 ppb
	No association in recent Canadian multicity study.	Stieb et al. (2009)	Mean 24-h avg: 21.4−41.2 ppb
	Coherence with increases in respiratory symptoms and decrements in lung function in populations with asthma in association with 24-h avg, 2-4 h avg NO_2, 1-h max, lags 0, 3 to 6-day avg. Panel studies of children examined representative populations recruited from schools. No reports of selective participation by particular groups.	Schildcrout et al. (2006), Gent et al. (2009), Zora et al. (2013), Greenwald et al. (2013), Holguin et al. (2007), Delfino et al. (2008a), McCreanor et al. (2007) Sections 5.2.2.2 and 5.2.2.3, Figure 5-3 and Figure 5-4	Outdoor school mean 1-week avg: 3.4−18.2 ppb Personal outdoor 2-h avg: 75.5 ppb Total personal mean 24-h avg: 28.6 ppb City mean 24-h avg: 17.8−26 ppb
Consistent evidence for NO_2 metrics with lower potential for exposure measurement error	Asthma-related effects associated with NO_2 measured in subjects' locations: total and outdoor personal, school outdoor. Better spatial alignment with subjects compared to central site NO_2.	Greenwald et al. (2013), Holguin et al. (2007), Delfino et al. (2008a), McCreanor et al. (2007), Sarnat et al. (2012), Zora et al. (2013), Delfino et al. (2006)	
Consistent evidence from multiple, high-quality controlled human exposure studies Rules out chance, confounding, and other biases with reasonable confidence	NO_2 increases airway responsiveness in adults with asthma exposed at rest following nonspecific or allergen challenge in several individual studies and meta-analyses. Clinical relevance supported by findings of a doubling reduction in provocative dose in response to NO_2.	Folinsbee (1992), Brown (2015) Section 5.2.2.1, Table 5-4, Table 5-5, and Table 5-6	Any change: 100 ppb for 1 h 200−300 ppb for 30 min Doubling reduction in PD: 100 ppb for 1 h, 140 ppb for 30 min

Table 5-45 (Continued): Summary of evidence for a causal relationship between short-term nitrogen dioxide (NO2) exposure and respiratory effects

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Epidemiologic evidence helps rule out chance, confounding, and other biases with reasonable confidence	NO_2 associations with lung function and pulmonary inflammation persist in copollutant models with a traffic-related copollutant: $PM_{2.5}$, EC/BC, OC, UFP, or VOCs in studies with exposure assessment in subjects' locations. Ambient and total personal NO_2 weakly-moderately correlated with other traffic-related pollutants in some studies (r = −0.43 to 0.49).	Delfino et al. (2006), Delfino et al. (2008a), Martins et al. (2012), McCreanor et al. (2007) Figure 5-16 and Figure 5-17, Table 5-44	Same as above
	Most central site NO_2 associations persist with adjustment for $PM_{2.5}$, EC/metals factor, UFP, or CO. Differential exposure measurement error limits inference from copollutant models based on central site NO_2 and copollutants.	Villeneuve et al. (2007), Jalaludin et al. (2008), Gent et al. (2009), von Klot et al. (2002)	
	Some associations were attenuated with adjustment for $PM_{2.5}$ or UFP.	Liu et al. (2009), von Klot et al. (2002)	
	Most associations for microenvironmental and central site NO_2 persist in copollutant models with PM_{10}, SO_2, or O_3.	Supplemental Figure S5-1 (U.S. EPA, 2014a)	
	Indoor NO_2 associated with increases in respiratory effects in children with asthma.	Sarnat et al. (2012), Lu et al. (2013), Hansel et al. (2008) No association in Greenwald et al. (2013)	Means of 3- to 7-day avg: 18.7−121 ppb 75th: 31 ppb Max: 394 ppb
	NO_2 associations persist with adjustment for meteorology, time trends, season, medication use.		
Evidence for Key Events in Mode of Action			
Allergic responses	Increases in eosinophil activation, IgE, Th2 cytokines in adults with asthma.	Barck et al. (2005a), Barck et al. (2002), Wang et al. (1995a), Ezratty et al. (2014) Sections 4.3.2.6, and 5.2.2.5 Figure 4-1	Humans: 260 ppb 15−30 min, 400 ppb 6 h, 581 ppb 30 min on 2 days

Table 5-45 (Continued): Summary of evidence for a causal relationship between short-term nitrogen dioxide (NO_2) exposure and respiratory effects

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Inflammation	Increases in PMNs and prostaglandins in healthy adults.	Section 5.2.7.4	1,500−3,500 ppb 20 min or 3−4 h
	Increases in eNO in children with asthma in association with 24-h avg NO_2.	Delfino et al. (2006), Sarnat et al. (2012), Martins et al. (2012) Section 5.2.7.4	Total personal mean 24-h avg: 24.3, 30.9 ppb Ambient mean 1-week avg: 4.5−20 ppb
Inconsistent effects on oxidative stress, pulmonary injury	See Respiratory Effects in Healthy Individuals below.		
COPD Exacerbation			
Inconsistent epidemiologic evidence and uncertainty regarding NO_2 independent effects	Increases in COPD hospital admissions and ED visits.	Faustini et al. (2013), Ko et al. (2007b), Arbex et al. (2009) Section 5.2.4.2	Mean 24-h avg: 24.1−63.0 ppb Mean 1-h max: 63.0 ppb
	Inconsistent associations with lung function decrements and symptoms in adults with COPD.	Section 5.2.4.1	
Inconsistent evidence from controlled human exposure studies	Decreased lung function not consistently found in adults with COPD.	Morrow et al. (1992), Vagaggini et al. (1996) Section 5.2.4.1	300 ppb for 1 h, 4 h
Weak evidence for key events in mode of action	Increased inflammation in healthy adults but not in adults with COPD.	Bruske et al. (2010) Sections 5.2.4.3 and 5.2.7.4	1,500−3,500 ppb 20 min or 3−4 h
Respiratory Infection			
Consistent animal toxicological evidence with relevant NO_2 exposures	Mortality from bacterial or viral infection in animals with relevant NO_2 exposures.	Ehrlich et al. (1977), Ehrlich et al. (1979), Ehrlich (1980), Graham et al. (1987) Section 5.2.5.1	1,500−5,000 ppb for 3 h 1,500 ppb with 4,500 ppb spike of 1−7.5 h
Inconsistent epidemiologic evidence and uncertainty regarding NO_2 independent effects	Associations with hospital admissions/ED visits for respiratory infections. All results based on central site NO_2, and some have wide 95% CIs. Inconsistent evidence for parental reports of infection or laboratory-confirmed infections.	Zemek et al. (2010), Mehta et al. (2013), Stieb et al. (2009), Faustini et al. (2013), Just et al. (2002), Stern et al. (2013) Sections 5.2.5.3, and 5.2.5.2.	Overall study mean 24-h avg: 11.7−28.6 ppb City mean 24-h avg: 9.3−34.6 ppb

Table 5-45 (Continued): Summary of evidence for a causal relationship between short-term nitrogen dioxide (NO_2) exposure and respiratory effects

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Limited Evidence for Key Events in Mode of Action			
Decreased alveolar macrophage function	Diminished superoxide production in AM function. No consistent effect on pulmonary clearance.	Section 5.2.5.4	
Respiratory Effects in Healthy Individuals			
Limited epidemiologic evidence and uncertainty regarding NO_2 independent effects	Consistent evidence for respiratory symptoms in children. All based on central site NO_2 and no examination of confounding by traffic-related copollutants.	Schwartz et al. (1994) Section 5.2.7.3, Table 5-39	Mean 24-h avg: 13 ppb
	Lung function not consistently associated with NO_2 measured at subjects' locations or central site NO_2 correlated (r = 0.61) with total personal. But, personal ambient NO_2 associations found with adjustment for $PM_{2.5}$, EC, OC, copper, iron, or UFP.	Strak et al. (2012), Moshammer et al. (2006), Linn et al. (1996) Section 5.2.7.2, Table 5-36	Max for 5-h avg: 96 ppb Max for 24-h avg: 96 ppb 75th for 24-h avg: 11.4 ppb
Limited and inconsistent evidence from controlled human exposure studies	Increases in airway responsiveness found in healthy adults above 1,000 ppb NO_2, not lower concentrations.	Folinsbee (1992), Kjaergaard and Rasmussen (1996) Section 5.2.7.1	1,000−2,000 ppb for 3 h
	Respiratory symptoms or lung function examined in adults; changes generally not found.	Sections 5.2.7.2 and 5.2.7.3	200−4,000 ppb for 2−5 h
Limited Evidence for Key Events in Mode of Action			
Inflammation	Increases in PMNs and prostaglandins in healthy adults.	Frampton et al. (2002), Frampton et al. (1989) Section 5.2.7.4	1,500−3,500 ppb for 3 h
	Limited epidemiologic evidence for associations of NO_2 measured in subjects' locations with increases in pulmonary inflammation in children and adults. Associations persist with adjustment for BC/EC, OC, UFP, or $PM_{2.5}$.	Strak et al. (2012), Steenhof et al. (2013), Lin et al. (2011) Section 5.2.7.4, Table 5-41	Mean 24-h avg: 9.3, 33 ppb Mean 5-h avg across sites with varying traffic: 20 ppb

Table 5-45 (Continued): Summary of evidence for a causal relationship between short-term nitrogen dioxide (NO2) exposure and respiratory effects

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Inconsistent effects on oxidative stress, pulmonary injury	Inconsistent changes in antioxidants in experimental studies but found in humans and rodents with lower dietary antioxidant vitamins.	Sections 4.3.2.3 and 5.2.7.4	Humans: 2,000 ppb for 4 h, 1 day or 4 days Rodents: 1,000−5,000 ppb for 3−7 days
	Increases in LDH, CC16, BAL fluid protein inconsistently found in humans, rodents. Limited evidence for impaired epithelial barrier function.	Sections 4.3.2.4 and 5.2.7.4	Humans: 600−2,000 ppb for 3−4 h, 1−4 days Animal models: 400−2,000 ppb for 1−3 weeks
Respiratory Mortality			
Consistent epidemiologic evidence but uncertainty regarding NO_2 independent effect	Multicity studies consistently observe associations of respiratory mortality with 24-h avg NO_2 at lag 0−1 days. Results based on NO_2 averaged across central sites. Potential confounding by traffic-related copollutants not assessed. NO_2 results robust to adjustment for PM_{10}, SO_2, O_3.	Wong et al. (2008), Chen et al. (2012b), Chiusolo et al. (2011), Bellini et al. (2007), Biggeri et al. (2005) Section 5.2.8	Means across cities for 24-h avg: 13.5−55.5 ppb
Uncertainty due to limited coherence with respiratory morbidity evidence	Evidence for asthma exacerbation in adults but limited coherence among lines of evidence for effects on COPD and respiratory infection. Uncertainty regarding spectrum of effects that can lead to respiratory mortality.		

CC16 = club cell protein, CI = confidence interval, CO = carbon monoxide, COPD = chronic obstructive pulmonary disease, EC = elemental carbon, ED = emergency department, eNO = exhaled nitric oxide, LDH = lactate dehydrogenase, NO_2 = nitrogen dioxide, O_3 = ozone, OC = organic carbon, PM = particulate matter, PMN = polymorphonuclear cells, SO_2 = sulfur dioxide, Th2 = T-derived lymphocyte helper 2, UFP = ultrafine particles, VOC = volatile organic compound.

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Tables I and II of the Preamble.

[b]Describes the key evidence and references, supporting or contradicting, contributing most heavily to causal determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where full body of evidence is described.

[c]Describes the NO_2 concentrations with which the evidence is substantiated (for experimental studies, ≤ 5,000 ppb).

5.3 Cardiovascular and Related Metabolic Effects

5.3.1 Introduction

The 2008 ISA for Oxides of Nitrogen concluded that the "available evidence on the effects of short-term exposure to NO_2 on cardiovascular health effects was inadequate to infer the presence or absence of a causal relationship" (U.S. EPA, 2008a). Multiple studies found associations between short-term exposure to NO_2 and rates of hospital admission or ED visits for cardiovascular diseases (CVDs), yet it was unclear at that time whether these results supported a direct effect of short-term NO_2 exposure on cardiovascular morbidity or were confounded by other correlated pollutants. Additionally, epidemiologic studies available at the time of the last review provided inconsistent evidence for associations between short-term NO_2 exposure and other cardiovascular events such as arrhythmia among patients with implanted cardioverter defibrillators and subclinical measures associated with cardiovascular events, such as heart rate variability (HRV) and electrocardiographic (ECG) markers of cardiac repolarization. Experimental studies available at the time of the 2008 ISA for Oxides of Nitrogen did not provide biological plausibility for the cardiovascular effects observed in epidemiologic studies. There was limited evidence from controlled human exposure studies demonstrating a reduction in hemoglobin and some evidence from toxicological studies for effects of NO_2 on various hematological parameters in animals, but these studies were limited and inconsistent. Overall, the experimental studies could not address the uncertainty related to copollutant confounding in epidemiologic studies of hospital admission or ED visits for CVDs in the 2008 ISA for Oxides of Nitrogen.

The following sections review the published studies pertaining to the cardiovascular and related metabolic effects of short-term exposure to oxides of nitrogen in humans, animals, and cells. When compared to the 2008 ISA for Oxides of Nitrogen, the recent epidemiologic and toxicological studies provide evidence for effects of NO_2 exposure on a broader array of cardiovascular effects and mortality. Still, substantial uncertainties remain concerning potential confounding by other traffic-related pollutants, exposure measurement error, and the limited mechanistic evidence to describe a role for NO_2 in the manifestation of cardiovascular diseases, including key events within the mode of action. The majority of the recent evidence is from epidemiologic studies, which suggest that exposure to NO_2 may result in the triggering of MI. To clearly characterize the evidence underlying causality, the discussion of the evidence is organized into groups of related outcomes [e.g., MI including ischemic heart disease (IHD), arrhythmia and cardiac arrest]. Evidence for subclinical effects (e.g., HRV, blood biomarkers of cardiovascular

effects) that potentially underlie the development, progression, or indication of various clinical events is discussed in Section 5.3.11, and may provide biological plausibility for multiple outcomes.

5.3.2 Myocardial Infarction

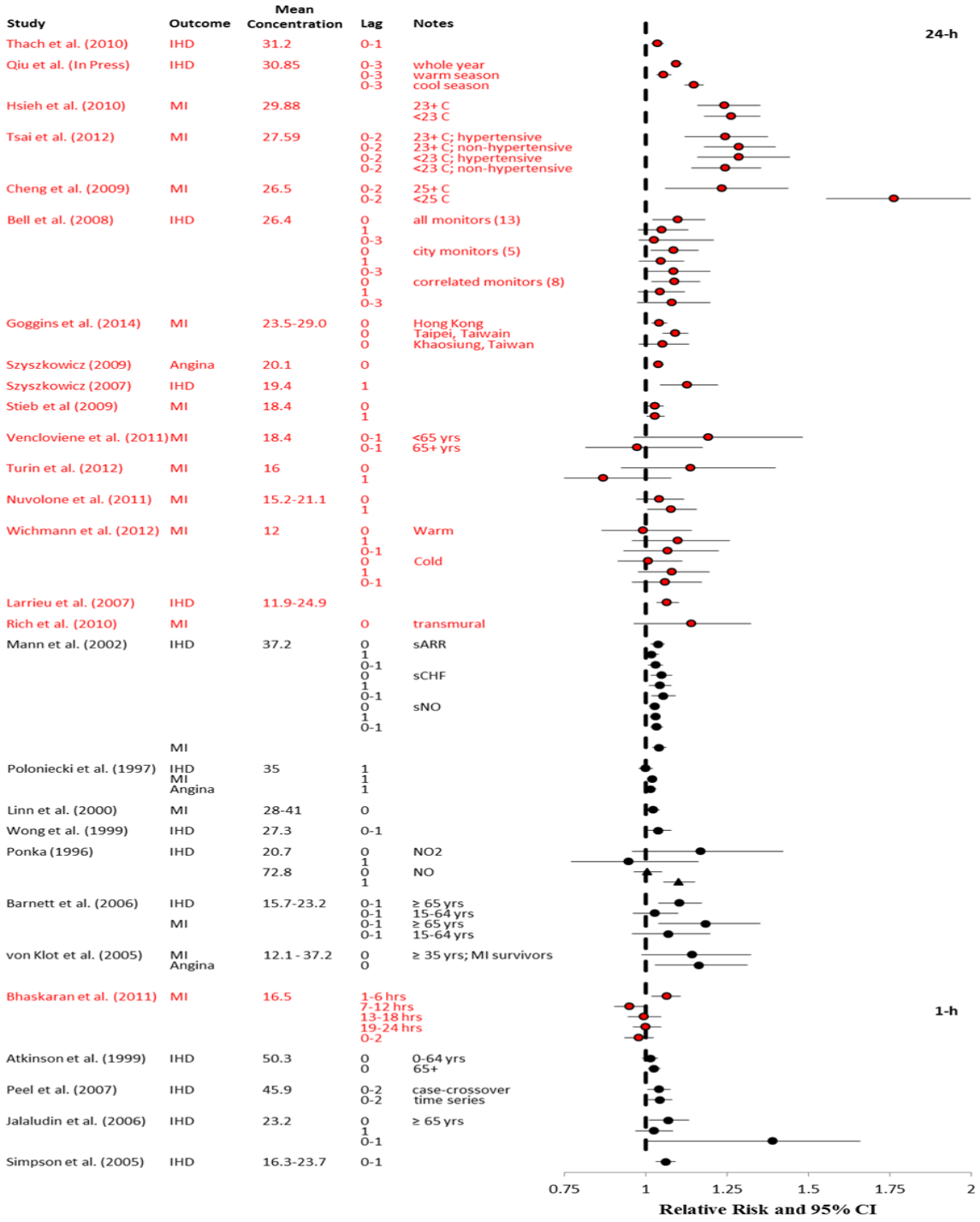
Several lines of evidence are discussed in support of a relationship between short-term NO_2 exposure and MI. An MI or heart attack occurs as a consequence of IHD, resulting in insufficient blood flow to the heart that overwhelms myocardial repair mechanisms and leads to muscle tissue death. ICD codes for MI are classified within the group of IHDs; thus, studies where IHD is evaluated will include any patients diagnosed with an MI. In addition, IHD includes the diagnosis of angina. Symptoms of MI are similar to those of angina; however, where MI results in damage to the heart muscle, angina does not result in myocardial necrosis. As angina may indicate an increased risk for future MI, studies analyzing outcomes of angina are discussed in support of a relationship to MI. Finally, acute MI may be characterized by ST segment depression, a nonspecific marker of myocardial ischemia. The evaluation of evidence supporting a relationship between short-term NO_2 exposure and triggering an MI includes hospitalization and ED visits for MI, IHD, or angina, and ST-segment amplitude changes.

5.3.2.1 Hospital Admissions and Emergency Department Visits for Myocardial Infarction and Ischemic Heart Disease

The 2008 ISA for Oxides of Nitrogen concluded that the epidemiologic evidence consistently supported the associations between short-term increases in ambient NO_2 concentrations and hospital admissions or ED visits for cardiac diseases (U.S. EPA, 2008a). This conclusion continues to be supported by studies published since the 2008 ISA, as reviewed below (Figure 5-18 and Table 5-46). However, potential copollutant confounding, especially from other traffic-related pollutants (e.g., EC, CO), and limited mechanistic evidence are still key uncertainties, and make it difficult to interpret the results of these studies. Additionally, all of the studies in this section use central site monitors to estimate ambient NO_2 exposure, which may result in misclassification of the exposure due to the high variability in NO_2 (Section 3.4.5.1).

A number of studies rely on clinical registries, which are generally less susceptible to misclassification of the outcome and exposure. The strongest evidence of an association between ambient NO_2 and the risk of MI comes from a study using clinical registry data from the U.K.'s Myocardial Ischaemia National Audit Project (Bhaskaran et al., 2011),

which found a 5.8% (95% CI: 1.7, 10.6) increase in risk of MI per 30-ppb increase in 1-h max NO_2 concentrations in the 6 hours preceding the event. This study is unique because it included detailed data on the timing of MI onset in more than 79,000 patients from 15 conurbations in England and Wales, which allowed examination of association with ambient NO_2 in the hours preceding MI. NO_2 results were robust to a number of sensitivity analyses that evaluated key aspects of study design and model specification (e.g., stricter diagnosis criteria, different time strata). Additionally, Bhaskaran et al. (2011) restricted analyses to urban areas to reduce heterogeneity that may have resulted in measurement bias from the use of fixed site monitors to assess NO_2 exposure. The findings for NO_2 were more pronounced in those aged between 60 and 80 years, among those with prior coronary heart disease, and for events occurring in the autumn and spring. Conversely, in a smaller study of only 429 MI events, Turin et al. (2012) did not observe a consistent positive association using data from the Takashima County Stroke and AMI Registry in Central Japan. Cases were cross-checked by research physicians, epidemiologists, and cardiologists, thereby minimizing potential misclassification of the outcome.



Note: Studies in red are recent studies. Studies in black were included in the 2008 ISA for Oxides of Nitrogen. Relative risks are standardized to a 20-ppb or 30-ppb increase in NO_2 or NO concentration for 24-h and 1-h averaging times, respectively. Studies are presented in descending order of mean NO_2 concentration, with the largest mean concentration (ppb) at the top and the smallest at the bottom of the figure (by averaging time and inclusion in previous ISA). Circles = NO_2; Triangles = NO.

Figure 5-18 Results of studies of short-term exposure to oxides of nitrogen and hospital admissions for ischemic heart disease.

Table 5-46 Corresponding risk estimates for hospital admissions for ischemic heart disease for studies presented in Figure 5-18.

Study	Location	Health Effect	Relative Risk[a] (95% CI)	Copollutant Examination[b]
†Thach et al. (2010)	Hong Kong, China	IHD	Lag 0−1: 1.04 (1.02, 1.05)	No copollutant models.
†Qiu et al., 2013	Hong Kong, China	IHD	All year, lag 0−3: 1.09 (1.08, 1.11) Warm season, lag 0−3: 1.05 (1.03, 1.08) Cool season, lag 0−3: 1.15 (1.12, 1.18)	No copollutant models. NO_2 and PM_{10} correlation: Pearson r = 0.76.
†Hsieh et al. (2010)	Taipei, Taiwan	MI	≥23°C: 1.24 (1.16, 1.35) <23°C: 1.26 (1.18, 1.35)	NO_2: robust to PM_{10}, SO_2, CO, or O_3 inclusion in copollutant models. Copollutants: all but O_3 attenuated by NO_2 adjustment. NO_2 correlations (Pearson r): PM_{10}: 0.55; SO_2: 0.51; CO: 0.71; O_3: 0.02.
†Tsai et al. (2012)	Kaohsiung, Taiwan	MI	Hypertension ≥23°C, lag 0−2: 1.24 (1.12, 1.38) <23°C, lag 0−2: 1.29 (1.16, 1.44) No Hypertension ≥23°C, lag 0−2: 1.29 (1.18, 1.40) <23°C, lag 0−2: 1.24 (1.14, 1.35)	No copollutant models examined. NO_2 correlations (Pearson r): PM_{10}: 0.48; SO_2: 0.45; CO: 0.77; O_3: −0.01.
†Cheng et al. (2009)	Kaohsiung, Taiwan	MI	≥25°C, lag 0-2: 1.23 (1.06, 1.44) <25°C, lag 0-2: 1.76 (1.55, 2.02)	NO_2: Attenuated by CO or O_3 adjustment on warm days and PM_{10} on cool days. Robust to SO_2 adjustment. Copollutants: All but CO and O_3 on warm days attenuated by NO_2 adjustment. NO_2 correlations (Pearson r): PM_{10}: 0.73; SO_2: 0.53; CO: 0.66; O_3: 0.09.
†Bell et al. (2008)	Taipei, Taiwan	IHD	All monitors Lag 0: 1.10 (1.02, 1.18) Lag 1: 1.05 (0.98, 1.13) Lag 0−3: 1.03 (0.98, 1.21) City monitors Lag 0: 1.09 (1.02, 1.16) Lag 1: 1.05 (0.98, 1.12) Lag 0−3:1.08 (0.99, 1.20) Correlated monitors Lag 0: 1.09 (1.02, 1.17) Lag 1: 1.05 (0.98, 1.12)	No copollutant models.

Table 5-46 (Continued): Corresponding risk estimates for hospital admissions for ischemic heart disease for studies presented in Figure 5-18.

Study	Location	Health Effect	Relative Risk[a] (95% CI)	Copollutant Examination[b]
			Lag 0−3: 1.08 (0.97, 1.20)	
†Goggins et al. (2013)	Hong Kong, China; Taipei, Taiwan; Kaohsiung, Taiwan	MI	Hong Kong Lag 0: 1.04 (1.02, 1.07) Taipei Lag 0: 1.09 (1.05, 1.13) Kaohsiung Lag 0: 1.05 (0.98, 1.13)	No copollutant models.
†Szyszkowicz (2009)	6 Canadian cities	Angina	Lag 0: 1.04 (1.03, 1.05)	No copollutant models.
†Szyszkowicz (2007)	Montreal, Canada	IHD	Lag 1: 1.13 (1.04, 1.22)	No copollutant models.
†Stieb et al. (2009)	7 Canadian cities	MI	Lag 0: 1.03 (1.00, 1.05) Lag 1: 1.03 (1.00, 1.06)	NO_2 association attenuated by CO.
†Vencloviene et al. (2011)	Kaunas, Lithuania	MI	<65 yrs, lag 0−1: 1.19 (0.96, 1.48) ≥65 yrs, lag 0−1: 0.97 (0.81, 1.17)	No results provided for other pollutants.
†Turin et al. (2012)	Takashima County, Japan	MI	Lag 0: 1.14 (0.92, 1.40) Lag 1: 0.87 (0.70, 1.08)	NO_2: robust to TSP or SO_2 adjustment. Attenuated by O_3 adjustment. Copollutants: TSP and SO_2 attenuated by NO_2 adjustment. No associations between O_3 and MI regardless of NO_2 adjustment.
†Nuvolone et al. (2011)	Tuscany, Italy	MI	Lag 0: 1.04 (0.97, 1.12) Lag 1: 1.08 (1.00, 1.15)	NO_2: robust to PM_{10} adjustment; attenuated by CO. Copollutants: PM_{10} no longer associated with MI after NO_2 adjustment. No association between CO and MI regardless of NO_2 adjustment. No correlations provided.
†Wichmann et al. (2012)	Copenhagen, Denmark	MI	Warm season Lag 0: 0.99 (0.86, 1.14) Lag 1: 1.10 (0.96, 1.26) Lag 0−1: 1.07 (0.93, 1.22) Cool season Lag 0: 1.01 (0.91, 1.11) Lag 1: 1.08 (0.98, 1.19) Lag 0−1: 1.06 (0.96, 1.17)	No copollutant models.
†Larrieu et al. (2007)	8 French cities	IHD	1.07 (1.03, 1.10)	No copollutant models.

Table 5-46 (Continued): Corresponding risk estimates for hospital admissions for ischemic heart disease for studies presented in Figure 5-18.

Study	Location	Health Effect	Relative Risk[a] (95% CI)	Copollutant Examination[b]
†Rich et al. (2010)	New Jersey, U.S.	MI	Lag 0, transmural: 1.14 (0.96, 1.32)	NO_2: slightly attenuated by PM2.5 adjustment. Copollutants: $PM_{2.5}$ association attenuated by adjustment for NO_2. NO_2 and $PM_{2.5}$ correlation: r = 0.44.
Mann et al. (2002)	Los Angeles, CA	IHD	With secondary arrhythmia Lag 0: 1.04 (1.02, 1.06) Lag 1: 1.02 (1.00, 1.04) Lag 0−1: 1.03 (1.01, 1.05) With secondary congestive heart failure Lag 0: 1.05 (1.01, 1.08) Lag 1: 1.04 (1.01, 1.08) Lag 0−1: 1.05 (1.02, 1.09) With no secondary disease Lag 0: 1.03 (1.01, 1.04) Lag 1: 1.03 (1.01, 1.04) Lag 0−1: 1.03 (1.02, 1.05)	No copollutant models.
		MI	1.04 (1.02, 1.06)	
Poloniecki et al. (1997)	London, U.K.	IHD, MI, angina	Lag1: 1.00 (0.98, 1.02) Lag 1: 1.02 (1.01, 1.03) Lag 1: 1.01 (1.00, 1.03)	NO_2: Robust to O; Attenuated by CO, SO_2, and BS.
Linn et al. (2000)	Los Angeles, CA	MI	Lag 0: 1.02 (1.00, 1.04)	No copollutant models.
Wong et al. (1999)	Hong Kong, China	IHD	Lag 0-1: 1.04 (1.00, 1.08)	No copollutant models.
Pönkä and Virtanen (1996)	Helsinki, Finland	IHD	NO_2 Lag 0: 1.17 (0.96, 1.42) Lag 1: 0.95 (0.77, 1.16) NO Lag 0: 1.01 (0.96, 1.05) Lag 1: 1.10 (1.05, 1.15)	No copollutant models.
Barnett et al. (2006)	7 Australian and New Zealand cities	IHD, MI	Lag 0−1, ≥65 yrs: 1.10 (1.04, 1.17) Lag 0−1, 15−64 yrs: 1.03 (0.96, 1.10) Lag 0−1, ≥65 yrs: 1.18 (1.04, 1.35) Lag 0−1, 15−64 yrs: 1.07 (0.96, 1.20)	No copollutant models.
Von Klot et al. (2005)	5 European cities	MI, angina	Lag 0: 1.14 (0.99, 1.32) Lag 0: 1.16 (1.03, 1.31) Ages > 35 yr, MI survivors	NO_2: Robust to PM_{10} or O_3 adjustment.

Table 5-46 (Continued): Corresponding risk estimates for hospital admissions for ischemic heart disease for studies presented in Figure 5-18.

Study	Location	Health Effect	Relative Risk[a] (95% CI)	Copollutant Examination[b]
†Bhaskaran et al. (2011)	England and Wales	MI	Lag 1−6 h: 1.06 (1.02, 1.11) Lag 7−12 h: 0.95 (0.90, 0.99) Lag 13−18 h: 0.99 (0.94, 1.05) Lag 19−24 h: 1.00 (0.96 (1.05) Lag 0−2 days: 0.98 (0.93, 1.02)	No copollutant models. NO_2 correlations: PM_{10}: 0.48; O_3: −0.58; CO: 0.61; SO_2: 0.31.
Atkinson et al. (1999)	London, U.K.	IHD	Lag 0; 0−64 yrs: 24.h (0.99, 1.04) Lag 0; 65+ yrs: 1.03 (1.01, 1.04)	No copollutant models analyzed for IHD.
Peel et al. (2007)	Atlanta, GA	IHD	Lag 0−2; case-crossover: 1.04 (1.00, 1.07) Lag 0−2; time-series: 1.04 (1.01, 1.08)	No copollutant models.
Jalaludin et al. (2006)	Sydney, Australia	IHD	Lag 0: 1.07 (1.01, 1.13) Lag 1: 1.02 (0.97, 1.08) Lag 0−1: 1.01 (0.99, 1.03) Ages > 65 yr	No copollutant models analyzed for IHD.
Simpson et al. (2005a)	4 Australian cities	IHD	Lag 0−1: 1.06 (1.03, 1.09)	No copollutant models analyzed for IHD.

CI = confidence interval, CO = carbon monoxide, IHD = ischemic heart disease, MI = myocardial infarction, NO = nitric oxide. NO_2 = nitrogen dioxide, O_3 = ozone, PM = particulate matter, SO_2 = sulfur dioxide, TSP = total suspended particles.
[a]Effect estimates are standardized to a 20-ppb or 30-ppb increase in NO_2 or NO for 24-h avg and 1-h max metrics, respectively.
[b]Relevant relative risks for copollutant models can be found in Figure S5-2, S5-3, S5-4, and S5-5 (U.S. EPA, 2014b, c, d, e).
†Studies published since the 2008 ISA for Oxides of Nitrogen.

A number of studies based on administrative data have also been published since the 2008 ISA for Oxides of Nitrogen. In six areas in central Italy, Nuvolone et al. (2011) found an 8% (95% CI: 0, 15) increase in risk of hospital admission for MI per 20-ppb increase in 24-h avg NO_2 on the previous day. Similar associations were seen in relation to lags 2 to 4 days prior to hospital admission. The finding at lag 2 was robust to adjustment for PM_{10} in a copollutant model, and remained positive, though somewhat attenuated, by adjustment for CO (Figure S5-2, (U.S. EPA, 2014b) and Figure S5-3, (U.S. EPA, 2014c)). The association with NO_2 was somewhat more pronounced among females and in the cold season. Using data from 14 hospitals in seven Canadian cities, Stieb et al. (2009) found a 2.8% (95% CI: 0.2, 5.4) increase in risk of ED visits for the composite endpoint of acute MI or angina per 20-ppb increase in 24-h avg NO_2 on the same day. However, the overall association was heavily influenced by the association observed in Edmonton, and exclusion of the data from Edmonton from the analysis attenuated the results. Furthermore, the association observed from the data including Edmonton was weakened in magnitude and precision (wider 95% CI) in a copollutant

model adjusting for CO (1.3% [95% CI: −2.9, 5.6] increase per 20 ppb increase in 24 h avg NO_2 on the same day). Larrieu et al. (2007) observed a positive association between hospital admissions for IHD and NO_2 concentrations in eight French cities. The magnitude of the association was higher for older adults (i.e., ≥65 years) than for the general population. In large single-city studies,Szyszkowicz (2007), Thach et al. (2010), and Franck et al. (2014) found that NO_2 was associated with increased risk of hospital admission for IHD in Montreal, Canada; Hong Kong, China; and Santiago, Chile, respectively. Qiu et al. (2013b) also reported an overall association between NO_2 concentrations risk of ED visits for IHD in Hong Kong that was stronger in the cool season and on low humidity days.

In New Jersey, Rich et al. (2010) found a relative risk of 1.14 (95% CI: 0.96, 1.32) per 20-ppb increase in 24-h avg NO_2 for hospitalization for transmural MIs, but that association was attenuated by adjustment for $PM_{2.5}$ in a copollutant model (1.05 [95% CI: 0.85, 1.28]). No results were reported for all MIs or for nontransmural infarcts. NO_2 was positively associated with hospital admissions for MI in Taipei, Taiwan (Goggins et al., 2013; Tsai et al., 2012; Hsieh et al., 2010), Kaohsiung, Taiwan (Tsai et al., 2012; Cheng et al., 2009), and Hong Kong (Goggins et al., 2013). The associations reported by Hsieh et al. (2010) remained relatively unchanged after adjustment for PM_{10}, SO_2, CO, or O_3 in copollutant models, as did the results from Cheng et al. (2009), with the exception of CO and O_3 on warm days. NO_2 was also positively associated with hospital admissions for IHD in Taipei, Taiwan (Bell et al., 2008). In an effort to reduce uncertainty related to the use of central site monitors, Bell et al. (2008) estimated NO_2 exposure over the entire Taipei area (average of 13 monitors), within Taipei City only (average of 5 monitors), and using a subset of monitors where all pairs of monitors had NO_2 correlations greater than 0.75 (8 monitors). The authors reported consistent results across the multiple exposure metrics, with the exception of stronger associations observed using the city or correlated monitors at lag 0−3 (Table 5-46). Wichmann et al. (2012) found that NO_2 was positively associated with risk of acute MI hospital admissions in Copenhagen, Denmark, but only in the warm months of the year. NO_2 was not associated with risk of hospital admission for acute coronary syndrome in Lithuania (Vencloviene et al., 2011).

5.3.2.2 Hospital Admissions and Emergency Department Visits for Angina Pectoris

The preceding epidemiologic evidence describing associations between short-term increases in ambient NO_2 concentrations and increased hospital admissions and ED visits for MI and IHD is supported by evidence for increases in hospital admissions and ED visits for angina. Angina pectoris results from an imbalance between the demand for

oxygen in the heart and the delivery by the coronary artery. Reduction in coronary blood flow due to atherosclerosis is a common cause of this imbalance. Unstable angina, where the coronary artery is not completely occluded, can lead to MI.

The 2008 ISA for Oxides of Nitrogen did not include specific discussion of angina but did report results from two studies that examined associations between ambient NO_2 concentrations and angina hospital admissions (U.S. EPA, 2008a). In a study of five European cities, Von Klot et al. (2005) examined the relationship between short-term air pollution and hospital readmissions of myocardial infarction survivors. The authors reported a 16% (95% CI: 3, 31) increase in risk of hospital readmissions for angina pectoris per 20-ppb increase in 24-h avg NO_2 on the same day. Poloniecki et al. (1997) observed a smaller, but statistically significant association between NO_2 concentrations on the previous day and angina hospital admissions in London, UK (Table 5-46). Neither study evaluated copollutant models.

More recent studies add to the limited, but consistent evidence of an association between ambient NO_2 exposure and angina hospital admissions and ED visits. Szyszkowicz (2009) found that NO_2 concentrations were associated with risk of ED visits for chest pain in six Canadian cities. The magnitude of association was stronger in the warm season, with a 5.9% increase in risk (95% CI: 3.3, 8.6) than in the cold season, with a 3.2% increase in risk (95% CI: 1.5, 5.0) at lag 1 per 20-ppb increase in 24-h avg NO_2. As discussed in Section 5.3.2.1, Stieb et al. (2009) examined the composite endpoint of acute MI or angina ED visits in a study of seven Canadian cities that included overlapping data with Szyszkowicz (2009). Stieb et al. (2009) observed a positive association between ambient NO_2 and MI/angina that was still positive, but attenuated, imprecise, and no longer statistically significant after adjustment for CO in a copollutant model. In addition to limited interpretability from using a composite endpoint, the results were also largely influenced by data from one city, as detailed in Section 5.3.2.1.

5.3.2.3 ST-Segment Amplitude

ST-segment changes (either ST-segment elevation or depression) on the electrocardiogram are considered a nonspecific marker of myocardial ischemia. While the 2008 ISA for Oxides of Nitrogen did not review any epidemiologic studies of ambient oxides of nitrogen concentrations and markers of myocardial ischemia (U.S. EPA, 2008a), a few recent studies report associations (Table 5-47). Chuang et al. (2008) conducted a repeated-measures study of Boston-area adults with a history of coronary heart disease and examined the association between ambient pollutants and ST-segment changes. The authors reported an OR of 3.29 (95% CI: 1.82, 5.92) for ST-segment

depression of ≥0.1 mm per 20-ppb increase in 24-h avg NO_2 concentrations over the previous 24 hours. This finding was robust to additional adjustment for $PM_{2.5}$ in a copollutant model (OR: 3.29 [95% CI: 1.65, 6.59]).

Delfino et al. (2011) used a similar design to study 38 older, nonsmoking adult residents of four retirement homes in the Los Angeles area with a documented history of coronary artery disease. A particular strength of this study is that the authors measured pollutant concentrations outside of the residence, which improved spatial matching of NO_2 concentrations to subjects' locations. The authors observed an OR of 10.13 (95% CI: 1.37, 74.23) for ST-segment depression ≥1.0 mm per 30-ppb increase in mean 1-hour NO_2 concentrations preceding measurement over the previous 3 days. Other averaging periods from 8 hours to 4 days gave similar or slightly weaker results. NO_2 was more strongly associated with ST depression than was NO_X. No copollutant models were evaluated.

Table 5-47 Epidemiologic studies of ST-segment amplitude.

Study	Location Sample Size	Mean NO_2 ppb	Exposure assessment	Selected Effect Estimates[a] (95% CI)
†Chuang et al. (2008)	Boston, MA (n = 48)	24-h avg NO_2 21.4 75th: 24.9 Max: 44.5	City-wide avg	ST segment change (mm): 12-h: −0.02 (−0.05, 0.00) 24-h: −0.08 (−0.12, −0.05) RR for ST-segment depression ≥0.1 mm: 12-h: 1.15 (0.72, 1.82) 24-h: 3.29 (95% CI: 1.82, 5.92
†Delfino et al. (2011)	Los Angeles, CA (n = 38)	1-h NO_2: 27.5 1-h NO_X: 46.6	Outdoor monitor at retirement community	OR for ST-segment depression ≥1.0 mm NO_2: 1-h: 1.33 (0.83, 2.11) 8-h: 2.37 (1.14, 4.92) 24-h: 4.75 (1.51, 14.84) 2-day: 7.51 (1.49, 37.87) 3-day: 10.13 (1.37, 74.23) 4-day: 5.47 (0.65, 45.89) NO_X: 1-h: 1.25 (0.96, 1.64) 8-h: 1.49 (0.94, 2.40) 24-h: 1.88 (0.83, 4.20) 2-day: 2.10 (0.67, 6.45) 3-day: 2.31 (0.45, 11.83) 4-day: 1.82 (0.21, 15.84)

CI = confidence interval, NO_2 = nitrogen dioxide, NO_X = sum of NO and NO_2, OR = odds ratio, RR = relative risk.
[a]Effect estimates are standardized to a 20-ppb or 30-ppb increase in NO_2 or 40 ppb or 60-ppb increase in NO_X concentration for 24-h avg and 1-h max metric, respectively.
†Studies published since the 2008 ISA for Oxides of Nitrogen.

5.3.2.4 Summary of Myocardial Infarction

In summary, the epidemiologic data available continue to support potential associations between ambient NO_2 concentrations and risk of triggering an MI. However, potential copollutant confounding by traffic-related pollutants was not examined extensively in these studies. In the studies that did analyze copollutant models to adjust for another traffic pollutant, the findings were generally inconsistent. Associations between ambient NO_2 and risk of hospital admissions or ED visits for MI and IHD were attenuated by adjustment for CO in two studies (Nuvolone et al., 2011; Stieb et al., 2009) but remained robust in three others (Hsieh et al., 2010; Cheng et al., 2009; Yang, 2008). Additionally, Rich et al. (2010) reported that an association between short-term NO_2 exposure and hospitalization for MI was attenuated by the inclusion of $PM_{2.5}$ in a copollutant model. There is limited, but consistent evidence of an association between NO_2 and angina pectoris (Stieb et al., 2009; Szyszkowicz, 2009; Von Klot et al., 2005; Poloniecki et al., 1997). However, only one study included a copollutant model, in which the association was attenuated in magnitude and precision after adjustment for CO (Stieb et al., 2009). None of the reviewed studies of MI, IHD, or angina utilized copollutant models to adjust for potential confounding by EC or VOCs. Additionally, all of the studies in this section used central site monitors to measure ambient NO_2, which have noted limitations in capturing the variation in NO_2 (Section 3.4.4.2).

In addition to hospital admission and ED visit studies, a few available epidemiologic studies report an association between short-term exposure to NO_2 and ST-segment changes on the electrocardiogram of older adults with a history of coronary artery disease, potentially indicating an association between NO_2 and increased risk of myocardial ischemia in this patient population. No studies from the previous ISA are available for comparison. Once again, there was limited assessment of potential confounding by traffic pollutants in copollutant models, though Chuang et al. (2008) reported that the association between NO_2 and ST-segment changes was robust to $PM_{2.5}$ adjustment.

5.3.3 Arrhythmia and Cardiac Arrest

5.3.3.1 Panel Epidemiologic Studies

The 2008 ISA for Oxides of Nitrogen found little epidemiologic evidence of an association between short-term changes in ambient NO_2 concentrations and cardiac

arrhythmias (U.S. EPA, 2008a). There continues to be limited epidemiologic evidence for such an association, either from panel studies of patients with ICDs or panel studies of arrhythmias detected on ambulatory ECG recordings (Table 5-48).

In a study of patients with ICDs, Ljungman et al. (2008) found that NO_2 was positively associated with increased risk of confirmed ventricular tachyarrhythmias (VT). The association with PM_{10} and $PM_{2.5}$ was stronger than the association for NO_2. The authors observed no evidence of effect modification by city, distance from the nearest ambient monitor at the time of the event, number of events, type of event (ventricular fibrillation vs. ventricular tachycardia), age, history of IHD, left ventricular ejection fraction, diabetes, body mass index, or use of beta blockers. They did, however, report effect modification depending on whether the patient was indoors or outdoors at the time of the event with a strong association between NO_2 and risk of VT among the 22 subjects that were outdoors at the time of ICD activation. Because the authors accounted for personal activity/behavior, exposure measurement error may have been reduced in the effect modification analysis, as more time spent outdoors is likely to correspond to a greater personal-ambient correlation (Section 3.4.4.1). In a similar study, Anderson et al. (2010) observed generally null associations between ICD activation and ambient NO, NO_2, or NO_X concentrations. Anderson et al. (2010) only had the study cardiologist review the electrocardiograms from about 60% of ICD activations (confirming 87% of those cases as VT), potentially leading to greater misclassification of the outcome than in the study by Ljungman et al. (2008). Recently, Link et al. (2013) examined a panel of patients with dual chamber ICDs. They observed positive associations between ICD-detected arrhythmias and atrial fibrillations ≥30 seconds and NO_2 concentrations that were generally stronger when the authors used a 2-hour lag compared to a 2-day lag. Finally, Metzger et al. (2007) observed generally null associations between NO_2 concentrations and VT events over a 10-year period in Atlanta, GA.

Using a different approach, Bartell et al. (2013) used ECG monitors to evaluate VT events in 50 older adult, non-smoking residents of four retirement communities in the greater Los Angeles area. The study reported a 35% (95% CI: −1, 82) increase in the daily rate of VT events per 40-ppb increase in 24-h avg NO_X. The estimated effect of 3- and 5-day avg NO_X on the daily rate of VT was somewhat stronger, though markedly less precise (i.e., wider confidence limits around the effect estimates). Bartell et al. (2013) measured pollutant concentrations outside of each of the retirement communities, which improved spatial matching of NO_2 concentrations to subjects' locations. Conversely, Barclay et al. (2009) generally observed weak and inconsistent associations between NO_2 or NO and incident arrhythmias detected on ambulatory ECG recordings in a repeated measures study of nonsmoking adults with stable heart failure.

Table 5-48 Epidemiologic studies of arrhythmia and cardiac arrest.

Study	Location Sample Size	Mean NO_2 (ppb)	Exposure Assessment	Selected Effect Estimates (95% CI)[a]
†Ljungman et al. (2008)	Gothenburg and Stockholm, Sweden n = 211 (266 events)	24-h avg NO_2 Gothenburg: 11.8 Stockholm: 8.3	Single monitor in Gothenburg, average of 2 monitors in Stockholm	Ventricular tachyarrhythmia (OR) 2-h avg: 1.37 (0.53, 3.64) 24-h avg: 1.26 (0.49, 3.32)
†Anderson et al. (2010)	London, U.K. n = 705 (5,462 device activations)	24-h avg NO_2: 12.1 24-h avg NO_X: 24.1 24-h avg NO: 19.4	City-wide avg	ICD activations (OR) NO_2; Lag 0−1: 0.93 (0.70, 1.24) NO_X; Lag 0−1: 0.92 (0.86, 1.08) NO: Lag 0−1: 0.96 (0.93, 1.04)
†Link et al. (2013)	Boston, MA n = 176 (328 atrial fibrillation episodes ≥30 sec)	24-h avg NO_2: 16.1	City-wide avg	ICD-detected arrhythmias (OR) 24-h lag: 1.23 (0.75, 2.10) 2-h lag: 1.57 (0.97, 2.47)
Metzger et al. (2007)	Atlanta, GA n = 518	1-h max NO_2: 44.9 90th: 68 Max: 181	Central monitor	All arrhythmia events (OR) All yr: 1.00 (0.95, 1.05) Warm season: 1.00 (0.93, 1.08) Cold season: 1.01 (0.94, 1.08) Events resulting in cardiac pacing or defibrillation: All yr: 1.01 (0.94, 1.10) Events resulting in defibrillation: All yr: 1.07 (0.93, 1.23)
†Bartell et al. (2013)	Los Angeles, CA n = 50 (302 subject h of VT observed)	24-h avg NO_X: 42.3 Max: 183.7	Monitors on trailers at each of 4 retirement communities	Ventricular tachyarrhythmia (RR) 24-h avg: 1.35 (0.99, 1.82) 3-day avg: 1.74 (0.47, 6.40) 5-day avg: 1.65 (0.56, 4.93)
†Barclay et al. (2009)	Aberdeen, Scotland n = 132	24-h avg NO_2: 30.1 NO: 14.7	Central monitor	All arrhythmias (regression coefficients) NO_2: 3.193 (−3.600, 9.985) NO: 3.524 (−3.059, 10.107) Ventricular ectopic beats NO_2: 3.642 (−4.837, 12.121) NO: 4.588 (−3.628, 12.803) Ventricular couplets NO_2: 0.356 (−7.395, 8.106) NO: −0.085 (−7.601, 7.431) Ventricular runs NO_2: 2.443 (−2.537, 7.422) NO: 2.219 (−2.618, 7.055) Supraventricular ectopic beats NO_2: 2.888 (−4.833, 10.608) NO: −2.688 (−10.170, 4.794) Supraventricular couplets NO_2: 5.209 (−1.896, 12.313) NO: 1.366 (−5.542, 8.274) Supraventricular runs NO_2: 3.441 (−1.760, 8.641) NO: 2.298 (−2.753, 7.348)

CI = confidence interval, ICD = implantable cardioverter defibrillators, NO = nitric oxide, NO_2 = nitrogen dioxide, NO_X = sum of NO and NO_2, OR = odds ratio, RR = relative risk, VT = ventricular tachyarrhythmias.
[a]Effect estimates are standardized to a 20-ppb or 30-ppb increase in NO_2 or NO or 40 ppb or 60-ppb increase in NO_X concentration for 24-h avg and 1-h max metrics, respectively.
†Studies published since the 2008 ISA for Oxides of Nitrogen.

5.3.3.2 Out-of-Hospital Cardiac Arrest

The majority of out-of-hospital cardiac arrests are due to cardiac arrhythmias. Dennekamp et al. (2010) observed generally positive, though weak, associations between NO_2 concentrations and risk of out-of-hospital cardiac arrest (Table 5-49). A similar approach was used by Silverman et al. (2010) using data from out-of-hospital cardiac arrests in New York City and observed generally null associations with NO_2 concentrations in all year and cold season analyses, and a positive association in the warm season analysis. More recently, Straney et al. (2014) also reported null associations between out-of-hospital cardiac arrest and ambient NO_2 concentrations from a case-crossover study in Perth, Australia. In two other studies of out-of-hospital cardiac arrest, (Ensor et al., 2013) found inconsistent and weak associations with ambient NO_2 concentrations in Houston, while (Wichmann et al., 2013) reported similarly inconsistent associations with NO_X in Copenhagen. However, (Wichmann et al., 2013) observed a positive association between ambient NO_X concentration and out-of-hospital cardiac arrest in females (46% [95% CI: 8%, 99] increase per 40-ppb increase in 24-h avg NO_X at lag 3), although there were slightly under two thirds the amount of cases observed in females compared to males. None of the out-of-hospital cardiac arrest studies examined potential copollutant confounding of NO_2 or NO_X associations. No studies from the previous ISA are available for comparison.

Table 5-49 Epidemiologic studies of out-of-hospital cardiac arrest.

Study	Location Sample Size	Mean NO_2 (ppb)	Exposure Assessment	Selected Effect Estimates[a] (95% CI)
†Dennekamp et al. (2010)	Melbourne, Australia n = 8,434	24-h avg NO_2: 12.0 75th: 15.16	Central monitor	% Change in out-of-hospital cardiac arrest Lag 0: 3.23 (−10.19, 18.51) Lag 1: 7.69 (−7.29, 25.11) Lag 2: −4.51 (−16.48, 10.56) Lag 3: 7.37 (−7.11, 24.13) Lag 0−1: 9.28 (−7.54, 29.14)
†Silverman et al. (2010)	New York City, NY n = 8,216	24-h avg NO_2: 50th: 27 75th: 32 95th: 43	City-wide avg	No quantitative results presented for NO_2.
†Straney et al. (2014)	Perth, Australia (n = 8,551)	1-h max NO_2: 50th: 3.0 75th: 8.1 95th: 19.8	Nearest monitor (avg and/or max distances not specified)	OR Lag 0-h: 1.008 (0.992, 1.030) Lag 1-h: 1.000 (0.979, 1.021) Lag 2-h: 0.987 (0.967, 1.004) Lag 3-h: 0.992 (0.971, 1.013) Lag 0−1-h: 1.004 (0.987, 1.026) Lag 0−3-h: 0.996 (0.975, 1.017) Lag 0−12-h: 0.996 (0.971, 1.026)
†Ensor et al. (2013)	Houston, TX (n = 11,677)	24-h avg NO_2: 9.11 75th: 11.66 95th: 16.87	City-wide avg	% Change in out-of-hospital cardiac arrests Lag 0: 3.2 (−20.3, 18.9) Lag 1: −2.5 (−14.7, 11.0) Lag 2: −1.4 (−13.8, 12.6) Lag 3: 3.2 (−9.6, 17.7) Lag 4: 1.1 (−11.5, 15.3) Lag 0−1: −0.4 (−14.4, 16.1) Lag 1−2: −2.8 (−16.3, 12.9)
†Wichmann et al. (2013)	Copenhagen, Denmark (n = 4,657)	24-h avg NO_X: 14.75 75th: 18.35	Central monitor	% Change in out-of-hospital cardiac arrests Lag 0: −13.5 (−28.6, 5.0) Lag 1: 5.4 (−12.7, 27.4) Lag 2: −9.2 (−25.0, 11.6) Lag 3: 16.0 (−4.0, 40.2) Lag 4: 8.7 (−10.2, 31.2) Lag 5: 5.4 (−13.1, 27.9) Males, lag 3: 2.2 (−19.7, 30.1) Females, lag 3: 46.1 (7.8, 98.7)

CI = confidene interval, NO_2 = nitrogen dioxide, NO_X = sum of NO and NO_2, OR = odds ratio.
[a]Effect estimates are standardized to a 20-ppb or 30-ppb increase in NO_2 or NO or 40 ppb or 60-ppb increase in NO_X concentration for 24- h avg and 1-h max metrics, respectively.
†Studies published since the 2008 ISA for Oxides of Nitrogen.

5.3.3.3 Hospital Admissions and Emergency Department Visits

There are a limited number of studies examining associations between short-term NO_2 exposure and hospital admissions with a primary discharge diagnosis related to arrhythmias. Using data from 14 hospitals in seven Canadian cities, Stieb et al. (2009) found no association between NO_2 and risk of hospital admission for arrhythmias. However, Tsai et al. (2009) reported a positive association in Taipei, Taiwan that was stronger on cool days (OR: 1.34 [95% CI: 1.25, 1.44] per 20 ppb increase in 24-h avg NO_2) than warm days (OR: 1.19 [95% CI: 1.10, 1.28] per 20 ppb increase in 24-h avg NO_2). Both cool and warm day associations remained robust in copollutant models controlling for PM_{10}, SO_2, CO, or O_3; however, they did not evaluate potential confounding by most of the traffic-related pollutants of concern.

5.3.3.4 Summary of Arrhythmia and Cardiac Arrest

In summary, there is currently inconsistent epidemiologic evidence for an association between 24-h avg NO_2, NO, or NO_X and risk of cardiac arrhythmias as examined in patients with ICDs, continuous ECG recordings, out-of-hospital cardiac arrest, and hospital admissions. The reviewed studies rarely adjusted for copollutant confounding by traffic pollutants, focused almost exclusively on ventricular arrhythmias, and are potentially limited by misclassification of the outcome. Additionally, the majority of studies used central site monitors to estimate ambient NO_2 exposure, which have noted limitations in capturing the variation in NO_2.

5.3.4 Cerebrovascular Disease and Stroke

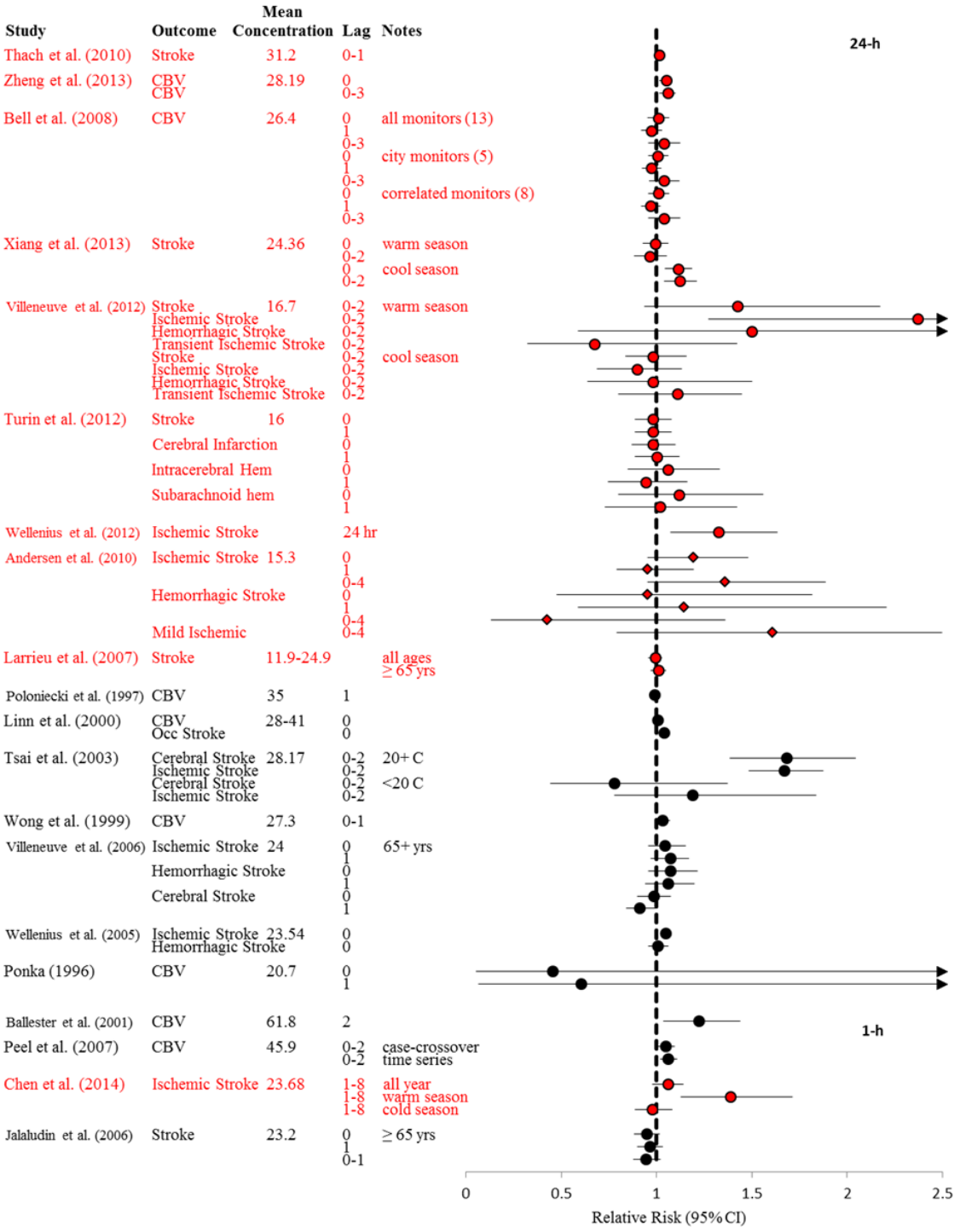
5.3.4.1 Hospital Admissions and Emergency Department Visits

The 2008 ISA for Oxides of Nitrogen found that the epidemiologic evidence for associations between short-term changes in NO_2 levels and hospital admissions or ED visits for cerebrovascular diseases was generally inconsistent and provided little support for an independent NO_2 effect (U.S. EPA, 2008a). Recent studies published since the 2008 ISA also provide inconsistent evidence (Figure 5-19 and Table 5-50).

Generally, studies based on clinical registries are less susceptible to misclassification of the outcome and exposure, which may explain the stronger evidence provided by these

studies than that based on administrative data. Wellenius et al. (2012) reviewed the medical records of 1,705 Boston-area patients hospitalized with neurologist-confirmed acute ischemic stroke and found an OR for ischemic stroke onset of 1.32 (95% CI: 1.08, 1.63) per 20-ppb increase in NO_2 concentration averaged over the 24 hours preceding hospitalization for stroke. A unique strength of this study was the availability of information on the date and time of stroke symptom onset in most patients, thereby potentially reducing misclassification of the exposure. Copollutant models were not evaluated.

Andersen et al. (2010) obtained data on strokes in Copenhagen, Denmark from the Danish National Indicator Project and found a positive association between ambient NO_X concentrations and risk of ischemic stroke but not hemorrhagic stroke. The strongest association was observed in relation to NO_X levels 4 days earlier and for those suffering a mild stroke, but the association was attenuated after adjustment for UFP. Using data from a stroke registry in Como, Italy, Vidale et al. (2010) found that NO_2 was associated with risk of ischemic stroke hospital admission. On the other hand, Turin et al. (2012) did not observe any association using data from the Takashima County Stroke and AMI Registry in Central Japan. Similarly, Oudin et al. (2010) found no association between modeled residential NO_X concentration and risk of ischemic or hemorrhagic stroke within the context of a Swedish quality register for stroke.



Note: Studies in red are recent studies. Studies in black were included in the 2008 ISA for Oxides of Nitrogen. Relative risks are standardized to a 20-ppb or 30-ppb increase in NO_2 concentration and 40 ppb or 60 ppb for NO_X concentrations for 24-h avg and 1-h max metrics, respectively. Studies are presented in descending order, with the largest mean concentration (ppb) at the top and the smallest at the bottom of the figure (by averaging time and inclusion in previous ISA). Circles = NO_2; Diamonds = NO_X.

Figure 5-19 Results of studies of short-term exposure to oxides of nitrogen and hospital admissions for cerebrovascular disease and stroke.

Table 5-50 Corresponding risk estimates for hospital admissions for cerebrovascular disease and stroke for studies presented in Figure 5-19.

Study	Location	Health Effect	Selected Relative Risks[a] 95% CI	Copollutant Examination[b]
†Thach et al. (2010)	Hong Kong, China	Stroke	Lag 0−1: 1.01 (1.00, 1.03)	No copollutant models.
†Zheng et al. (2013)	Lanzhou, China	Cerebrovascular disease	Lag 0: 1.05 (1.02, 1.08) Lag 0−3: 1.06 (1.02, 1.10)	NO_2: associations were robust to adjustment for SO_2; associations increased with adjustment for PM_{10}. Copollutants: SO_2 (positive) associations and PM_{10} (negative) associations robust to adjustment for NO_2. NO_2 correlations (Spearman *r*): PM_{10}: 0.64; SO_2: 0.64.
†Bell et al. (2008)	Taipei, Taiwan	Cerebrovascular disease	All monitors: Lag 0: 1.01 (0.95, 1.07) Lag 1: 0.97 (0.92, 1.03) Lag 0−3: 1.04 (0.96, 1.12) City monitors: Lag 0: 1.01 (0.96, 1.06) Lag 1: 0.97 (0.92, 1.02) Lag 0−3: 1.04 (0.96, 1.12) Correlated monitors: Lag 0: 1.01 (0.96, 1.06) Lag 1: 0.97 (0.92, 1.02) Lag 0−3: 1.04 (0.96, 1.12)	No copollutant models.
†Xiang et al. (2013)	Wuhan, China	Stroke	Warm season: Lag 0: 0.99 (0.93, 1.06) Lag 0−2: 0.96 (0.88, 1.05) Cool season: Lag 0: 1.11 (1.05, 1.18) Lag 0−2: 1.12 (1.04, 1.21)	NO_2: cold season association robust to PM_{10} adjustment. Copollutants: PM_{10} no longer associated with stroke hospital admissions in the cold season after NO_2 adjustment. No correlations provided.

Table 5-50 (Continued): Corresponding risk estimates for hospital admissions for cerebrovascular disease and stroke for studies presented in Figure 5-19.

Study	Location	Health Effect	Selected Relative Risks[a] 95% CI	Copollutant Examination[b]
†Villeneuve et al. (2012)	Edmonton, Canada	Stroke, ischemic stroke, hemorrhagic stroke, transient ischemic stroke	Stroke (Lag 0−2): Warm: 1.42 (0.94, 2.17) Cool: 0.98 (0.84, 1.15) Ischemic stroke (Lag 0−2): Warm: 2.37 (1.27, 4.42) Cool: 0.90 (0.69, 1.13) Hemorrhagic stroke (Lag 0−2): Warm: 1.50 (0.59, 4.32) Cool: 0.98 (0.64, 1.50) Transient ischemic stroke (Lag 0−2): Warm: 0.67 (0.33, 1.42) Cool: 1.11 (0.80, 1.45)	Ischemic stroke during warm season NO_2: associations robust to adjustment for SO_2; slightly attenuated but positive after adjustment for CO, O_3, or $PM_{2.5}$. Copollutants: CO, O_3, and $PM_{2.5}$ associations attenuated by adjustment for NO_2. No association between SO_2 and ischemic stroke. Hemorrhagic stroke during warm season NO_2: associations attenuated after adjustment for SO_2 and O_3, but increased after adjustment for CO or $PM_{2.5}$. Copollutants: SO_2 and O_3 associations robust to NO_2 adjustment; CO no longer associated with hemorrhagic stroke after NO_2 adjustment.
†Turin et al. (2012)	Takashima County, Japan	Stroke, cerebral infarction, intracerebral hemorrhage, subarachnoid hemorrhage	Stroke: Lag 0: 0.98 (0.89, 1.08) Lag 1: 0.98 (0.89, 1.08) Cerebral infarction: Lag 0: 0.98 (0.87, 1.10) Lag 1: 1.00 (0.89, 1.12) Intracerebral hemorrhage: Lag 0: 1.06 (0.85, 1.33) Lag 1: 0.94 (0.75, 1.16) Subarachnoid hemorrhage: Lag 0: 1.12 (0.80, 1.56) Lag 1: 1.02 (0.73, 1.42)	No evidence of an association between NO_2 and stroke. Copollutant models did not change the results.
†Wellenius et al. (2012)	Boston, MA	Ischemic stroke	Lag 24 h preceding event: 1.32 (1.08, 1.63)	No copollutant models.
†Andersen et al. (2010)	Copenhagen, Denmark	Ischemic stroke, hemorrhagic stroke, mild ischemic stroke	NO_X: Ischemic stroke: Lag 0: 1.20 (0.96, 1.48) Lag 1: 0.96 (0.79, 1.20) Lag 0−4: 1.36 (0.96, 1.89) Hemorrhagic stroke: Lag 0: 0.96 (0.48, 1.81) Lag 1: 1.14 (0.59, 2.20) Lag 0−4: 0.43 (0.13, 1.36) Mild ischemic stroke : Lag 0−4: 1.61 (0.79, 3.30)	NO_X: no longer associated with ischemic stroke after adjustment for UFPs. Copollutants: UFP association robust after adjustment for NO_X.
†Larrieu et al. (2007)	8 French cities	Stroke	All ages: 0.99 (0.96, 1.03) ≥65 yrs: 1.01 (0.97, 1.05)	No copollutant models.

Table 5-50 (Continued): Corresponding risk estimates for hospital admissions for cerebrovascular disease and stroke for studies presented in Figure 5-19.

Study	Location	Health Effect	Selected Relative Risks[a] 95% CI	Copollutant Examination[b]
Poloniecki et al. (1997)	London, U.K.	Cerebrovascular disease	Lag 1: 0.99 (0.98, 1.00)	No copollutant models examined.
Linn et al. (2000)	Los Angeles, CA	Cerebrovascular disease, occlusive stroke	Cerebrovascular disease: Lag 0: 1.01 (0.99, 1.02) Occlusive stroke: Lag 0: 1.04 (1.02, 1.06)	No copollutant models. NO_2 correlations: PM_{10}: 0.67 to 0.88; O_3: −0.23 to 0.35; CO: 0.84 to 0.94
Tsai et al. (2003)	Kaohsiung, Taiwan	Cerebral stroke, ischemic stroke	Cerebral stroke: Lag 0−2; 20+°C: 1.68 (1.38, 2.04) Lag 0−2; <20°C: 0.78 (0.44, 1.37) Ischemic stroke: Lag 0−2; 20+°C: 1.67 (1.48, 1.87) Lag 0−2; <20°C: 1.19 (0.78, 1.84)	NO_2: Ischemic stroke and hemorrhagic stroke associations robust to SO_2, CO, or O_3 adjustment. Attenuated, but positive after PM_{10} adjustment. Copollutants: PM_{10}, SO_2, CO, and O_3 ischemic stroke and hemorrhagic stroke associations attenuated by adjustment for NO_2.
Wong et al. (1999)	Hong Kong, China	Cerebrovascular disease	Lag 0−1: 1.03 (0.99, 1.07)	No copollutant models.
Villeneuve et al. (2006a)	Edmonton, Canada	Ischemic stroke, hemorrhagic stroke, cerebral stroke	Ages 65 yr and older Ischemic stroke: Lag 0: 1.04 (0.96, 1.15) Lag 1: 1.07 (0.97, 1.17) Hemorrhagic stroke: Lag 0: 1.07 (0.96, 1.21) Lag 1: 1.06 (0.94, 1.20) Cerebral stroke: Lag 0: 0.99 (0.90, 1.07) Lag 1: 0.91 (0.84, 1.00)	Ischemic stroke during warm season NO_2: warm season associations robust to adjustment for SO_2 or CO; increase with adjustment for PM_{10} or $PM_{2.5}$; attenuated with adjustment for O_3. Hemorrhagic stroke during warm season NO_2: warm season associations robust to SO_2, O_3, $PM_{2.5}$, or PM_{10} adjustment (large increases in CIs in models with PM); but attenuated with adjustment for CO. NO_2 warm season correlations (Pearson *r*): SO_2: 0.22; O_3: −0.09; CO: 0.59; $PM_{2.5}$: 0.52; PM_{10}: 0.57.
Wellenius et al. (2005)	9 U.S. cities	Ischemic stroke, hemorrhagic stroke	Lag 0: 1.05 (1.03, 1.07) Lag 0: 1.01 (0.96, 1.06)	No copollutant models.
Pönkä and Virtanen (1996)	Helsinki, Finland	Cerebrovascular disease	Lag 0: 0.96 (0.87, 1.07) Lag 1: 0.98 (0.87, 1.09)	No copollutant models.
Ballester et al. (2001)	Valencia, Spain	Cerebrovascular disease	Lag 2: 1.22 (1.04, 1.44)	NO_2: associations were robust to adjustment for SO_2 or BS.
Peel et al. (2007)	Atlanta, GA	Cerebrovascular disease	Lag 0–2; case-crossover: 1.05 (1.01, 1.09) Lag 0−2; time-series: 1.06 (1.02, 1.11)	No copollutant models.

Table 5-50 (Continued): Corresponding risk estimates for hospital admissions for cerebrovascular disease and stroke for studies presented in Figure 5-19.

Study	Location	Health Effect	Selected Relative Risks[a] 95% CI	Copollutant Examination[b]
†Chen et al. (2014)	Edmonton, Canada	Ischemic stroke	All Year: Lag 1−8 h: 1.06 (0.98, 1.14) Warm Season: Lag 1−8 h: 1.39 (1.13, 1.71) Cold Season: Lag 1−8 h: 0.98 (0.89, 1.08)	No copollutant models.
Jalaludin et al. (2006)	Sydney, Australia	Stroke	Ages 65 yr and older Lag 0: 0.95 (0.88, 1.02) Lag 1: 0.96 (0.90, 1.03) Lag 0−1: 0.95 (0.88, 1.02)	No copollutant models analyzed for cerebrovascular disease.

CI = confidence interval, CO = carbon monoxide, NO_2 = nitrogen dioxide, NO_X = sum of NO and NO_2, O_3 = ozone, PM = particulate matter, SO_2 = sulfur dioxide, UFP = ultrafine particles.
[a]Effect estimates are standardized to a 20-ppb or 30-ppb increase in NO_2 or 40 ppb or 60-ppb increase in NO_X concentration for 24-h avg and 1-h max metrics, respectively.
[b]Relevant relative risks for copollutant models can be found in Figure S5-2, S5-3, S5-4, and S5-5 (U.S. EPA, 2014b, c, d, e).
†Studies published since the 2008 ISA for Oxides of Nitrogen.

Additional studies based on administrative data are also available. A number of the administrative data studies were conducted in Edmonton, Canada and used similar or identical data sources. The most thorough Edmonton study observed an association between NO_2 and ED visits for ischemic stroke in the warm season (OR: 2.37 [95% CI: 1.27, 4.41] per 20-ppb increase in 3 day average NO_2) and a weak association between hemorrhagic stroke in the warm season (OR: 1.50 [95% CI: 0.59, 3.82] per 20-ppb increase in 3 day average NO_2) (Villeneuve et al., 2012). Villeneuve et al. (2012) also examined copollutant models in which ischemic stroke associations were robust to adjustment for SO_2 (OR: 2.34 [95% CI: 1.25, 4.37]), and remained positive, but slightly attenuated after adjustment for CO (OR: 2.05 [95% CI: 0.92, 4.57]), O_3 (OR: 1.92 [95% CI: 0.98, 3.78]), and $PM_{2.5}$ (OR: 1.98 [95% CI: 0.94, 4.20]). The hemorrhagic stroke associations were attenuated after adjustment for SO_2 or O_3 but were robust to adjustment for the traffic-related pollutant CO or $PM_{2.5}$. There is the potential for misclassification of the exposure due to differences in the timing of stroke symptoms and the corresponding ED visit; however, after surveying a subset of the study population, Villeneuve et al. (2012) observed that roughly 75% of patients visited the emergency room on the same day that their symptoms presented. Further, when the authors adjusted the assigned pollution levels from the day of the ED visit to the day of symptom presentation, they observed no systematic differences in assigned pollution levels. Szyszkowicz (2008) observed a positive association between 24-h avg NO_2 and ED visits for ischemic stroke in Edmonton, Canada but only within specific subgroups according to sex, season, and age. In a recent related study, Chen et al. (2014) used the same data, but applied hourly NO_2 values to their models. The authors reported an association between NO_2 and ED

visits for acute ischemic stroke that remained relatively consistent across lag days. However, that association was almost entirely influenced by the association observed in the warm season, as the association in the cold season was null.

Zheng et al. (2013) conducted a time-series study in Lanzhou, China and found a positive association between NO_2 and all cerebrovascular hospital admissions. The strongest relationships were observed on same-day and 3-day cumulative lags. Zheng et al. (2013) also reported stronger associations in women and the elderly. Xiang et al. (2013) observed a positive association between NO_2 and hospital admissions for all strokes in the cold season in Wuhan, China that was robust in a copollutant model including PM_{10}. Conversely, in Taipei, Taiwan, Bell et al. (2008) did not observe an association between NO_2 and cerebrovascular disease. As mentioned in Section 5.3.2.1, Bell et al. (2008) attempted to reduce uncertainty related to the use of central site monitors by estimating NO_2 exposure over the entire Taipei area (average of 13 monitors), within Taipei City only (average of 5 monitors), and using a subset of monitors where all pairs of monitors had NO_2 correlations greater than 0.75 (8 monitors). The null findings were consistent across the three exposure assignment techniques. In a 7-year study of Hong Kong residents, Thach et al. (2010) also reported no association between NO_2 and all cerebrovascular hospital admissions.

5.3.4.2 Summary of Cerebrovascular Disease and Stroke

In summary, the epidemiologic data provide generally inconsistent evidence for a potential association between ambient NO_2 concentrations and risk of hospital admission for cerebrovascular disease and stroke. Clinical registry studies reported both positive (Wellenius et al., 2012; Andersen et al., 2010; Vidale et al., 2010) and null (Turin et al., 2012; Oudin et al., 2010) associations, while the majority of administrative database evidence of an association came from studies using similar or identical data sets (Chen et al., 2014; Villeneuve et al., 2012; Szyszkowicz, 2008). There were a limited number of studies that evaluated potential confounding by traffic pollutants (UFP, CO, $PM_{2.5}$, BS, and PM_{10}), and the results were, again, inconsistent. Additionally, the majority of the studies in this section used central site monitors to estimate ambient NO_2 exposure, which have noted limitations in capturing the variation in NO_2 (Section 3.4.4.2).

5.3.5 Decompensation of Heart Failure

Two recent studies found associations between short-term increases in ambient NO_2 concentration and hospital admissions or ED visits for heart failure. In the study of seven

Canadian cities described above, Stieb et al. (2009) observed a 5.1% (95% CI: 1.3, 9.2) increase in risk of ED visits for heart failure per 20-ppb increase in 24-h avg NO_2. Unlike the results for the composite endpoint of MI or acute angina, the increased risk of ED visits for heart failure was not dominated by results from a single city. In Taipei, Taiwan, Yang (2008) found that risk of hospital admission for heart failure were associated with NO_2 concentrations but only on days where the mean ambient temperature was ≥20°C. The association on warm days remained relatively unchanged after copollutant adjustment for PM_{10}, SO_2, CO, or O_3.

5.3.6 Increased Blood Pressure and Hypertension

5.3.6.1 Epidemiologic Studies

The 2008 ISA for Oxides of Nitrogen did not review any epidemiologic studies of ambient oxides of nitrogen and blood pressure (BP) (U.S. EPA, 2008a). Several studies are now available for review (Table 5-51). There is little evidence from longitudinal studies of the association between NO_2 and BP. A number of longitudinal studies measured BP in subjects in Beijing before, during, and after the 2008 Beijing Olympics when city-wide air pollution control measures substantially reduced ambient levels of most criteria pollutants. One study reported that NO_2 concentrations during the Olympics were reduced by close to 22% versus the previous month and 13% versus the same period the previous summer (Huang et al., 2012a). Other ambient pollutants (except O_3) were reduced by similar or larger amounts. Huang et al. (2012a) measured BP repeatedly in participants with pre-existing cardiovascular disease in Beijing and found no association between NO_2 and either systolic or diastolic BP. Focusing on healthy young adults, Zhang et al. (2013) and Rich et al. (2012) each observed no clear association between NO_2 and either systolic or diastolic BP among participants assessed before, during, and after the 2008 Beijing Olympics.

Table 5-51 Epidemiologic studies of blood pressure.

Study	Location Sample Size	Mean NO_2 (ppb)	Exposure Assessment	Selected Effect Estimates[a] (95% CI)
†Williams et al. (2012a)	Detroit, MI n = 65	24-h avg NO_2: 24.0 75th: 28.0 Max: 100.0	Personal monitoring and central monitor	No quantitative results presented.
†Huang et al. (2012a)	Beijing, China n = 40	2007, Visit 1: 33.8 2007, Visit 2: 26.3 2008, Visit 3: 29.2 2008, Visit 4: 22.9	Central monitor	Change in SBP (mmHg) 30-min: 3.73 (−1.04, 8.28) 2-h: 0.00 (−5.89, 5.89) 12-h: 1.69 (−7.73, 11.11) 24-h: −2.32 (−19.10, 14.47) Change in DBP (mmHg) 30-min: 2.28 (−1.86, 6.221) 2-h: −0.19 (−4.45, 6.00) 12-h: 2.90 (−5.07, 10.87) 24-h: 4.34 (−9.84, 18.52)
†Rich et al. (2012) and †Zhang et al. (2013)	Beijing, China n = 125	24-h avg NO_2: Entire study: 27.0 Before: 26.0 During: 13.9 After: 41.4	Central monitor	No quantitative results presented; results presented graphically. Generally inconsistent results with SBP: positive and negative associations across lags. Generally null and inconsistent associations with DBP across lags 0−6.
†Liu et al. (2014b)	Sault Ste. Marie. Ontario, Canada n = 61	1-h max NO_2: Site 1: 3.9 95th: 9.5 Site 2: 5.8 95th: 13.8	Central monitor	Change in SBP (mmHg) Lag 0: −1.04 (−4.20, 2.12) Lag 1: 2.08 (−1.36, 5.52) Change in DBP (mmHg) Lag 0: −1.32 (−3.88, 1.28) Lag 1: 1.64 (−1.36, 4.60)
†Cakmak et al. (2011a)	Canada n = 5,604	24-h avg NO_2: 12.6	City-wide avg	Change in resting SBP (mmHg) Lag 0: 1.76 (0.35, 3.17) Change in resting DBP (mmHg) Lag 0: 2.11 (1.12, 3.10)
†Chuang et al. (2010)	Taiwan n = 7,578	24-h avg NO_2: 22.4 Max: 65.5	Nearest monitor (within 10 km)	No quantitative results presented for NO_2.
†Chen et al. (2012c)	Taiwan (n = 9,238)	24-h avg NO_2: 13.9 to 26.1 across locations Max: 34.3 to 49.1	City-wide avg	Change in SBP (mmHg) Lag 0: −0.81 (−2.16, 0.55) Lag 0−1: −1.17 (−2.34, −0.01) Lag 0−2: −4.20 (−5.22, −3.17) Change in DBP (mmHg) Lag 0: 1.03 (0.11, 1.95) Lag 0−1: 1.54 (0.75, 2.32) Lag 0−2: −0.01 (−0.71, 0.68) Pulse Pressure Change Lag 0: −2.55 (−3.62, −1.48) Lag 0−1: −2.09 (−3.02, −1.18) Lag 0−2: −3.22 (−4.04, −2.40)

Table 5-51 (Continued): Epidemiologic studies of blood pressure.

Study	Location Sample Size	Mean NO_2 (ppb)	Exposure Assessment	Selected Effect Estimates[a] (95% CI)
†Choi et al. (2007)	Incheon, South Korea (n = 10,459)	24-h avg NO_2: Warm season: 22.5 75th: 26.9 Max: 49.3 Cool season: 29.2 75th: 34.7 Max: 74.0	City wide avg	Warm season Change in SBP (mmHg) Lag 0: 2.24 (p = 0.002) Lag 1: 2.40 (p < 0.001) Lag 2: −0.04 (p = 0.534) Change in DBP (mmHg) Lag 0: 2.02 (p = 0.645) Lag 1: 2.12 (p = 0.016) Lag 2: −0.04 (p = 0.331) Cool season Change in SBP (mmHg) Lag 0: 2.06 (p = 0.181) Lag 1: 2.06 (p = 0.195) Lag 2: −0.06 (p = 0.223) Change in DBP (mmHg) Lag 0: −0.02 (p = 0.573) Lag 1: 2.00 (p = 0.445) Lag 2: 2.02 (p = 0.445)

CI = confidence interval, DBP = diastolic blood pressure, NO_2 = nitrogen dioxide, SBP = systolic blood pressure.
[a]Effect estimates are standardized to a 20-ppb or 30-ppb increase in NO_2 for 24-h avg and 1-h max metrics, respectively.
†Studies published since the 2008 ISA for Oxides of Nitrogen.

In the Detroit area, Williams et al. (2012a) measured BP up to 10 times in each of 65 adult participants and found no association between BP and either total personal or ambient NO_2 concentrations. A strength of this study was the authors' use of personal exposure measurements, which are less susceptible to exposure misclassification due to the variability in NO_2 concentrations and variation in time-activity patterns than central site monitoring (Section 3.4.4). Similarly, in a randomized cross-over study designed to examine the cardiovascular effects of exposure to steel plant emissions in Ontario, Liu et al. (2014b) measured NO_2 exposure near subjects' randomized exposure location and reported no association between NO_2 and either systolic or diastolic BP.

Results of cross-sectional studies of the association between NO_2 and BP measured on the same day or with the NO_2 measurement lagged 1−3 days before the BP measurement have also been mixed. Cakmak et al. (2011a) used cross-sectional data from a national population-based survey of children and adults in Canada and found a 1.76 mmHg (95% CI: 0.35, 3.17 mmHg) increase in systolic BP and a 2.11 mmHg (95% CI: 1.12, 3.10 mmHg) increase in diastolic BP per 20-ppb increase in 24-h avg NO_2 on the same day. Chuang et al. (2010) used cross-sectional data from a national population-based health screening of adults in Taiwan and reported finding no association between BP and NO_2 levels, although quantitative results were not presented. On the other hand, Chen et al. (2012c) used cross-sectional data from a different population-based health screening in adults across six townships in Taiwan and found a 4.20 mmHg decrease (95% CI: −5.22, −3.17 mmHg) in systolic BP per 20-ppb increase in 24-h avg NO_2 at lag 3 and a

1.54 mmHg increase (95% CI: 0.75, 2.32 mmHg) in diastolic BP per 20-ppb increase in 24-h avg NO_2 at lag 2. Choi et al. (2007) observed positive associations between NO_2 concentrations and systolic BP during the warm and cold seasons at lags 0 and 1, though the associations for diastolic BP were generally null.

5.3.6.2 Controlled Human Exposure Studies

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a) reviewed controlled human studies of cardiac output and BP (Table 5-56); several of these studies also examined heart rate (HR) as described in Section 5.3.11.1. NO_2 exposure generally did not increase cardiac output or BP in healthy adults or those with COPD. These endpoints have not been evaluated in recent controlled human exposure studies of NO_2.

Cardiac output is the volume of blood pumped out by each of the two ventricles per minute. It is directly related to HR, as the output of each ventricle is the product of the HR (beats/minute) and the stroke volume (mL of blood/beat). BP is the product of cardiac output and vascular resistance. Cardiac output, vascular resistance, and BP interact moment-to-moment to ensure systemic circulatory demands are met.

Folinsbee et al. (1978) exposed three groups of five young healthy adult males to 600 ppb NO_2 for 2 hours with intermittent exercise. The authors reported no changes in cardiac output or BP. Drechsler-Parks (1995) exposed eight older healthy adults to filtered air, 600 ppb NO_2, 450 ppb O_3, and NO_2 + O_3 for 2 hours with intermittent exercise. There was no change in stroke volume or cardiac output following exposure to NO_2 or O_3 alone compared to filtered air; however, a decrease in cardiac output was observed following NO_2 + O_3 exposure compared to O_3 and filtered air exposures ($p < 0.05$). Gong et al. (2005) reported no change in BP after exposure to 400 ppb NO_2 for 2 hours with intermittent exercise in volunteers with COPD and healthy volunteers. One controlled human exposure study examined exposure to higher concentrations of NO_2. Linn et al. (1985b) reported a small, but statistically significant decrease in BP after exposure to approximately 4,000 ppb NO_2 for 75 minutes with exercise. In both healthy volunteers and those with asthma, the mean BP decrease was about 5 mmHg relative to controls.

5.3.6.3 Hospital Admissions and Emergency Department Visits

In contrast with findings for changes in BP, the limited number of available studies report associations between NO_2 and ED visits for hypertension. In Beijing, China, Guo et al. (2010) found that NO_2 was associated with ED visits for hypertension, and the association remained relatively unchanged in copollutant models adjusting for PM_{10} or

SO_2. Similarly, in Edmonton, Canada, Szyszkowicz et al. (2012) found that ED visits for hypertension were positively associated with NO_2 in single-pollutant models. The association was attenuated in a multipollutant model adjusting for SO_2 and PM_{10}, but results from these models are difficult to interpret given the potential for multicollinearity among pollutants.

5.3.6.4 Summary of Blood Pressure and Hypertension

In summary, there is little evidence from available epidemiologic studies to suggest that short-term exposure to ambient NO_2 is associated with increased BP in the population overall. There is no evidence of an association from longitudinal studies and mixed evidence from cross-sectional studies. However, cross-sectional studies do not assess temporal relationships and are more prone to confounding by factors that differ between individual participants. Controlled human exposure studies show no evidence to suggest that short-term exposure to ambient relevant concentrations of NO_2 alone alter BP or cardiac output.

5.3.7 Venous Thromboembolism

Venous thromboembolism is a term that includes both deep vein thrombosis (DVT) and pulmonary embolism (PE). DVT occurs when a blood clot develops in the deep veins, most commonly in the lower extremities. A part of the clot can break off and travel to the lungs, causing a PE, which can be life threatening.

Two recent studies found associations between NO_2 and venous thrombosis and/or PE; however, both studies were small, and neither evaluated potential copollutant confounding. A study covering the metropolitan region of Santiago, Chile, found a 9.7% (95% CI: 4.1, 15.4) and 8.4% (95% CI: 5.0, 11.8) increase in hospital admissions for venous thrombosis and PE, respectively, per 20-ppb increase in 24-h avg NO_2 concentrations (Dales et al., 2010). Spiezia et al. (2014) also examined the association between ambient air pollution and PE hospital admissions in a small case-control study of 105 adults in Padua, Italy. The authors observed an increase in the risk of unprovoked PE for subjects who were in the upper tertile of NO_X exposure (average exposure for the month leading up to hospitalization $\geq$124 $\mu g/m^3$) compared to those in the bottom two exposure tertiles (OR: 2.35 [95% CI: 0.76, 7.25]).

5.3.8 Cardiometabolic Effects

There were no epidemiologic studies of diabetes or insulin deficiency available for the 2008 ISA for Oxides of Nitrogen. Two recent studies reported contrasting findings regarding short-term associations between air pollutants and measures of insulin resistance, which plays a key role in the development of type two diabetes mellitus (DM). In a panel study of older adults in Korea, Kim and Hong (2012) observed a 1.33 μU/mL (95% CI: 0.54, 2.11) increase in insulin resistance and a 0.52 mean (95% CI: 0.24, 0.77) increase in the homeostatic model assessment index of insulin resistance [fasting insulin * (fasting glucose ÷ 22.5)] per 20-ppb increase in 24-h avg NO_2 at lag 7. The association was stronger in participants with a history of DM but still present for those without. On the other hand, Kelishadi et al. (2009) reported a lack of an association between 24-h avg NO_2 and insulin resistance in a study of 374 Iranian children aged 10−18 years. Both of the recent studies relied on central site monitoring for exposure estimation, and neither evaluated potential confounding by other traffic-related pollutants. There are a number of recent prospective studies reporting associations between diabetes and long-term exposure to oxides of nitrogen (Section 6.3.3).

5.3.9 Aggregated Cardiovascular Effects

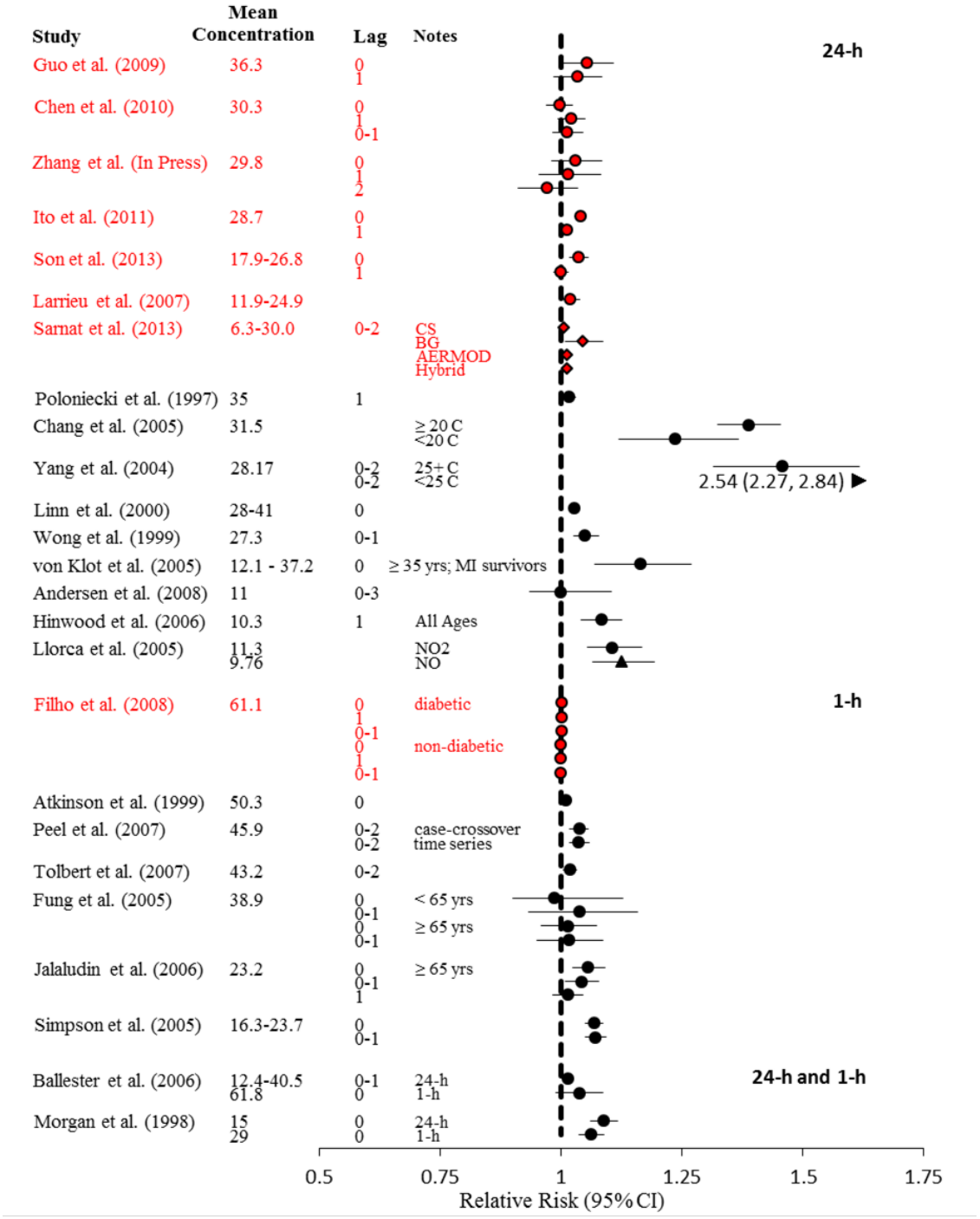
Many epidemiologic studies consider the composite endpoint of all cardiovascular diseases, which typically includes all diseases of the circulatory system (e.g., heart diseases and cerebrovascular diseases). Most studies reviewed in the 2008 ISA for Oxides of Nitrogen found positive associations between ambient NO_2 concentrations and risk of hospital admissions or ED visits for all cardiovascular diseases (U.S. EPA, 2008a) (Figure 5-20 and Table 5-52). However, it was unclear at that time whether these results truly indicated effects of NO_2 or were confounded by other correlated pollutants. Several additional studies are now available with broadly consistent results, though some uncertainty still remains with regard to potential copollutant confounding.

Ito et al. (2011) observed that risk of CVD hospital admission was associated with NO_2 concentrations at lag 0 in New York City. Results from copollutant models were not reported. Zheng et al. (2013) and Son et al. (2013) observed seasonal variation in the strength of association between NO_2 and CVD hospital admission in Lanzhou, China and Korea, respectively. In contrast, Ito et al. (2011) did not find any seasonal differences in New York City. A study in Santiago, Chile reported an increase in risk of CVD hospital admissions per increase in 24-h avg NO_2, which remained relatively unchanged after adjustment for highly correlated traffic-related copollutants, CO (r = 0.94) or $PM_{2.5}$ (r = 0.87) (no quantitative results; results presented graphically) (Franck et al., 2014). In

Beijing, China, Guo et al. (2009) reported an association between ambient NO_2 concentrations and risk of CVD hospital admissions at lag 0 (OR: 1.05 [95% CI: 1.00, 1.11] per 20-ppb increase in 24-h avg NO_2), but this association was attenuated and had a wide 95% CI in copollutant models adjusting for either $PM_{2.5}$ (OR: 1.02 [95% CI: 0.96, 1.09]) or SO_2 (OR: 1.01 [95% CI: 0.94, 1.08]). Sarnat et al. (2013b) reported a positive association between NO_X concentrations and CVD ED visits in Atlanta. This study compared the strength of the association across exposure assessment techniques and estimated larger effects using spatially refined ambient concentration metrics (AERMOD, Air Pollution Exposure model, and a hybrid model of background concentrations and AERMOD) in contrast to central site monitoring data. However, there is uncertainty regarding the extent to which an association with NO_X reflects an association with NO_2 (Sections 1.1 and 2.5).

In Shanghai, China, Chen et al. (2010) found a 1.02% (95% CI: −2.0, 4.0) increased risk of hospital admission for CVD per 20-ppb increase in 24-h avg NO_2 concentrations (lag 0−1 days). This association was robust to additional adjustment for PM_{10} but was attenuated after adjustment for SO_2 (Table S5-5). A study in São Paulo, Brazil, also found a positive association with some evidence that the association was stronger among patients with a secondary diagnosis of diabetes mellitus (Filho et al., 2008). (Jevtić et al., 2014) reported a positive association that was robust to the inclusion of SO_2 in a copollutant model in Novi Sad, Serbia. Studies from Copenhagen, Denmark (Andersen et al., 2008b); Madrid, Spain (Linares and Diaz, 2010); Reykjavik, Iceland (Carlsen et al., 2013); and Taipei, Taiwan (Chan et al., 2008) reported null or negative associations between NO_2 concentrations and risk of hospital admission for CVD. A study in Guangzhou, China also found no clear association between NO_2 and CVD hospital admissions, with observed associations alternating between positive and negative depending on the lags examined (Zhang et al., 2014).

In summary, consistent evidence reported in the 2008 ISA for Oxides of Nitrogen combined with recent epidemiologic data continue to support the presence of an association between ambient NO_2 concentrations and risk of hospital admission for cardiovascular diseases (Figure 5-20 and Table 5-52). However, despite generally consistent associations, there were a limited number of studies that evaluated potential confounding by correlated traffic-related copollutants, particularly EC, $PM_{2.5}$, UFPs, and VOCs, resulting in some uncertainty of the independent effect of NO_2 on cardiovascular disease hospital admissions and ED visits (Table 5-52).



Note: Studies in red are recent studies. Studies in black were included in the 2008 ISA for Oxides of Nitrogen. Relative risks are standardized to a 20-ppb or 30-ppb increase in NO_2 or NO concentrations and 40 ppb or 60 ppb for NO_X concentrations for 24-h avg and 1-h max metrics, respectively. Studies are presented in descending order of mean concentration, with the largest mean concentration (ppb) at the top and the smallest at the bottom of the figure (by averaging time and inclusion in previous ISA). Circles = NO_2; Triangles = NO; Diamonds = NO_X. Franck et al. (2014) not presented in table due to lack of quantitative results.

Figure 5-20 Results of studies of short-term exposure to oxides of nitrogen and hospital admissions for all cardiovascular disease.

Table 5-52 Corresponding effect estimates for hospital admissions for all cardiovascular disease studies presented in Figure 5-20.

Study	Location	Relative Risk[a] (95% CI)	Copollutant Examination[b]
†Guo et al. (2009)	Beijing, China	Lag 0: 1.05 (1.00, 1.11) Lag 1: 1.03 (0.985, 1.09)	NO_2: associations attenuated by $PM_{2.5}$ or SO_2 adjustment. Copollutants: $PM_{2.5}$ and SO_2 associations robust to adjustment for NO_2. NO_2 correlations (Pearson *r*): $PM_{2.5}$: 0.67; SO_2: 0.53.
†Chen et al. (2010)	Shanghai, China	Lag 0: 0.997 (0.970, 1.025) Lag 1: 1.02 (0.99, 1.05) Lag 0−1: 1.01 (0.98, 1.04)	NO_2: associations robust to adjustment for PM_{10}; attenuated by SO_2 adjustment. Copollutants: PM_{10} and SO_2 associations attenuated by adjustment for NO_2. NO_2 correlations (Pearson *r*): PM_{10}: 0.70; SO_2: 0.76.
†Zhang et al. (2014)	Guangzhou, China	Lag 0: 1.03 (0.98, 1.08) Lag 1: 1.02 (0.95, 1.08) Lag 2: 0.97 (0.91, 1.03)	No copollutant models. NO_2 correlations (Spearman *r*): PM_{10}: 0.82; SO_2: 0.60.
†Ito et al. (2011)	New York City, NY	Lag 0: 1.04 (1.03, 1.05) Lag 1: 1.01 (1.00, 1.02)	No copollutant models.
†Son et al. (2013)	8 Korean cities	Lag 0: 1.04 (1.02, 1.06) Lag 1: 1.00 (0.98, 1.01)	No copollutant models. NO_2 correlations (Pearson *r*): PM_{10}: 0.5; SO_2: 0.6; CO: 0.7; O_3: −0.1.
†Larrieu et al. (2007)	8 French cities	1.02 (1.00, 1.04)	No copollutant models.
†Sarnat et al. (2013b)	Atlanta, GA	Central site Lag 0−2: 1.01 (1.00, 1.01) Background (distance^2 weighting) Lag 0−2: 1.05 (1.01, 1.09) AERMOD Lag 0−2: 1.01 (1.00, 1.02) Hybrid—background and AERMOD Lag 0−2: 1.01 (1.0, 1.02)	No copollutant models.
Poloniecki et al. (1997)	London, U.K.	Lag 1: 1.02 (1.00, 1.04)	No copollutants models analyzed for CVD.
Chang et al. (2005)	Taipei, Taiwan	≥20°C: 1.39 (1.32, 1.45) <20°C: 1.23 (1.12, 1.37)	NO_2: associations robust to adjustment for PM_{10}, SO_2, CO, or O_3, with the exception of PM_{10} on cold days.
Yang et al. (2004)	Kaohsiung, Taiwan	≥25°C, lag 0-2: 1.46 (1.31, 1.62) <25°C, lag 0-2: 2.54 (2.27, 2.84)	NO_2: associations robust to adjustment for PM_{10}, SO_2, CO, or O_3 on cold days. Somewhat attenuated after adjustment on warm days, but still positive (except SO_2, still robust).
Linn et al. (2000)	Los Angeles, CA	Lag 0: 1.03 (1.02, 1.04)	No copollutant models. NO_2 correlations: PM_{10}: 0.67 to 0.88; O_3: −0.23 to 0.35; CO: 0.84 to 0.94.

Table 5-52 (Continued): Corresponding effect estimates for hospital admissions for all cardiovascular disease studies presented in Figure 5-20.

Study	Location	Relative Risk[a] (95% CI)	Copollutant Examination[b]
Wong et al. (1999)	Hong Kong, China	Lag 0−1: 1.05 (1.03, 1.08)	No copollutant models.
Von Klot et al. (2005)	5 European cities	Lag 0: 1.16 (1.07, 1.27)	NO_2: associations robust to adjustment for PM_{10} or O_3.
Andersen et al. (2008b)	Copenhagen, Denmark	Lag 0−3: 1.00 (0.93, 1.10)	No evidence of an association between NO_2 and CVD. Copollutant models did not change the results.
Hinwood et al. (2006)	Perth, Australia	Lag 1: 1.08 (1.04, 1.13)	No copollutant models.
Llorca et al. (2005)	Torrelavega, Spain	NO_2: 1.11 (1.05, 1.17) NO: 1.13 (1.07, 1.19)	No copollutant models.
†Filho et al. (2008)	São Paulo, Brazil	Diabetics Lag 0: 1.00 (1.00, 1.00) Lag 1: 1.00 (0.99, 1.00) Lag 0−1: 1.00 (1.00, 1.00) Non-diabetics Lag 0: 1.00 (1.00, 1.00) Lag 1: 1.00 (0.99, 1.00) Lag 0−1: 1.00 (1.00, 1.00)	No copollutant models. NO_2 correlations (Pearson *r*): PM_{10}: 0.68; SO_2: 0.62; CO: 0.58; O_3: 0.41.
Atkinson et al. (1999)	London, U.K.	Lag 0: 1.01 (1.00, 1.02)	NO_2: association attenuated by adjustment for BS. Copollutants: BS robust to adjustment for NO_2.
Peel et al. (2007)	Atlanta, GA	Case-crossover; lag 0−2: 1.04 (1.02, 1.06) Time-series; lag 0−2: 1.04 (1.02, 1.06)	No copollutant models.
Tolbert et al. (2007)	Atlanta, GA	Lag 0−2: 1.02 (1.01, 1.03)	NO_2: association attenuated after adjustment for CO or $PM_{2.5}$ TC. Copollutants: CO and $PM_{2.5}$ TC associations robust to adjustment for NO_2. NO_2 correlations (Spearman *r*): CO: 0.70; $PM_{2.5}$ TC: 0.65.
Fung et al. (2005)	Windsor, Ontario, Canada	<65 yr Lag 0: 0.99 (0.90, 1.13) Lag 0−1: 1.04 (0.93, 1.16) ≥65 yr Lag 0: 1.02 (0.96, 1.07) Lag 0−1: 1.02 (0.98, 1.05)	Copollutant results not reported for NO_2.

Table 5-52 (Continued): Corresponding effect estimates for hospital admissions for all cardiovascular disease studies presented in Figure 5-20.

Study	Location	Relative Risk[a] (95% CI)	Copollutant Examination[b]
Jalaludin et al. (2006)	Sydney, Australia	Lag 0: 1.06 (1.02, 1.09) Lag 0−1: 1.01 (0.98, 1.05) Lag 1: 1.04 (1.01, 1.08)	NO_2: associations robust to adjustment for PM_{10}, $PM_{2.5}$, SO_2, O_3, or BS in adults aged 65 yr and older. Attenuated after CO adjustment. Copollutants: CO, $PM_{2.5}$, SO_2, O_3, and BS associations robust to NO_2 adjustment; PM_{10} association attenuated. NO_2 correlations: BS: 0.35 to 0.59; PM_{10}: 0.44 to 0.67; $PM_{2.5}$: 0.45 to 0.68; O_3: 0.21 to 0.45; CO: 0.55 to 0.71; SO_2: 0.52 to 0.56.
Simpson et al. (2005a)	4 Australian cities	Lag 0: 1.07 (1.05, 1.09) Lag 0−1: 1.07 (1.05, 1.09)	NO_2: associations robust to adjustment for BS; attenuated, but positive after O_3 adjustment. Copollutants: O_3 negative association robust to adjustment for NO_2; BS association attenuated, but positive after adjustment for NO_2.
Ballester et al. (2006)	Spain	24-h NO_2, lag 0: 1.01 (1.00, 1.03) 1-h NO_2, lag 0: 1.04 (0.99, 1.09)	NO_2: associations robust to adjustment for O_3; attenuated but positive with CO or SO_2 adjustment. Copollutants: CO, BS, PM_{10}, SO_2, and O_3 associations robust to NO_2 adjustment.
Morgan et al. (1998)	Sydney, Australia	24-h NO_2, lag 0: 1.09 (1.06, 1.12) 1-h NO_2, lag 0: 1.06 (1.04, 1.09)	No copollutant models. NO_2 correlations: O_3: −0.09; PM: 0.53.

CI = confidence interval, CO = carbon monoxide, CVD = cardiovascular disese, ED = emergency department, NO = nitric oxide, NO_2 = nitrogen dioxide, O_3 = ozone, PM = particulate matter, SO_2 = sulfur dioxide.
[a]Relative risks are standardized to a 20-ppb or 30-ppb increase in NO_2 or NO or 40 ppb or 60-ppb increase in NO_X concentration for 24-h avg and 1-h max metrics, respectively.
[b]Relevant relative risks for copollutant models can be found in Figure S5-2, S5-3, S5-4, and S5-5 (U.S. EPA, 2014b, c, d, e).
†Studies published since the 2008 ISA for Oxides of Nitrogen.

5.3.10 Cardiovascular Mortality

Studies evaluated in the 2008 ISA for Oxides of Nitrogen that examined the association between short-term NO_2 exposure and cause-specific mortality consistently reported positive associations with cardiovascular mortality. Across studies, there was evidence that the magnitude of the NO_2-cardiovascular mortality relationship was similar or slightly larger than that for total mortality. Recent multicity studies as well as a meta-analysis of studies conducted in Asian cities Atkinson et al. (2012) provide evidence that is consistent with those studies evaluated in the 2008 ISA for Oxides of Nitrogen (Section 5.4, Figure 5-23).

The NO_2-cardiovascular mortality relationship was further examined in a few studies that analyzed copollutant models. It is important to note that it is difficult to examine whether NO_2 is independently associated with cardiovascular mortality because NO_2 often is highly correlated with other traffic-related pollutants. In the 17 Chinese cities study (CAPES), Chen et al. (2012b) found that NO_2 risk estimates for cardiovascular mortality were slightly attenuated but remained positive in copollutant models with PM_{10} or SO_2 (6.9% [95% CI: 3.8, 10.1] for a 20-ppb increase in 24-h avg NO_2 concentrations at lag 0−1; 4.6% [95% CI: 1.1, 8.1] with PM_{10}; 5.7% [95% CI: 2.5, 9.0] with SO_2). Chen et al. (2013) reported similar results when examining stroke mortality in a subset of 8 CAPES cities, i.e., 5.6% increase in stroke mortality (95% CI: 3.4, 8.0) at lag 0−1 for a 20-ppb increase in 24-h avg NO_2 concentrations with a slight attenuation of the association in copolluant models with PM_{10} (4.5% [95% CI: 1.8, 7.3]) or SO_2 (5.2% [95% CI: 2.1, 8.3]). Also, Chiusolo et al. (2011) found evidence that associations between short-term NO_2 exposure and cardiovascular mortality remained robust in copollutant models in a study of 10 Italian cities. In an all-year analysis, a 20-ppb increase in NO_2 at lag 0−5 was associated with a 10.5% (95% CI: 5.9, 14.8) increase in cardiovascular mortality and a 10.1% (95% CI: 4.0, 16.4) increase adjusted for PM_{10}. In a warm season analysis (April−September), the NO_2 effect estimate was 19.2% (95% CI: 11.4, 27.4) and 18.8% (95% CI: 10.7, 27.5) with adjustment for O_3. Overall, the limited number of studies that have examined the potential confounding effects on the NO_2-cardiovascular mortality relationship indicate that associations remain relatively unchanged with adjustment for PM_{10} or SO_2, but it remains difficult to disentangle the independent effects of NO_2 as confounding by more highly traffic-related copollutants has not been examined.

Of the multicity studies evaluated, only the studies conducted in Italy examined potential seasonal differences in the NO_2-cause-specific mortality relationship (Chiusolo et al., 2011; Bellini et al., 2007). Additional information with regard to whether there is evidence of seasonal differences in NO_2-cardiovascular mortality associations is provided by single-city studies conducted in the U.S. (Sacks et al., 2012; Ito et al., 2011). In a study of 15 Italian cities, Bellini et al. (2007) found that risk estimates for cardiovascular mortality were dramatically increased in the summer from 1.5 to 7.3% for a 20-ppb increase in 24-h avg NO_2 concentrations at lag 0−1, respectively, with no evidence of an association in the winter. These results were corroborated in a study of 10 Italian cities (Chiusolo et al., 2011), which observed an increase in risk estimates for cardiovascular mortality in the warm season (i.e., April−September) compared to all-year analyses. Chiusolo et al. (2011) did not conduct analyses with only the winter season. U.S. studies conducted in New York, NY (Ito et al., 2011) and Philadelphia, PA (Sacks et al., 2012) do not provide consistent evidence indicating seasonal differences (Section 5.4.6). Overall, the cardiovascular mortality results from the multicity studies conducted in Italy are consistent with those observed in the total mortality analyses conducted by Bellini et

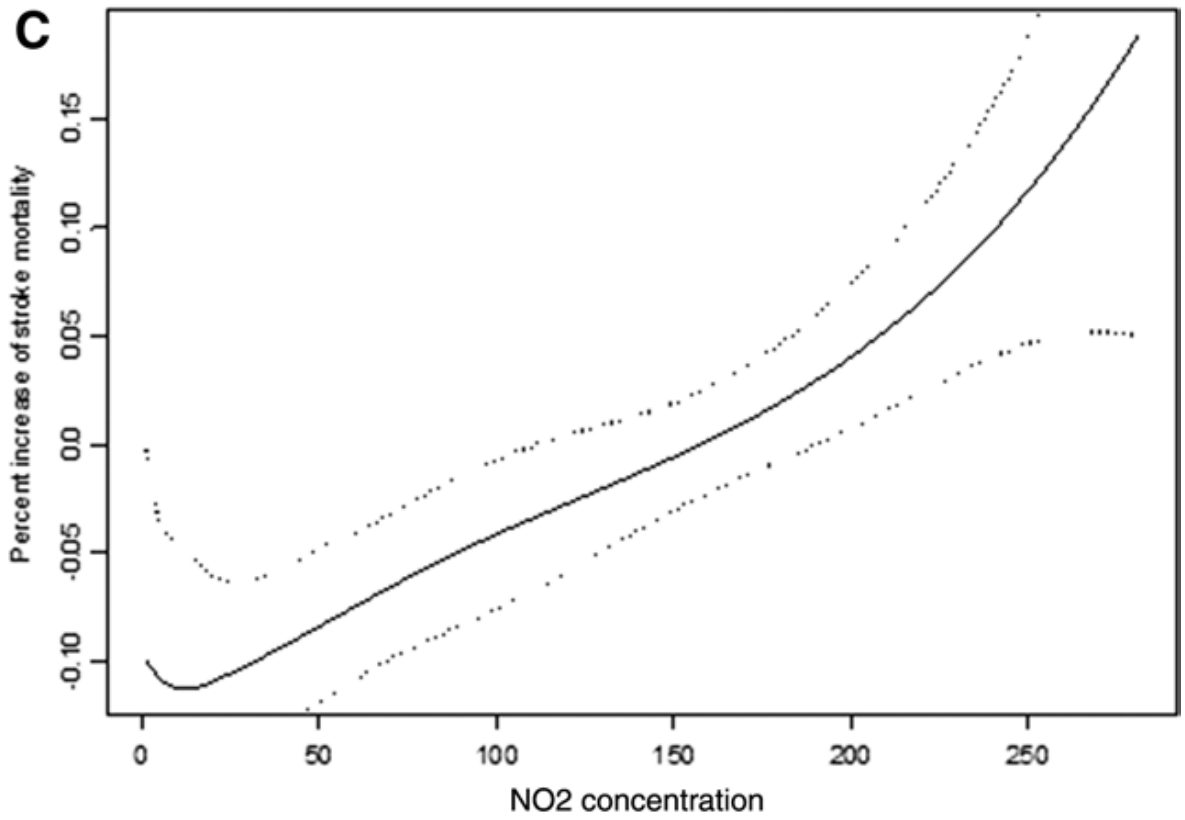
al. (2007) and Chiusolo et al. (2011). However, as discussed in Section 5.4.3, studies conducted in Asian cities observed very different seasonal patterns, and it remains unclear if the seasonal patterns observed for total mortality would be similar to those observed for cardiovascular mortality in these cities.

An uncertainty that often arises when examining the relationship between short-term air pollution exposures and cause-specific mortality is whether analyses that examine statistical modeling parameters, the lag structure of associations, and the C-R relationship provide results that are consistent with those observed for total mortality. In a study conducted in Philadelphia, PA, Sacks et al. (2012) examined whether the various modeling approaches to control for both temporal trends/seasonality and weather used in a number of multicity studies [e.g., National Morbidity, Mortality, and Air Pollution Study (NMMAPS), Air Pollution and Health: A European Approach (APHEA)] influence air pollution-cardiovascular mortality associations when using the same data set. Across models, the authors reported that associations of NO_2 with cardiovascular mortality were relatively consistent, with the percentage increase in cardiovascular mortality for a 20-ppb increase in 24-h avg NO_2 concentrations ranging from 1.4 to 2.0%. The results of Sacks et al. (2012) support those of Chen et al. (2013), which found that NO_2-stroke mortality associations were robust to using 4 to 10 df per year to control for temporal trends.

Studies that examined the lag structure of associations for cardiovascular mortality reported results consistent with those observed for total mortality (see Section 5.4.7). In a study of 10 Italian cities, Chiusolo et al. (2011) reported evidence of an immediate effect of NO_2 at lag 0−1 on cardiovascular mortality but also provided evidence for a prolonged effect due to the magnitude of the association being larger at lag 0−5 (Figure 5-24). These results are consistent with those of Chen et al. (2012b) in the CAPES study. The authors found the largest effect at single day lags of 0 and 1 and the average of lag 0−1 days providing support for an immediate effect of NO_2 on cardiovascular mortality (Figure 5-25). However, when examining longer lags Chen et al. (2012b) reported that the magnitude of the association was slightly larger for a 0−4 day lag suggesting a potential prolonged effect. In an analysis of stroke mortality Chen et al. (2013) reported similar results in a subset of eight Chinese cities from the CAPES study.

To date, analyses detailing the C-R relationship between air pollution and cause-specific mortality have been limited. In the analysis of eight Chinese cities, Chen et al. (2013) also examined the air pollution and stroke mortality C-R relationship. To examine the assumption of linearity, the authors fit both a linear and spline model to the NO_2-stroke mortality relationship. Chen et al. (2013) then computed the deviance between the two models to determine if there was any evidence of non-linearity. An examination of the

deviance did not indicate that the spline model improved the overall fit of the NO_2-stroke mortality relationship (Figure 5-21).



Source: Reprinted with permission of Wolters Kluwer Health Chen et al. (2013).
Note: The black line represents the mean estimate, and the dotted lines are 95% confidence intervals.

Figure 5-21 Pooled concentration-response curve for nitrogen dioxide (NO_2) and daily stroke mortality in eight Chinese cities for lag 0−1 day.

5.3.11 Subclinical Effects Underlying Cardiovascular Effects

The following subsections review studies of subclinical effects that serve as useful measures of physiological and biochemical responses and could provide mechanistic evidence to describe a role for NO_2 in the manifestation of cardiovascular diseases. These subclinical effects are not widely validated markers of specific clinical cardiovascular outcomes but could potentially underlie the development, progression, or indication of various clinical events and provide biological plausibility for multiple outcomes.

5.3.11.1 Heart Rate and Heart Rate Variability

HRV provides a noninvasive marker of cardiac autonomic nervous system function. The rhythmic variation in the intervals between heart beats can be quantified in either the time domain or the frequency domain (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Common time-domain measures of HRV include the standard deviation of all normal-to-normal intervals (SDNN, an index of total HRV) and the root-mean-square of successive differences (rMSSD, an index influenced mainly by the parasympathetic nervous system). In the frequency domain, HRV is usually divided into the high frequency (HF) and low frequency (LF) components, as well as the ratio of the LF to HF components (LF/HF) (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Decreases in indices of HRV have been associated with increased risk of cardiovascular events in prospective cohort studies (La Rovere et al., 2003; Kikuya et al., 2000; Tsuji et al., 1996; Tsuji et al., 1994).

Epidemiologic Studies

The 2008 ISA for Oxides of Nitrogen reported that there was insufficient evidence to determine whether exposure to oxides of nitrogen was associated with changes in cardiac autonomic control as assessed by indices of HRV (U.S. EPA, 2008a). Additional studies are now available for review (Table 5-53) that provide evidence for an association between exposure to NO_2 and HRV among those with pre-existing disease but not in healthy individuals.

The multicountry ULTRA study assessed the longitudinal association between ambient pollution and HRV among older adults with stable coronary artery disease in Amsterdam, the Netherlands; Erfurt, Germany; and Helsinki, Finland (Timonen et al., 2006). In each participant, HRV was assessed multiple times over a 6-month period, resulting in a total of 1,266 repeated measures. Pooling results across the three cities, the authors found a 3.01 msec (95% CI: −5.94, −0.11) decrease in SDNN and a 17.67% (95% CI: −31.95, −3.01) decrease in LF/HF associated with a 20-ppb increase in 24-hour average NO_2 concentrations at lag day 2. The magnitudes of these associations were somewhat larger in relation to the 5-day moving average of NO_2. The authors report that these effects were robust to adjustment for other pollutants in copollutant models, including UFPs, $PM_{2.5}$, or CO, but detailed results were not provided. These results were reportedly similar in men and women and after exclusion of those exposed to environmental tobacco smoke at home. Most associations with HF were positive.

Table 5-53 Epidemiologic studies of heart rate/heart rate variability.

Study	Location Sample Size	Pre-Existing Condition	Mean NO_2 ppb	Exposure Assessment	Selected Effect Estimates[a] (95% CI)	
Timonen et al. (2006)	Amsterdam, Netherlands; Erfurt, Germany; Helsinki, Finland n = 131	Coronary artery disease	24-h avg NO_2 Amsterdam: 22.7 Erfurt: 15.4 Helsinki: 16.5	Central monitor	SDNN (msec) Lag 0: −1.05 (−3.50, 1.39) Lag 1: −1.28 (−3.98, 1.43) Lag 2: −3.01 (−5.94, −0.11) Lag 3: −0.68 (−3.42, 2.07) Lag 0−4: −4.59 (−9.32, 0.15) HF (%) Lag 0: 4.51 (−33.46, 18.42) Lag 1: 4.51 (−10.15, 19.55) Lag 2: 1.88 (−13.91, 18.05) Lag 3: −1.88 (−16.92, 13.16) Lag 0−4: 4.51 (−22.18, 30.83)	LF/HF (%) Lag 0: −3.01 (−15.41, 9.77) Lag 1: −16.54 (−30.08, −3.01) Lag 2: −17.67 (−31.95, −3.01) Lag 3: −1.88 (−15.41, 11.65) Lag 0−4: −25.9 (−50.0, −1.88)
†Zanobetti et al. (2010)	Boston, MA n = 46 (aged 43−75 yr)	Coronary artery disease	2-h avg NO_2 50th: 21 75th: 27 95th: 36 72-h avg NO_2 50th: 21 75th: 25 95th: 31	City-wide avg	HF (% Change) 2-h: −18.27 (−29.45, −6.82) Lag 0−4: −47.00 (−70.50, −22.00) All other results presented graphically, no quantitative results.	
†Bartell et al. (2013)	Los Angeles, CA n = 50	Coronary artery disease	24-h avg NO_X: 42.3 Max: 183.7	Monitors on trailers parked at each of 4 retirement communities	No quantitative results presented; results presented graphically. Generally null associations between NO_X and SDNN medication use in participants with and without acetylcholine esterase inhibitors.	

Table 5-53 (Continued): Epidemiologic studies of heart rate/heart rate variability.

Study	Location Sample Size	Pre-Existing Condition	Mean NO_2 ppb	Exposure Assessment	Selected Effect Estimates[a] (95% CI)	
†Barclay et al. (2009)	Aberdeen, Scotland, U.K. n = 132	Stable heart failure	24-h avg NO_2: 30.1 24-h avg NO: 14.7	Central monitor	HR NO_2: 0.398 (−0.003, 0.799) NO: 0.353 (−0.036, 0.742) SDNN (msec) NO_2: 0.619 (−0,588, 1.826) NO: 0.608 (−0.562, 1.778) SDANN (msec) NO_2: 0.512 (−0.865, 1.890) NO:0.570 (−0.766, 1.906) rMSSD (msec) NO_2: 0.398 (−0.003, 0.799) NO: 0.353 (−0.036, 0.742) pNN50 (%) NO_2: 1.568 (−3.851, 6.986) NO: 0.909 (−4.347, 6.165)	LF power NO_2: 2.353 (−1.052, 5.757) NO: 1.940 (−1.328, 5.207) LF normalized NO_2: −0.857 (−2.817, 1.102) NO: −0.183 (−2.066, 1.699) HF power NO_2: 3.365 (−1.169, 7.900) NO: 2.895 (−1.457, 7.247) HF normalized NO_2: 0.722 (−1.554, 2.998) NO: 1.407 (−0.775, 3.558) LF/HF ratio NO_2: −1.089 (−3.930, 1.753) NO: −1.054 (−3.779, 1.672)
†Goldberg et al. (2008)	Montreal, Quebec, Canada n = 31	Stable heart failure	24-h avg NO_2 17.9 Max: 54.1	City-wide avg	Pulse Rate (mean difference) Lag 0: −0.07 (−0.09, 0.80) Lag 1: 0.78 (−0.14, 1.71) Lag 0−2: 0.99 (−0.34, 2.32)	
†Suh and Zanobetti (2010b)	Atlanta, GA (n = 30)	MI or COPD	24-h avg NO_2 Ambient: 17.1 Personal: 11.6	City-wide avg Personal	SDNN (% change) Ambient: −0.64 −11.06, 10.43) Personal: −3.48 (−10.69, 3.89) rMSSD (% change) Ambient: −6.60 (−30.64, 20.85) Personal: −14.52 (−29.87, 1.70) pNN50 (% change) Ambient: 0.30 (−38.28, 47.38) Personal: −32.30 (−56.49, −5.65)	HF (% change) Ambient: −1.49 (−37.09, 41.32) Personal: −21.35 (−44.92, 4.48) LF/HF (% change) Ambient: 13.74 (−4.11, 33.13) Personal: 9.69 (−2.34, 22.20)

Table 5-53 (Continued): Epidemiologic studies of heart rate/heart rate variability.

Study	Location Sample Size	Pre-Existing Condition	Mean NO_2 ppb	Exposure Assessment	Selected Effect Estimates[a] (95% CI)	
†Huang et al. (2012a)	Beijing, China n = 40	CVD	1-h max NO_2 2007, visit 1: 33.8 2007, visit 2: 26.3 2008, visit 3: 29.2 2008, visit 4: 22.9	Central monitor	SDNN (% change) 1-h: -1.9 (-3.4, -0.3) 4-h: −3.9 (−5.7, −2.2) 12-h: −3.6 (-5.5, −1.6) *r*-MSSD (% change) 1-h: 1.4 (−1.1, 3.9) 4-h: −2.2 (−5.7, 1.5) 12-h: −2.2 (−6.1, 2.0)	LF (% change) 1-h: -5.4 (-9.3, -1.4) 4-h: −8.9 (−13.2, −4.3) 12-h: −7.9 (−12.8, −2.8) HF (% change) 1-h: -3.5 (-8.2, 1.4) 4-h: −5.1 (-11.0, 1.3) 12-h: −3.7 (-10.4, 3.5)
†Williams et al. (2012b)	Detroit, MI n = 65	CV risk factors (i.e., hypertension, hyperlipidemia, diabetes)	24-h avg NO_2 24.0 75th: 28.0 Max: 100.0	Personal monitor	HR (bpm) bpm: −2.95 (−4.82, −0.80)	
†Laumbach et al. (2010)	New Brunswick, NJ n = 21	Diabetes	NO_2: 50th: 25.9 75th: 32.8 Max: 61.1	In-vehicle mean	HF (% change) −11.92 (−104.64, 80.79) LF/HF ratio (% change) −107.28 (−298.01, 83.44)	
†Peel et al. (2011)	Atlanta, GA n = 4,277	Healthy infants	1-h max NO_2 41.7 90th: 65.6 Max: 109.2	Central monitor	Bradycardia (OR) 1.04 (1.00, 1.08)	
†Chuang et al. (2007a)[b]	Taipei, Taiwan n = 76	Healthy	24-h avg NO_2 17.3 Max: 53.1	Central monitor	"We found no associations between HRV indices and NO_2." No quantitative results presented.	
†Jia et al. (2011)	Beijing, China n = 41	Healthy	24-h avg NO_X 35.0	Central monitor	"No significant effects are found between daily average…NO_X on HRV indices." No quantitative results presented.	
†Park et al. (2010)	6 U.S. communities: Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles, CA; New York, NY; St. Paul, MN n = 5,465	Healthy	24-h avg NO_2 Lag 0−1: 23.5	City-wide avg	"There were no significant associations of HRV with gaseous pollutants (data not shown)." No quantitative results presented.	

Table 5-53 (Continued): Epidemiologic studies of heart rate/heart rate variability.

Study	Location Sample Size	Pre-Existing Condition	Mean NO_2 ppb	Exposure Assessment	Selected Effect Estimates[a] (95% CI)	
†Chuang et al. (2007b)	Taipei, Taiwan n = 46	Healthy	1-h max NO_2 38.4	Avg of monitors within 1 km of residence	"…NO_2…exposures were not associated with any HRV indices in our study participants (data not shown)." No quantitative results presented.	
†Min et al. (2008)	Taein Island, South Korea n = 1,349	Healthy	24-h avg NO_2 24 75th: 30 Max: 119	Central monitor	SDNN (% change) 6-h: −2.45 (−6.28, 1.53) 9-h: −3.89 (−8.31, 0.71) 12-h: −3.81 (−8.75, 1.34) 24-h: −1.72 (−6.71, 3.51) 48-h: 2.93 (−2.33, 8.42) 72-h: 1.20 (−3.81, 6.42) LF (% change) 6-h: −8.61 (−16.85, 0.31) 9-h:- −12.24 (−21.48, −2.11) 12-h: −12.28 (−22.58, −0.88) 24-h: −5.71 (−16.58, 6.33) 48-h: 3.69 (−8.22, 16.92) 72-h:5.84 (−6.19, 18.45)	HF (% change) 6-h: −1.08 (−10.75, 9.47) 9-h: −3.31 (−14.32, 8.88) 12-h: −2.38 (−14.73, 11.45) 24-h: −4.53 (−16.58, 8.94) 48-h: 4.42 (−8.72, 19.14) 72-h: 4.18 (−8.52, 18.35)
†Weichenthal et al. (2011)[b]	Ottawa, Canada (n = 42)	Healthy	1-h max NO_2 4.8	Central Monitor	ΔLF (ms^2) 1-h: −532.5 (−2872.5, 1807.5) 2-h: 12.0 (−2467.5, 2490.0) 3-h: 577.5 (−2055.0, 3217.5) 4-h: −397.5 (−3532.5, 2025.0) ΔHF (ms^2) 1-h: −420.0 (−1785.0, 952.5) 2-h: −487.5 (−1612.5, 637.5) 3-h: −24.0 (−1020.0, 975) 4-h: −247.5 (−1417.5, 922.5) ΔLF:HF 1-h: 5.70 (−2.10, 13.50) 2-h: 10.50 (2.63, 18.75) 3-h: 12.75 (4.20, 21.75) 4-h: 7.50 (−1.80, 17.25)	ΔSDNN (msec) 1-h: −18.75 (−112.50, 72.00) 2-h: −75.0 (−150.0, −2.55) 3-h: −39.75 (−120.0, 40.50) 4-h: −12.00 (−82.50, 61.50) ΔrMSSD (msec) 1-h: −12.00 (−48.75, 24.75) 2-h: −12.00 (−41.25, 17.25) 3-h: 2.33 (−30.0, 34.5) 4-h: −2.10 (−33.0, 29.25) ΔpNN50 (%) 1-h: −3.30 (−31.50, 24.75) 2-h: −8.25 (−33.00, 15.75) 3-h: −3.23 (−29.25, 22.50) 4-h: 1.28 (−26.25, 29.25)
†Shields et al. (2013)	Mexico City, Mexico (n = 16)	Healthy	1-h max NO_2 130	In-Vehicle Monitor	LF (% Change) -0.69 (-1.91, 0.57) HF (% Change) -0.24 (-1.80, 1.47)	LF/HF (% Change) -0.45 (-1.53, 0.64) SDNN (% Change) -1.03 (-1.55, -0.49)

Table 5-53 (Continued): Epidemiologic studies of heart rate/heart rate variability.

Study	Location Sample Size	Pre-Existing Condition	Mean NO_2 ppb	Exposure Assessment	Selected Effect Estimates[a] (95% CI)
†Rich et al. (2012)[b]	Beijing, China (n = 125)	Healthy	24-h avg NO_2 Entire study: 27.0 Before: 26.0 During: 13.9 After: 41.4	Central monitor	No quantitative results presented; results presented graphically. Positive, but statistically nonsignificant increase in heart rate, generally consistent across lags from 0 to 6.
†Zhang et al. (2013)[b]	Beijing, China (n = 125)	Healthy	24-h avg NO_2 Before: 25.6 During: 14.6 After: 41.4	Central monitor	No quantitative results presented; results presented graphically. Statistically significant decreases in SDNN and rMSSD in the early lags (0 to 1); measurable but non-statistically significant decreases across lags 2 and 3; and generally null associations in lags 4, 5, and 6.

CI = confidence interval, COPD = chronic obstructive pulmonary disease, CVD = cardiovascular disease, HF = high frequency, HR = heart rate, HRV = heart rate variability, LF = low frequency, LF/HF = LF to HF components, MI = myocardial infarction, NO = nitric oxide, NO_2 = nitrogen dioxide, NO_X = sum of NO and NO_2, OR = odds ratio, pNN50 = proportion of pairs of successive normal simus intervals that exceeds 50 milliseconds divided by the total number of successive pairs of normal simus intervals, rMSSD = root-mean-square of successive differences, SDNN = standard deviation of all normal-to-normal intervals.

[a]Relative risks are standardized to a 20-ppb or 30-ppb increase in NO_2 or NO or 40 ppb or 60-ppb increase in NO_X concentration for 24-h avg and 1-h max metrics, respectively.

†Studies published since the 2008 ISA for Oxides of Nitrogen.

Huang et al. (2012a) measured HRV repeatedly in participants with pre-existing cardiovascular disease in Beijing in the summer of 2007 and again in the summer of 2008, including one measurement period during the 2008 Beijing Olympics when city-wide air pollution control measures substantially reduced ambient concentrations of most criteria pollutants as described in more detail in Section 5.3.6.1. In single-pollutant models, an unspecified IQR increase in 1-h max NO_2 was associated with a 3.6% decrease (95% CI: −5.5, −1.6) in SDNN, and a 7.9% decrease (95% CI: −12.8, −2.8) in LF. The association with SDNN was stronger among those with a higher C-reactive protein (CRP), women, and those without a history of diabetes, but BMI did not appear to modify the association. Rich et al. (2012) also examined the association between heart rate and NO_2 concentrations before, during and after the 2008 Beijing Olympics. The authors observed increases in heart rate that were generally consistent in magnitude across lags from 0 to 6 days. In expanded results from the same protocol, Zhang et al. (2013) reported that NO_2 was inversely associated with SDNN and rMSSD, with stronger associations in the earlier lags (0 to 3). The HR association with NO_2 was somewhat attenuated, but still positive after adjustment for $PM_{2.5}$, CO, SO_2, or OC, and no longer positive after adjustment for EC. The decrements in rMSSD and SDNN associated with increased ambient NO_2 remained relatively unchanged after adjustment for $PM_{2.5}$, CO, SO_2, OC, or EC.

Several studies (Weichenthal et al., 2011; Laumbach et al., 2010; Suh and Zanobetti, 2010a) used personal exposure assessment techniques that tend to reduce uncertainty in the NO_2 exposure estimate when compared to the use of city-wide averages, decreasing the distance between the monitor and subject (Section 3.4.4.2). Suh and Zanobetti (2010b) examined the association between HRV and short-term exposure to NO_2 among people that had either recently experienced an MI or had COPD. Same-day total personal exposures to NO_2 were associated with decreased HRV. Decreases in pNN50 (proportion of pairs of successive normal simus intervals exceeds 50 milliseconds divided by the total number of successive pairs of normal simus intervals) were the largest among the individuals with COPD, while NO_2-associated decrements in HF were the largest among individuals with a recent MI, but were less precise when all individuals or individuals with COPD were included in the analysis. Associations were most pronounced when examining personal as opposed to ambient measures of NO_2. Copollutant confounding was not assessed. Laumbach et al. (2010) studied the effects of in-vehicle exposure to traffic-related pollutants among a group of individuals with diabetes. The authors did not observe any strong evidence of an association between HF HRV and in-vehicle exposure to NO_2. Weichenthal et al. (2011) carried out a cross-over trial with 42 healthy adults who cycled for 1 hour on high- and low-traffic routes as well as indoors. Mean

concentrations of NO_2 measured at nearby stationary monitors were associated with decreases in SDNN and increases in LF/HF.

In a repeated-measures study of Boston-area patients with clinically significant coronary artery disease, Zanobetti et al. (2010) found that HF was inversely associated with ambient NO_2 concentrations. This association remained robust after adjustment for $PM_{2.5}$ in a copollutant model. Among a population reporting a substantial prevalence of cardiovascular risk factors (i.e., hypertension, diabetes, hyperlipidemia), Williams et al. (2012a) observed a strong association between NO_2 concentrations and reduced heart rate. On the other hand, Barclay et al. (2009) reported no association between NO_2 or NO and indices of HRV in a repeated-measures study of non-smoking patients with stable heart failure. (Bartell et al., 2013) observed generally null associations between NO_2 and SDNN medication use in retirement residents with coronary artery disease in the greater Los Angeles area. Also, Goldberg et al. (2008) followed 31 Montreal-area participants with stable heart failure for 2 months and found no association between pulse rate and NO_2 concentrations.

Infants are potentially at greater risk of pollution-related health effects (American Academy of Pediatrics, 2004). Peel et al. (2011) examined data from 4,277 Atlanta-area infants prescribed home cardiorespiratory monitors and observed a slightly elevated risk of bradycardia (OR: 1.04 [95% CI: 1.00, 1.08]) per 30-ppb increase in 1-h max NO_2 concentrations averaged over the previous 2 days and measured at a central site monitor. The clinical or public health significance of this finding is unclear.

The majority of the above studies focused on infants or participants with a documented history of heart disease, with the exception of the Beijing Olympics studies (Zhang et al., 2013; Rich et al., 2012). In contrast to the pre-existing disease studies, there is little evidence that HRV is associated with NO_2 concentrations in healthy participants. For example, a repeated-measures study of young healthy participants in Taipei, Taiwan found no association between NO_2 and HRV indices (Chuang et al., 2007a). Another repeated-measures study in Mexico City observed small decrements in SDNN associated with increases in NO_2, but no association between NO_2 and LF/HF (Shields et al., 2013). In Beijing, Jia et al. (2011) assessed HRV two times in each of 20 healthy participants and reported no association between oxides of nitrogen and HRV. However, this study was quite small, and detailed results were not shown.

Cross-sectional analyses of populations with or without a history of heart disease have also tended to yield null results. In a cross-sectional analysis of 5,465 participants, ages 45−84 years, from the multicity Multiethnic Study of Atherosclerosis, Park et al. (2010) found no association between NO_2 concentrations and indices of HRV. A cross-sectional study from Taipei also observed no association between NO_2 and HRV among 46 older

adults with cardiovascular disease risk factors (Chuang et al., 2007b). A cross-sectional study of 1,349 healthy participants in Taein Island, South Korea by Min et al. (2008), found that NO_2 was associated with decreases in the LF component of HRV, but not with changes in SDNN or the HF component.

In summary, current evidence suggests that among participants with pre-existing or elevated risk for cardiovascular disease, ambient NO_2 concentrations are associated with alterations in cardiac autonomic control as assessed by indices of HRV; however, evidence for differential effects between populations with and without pre-existing diseases and conditions is limited. In this specific subgroup of the population, NO_2 seems to be associated with changes in HRV, which is consistent with relative increases in sympathetic nervous system activity and/or decreases in parasympathetic nervous system activity. In contrast, this association has not been commonly apparent among healthier participants. Additionally, the observed associations in the pre-existing disease populations were generally persistent after adjustment for UFPs, $PM_{2.5}$, CO, SO_2, or OC in limited traffic pollutant models.

Controlled Human Exposure Studies

Controlled human exposure studies evaluating HRV were not available for review in the 2008 ISA for Oxides of Nitrogen; since then, two new studies have become available (Table 5-56). Huang et al. (2012b) evaluated changes in various HRV parameters following NO_2 exposure in healthy adult volunteers performing intermittent exercise. Exposure to 500 ppb NO_2 did not alter HRV time domain intervals, but did slightly increase LF/HF, although this change was not statistically significant. The authors reported an 11.6 and 13% decrease in the HF domain normalized for heart rate (HFn) 1 and 18 hours after exposure, respectively. Combined exposure to NO_2 and $PM_{2.5}$ CAPs increased LF/HF (1 hour; $p = 0.062$), as well as the low frequency domain normalized for heart rate (1 hour; $p = 0.021$) and cardiac t-wave amplitude (18 hour; $p = 0.057$). CAPs exposure alone did not induce such changes. Epidemiologic studies found NO_2-associated decreases in HRV primarily in adults with or at risk for cardiovascular disease. However, a recent study in resting adults with stable coronary heart disease and impaired left ventricular systolic function showed no statistically significant changes in HRV after exposure to 400 ppb NO_2 for 1 hour while seated (Scaife et al., 2012); however, it should be noted that the study had only 75% power to detect significant differences in the HF domain of 50% or less.

The few studies reviewed in the previous assessments of oxides of nitrogen (U.S. EPA, 2008a, 1993) reported mixed effects of NO_2 exposure on HR; a recent study shows no effect. Folinsbee et al. (1978) and Drechsler-Parks (1995) exposed healthy adult males

and healthy older adults, respectively, to approximately 600 ppb NO_2 for 2 hours and reported no changes in HR. Changes in HR were also examined in potentially at-risk populations exposed to NO_2. Exposure to 400 ppb NO_2 did not alter HR in adults with coronary heart disease (Scaife et al., 2012) and resulted in a statistically nonsignificant increase in adults with COPD and healthy volunteers, (Gong et al., 2005). Among healthy volunteers and those with asthma, NO_2 exposure resulted in no change in HR (Linn et al., 1985a).

In summary, there is limited, inconsistent evidence from controlled human exposure studies to suggest NO_2 alone or in combination with CAPs exposure during exercise alters HRV. Additionally, there appears to be no evidence from controlled human exposure studies to suggest NO_2 alters HR.

Toxicological Studies

Toxicology studies examining HRV changes were not available for review in the 2008 ISA for Oxides of Nitrogen. Similar to controlled human exposure studies, a recent study in rats found mixed evidence for changes in HR and HRV (Table 5-57). Ramos-Bonilla et al. (2010) examined body weight, HR, and HRV, following exposure of aged inbred mice to an ambient mixture consisting of PM, CO, and NO_2. Animals were exposed to either filtered or unfiltered outdoor Baltimore air for 6 hours daily for 40 weekdays. Health effects associated with daily exposure to each pollutant were ascertained with multipollutant models and lagged covariates. Statistically significant declines in HR were associated with NO_2 at lag 3 and the 7-day cumulative concentration with adjustment for PM and CO. However, HRV changes were not associated with NO_2 exposure. The independent effects of each pollutant are difficult to distinguish in this multipollutant model because of multicollinearity among pollutants.

5.3.11.2 QT-Interval Duration

The QT interval provides an ECG marker of ventricular repolarization. Prolongation of the QT interval is associated with increased risk of life-threatening ventricular arrhythmias. Limited evidence from the epidemiologic study reviewed in the 2008 ISA for Oxides of Nitrogen and the single recent study does not clearly indicate an association between short-term NO_2 exposure and markers of ventricular repolarization (U.S. EPA, 2008a). Epidemiologic evidence is similarly inconsistent for associations of NO_2 exposure and arrhythmias (Section 5.3.3).

Within the context of the Veterans Administration Normative Aging Study, Baja et al. (2010) found imprecise associations between heart-rate corrected QT interval (QTc) and 10-hour moving average of NO_2 concentrations among older, generally white men, but was associated with NO_2 concentrations at lags 3 and 4 hours (longer lags or moving averages were not considered) (Table 5-54). The only prior study available for comparison found that 24-h avg NO_2 concentrations were positively associated with increased QTc duration, but this association was imprecise (i.e., had wide confidence intervals), and the 6-hour moving average of NO_2 was not associated with an increase in QTc duration (Henneberger et al., 2005).

Table 5-54 Epidemiologic studies of QT-interval duration.

Study	Location Sample Size	Mean Concentration ppb	Exposure Assessment	Selected Effect Estimates[a] (95% CI)
†Baja et al. (2010)	Boston, MA n = 580	1-h max NO_2 19 during ECG monitoring 21 ppb 10 h before monitoring	Central site	Change in QTc (msec) 10-h lag: 5.91 (−2.03, 13.85) 4-h lag: 6.28 (−0.02, 12.55)
Henneberger et al. (2005)	Erfurt, Germany n = 56	24-h avg NO_2: 18.2 75th: 22.6 Max: 36.4 24-h avg NO: 19.4 75th: 24.2 Max: 110.1	City-wide avg	QTc (msec) NO_2, lag 6−11 h: 9.77 (2.23, 17.33) T-wave complexity (%) NO, lag 0−23: 0.15 (0.02, 0.28) T-wave amplitude (μV) NO, lag 0−5 h: −2.10 (−4.16, −0.03)

CI = confidence interval, ECG = electrocardiographic, NO = nitric oxide, NO_2 = nitrogen dioxide, QTc = corrected QT interval.
[a]Effect estimates are standardized to a 20-ppb or 30-ppb increase in NO_2 or NO for 24-h avg and 1-h max metrics, respectively.
†Study published since the 2008 ISA for Oxides of Nitrogen.

Controlled Human Exposure Studies

There were no available controlled human exposure studies evaluating changes in the QT interval for the 2008 ISA for Oxides of Nitrogen; since then, one new study has become available. Huang et al. (2012b) found a near statistically significant (quantitative results not reported) decrease in QTc at 1 and 18 hours after a 2-hour exposure to 500 ppb NO_2 in healthy exercising adults (Table 5-56). NO_2 exposure also induced a 29.9% decrease (p = 0.001) in the QT variability index (QTVI). However, when volunteers were exposed to both $PM_{2.5}$ and NO_2, the QTVI synergistically increased.

5.3.11.3 Vascular Reactivity

The vascular endothelium plays a fundamental role in the maintenance of vascular tone that is involved in the regulation of blood pressure and blood flow. In a controlled human exposure study, Langrish et al. (2010) examined the effects of NO_2 on vascular endothelial tone and fibrinolytic function. In a random crossover double-blind study, healthy male volunteers were exposed to 4,000 ppb of NO_2 for 1 hour with intermittent exercise. This study employed infusion of endothelial-dependent vasodilators, bradykinin and acetylcholine, and endothelial-independent vasodilators, sodium nitroprusside and verapamil, to examine vascular endothelial tone. The results demonstrated that NO_2 did not attenuate the vasodilator response to these vasoactive agents.

Epidemiologic studies provide inconsistent evidence regarding a potential association between NO_2 and vascular function. In an analysis of data from the EPA's Detroit Exposure and Aerosol Research Study, Williams et al. (2012a) found that total personal NO_2 concentrations were associated with changes in brachial artery diameter (positive association at lag 1 and negative association at lag 2) and positive (i.e., presumably beneficial) changes in flow-mediated dilation. No associations were observed in relationship to ambient measures of NO_2. Ljungman et al. (2014) reported no consistent associations between 1-, 2-, 3-, 5-, and 7-day moving averages of NO_X and peripheral arterial tonometry ratio in the Offspring and Third Generation Cohorts of the Framingham Heart Study.

5.3.11.4 Blood Biomarkers of Cardiovascular Effects

Several epidemiologic and toxicological studies have explored the potential relationship between NO_2 and biomarkers of cardiovascular risk. In particular, markers of inflammation, cell adhesion, coagulation, and thrombosis have been evaluated in a number of epidemiologic studies published since the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a) (Table 5-55). These biomarkers also have been examined in controlled human exposure and animal toxicological studies.

Table 5-55 Epidemiologic studies of biomarkers of cardiovascular effects.

Study	Location Sample Size	Pre-existing Condition	Mean NO_2 ppb	Exposure Assessment	Selected Effect Estimates[a] (95% CI)	
†Delfino et al. (2008b)	Los Angeles, CA n = 29	Coronary artery disease	24-h avg NO_2 Outdoor: 33.1 Max: 59.8 Indoor: 32.3 Max: 53.5	Indoor and outdoor home measurements	Outdoor: CRP (ng/mL) Lag 0: 1,125 (−314, 2,565) Lag 0−2: 1,027 (−465, 2,520) Fibrinogen (µg/mL) Lag 0: −110.31 (−504, 283) Lag 0−2: −110 (−502, 281) IL-6 (pg/mL) Lag 0: 1.32 (0.48, 2.18) Lag 0−2: 1.17 (0.28, 2.08) IL-6R (pg/mL) Lag 0: −493 (−9,387, −249) Lag 0−2: −3,212 (−7,789, 1,365) TNF-α (pg/mL) Lag 0: 0.13 (−0.26, 0.52) Lag 0−2: 0.15 (−0.22, 0.54) TNF-RII (pg/mL) Lag 0: 290 (−41, 623) Lag 0−2: 240 (−82, 562)	P-selectin (ng/mL) Lag 0: 5.13 (−1.02, 11.27) Lag 0−2: 1.49 (−5.04, 8.02) VCAM-1 (pg/mL) Lag 0: 53,734 (−11,381, 118,849) Lag 0−2: 18,266 (−45,532, 82,062) ICAM-1 (pg/mL) Lag 0: 5,381 (−8,987, 19,748) Lag 0−2: 575 (−13,495, 14,643) SOD (U/g Hb) Lag 0: −541 (−1,021, −63) Lag 0−2: −571 (−1,036, −106) GPx (U/g Hb) Lag 0: −1.99 (−3.68, −0.26) Lag 0−2: 1.15 (−2.81, 0.58) MPO (ng/mL) Lag 0: −5.34 (−14.92, 4.33) Lag 0−2: −1.15 (−10.81, 8.44)
†Delfino et al. (2009)	Los Angeles, CA n = 60	Coronary artery disease	24-h avg NO_2 Phase 1: 26.4 Phase 2: 28.3 24-h avg NO_X Phase 1: 37.2 Phase 2: 53.9	Hourly outdoor home air measurements	NO_X: IL-6 (pg/mL) Lag 0: 0.23 (0.12, 0.35) Lag 0−2: 0.23 (0.10, 0.35) P-selectin (ng/mL) Lag 0: 1.52 (0.09, 2.94) Lag 0−2: 2.29 (0.68, 3.90) TNF-RII (pg/mL) Lag 0.66 (7.93, 124.76) Lag 0−2: 86.54 (18.75, 155.05) TNF-α (pg/mL) Lag 0: 0.01 (−0.06, 0.07) Lag 0−2: 0.04 (−0.03, 0.12)	NO_X: CRP (ng/mL) Lag 0: 469.47 (212.74, 726.92) Lag 0−2: 408.17 (111.06, 705.29) SOD (U/g Hb) Lag 0: −100.24 (−201.92, 2.16) Lag 0−2: −95.91 (−214.90, 22.36) GPx (U/g Hb) Lag 0: −0.17 (−0.61, 0.26) Lag 0−2: −0.14 (−0.63, 0.36)

Table 5-55 (Continued): Epidemiologic studies of biomarkers of cardiovascular effects.

Study	Location Sample Size	Pre-existing Condition	Mean NO_2 ppb	Exposure Assessment	Selected Effect Estimates[a] (95% CI)	
†Delfino et al. (2010)	Los Angeles, CA n = 60	Coronary artery disease	Warm season 24-h avg NO_2: 26.4 24-h avg NO_X: 37.2 Cool season 24-h avg NO_2: 28.3 24-h avg NO_X: 53.9	Hourly outdoor home air measurements	IL-6 (pg/mL) NO_2: 0.48 (−0.06, 1.05) NO_X: 0.60 (0.26, 0.96)	
†Wittkopp et al. (2013)	Los Angeles, CA n = 36	Coronary artery disease	24-h avg NO_X: 45.35 Max: 188.00	Hourly outdoor home air measurements	No quantitative results presented; results presented graphically. Statistically significant positive associations between 1-, 2-, 3-, and 5-day avg NO_X and IL-6 (pg/mL) and TNF-α (pg/mL) in haplogroup H participants. Statistically nonsignificant, but negative associations between 1-, 2-, 3-, and 5-day avg NO_X and IL-6 (pg/mL) and TNF-α (pg/mL) in haplogroup U participants.	
†Ljungman et al. (2009)	6 European cities n = 955 (total n = 5,539 measurements)	MI	24-h avg NO_2 22.6	City-wide avg	IL-6 (% change) Overall: 4.02 (0.47, 8.04) IL-6 genetic variants IL6 rs2069832 (1,1): 7.33 (2.13, 12.77) IL6 rs2069832 (1,2): 2.84 (−1.18, 7.09) IL6 rs2069832 (2,2): −1.18 (−8.27, 5.91) IL6 rs2069840 (1,1): 4.26 (−0.95, 9.46) IL6 rs2069840 (1,2): 4.02 (0.00, 8.04) IL6 rs2069840 (2,2): 4.02 (−3.55, 11.58)	IL6 rs2069845 (1,1): 6.62 (1.18, 12.06) IL6 rs2069845 (1,2): 3.07 (−0.95, 7.33) IL6 rs2069845 (2,2): 0.47 (−6.15, 7.57) IL6 rs2070011 (1,1): 4.96 (−0.24, 10.17) IL6 rs2070011 (1,2): 3.78 (−0.24, 7.80) IL6 rs2070011 (2,2): 2.60 (−4.26, 9.69) IL6 rs1800790 (1,1): 2.36 (−2.13, 6.86) IL6 rs1800790 (1,2): 6.62 (1.42, 11.82) IL6 rs1800790 (2,2): 10.40 (0.24, 21.04)
†Brüske et al. (2011)	Augsburg, Germany n = 200	MI	24-h avg NO_2 20.8 75th: 24.7 Max: 38.2	Central site monitor	Lp-PLA_2 (% Change) NO_2 Lag 4: 7.28 (3.00, 11.56) NO Lag 4: 2.74 (−0.21, 5.70)	

Table 5-55 (Continued): Epidemiologic studies of biomarkers of cardiovascular effects.

Study	Location Sample Size	Pre-existing Condition	Mean NO_2 ppb	Exposure Assessment	Selected Effect Estimates[a] (95% CI)	
			24-h NO 24.0 75th: 25.8 Max: 141.1		"Inverse associations were observed for … NO_2 with Lp-PLA_2 at lag days 1−2 and positive associations were estimated …with Lp-PLA_2 lagged 4 and 5 days."	
†Barclay et al. (2009)	Aberdeen, Scotland n = 132	Stable chronic heart failure	24-h avg NO_2: 30.1 24-h avg NO: 14.7	Central monitor	Hemoglobin NO_2: 0.035 (−0.291, 0.361) NO: −0.011 (−0.331, 0.310) Mean corpuscular hemoglobin NO_2: 0.050 (−0.158, 0.257) NO: −0.039 (−0.243, 0.165) Platelets NO_2: −0.049 (−0.867, 0.768) NO: 0.247 (−0.556, 1.050) Hematocrit NO_2: −0.017 (−0.350, 0.316) NO: 0.101 (−0.226, 0.428) WBC NO_2: −0.722 (−1.670, 0.226) NO: −0.708 (−1.640, 0.224) CRP NO_2: 0.423 (−5.263, 6.108) NO: 0.890 (−4.694, 6.473)	IL-6 NO_2: 6.276 (0.594, 11.940) NO: 2.767 (−2.810, 8.344) vWF NO_2: 2.164 (−0.328, 4.655) NO: 3.522 (1.091, 5.954) E-selectin NO_2: 1.162 (−0.372, 2.696) NO: 0.483 (−1.022, 1.989) Fibrinogen NO_2: −0.219 (−1.759, 1.322) NO: 0.195 (−1.320, 1.709) Factor VII NO_2: 0.273 (−1.441, 1.987) NO: 0.335 (−1.348, 2.018) d-dimer NO_2: −0.243 (−2.781, 2.294) NO: −0.316 (−2.807, 2.175)
Wellenius et al. (2007)	Boston, MA n = 28	Stable chronic heart failure	24-h avg NO_2 20.7	City-wide avg	"No significant associations were observed between (NO_2) and BNP levels at any of the lags examined." No quantitative results presented.	
†Hildebrandt et al. (2009)	Erfurt, Germany n = 38	COPD	24-h avg NO_2 13.5 24-h NO 10.7	Central monitor	Increases in fibrinogen and prothrombin fragment 1 + 2 associated with NO concentrations. A decrease in vWF was associated with NO_2 concentrations. No quantitative results presented for NO or NO_2.	
†Dadvand et al. (2014)	Barcelona, Spain n = 242	COPD	24-h avg NO_2: 30.7	Residential land-use regression	CRP (% change) Lag 1: 2.99 (−21.6, 34.16) Lag 2: 26.05 (−3.47, 64.08) Lag 5: 54.92 (23.21, 94.47) TNF-α (% change) Lag 1: 3.90 (−24.6, 41.5) Lag 2: 10.61 (−19.43, 51.37) Lag 5: (26.54 (−4.51, 66.85) IL-6 (% change) Lag 1: 10.47 (−13.46, 40.14)	IL-8 (% change) Lag 1: 7.94 (−2.07, 18.98) Lag 2: 8.09 (−2.00, 19.12) Lag 5: 3.44 (−5.37, 13.11) Fibrinogen (% change) Lag 1: 3.57 (−1.84, 9.09) Lag 2: 3.26 (−2.00, 8.72) Lag 5: 10.42 (5.59, 15.58) HGF (% change) Lag 1: 3.11 (−3.91, 10.58)

Table 5-55 (Continued): Epidemiologic studies of biomarkers of cardiovascular effects.

Study	Location Sample Size	Pre-existing Condition	Mean NO_2 ppb	Exposure Assessment	Selected Effect Estimates[a] (95% CI)	
					Lag 2: 4.31 (−18.28, 32.88) Lag 5: 21.28 (−2.47, 50.40)	Lag 2: 5.57 (−1.58, 13.24) Lag 5: 9.99 (3.44, 16.98)
†Khafaie et al. (2013)	Pune City, India n = 1,392	Type 2 diabetes	24-h avg NO_X: 21.1	City-wide avg	No quantitative results presented; results presented graphically. NO_X was statistically significantly associated with increases in CRP at lags 0, 1, 2, 4, and 0−7. There were no measurable differences between winter and summer associations.	
†Bind et al. (2012)	Boston, MA n = 704	Healthy	24-h avg NO_2 18 95th: 35	City-wide avg	Fibrinogen (% change) Lag 0−2: 8.18 (4.73, 11.64)	
†Ren et al. (2011)	Boston, MA n = 320	Healthy	24-h avg NO_2 17.8	Central site monitor	8-OhdG (% change) Lag 0: 28.48 (−19.39, 76.36) Lag −6: 90.00 (−12.22, 191.67)	Lag 0−13: 166.88 (28.75, 305.63) Lag 0−20: 195.15 (44.85, 344.85)
†Thompson et al. (2010)	Toronto, Canada n = 45	Healthy	24-h avg NO_2 23.8	Central site monitor	Quantitative results not presented.	

Table 5-55 (Continued): Epidemiologic studies of biomarkers of cardiovascular effects.

Study	Location Sample Size	Pre-existing Condition	Mean NO_2 ppb	Exposure Assessment	Selected Effect Estimates[a] (95% CI)	
†Rudez et al. (2009)	Rotterdam, the Netherlands n = 40	Healthy	24-h avg NO_2 50th: 19.7 75th: 25.5 Max: 43.1 24-h NO: 50th: 5.6 75th: 12 Max: 130.4	Central site monitor	Maximal platelet aggregation (% change) NO; NO_2 Lag 0−6 h: 5.42 (−18.33, 29.58); −4.11 (−13.04, 4.82) Lag 0−12 h: 2.92 (−22.50, 28.33); −4.64 (−15.00, 5.89) Lag 0−24 h: 7.92 (−12.50, 28.75); −5.36 (−18.39, 7.68) Lag 24−48 h: 5.00 (−17.08, 27.08); −1.07 (−11.79, 9.46) Lag 48−72 h: 25.42 (10.00, 40.42); 10.00 (2.68, 17.32) Late aggregation (% change) NO; NO_2 Lag 0−6 h: 33.75 (−5.00, 72.08); 5.89 (−9.46, 21.07) Lag 0−12 h: 35.42 (−2.92, 73.33); 13.39 (−4.11, 30.71) Lag 0−24 h: 37.08 (4.67, 69.17); 17.68 (−4.46, 39.82) Lag 24−48 h: 22.92 (−6.25, 51.67); 3.39 (−16.07, 22.68) Lag 48−72 h: 32.92 (9.58, 55.83); 15.89 (4.64, 27.14) Lag 72−96 h: 14.17 (−23.75, 52.50); 8.57 (−7.68, 24.82) Lag 0−96 h: 54.17 (20.42, 87.92); 28.75 (8.93, 48.57)	Thrombin generation—Peak (% change) NO; NO_2 Lag 0−6 h: −1.67 (−15.00, 11.67); −2.68 (−9.82, 4.46) Lag 0−12 h: −1.67 (−12.92, 9.58); −1.25 (−9.11, 6.61) Lag 0−24 h: −2.50 (−16.25, 10.83); −1.07 (−9.46, 7.32) Lag 24−48 h: 17.08 (4.58, 30.00); 14.29 (4.29, 24.29) Lag 48−72 h: 5.00 (−6.67, 16.67); 6.61 (−2.68, 16.07) Lag 72−96 h: 14.58 (1.67, 27.92); −0.36 (−8.57, 7.86) Lag 0−96 h: 12.92 (−7.08, 32.50); 1.79 (−7.32, 10.71)

Table 5-55 (Continued): Epidemiologic studies of biomarkers of cardiovascular effects.

Study	Location Sample Size	Pre-existing Condition	Mean NO_2 ppb	Exposure Assessment	Selected Effect Estimates[a] (95% CI)	
†Rudez et al. (2009) (continued)	Rotterdam, the Netherlands n = 40	Healthy	24-h avg NO_2 50th: 19.7 75th: 25.5 Max: 43.1 24-h NO: 50th: 5.6 75th: 12 Max: 130.4	Central site monitor	Thrombin generation—ETP (% change) NO; NO_2 Lag 0−6 h: −1.67 (−9.58, 6.25); −2.14 (−6.43, 2.14) Lag 0−12 h: −1.67 (−8.33, 4.58); −0.36 (−5.00, 4.29) Lag 0−24 h: −1.25 (−9.17, 6.67); 0.54 (−4.46, 5.54) Lag 24−48 h: 7.92 (0.42, 15.42); 6.25 (0.36, 12.14) Lag 48−72 h: 1.43 (−3.39, 6.25); 7.08 (−4.58, 18.75) Lag 72−96 h: 8.75 (0.83, 16.67); 1.43 (−3.39, 6.25) Lag 0−96 h: 7.08 (−4.58, 18.75); 1.96 (−3.04, 7.14)	Thrombin generation—Lag time (% change) NO; NO_2 Lag 0−6 h: −0.42 (−5.83, 4.58); 0.00 (−2.86, 2.86) Lag 0−12 h: 0.00 (−4.58, 4.17); 0.00 (−3.21, 3.04) Lag 0−24 h: 2.50 (−2.50, 7.50); 0.36 (−2.86, 3.57) Lag 24−48 h: −7.50 (−12.08, −2.92); −5.54 (−9.11, −1.79) Lag 48−72 h: −3.33 (−7.50, 1.25); −4.46 (−7.68, 1.07) Lag 72−96 h: −5.83 (−10.83, −0.83); 0.00 (−3.21, 3.04) Lag 0−96 h: −4.58 (−11.67, 2.08); −1.25 (−4.46, 1.96) Fibrinogen—lag time (% change) NO; NO_2 Lag 24−48 h: 0.42 (−4.17, 5.42); 0.71 (−3.04, 4.46) Lag 48−72 h: 1.25 (−3.33, 5.83); 2.50 (−1.07, 6.07) Lag 72−96 h: 0.42 (−4.58, 5.83); −0.71 (−4.11, 2.50) CRP—lag time (% change) NO; NO_2 Lag 24−48 h: 15.00 (−12.08, 41.67); 11.61 (−8.75, 31.79) Lag 48−72 h: 0.42 (−27.08, 27.92); −0.18 (−19.64, 19.29) Lag 72−96 h: −19.17 (−50.00, 12.08); −12.32 (−30.71, 6.25)
†Steenhof et al. (2014)	Utrecht, the Netherlands n = 31	Healthy	5-h avg NO_2: 20	Central monitors at each of 5 sites	No quantitative results presented; results presented graphically. NO_2 was statistically significantly associated with decreases in eosinophils and lymphocytes 2-h after exposure. Null associations were observed between NO_2 and WBC count, neutrophils, or monocytes.	

Table 5-55 (Continued): Epidemiologic studies of biomarkers of cardiovascular effects.

Study	Location Sample Size	Pre-existing Condition	Mean NO_2 ppb	Exposure Assessment	Selected Effect Estimates[a] (95% CI)	
†Strak et al. (2013)	Utrecht, the Netherlands n = 31	Healthy	5-h avg NO_2: 20	Central monitors at each of 5 sites	Endogenous thrombin [in FXII-mediated thrombin generation pathway (% Change)]. All Sites: 65.47 (7.63, 144.68) Outdoor: 76.14 (−2.23, 154.51)	
Steinvil et al. (2008)	Tel Aviv, Israel n = 3,659	Healthy	24-h avg NO_2 19.5 75th: 25.3	City-wide avg	CRP (% change) men; women Lag 0: 0.31 (−7.87, 12.60); −4.72 (−17.32, 9.45) Lag 1: −7.87 (−17.32, 9.45); −3.15 (−15.75, 11.02) Lag 2: −1.57 (−11.02, 11.02); 0.00 (−12.60, 15.75) Fibrinogen (mg/dL) men; women Lag 0: −9.92 (−15.59, −4.25); −12.44 (−19.84, −5.20) Lag 1: −7.87 (−13.86, −2.05); −5.51 (−12.91, 1.89) Lag 2: −7.09 (−13.07, −1.10); −1.42 (−9.45, 6.46)	WBC (cells/µL) men; women Lag 0: 22.05 (−155.91, 200.00); −83.46 (−305.51, 138.58) Lag 1: 39.37 (−146.46, 223.62); −20.47 (−244.09, 203.15) Lag 2: −36.22 (−226.77, 154.33); 18.90 (−218.90, 255.12)
†Hildebrandt et al. (2009)	Erfurt, Germany n = 38	Healthy	24-h avg NO_2 13.5 24-h NO 10.7	Central monitor	Increases in fibrinogen and prothrombin fragment 1 + 2 associated with NO concentrations. A decrease in vWF was associated with NO_2 concentrations. No quantitative results presented for NO or NO_2.	
†Khafaie et al. (2013)	Pune City, India n = 1,392	Healthy	24-h avg NO_X: 21.1	City-wide avg	No quantitative results presented; results presented graphically. NO_X was statistically significantly associated with increases in CRP at lags 0, 1, 2, 4, and 0−7. There were no measurable differences between winter and summer associations.	
†Kelishadi et al. (2009)	Isfahan, Iran 2004-2005 (n = 374)	Healthy	24-h avg: 35.8 75th %: 47.2 Max: 271	City-wide avg	NO_2 positively associated with CRP and markers of oxidative stress (oxidized-LDL, malondialdehyde, and conjugated diene).	
†Lee et al. (2011)	Allegheny County, PA 1997-2001 (n = 2,211)	Healthy	7-d avg: 8.4 75th %: 10.1 Max: 25.4	City-wide avg	No quantitative results presented. "… NO_2 … associations [with CRP] were negligible for both the entire population and nonsmokers only."	
Chuang et al. (2007a)	Taipei, Taiwan n = 76	Healthy	24-h avg NO_2 17.3 Max: 53.1	Central monitor	"There was no association between NO_2 and any of the blood markers." No quantitative results presented.	

Table 5-55 (Continued): Epidemiologic studies of biomarkers of cardiovascular effects.

Study	Location Sample Size	Pre-existing Condition	Mean NO_2 ppb	Exposure Assessment	Selected Effect Estimates[a] (95% CI)	
Baccarelli et al. (2007)	Lombardia, Italy n = 1,213	Healthy	24-h avg NO_2 Median: 22.7 75th: 33.7 Max: 194.2	City-wide avg	Homocysteine difference (% change) Lag 24 h: 0.24 (−2.86, 3.57) Lag 0−6 days: −2.21 (−6.01, 1.72)	Homocysteine, post-methionine-load (% change) Lag 24 h: 0.00 (−2.86, 2.86) Lag 0−6 days: 0.49 (−2.94, 4.17)
†Rich et al. (2012)	Beijing, China n = 125	Healthy	24-h avg NO_2 Entire study: 27.0 Before: 26.0 During: 13.9 After: 41.4	Central monitor	No quantitative results presented; results presented graphically. Positive and statistically significant increase in sCD62P, generally consistent across lags from 0 to 6. Generally null associations with sCD40L across lags from 0−6. Positive and statistically significant increases in vWF and fibrinogen at early lags (lag 0, lag 1) but null, or negative at later lags. Generally null or negative associations with WBC across lags 0−6.	
†Zhang et al. (2013)	Beijing, China n = 125	Healthy	24-h avg NO_2 Before: 25.6 During: 14.6 After: 41.4	Central monitor	No quantitative results presented; results presented graphically. Statistically significant increase in fibrinogen at lag 0. Positive, but statistically nonsignificant at lags 1, 2, 3, and 6.	

8-OhdG = urinary 8-hydroxy-29-deoxyguanosine, CI = confidence interval, COPD = chronic obstructive pulmonary disease, CRP = C-reactive protein, GPx = glutathione peroxidase, HGF = hepatocyte growth factor, ICAM-1 = inter-cellular adhesion molecule 1, IL = interleukin, Lp-PLA_2 = lipoprotein-associated phospholipase A_2, MI = myocardial infarction, NO = nitric oxide, NO_2 = nitrogen dioxide, NO_X = sum of NO and NO_2, SOD = superoxide dismutase, TNF-α = tumor necrosis factor alpha, VCAM-1 = vascular adhesion molecule-1, vWF = von Willebrand factor, WBC = white blood cells.

[a]Effect estimates are standardized to a 20-ppb or 30-ppb increase in NO_2 or NO or 40-ppb or 60-ppb increase in NO_X concentration for 24-h avg and 1-h max metrics, respectively.

†Studies published since the 2008 ISA for Oxides of Nitrogen.

Epidemiologic Studies

Levels of some circulating systemic inflammatory markers appear to be related to NO_2 concentrations among participants with a history of heart disease. Delfino et al. (2008b) followed nonsmoking elderly subjects with a history of coronary artery disease living in retirement communities in Los Angeles, CA and measured plasma biomarkers weekly over a 12-week period. The authors observed that indoor and/or outdoor NO_2 concentrations measured at the retirement homes were associated with increases IL-6 and the soluble tumor necrosis factor α receptor II (sTNFα-RII), markers of systemic inflammation, but not associated with a number of other biomarkers of inflammation and vascular injury including CRP, P-selectin, D-dimer, TNF-α, soluble intercellular adhesion molecule-1 (sICAM-1), or soluble vascular adhesion molecule-1 (sVCAM-1). In subsequent analysis of overlapping populations, Delfino et al. (2009) and Delfino et al. (2010) found that NO_2 and NO_X were both associated with circulating levels of IL-6. Delfino et al. (2009) also observed positive associations with P-selectin, TNF-RII, and CRP. Working with the same study population, Wittkopp et al. (2013) also found an association between NO_X concentrations and increases in IL-6 and TNF-α, but only for participants with mitochondrial haplogroup H, which has been linked to oxidative damage and increased risk of age-related diseases. Similarly, Ljungman et al. (2009) repeatedly measured plasma IL-6 in 955 MI survivors from six European cities and found that NO_2 was associated with increased levels of IL-6, and that the strength of the association varied in individuals with specific variants of inflammatory genes. However, in studies conducted among patients with stable chronic heart failure, no associations were observed between any biomarkers (including hematological markers and markers of inflammation) and NO_2 concentrations (Barclay et al., 2009; Wellenius et al., 2007). None of these studies examined potential confounding by traffic-related copollutants.

In Augsburg, Germany, Brüske et al. (2011) measured lipoprotein-associated phospholipase A_2 (Lp-PLA_2), a marker of vascular inflammation and an independent predictor of coronary heart disease events and stroke, up to six times in 200 participants with a history of myocardial infarction. They found that Lp-PLA_2 was associated with both NO and NO_2. However, the association was negative at short lags and positive at longer lags, making interpretation of these results difficult.

The results have been more heterogeneous in participants without a history of heart disease. One semi-experimental design assessed changes in blood biomarker levels in healthy participants exposed to ambient air pollution at five locations in the Netherlands with contrasting air pollution characteristics (Steenhof et al., 2014; Strak et al., 2013). A particular strength of these studies is the measurement of pollutants at the location of participants' outdoor exposure, which minimizes measurement error from time-activity

patterns and variability in NO_2 concentration (Sections 3.4.4.1 and 3.4.4.2). Steenhof et al. (2014) reported that NO_2 was negatively associated with both eosinophil and lymphocyte counts. Importantly, this could either be due to eosinophils and lymphocytes leaving the blood and infiltrating stressed tissue, or a decrease in formation of eosinophils and lymphocytes. The associations were relatively unchanged after adjustment for $PM_{2.5}$, EC, OC, or PM_{10} in copollutant models. Strak et al. (2013) observed an increase in thrombin generation in the endogenous pathway (FXII-mediated) associated with ambient outdoor NO_2, that was robust to adjustment for EC or OC, and slightly attenuated after adjustment for $PM_{2.5}$.

Among older men participating in the Normative Aging Study, Bind et al. (2012) found that NO_2 was associated with fibrinogen, sVCAM-1, and sICAM-1, but not CRP. In this same cohort, Ren et al. (2011) found that NO_2 was positively linked with urinary 8-hydroxy-29-deoxyguanosine (8-OhdG) concentrations, a marker of oxidative stress resulting in DNA damage. Thompson et al. (2010) analyzed the baseline data on IL-6 and fibrinogen from 45 non-smoking subjects who participated in a controlled human exposure study in Toronto, Canada. Using baseline blood samples allowed the authors to measure the association between systemic inflammation and ambient NO_2, prior to controlled exposure. The authors found that NO_2 concentrations were not associated with either IL-6 or fibrinogen overall, but IL-6 was associated with NO_2 in the winter months. In Rotterdam, the Netherlands, Rudez et al. (2009) measured CRP, fibrinogen, and markers of platelet aggregation and thrombin generation up to 13 times in 40 healthy participants. Both NO_2 and NO were associated with markers of platelet aggregation and thrombin generation, but neither NO_2 nor NO was associated with CRP or fibrinogen.

During the Beijing Olympics, NO_2 was positively associated with increases in biomarkers indicative of the thrombosis-endothelial dysfunction mechanism (i.e., sCD62P) and increases in fibrinogen among healthy young adults (Zhang et al., 2013; Rich et al., 2012). The association with sCD62P was attenuated, but remained positive after adjustment for $PM_{2.5}$, CO, O_3, SO_2, EC, or OC; whereas the association between NO_2 and fibrinogen was generally robust to the above pollutants, with the exception of EC and OC. Among 3,659 individuals in Tel-Aviv, Steinvil et al. (2008) found a null association between NO_2 concentrations and CRP and a negative association with fibrinogen and white blood cell counts. Baccarelli et al. (2007) observed generally null associations between NO_2 concentrations and total homocysteine among subjects in Lombardia, Italy. Similarly, Chuang et al. (2007a) observed no association between NO_2 and any blood markers, including markers of systemic inflammation and oxidative stress, as well as fibrinolytic and coagulation factors.

Other subgroups that might be at increased risk of pollution-related health effects have also been studied. In a cross-sectional study of COPD patients in Barcelona, Spain, there was evidence of a positive association between NO_2 and multiple biomarkers of inflammation and tissue repair, including CRP, TNFα, IL-6, IL-8, fibrinogen, and hepatocyte growth factor (HGF) (Dadvand et al., 2014). These associations were generally strongest at lags of 4 or 5 days. A particular strength of this study is that the authors used validated land use regression (LUR) models to estimate ambient NO_2 exposure at residential locations. In a repeated-measures study of male patients with chronic pulmonary disease in Germany, Hildebrandt et al. (2009) reported that NO was positively associated with fibrinogen and prothrombin levels but not other markers of coagulation; however, detailed results were not presented in the paper. Khafaie et al. (2013) observed a positive association between NO_2 and CRP in a cross-sectional study of type 2 diabetes patients in Pune City, India. In another cross-sectional analysis of pregnant women in Allegheny County, PA, there was no association between NO_2 and CRP (Lee et al., 2011). Among 374 Iranian children aged 10−18 years, Kelishadi et al. (2009) found that NO_2 was associated with CRP and markers of oxidative stress.

Controlled Human Exposure Studies

Markers of inflammation, oxidative stress, cell adhesion, coagulation, and thrombosis have been evaluated in a few controlled human exposure studies published since the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a) (Table 5-56). Similar to epidemiologic studies, controlled human exposure studies also report evidence for increases in some inflammatory markers, but not consistently across all studies. There is also evidence for hematological changes following NO_2 exposure, and a recent study reported endothelial cell activation.

Table 5-56 Controlled human exposure studies of short-term nitrogen dioxide (NO_2) exposure and cardiovascular effects.

Study	Age; Sex; n	Exposure Details Concentration; Duration	Endpoints Examined
Channell et al. (2012)	Adult (25.3 ± 5.5 yr); M/F; n = 7 Primary hCAECs	Adults were exposed to 500 ppb NO_2; 2 h; intermediate intermittent exercise (15 min on/off; $\dot{V}_E$ = 25 L/min per m^2 of BSA). Plasma samples were collected before exposures, immediately after, and 24-h post-exposure. hCAECs were treated with a dilution of these plasma samples (10 or 30% in media) for 24 h.	LOX-1 protein measured from plasma pre, immediately post, and 24-h post-exposure ICAM-1 and VCAM-1 mRNA from hCAECs and IL-8 and MCP-1 protein from cell supernatant measured immediately post-exposure to plasma.
Drechsler-Parks (1995)	Adult (65.9 ± 9 yr); M/F; n = 8	600 ppb; 2 h; intermittent exercise (20 min on/off) $\dot{V}_E$ = 26−29 L/min	HR was calculated throughout exposure, cardiac output was measured during the last 2 min of each exercise period.
Folinsbee et al. (1978)	Adult (20−25 yr); M; n = 5/group	600 ppb; 2 h; exercise (15, 30, or 60 min; $\dot{V}_E$ = 33 L/min)	HR, BP, and cardiac output were measured during exposure.
Frampton et al. (2002)	Adult; M (26.9 ± 4.5 yr); n = 12 F (27.1 ± 4.1 yr); n = 9	600 and 1,500 ppb; 3 h; intermittent exercise (10 min on/20 min off); $\dot{V}_E$ = 40 L/min	Venous blood collected for hematocrit, hemoglobin, and red blood cell count 3.5 h after exposure.
Gong et al. (2005)	Older adult: Healthy nonsmokers: 68 ± 11 yr; n = 6 Ex-smokers with COPD: 72 ± 7 yr; n = 18	400 ppb NO_2; 2 h; intermittent exercise (15 min on/off); $\dot{V}_E$ = 22−26 L/min	HR and BP were measured immediately post, 4-h post, and Day 2.
Huang et al. (2012b)	Adult; M/F (24.56 ± 4.28 yr); n = 23	500 ppb NO_2 and 500 ppb NO_2 + 73.4 ± 9.9 µg/m^3 CAPs; 2 h; intermittent exercise (15 min on/off); $\dot{V}_E$ = 25 L/min per m^2 of BSA	IL-6, coagulation factors, and lipid panel in peripheral blood; HRV; and HR measured 1 and 18 h after exposure.

Table 5-56 (Continued): Controlled human exposure studies of short-term nitrogen dioxide (NO_2) exposure and cardiovascular effects.

Study	Age; Sex; n	Exposure Details Concentration; Duration	Endpoints Examined
Langrish et al. (2010)	Adult; M; (median age 24 yr); n = 10	4,000 ppb NO_2; 1 h; intermittent exercise; ($\dot{V}_E$ = 25 L/min) 4 h after exposure 5,10, and 20 µg/min acetylcholine; 100, 300, and 1,000 pmol/min bradykinin; 2,4, and 8 µg/min sodium nitroprusside; 10, 30, and 100 µg/min verapamil were infused in the brachial artery for 6 min/dose during forearm venous occlusion plethysmography. Each vasodilator administration was separated by a 20-min washout period.	Hemoglobin concentration was measured 4 and 6 h after exposure, Forearm blood flow and tissue-plasminogen activator and plasminogen-activator inhibitor Type 1 were measured 4 h after exposure.
Linn et al. (1985a)	Adult; M/F With asthma (18−34 yr); n = 23, Without asthma (20−36 yr); n = 25	3,850−4,210 ppb NO_2; 75 min; intermittent exercise; light and heavy exercise $\dot{V}_E$ = 25 and 50 L/min (15 min of each; light minute ventilation 25 L/min and heavy minute ventilation 50 L/min)	HR and BP were measured throughout exposure.
Posin et al. (1978)	Adult; NR; NR; n = 8−10	1,000 or 2,000 ppb NO_2; 2.5 h; light intermittent exercise (15 min on/off)	Acetylcholinesterase, glutathione, glucose-6-phosphate dehydrogenase, lactate dehydrogenase, erythrocyte glutathione reductase, erythrocyte glutathione peroxidase, alpha-tocopherol, TBARS, serum glutathione reductase, 2,3 diphosphoglycerate, hemoglobin, hematocrit.
Riedl et al. (2012)	Adult; M/F (1) 37.33 ± 10.91 yr; n = 10 M, 5 F (2) 36.13 ± 2.52 yr; n = 6 M, 9 F	(1−2) 350 ppb NO_2; 2 h; intermittent exercise (15 min on/off); $\dot{V}_E$ = 15−20 L/min × m^2 BSA (1) Methacholine challenge after exposure. (2) Cat allergen challenge after exposure.	Serum levels of IL-6, ICAM-1, fibrinogen, Factor VII, and vWF. Serum collected 22.5 h after exposure.
Scaife et al. (2012)	Adult (median age 68 yr); with stable coronary heart disease or impaired left ventricular systolic function; M/F; n = 18	400 ppb NO_2; 1 h	HR and HRV monitored continuously for 24 h after exposure.

BSA = body surface area, COPD = chronic obstructive pulmonary disease, F = female, hCAEC = human coronary artery endothelial cells, HR = heart rate, HRV = heart rate variability, ICAM-1 = inter-cellular adhesion molecule 1, IL = interleukin M = male, MCP-1 = monocyte chemoattractant protein-1, mRNA = messenger RNA, NO_2 = nitrogen dioxide, NR = not reported, TBARS - thiobarbituric acid reactive substances, VCAM-1 = vascular adhesion molecule-1, vWF = von Willebrand factor.

In healthy adults, exposure to 500 ppb NO_2 for 2 hours with intermittent exercise did not alter circulating IL-8, a pro-inflammatory cytokine, or coagulation factors but induced a statistically nonsignificant increase in the pro-inflammatory cytokine, IL-6 (Huang et al., 2012b). Lipid profile changes were also reported. There was a 4.1% increase in blood total cholesterol (p = 0.059) and a 5.9% increase in high density lipoprotein cholesterol (p = 0.036) 18 hours after exposure, but no changes in low density lipoprotein or very low density lipoprotein cholesterol or triglycerides.

The controlled human exposure study by Langrish et al. (2010) examined the effects of NO_2 on fibrinolytic function. The endogenous fibrinolytic pathway was assessed by sampling venous concentrations of tissue-plasminogen activator and plasminogen-activator inhibitor Type I at baseline and 4 hours after exposure. Concentrations of these proteins were not affected by exposure to NO_2.

Atherosclerosis is a chronic inflammatory disease; early stages of the disease include inflammatory activation of endothelial cells and adhesion of leukocytes to the vascular endothelium. Channell et al. (2012) reported endothelial cell activation in an in vitro model following NO_2 exposure. Plasma samples were collected from healthy volunteers exposed to filtered air or 500 ppb NO_2 for 2 hours with intermittent exercise. Primary human coronary artery endothelial cells (hCAECs) were then treated with a dilution of these plasma samples (10 or 30% in media) for 24 hours. Increases in messenger RNA (mRNA) expression levels of endothelial cell adhesion molecules, vascular adhesion molecule-1 (VCAM-1) and ICAM-1, from hCAECs were observed for both post-exposure time points compared to control. Cells treated with plasma (30%) collected immediately post NO_2 exposure had a statistically significant greater release of IL-8 but not monocyte chemoattractant protein-1 (MCP-1). In addition, plasma collected 24 hours post NO_2 exposure had a significant increase (30%) in soluble lectin-like oxLDL receptor (LOX-1) levels, a protein recently found to play a role in the pathogenesis of atherosclerosis (Sections 4.3.2.9 and 4.3.5).

Riedl et al. (2012) reported on the cardiovascular effects of healthy volunteers and individuals with asthma exposed to filtered air, diesel exhaust, or 350 ppb NO_2 for 2 hours with intermittent exercise. No statistically significant differences were found in IL-6, ICAM-1, and blood coagulation factors [i.e., factor VII, fibrinogen, and von Willebrand factor (vWF)] the morning after NO_2 exposure.

Studies from the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a) reported NO_2-induced hematological changes. Frampton et al. (2002) reported decreases in hematocrit, hemoglobin, and red blood cell count in healthy volunteers 3.5 hours after exposure to 600 and 1,500 ppb NO_2 for 3 hours with intermittent exercise. Results from this study support those of Posin et al. (1978), in which hematocrit and hemoglobin levels

were decreased in young males exposed to 1,000 and 2,000 ppb NO_2 for approximately 2.5 hours with intermittent exercise. However, a recent study reported no change in hemoglobin levels 4 and 6 hours post-exposure to 4,000 ppb NO_2 for 1 hour (Langrish et al., 2010).

Toxicological Studies

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a) reported on various hematological parameters in animals including oxidative stress, red blood cell turnover, and methemoglobin levels. Similar to epidemiologic and controlled human exposure studies, several recently published toxicological studies have examined the potential association between short-term NO_2 exposure and biomarkers of cardiovascular effects, including markers of oxidative stress, inflammation, and cell adhesion (Table 5-57).

Recently, the effects of NO_2 on markers of oxidative stress were examined by Li et al. (2011a). Rats exposed to 2,660 or 5,320 ppb NO_2 for 7 days had a small, but statistically significant decrease in the activity of the antioxidant enzyme Cu/Zn-SOD and, at the higher dose, an increase in malondialdehyde (MDA, an indicator of lipid peroxidation) in heart tissue. These changes were accompanied by mild pathological changes in the heart. However, there were no changes in Mn-SOD or GPx activity or protein carbonyl (PCO) levels at either exposure concentration. Campen et al. (2010) reported Apolipoprotein E knockout mice ($ApoE^{-/-}$) exposed to 200 and 2,000 ppb NO_2 had a concentration-dependent decrease (statistically significant linear trend) in the expression of the antioxidant enzyme HO-1 in the aorta. Together, these results demonstrate the ability of NO_2 inhalation to perturb the oxidative balance in the heart and aorta.

The effects of NO_2 on antioxidant capacity were also examined in the context of diet (de Burbure et al., 2007). Rats were placed on low (Se-L) or supplemented (Se-S) selenium (Se) diets and were exposed to 5,000 ppb NO_2 for 5 days. Se is an integral component of the antioxidant enzyme GPx. GPx levels in red blood cells (RBC) increased in both groups immediately and 48 hours after exposure; however, plasma levels were decreased in Se-L diet rats at both time points. SOD activity in RBCs also decreased in Se-L diet rats at both time points but increased in Se-S diet rats 48 hours after exposure. Overall, NO_2 exposure stimulated oxidative stress protective mechanisms with high Se but were mixed with low Se.

Table 5-57 Animal toxicological studies of short-term nitrogen dioxide (NO_2) exposure and cardiovascular effects.

Study	Species (Strain); Age; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Campen et al. (2010)	Mice ($ApoE^{-/-}$); 8 weeks; M; n = 5−10/group	High fat diet; 200 ppb, 2,000 ppb NO_2; 6 h/day for 7 days	ET-1, MMP-9, HO-1, and TIMP-1 mRNA expression in aorta; TBARS in aorta; MMP-2/9 activity in aorta. Endpoints measured 18 h after exposure.
de Burbure et al. (2007)	Rats (Wistar); 8 weeks; M; n = 8/group	Selenium: 6 µg/day or 1.3 µg/day: 1,000 ppb NO_2; 28 day, 6 h/day, 5 days/week (Se+/Se−) 10,000 ppb NO_2; 28 day, 6 h/day, 5 days/week; 5,000 ppb NO_2; 5 days, 6 h/day 50,000 ppb; 30 min	GPx in plasma and red blood cell lysate; SOD activity in red blood cell lysate; GST activity in red blood cell lysate; TBARS in plasma. Endpoints examined immediately and 48 h after exposure.
Kunimoto et al. (1984)	Rats (Wistar); 16−20 weeks; M; n = 6/group	4,000 ppb NO_2; continuously for 1−10 days	ATPase activity, sialic acid, and hexose in red blood cell membranes measured after 1, 4, 7, and 10 days of exposure.
Li et al. (2011a)	Rats (Wistar); Adults; M; n = 6/group	2,660, 5,320, and 10,640 ppb NO_2; 6 h/day for 7 days	H&E staining of heart tissue; Cu/Zn-SOD, Mn-SOD activity, GPx activity, MDA level, and PCO level in heart tissue; ET-1, eNOS, TNF-α, IL-1, and ICAM-1 mRNA and protein levels in heart tissue; cardiac myocyte apoptosis. Endpoints examined 18 h after exposure.
Mersch et al. (1973)	Guinea pigs; NR; n = 8	360 ppb NO_2; continuously for 7 days	D-2,3-diphosphoglycerate content in red blood cells; collection time NR.
Mochitate and Miura (1984)	Rats (Wistar); 16−20 weeks; M; n = 6	4,000 ppb NO_2; continuously for 1−10 days	PK and PFK activity and hemoglobin content in red blood cells was measured after 1, 3, 5, 7, and 10 days of exposure.
Nakajima and Kusumoto (1968)	Mice (ICR); 4 weeks; M; n = NR	800 ppb NO_2; continuously for 5 days	Metahemoglobin in blood from the heart taken immediately after exposure.
Ramos-Bonilla et al. (2010)	Mice (AKR/J); 180 days; M; n = 3/group	Low-pollution chamber: (21.2 ppb NO_2, 465 ppb CO, 11.5 µg/m^3 PM); High-pollution chamber: (36.1 ppb NO_2, 744 ppb CO, 36.7 µg/m^3 PM); 6 h/day, 5 days/week, 40 weekdays	ECG (HR, SDNN, *r*-MSSD, TP, LF, HF, LH:HF), BW; Endpoints measured throughout the exposure.

CO = carbon monoxide, ECG = electrocardiographic, eNOS = endothelial nitric oxide synthase, ET-1 = endothelin-1, GPx = glutathione peroxidase. GST = Glutathione-S-transferase, HF = high frequency, HR = heart rate, ICAM-1 = inter-cellular adhesion molecule 1, IL = interleukin, LF = low frequency, M = male, MDA = malondialdehyde, mRNA = messenger RNA, NO_2 = nitrogen dioxide, NR = not reported, PCO = protein carbonyl, PFK = phosphofructokinase, PK = pyruvate kinase, PM = particulate matter, SDNN = standard deviation of all normal-to-normal intervals, Se = selenium, SOD = superoxide dismutase, TBARS - thiobarbituric acid reactive substances.

The effects of NO_2 on vascular tone modifiers, endothelin-1 (ET-1), and endothelial nitric oxide synthase (eNOS) were recently examined in two studies (Li et al., 2011a; Campen et al., 2010). ET-1 is a potent vasoconstrictor while the enzyme eNOS catalyzes the production of NO, which induces vasodilation. Campen et al. (2010) did not see a statistically significant increase in ET-1 expression level in the aorta after exposure of mice to 200 and 2,000 ppb NO_2. However, exposure to higher NO_2 concentrations induced a statistically significant increase in ET-1 in the heart at the mRNA (10,640 ppb) and protein level (5,320 and 10,640 ppb) (Li et al., 2011a). eNOS mRNA and protein levels were increased at both 2,660 and 5,320 ppb NO_2 and decreased to control levels at 10,640 ppb NO_2. At ambient-relevant concentrations of NO_2 exposure, there was an increase in eNOS, while higher concentrations elicited an increase in the vasoconstrictor, ET-1.

Studies have also reported changes in some inflammatory markers and adhesion molecules after NO_2 exposure in animals. Li et al. (2011a) observed a statistically significant increase in TNF mRNA levels in the heart at 5,320 ppb NO_2. In addition, IL-1 expression and protein levels were increased; however, this effect was in response to a higher NO_2 concentration. ICAM-1 transcription and protein levels were increased in the heart after both the 2,660 and 5,320 ppb NO_2 exposures. These results are consistent with the increase in ICAM-1 mRNA Channell et al. (2012) found in an in vitro model described above.

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a) reported on several animal studies examining hematological parameters. Three studies indicate elevated levels of a younger population of red blood cells following NO_2 exposure. Red blood cell D-2,3-diphosphoglycerate levels, important in hemoglobin-oxygen dissociation, were increased in guinea pigs following a 7-day continuous exposure to 360 ppb NO_2 (Mersch et al., 1973). Kunimoto et al. (1984) reported an increase in red blood cell sialic acid after 24 hours of exposure to 4,000 ppb NO_2. Similarly, Mochitate and Miura (1984) reported an elevation of the glycolytic enzymes pyruvate kinase and phosphofructokinase after a 7-day continuous exposure to 4,000 ppb NO_2. These results suggest an increase in red blood cell turnover after NO_2 exposure. Nakajima and Kusumoto (1968) reported that mice exposed to 800 ppb NO_2 continuously for 5 days had no change in the oxygen-carrying metalloprotein hemoglobin, methemoglobin.

Summary of Blood Biomarkers of Cardiovascular Effects

In summary, the evidence across disciplines for changes in blood biomarkers of cardiovascular effects is inconsistent; however, there is limited but supportive evidence for measures of NO_2-induced systemic inflammation. Some epidemiologic evidence

suggests the presence of an association between NO_2 concentrations and some markers of systemic inflammation among participants with a history of heart disease. However, potential copollutant confounding was not evaluated in these studies, so the possibility remains that the associations observed were the artifact of correlated pollutants. This association is not consistently observed in healthy individuals. Other potentially at-risk populations have not been clearly identified due to contrasting or limited evidence. Controlled human exposure studies evaluating systemic inflammation demonstrated inconsistent results; however this may be due to a dose-dependent effect. Toxicological studies reported an increase in some inflammatory mediators, as well as oxidative stress effects in RBC, the heart, and aorta of rodents. Other biological markers of cardiovascular effects, also discussed in Section 4.3.2.9, demonstrated that short-term NO_2 exposure causes a slight reduction in hematocrit and hemoglobin levels associated with a decrease in RBC levels in controlled human exposure studies. Toxicological studies reported an increase in RBC turnover. The clinical significance of these findings is unknown (Section 4.3.2.9). Evidence has not shown NO_2 to alter circulating blood coagulation factors or modify the body's response to vasodilators in controlled human exposure studies. However, in toxicological studies at higher concentrations, NO_2 was found to induce the expression and production of the vasoconstrictor ET-1.

Overall, there is preliminary evidence, albeit not entirely consistent, from controlled human exposure and toxicological studies that suggests systemic inflammation and oxidative stress can occur after exposure to NO_2. However, changes in other blood biomarkers, such as coagulation or vasomotor response, are not observed in relation to NO_2 exposure.

5.3.12 Summary and Causal Determination

Available evidence is suggestive of, but not sufficient to infer, a causal relationship between short-term exposure to oxides of nitrogen and cardiovascular health effects. The strongest evidence comes from epidemiologic studies of adults and consistently demonstrates a relationship between short-term exposure to NO_2 and triggering of an MI. This is supported by epidemiologic studies reporting NO_2-associated hospitalizations and ED visits for MI, IHD, and angina, ST-segment alterations, and mortality from cardiovascular disease. There is a lack of experimental studies that evaluate similar clinical outcomes in order to assess the coherence across disciplines. However, some controlled human exposure and toxicological studies provide limited evidence for potential biologically plausible mechanisms, including inflammation and oxidative stress. Evidence for other cardiovascular and related metabolic effects is inconsistent.

This conclusion represents a change from the 2008 ISA for Oxides of Nitrogen, which concluded the "available evidence on the effects of short-term exposure to NO_2 on cardiovascular health effects was inadequate to infer the presence or absence of a causal relationship at this time" (U.S. EPA, 2008a). Specifically, the epidemiologic panel studies and toxicological studies available at the time of the last review were inconsistent. Most epidemiologic studies reviewed in the 2008 ISA for Oxides of Nitrogen found positive associations between ambient NO_2 concentrations and risk of hospital admissions or ED visits for all cardiovascular diseases (U.S. EPA, 2008a). However, it was unclear at that time whether these results supported a direct effect of short-term NO_2 exposure on cardiovascular morbidity or were confounded by other correlated pollutants. Recent epidemiologic studies have further evaluated this uncertainty using copollutant models and comparing associations of NO_2 with those of other criteria pollutants. While the recently reviewed studies provide suggestive evidence for independent associations of NO_2 with cardiovascular effects after adjusting for some pollutants, uncertainties still remain regarding the potential for NO_2 to serve as an indicator for other combustion-related pollutants or mixtures. Specifically, there is a lack of epidemiologic studies evaluating traffic-related pollutants (i.e., $PM_{2.5}$, BC/EC, UFPs, or VOCs) in copollutant models with NO_2.

There continues to be a lack of experimental evidence in coherence with the epidemiologic studies to strengthen the inference of causality for NO_2-related cardiovascular effects, including MI. Further, the limited mechanistic evidence to describe a role for NO_2 in the triggering of cardiovascular diseases, including key events within the mode of action, remains from the 2008 ISA for Oxides of Nitrogen. The evidence for cardiovascular effects, with respect to the causal determination for short-term exposure to NO_2, is detailed below using the framework described in the Preamble (Tables I and II). The key evidence, supporting or contradicting, as it relates to the causal framework, is summarized in Table 5-58.

5.3.12.1 Evidence on Triggering a Myocardial Infarction

The causal determination for the relationship between short-term NO_2 exposure and cardiovascular effects is based on the evidence for effects related to triggering an MI, including findings for hospital admissions and ED visits for IHD, MI, or angina and ST-segment amplitude changes. Time-series studies of adults in the general population consistently report positive associations between 24-h avg and 1-h max NO_2 concentrations and hospital admissions and ED visits for IHD and MI among adults (Figure 5-18, Section 5.3.2.1). Risk estimates ranged from 0.87 to 1.76 per a 20 or 30 ppb increase in NO_2, with the magnitude of most of the risk estimates greater than 1.00.

Symptoms of MI are similar to those of angina; however, where MI results in damage to the heart muscle, angina does not result in myocardial necrosis. However, angina may indicate an increased risk for future MI. IHD is an over-arching category of related ischemic events that includes both acute MI and angina, as well as events related to older MI and other IHD-related events. Increased observations of hospital admissions and ED visits for MI and IHD are coherent with epidemiologic studies reporting increased hospital admissions and ED visits for angina (Section 5.3.2.2). Among those hospitalized, ST-segment decreases are considered a nonspecific marker of myocardial ischemia. A small number of epidemiologic panel studies have reported associations between short-term exposure to NO_2 and ST-segment changes on the electrocardiogram of older adults with a history of coronary artery disease (Section 5.3.2.3).

Coherent with the increase in hospital admissions and ED visits for IHD, MI, and angina, single-city studies from the U.S. (Ito et al., 2011; Peel et al., 2007; Tolbert et al., 2007) and multicity studies conducted in Europe and Australia and New Zealand (Larrieu et al., 2007; Ballester et al., 2006; Barnett et al., 2006; Von Klot et al., 2005) report positive associations with all CVD hospital admissions in adults with adjustment for numerous potential confounding factors, including weather and time trends (Section 5.3.9). Additionally, the evidence for associations observed in time-series studies is coherent with positive associations reported in epidemiologic studies of short-term NO_2 exposure and cardiovascular mortality in adults (Section 5.3.10). These include studies reviewed in the 2008 ISA for Oxides of Nitrogen and recent multicity studies that generally report a similar or slightly larger magnitude for the NO_2 cardiovascular mortality relationship compared to total mortality.

Recent controlled human exposure and animal toxicological studies provide preliminary evidence for a potentially biologically plausible mechanism for short-term exposure to NO_2 leading to cardiovascular disease, including IHD. Reactive intermediates or inflammatory mediators that have "spilled over" from the respiratory tract into the circulation may result in systemic inflammation and/or oxidative stress, which may mediate effects in the heart and vasculature (Sections 4.3.2.9 and 4.3.5). These nonspecific effects may promote the triggering of an MI. There is limited and supportive evidence in humans and animals for increased systemic and tissue specific oxidative stress (Channell et al., 2012; Li et al., 2011a). In addition, evidence in animal and cell models and in some controlled human exposure studies report NO_2-mediated increases in inflammatory markers (Channell et al., 2012; Huang et al., 2012b; Li et al., 2011a).

A key uncertainty that remains since the 2008 ISA for Oxides of Nitrogen is the potential for confounding by other correlated traffic-related pollutants given a common source and moderate to high correlations with NO_2. Recent studies have evaluated this uncertainty

using copollutant models and comparing associations of NO_2 with those of other pollutants. A number of studies examined associations between short-term exposure to NO_2 and cardiovascular disease adjusted for PM_{10} or TSP (Figure S5-2, (U.S. EPA, 2014b)), CO (Figure S5-3, (U.S. EPA, 2014c)), O_3 (Figure S5-4; (U.S. EPA, 2014d)), and SO_2 (Figure S5-5; (U.S. EPA, 2014e)), and reported that the effect estimate was generally robust to the inclusion of the copollutant in the models. However, not all analyses reported NO_2 as the strongest predictor of cardiovascular effects. One study reported that associations with cardiovascular hospital admissions were not robust in models adjusting for CO exposure (Barnett et al., 2006) and another reported associations with CO, total carbon, and EC and OC components of $PM_{2.5}$ that were stronger or similar in magnitude to those for NO_2 (Tolbert et al., 2007). However, other traffic-related pollutants that may be potentially correlated with NO2 (i.e., $PM_{2.5}$, EC, BC, VOCs) were generally not examined in copollutant models, resulting in the potential for unmeasured confounding. A limited number of studies that examined copollutant confounding on the NO_2 cardiovascular mortality relationship indicate that associations remain robust to adjustment for PM_{10}, SO_2, or O_3 (Chen et al., 2012b; Chiusolo et al., 2011). Finally, while copollutant models are a common statistical tool used to evaluate the potential for copollutant confounding, inferences from their results can be limited (Section 5.1.2.2). Until more reliable methods to adjust for multiple copollutants simultaneously become available, there is potential for residual confounding due to unmeasured copollutants (Section 5.1.2.2). Without consistent and reproducible experimental evidence that is coherent with the effects observed in epidemiologic studies, uncertainty still exists concerning the role of correlated pollutants in the associations observed with NO_2. Additionally, the lack of studies with copollutant models evaluating $PM_{2.5}$, BC/EC, UFPs, or VOCs in relation to NO_2 raises the concern that these associations could be a result of NO_2 serving as a marker for effects of other traffic-related pollutants or mixtures of pollutants.

5.3.12.2 Evidence on Other Cardiovascular and Related Metabolic Effects

There is inconclusive evidence from epidemiologic, controlled human exposure, and animal toxicological studies for other cardiovascular and related metabolic effects from short-term exposure to NO_2. There are a number of epidemiologic studies that provide inconsistent evidence for an association between 24-h avg NO_2, NO, or NO_X and risk of cardiac arrhythmias as examined in patients with ICDs, continuous ECG recordings, out-of-hospital cardiac arrest, and hospital admissions (Section 5.3.3). Similarly, epidemiologic studies provide inconsistent evidence for a potential association between ambient NO_2 concentrations and risk of hospital admission for cerebrovascular disease

and stroke (Section 5.3.4). Both epidemiologic and controlled human exposure studies provide little to no evidence to indicate that short-term exposure to ambient NO_2 is associated with increased BP or hypertension (Section 5.3.6). Other outcomes have an insufficient quantity of studies to evaluate the effects. A small number of epidemiologic studies have found associations between NO_2 concentrations and hospital admissions or ED visits for heart failure (Section 5.3.5) and hospital admission for venous thrombosis and pulmonary embolism (Section 5.3.7). One recent epidemiologic study reported a lack of an association between 24-h avg NO_2 and insulin resistance (Section 5.3.8).

Various subclinical effects have been investigated that are not clearly associated with a particular clinical event observed in the population but may be key events within a mode of action for cardiovascular effects other than MI. There is limited evidence from epidemiologic and controlled human exposure studies to suggest that NO_2 exposure results in alterations of cardiac autonomic control. Recent epidemiologic studies generally reported associations between ambient NO_2 levels and decreases in indices of HRV (Section 5.3.11.1) and changes in ventricular repolarization (Section 5.3.11.2) among populations with pre-existing or at elevated risk for cardiovascular disease. Experimental studies also evaluated changes in HRV and ventricular repolarization parameters. Although changes were not observed across all endpoints, a recent controlled human exposure study reported decreased HFn and QTVI in healthy exercising adults exposed to NO_2, indicating a potential disruption in the normal cardiac autonomic control (Huang et al., 2012b). However, similar measures of autonomic control in another controlled human exposure study showed statistically nonsignificant increases after exposure to NO_2 (Scaife et al., 2012).

5.3.12.3 Conclusion

In conclusion, consistent epidemiologic evidence from multiple studies at relevant NO_2 concentrations is suggestive of, but not sufficient to infer, a causal relationship between short-term NO_2 exposure and cardiovascular health effects. The strongest evidence supporting this determination comes from studies of triggering an MI. However, uncertainty remains regarding exposure measurement error and potential confounding by traffic-related copollutants. Experimental studies provide some evidence describing key events within the mode of action but do not provide evidence that is coherent with the epidemiologic studies to help rule out chance, confounding, and other biases. Evidence for other cardiovascular and related metabolic effects is inconclusive, including effects on cardiac arrest and arrhythmia, cerebrovascular disease and stroke, increased blood pressure and hypertension, decompensation of heart failure, and diabetes. Studies of adults consistently demonstrate NO_2-associated hospital admissions and ED visits for

IHD, MI, and angina, as well as all cardiovascular diseases. This is coherent with evidence for NO_2-related ST segment decrements and mortality from cardiovascular disease. These studies have been replicated by different researchers in different locations and have adjusted for numerous potential confounding factors including meteorological factors and time trends. However, due to limited analysis of potentially correlated pollutants and recognized limitations of copollutant models, some uncertainty remains regarding the extent to which NO_2 is independently associated with cardiovascular effects or if NO_2 serves as a marker for the effects of another traffic-related pollutant or mix of pollutants. Thus, the combined evidence from epidemiologic and experimental studies is suggestive of, but not sufficient to infer, a causal relationship between short-term NO_2 exposure and cardiovascular effects.

Table 5-58 Summary of evidence, which is suggestive, but not sufficient, to infer a causal relationship between short-term nitrogen dioxide (NO_2) exposure and cardiovascular and related metabolic effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Triggering a myocardial infarction			
Consistent epidemiologic evidence from multiple, high-quality studies at relevant NO_2 concentrations	Increases in hospital admissions and ED visits for IHD and MI in adults in multiple studies, including multicity studies, in diverse locations.	Larrieu et al. (2007); Stieb et al. (2009); Peel et al. (2007); Von Klot et al. (2005); Mann et al. (2002) Figure 5-13; Section 5.3.2.1	Mean 24-h avg: 11.9−37.2 ppb Mean 1-h max: 45.9 ppb
	Coherence with limited evidence for increased hospital admissions and ED visits for angina in adults in multiple studies, including multicity studies.	Szyszkowicz (2009); Poloniecki et al. (1997); Von Klot et al. (2005) Section 5.3.2.2	
	Increases in hospital admissions and ED visits for all CVD in adults in multiple studies, including multicity studies, in diverse locations.	Larrieu et al. (2007); Ito et al. (2011); Peel et al. (2007); Tolbert et al. (2007); Von Klot et al. (2005); Ballester et al. (2006); Barnett et al. (2006) Section 5.3.9	Mean 24-h avg: 11.9−40.5 ppb Mean 1-h max: 43.2−45.9 ppb
	Coherence with ST-segment depression in adults with pre-existing coronary heart disease in association with 24-h avg and 1-h avg NO_2.	Chuang et al. (2008); Delfino et al. (2011) Section 5.3.2.3	24 h avg: 21.4 ppb Mean 1-h max: 27.5 ppb
	Consistent evidence for increased risk of cardiovascular mortality in adults applying differing model specifications in diverse locations.	Bellini et al. (2007); Wong et al. (2008); Chen et al. (2012b); Chiusolo et al. (2011) Section 5.3.10	Mean 24-h avg: 13.5−35.4 ppb
Uncertainty regarding exposure measurement error	Majority of evidence from time-series studies that rely on central site exposure estimates.		

Table 5-58 (Continued): Summary of evidence, which is suggestive, but not sufficient, to infer a causal relationship between short-term nitrogen dioxide (NO2) exposure and cardiovascular and related metabolic effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Uncertainty regarding potential confounding by traffic-related copollutants	NO_2 associations with ED visits and hospital admissions are generally robust in copollutant models containing PM_{10}, CO, O_3, or SO_2.	Supplemental Figures S5-2, S5-3, S5-4, and S5-5 (U.S. EPA, 2014b, c, d, e)	
	Inability to disentangle the effects of traffic-related pollutants because of lack of examination (e.g., $PM_{2.5}$, BC/EC, UFPs, or VOCs).		
	NO_2 associations with ED visits, hospital admissions, and mortality found with adjustment for numerous potential confounding factors including meteorological factors and time trends.		
Some evidence for key events within the mode of action			
Oxidative stress	Limited and supportive evidence of increased oxidative stress in heart tissue in rats with relevant NO_2 exposures (i.e., MDA) and plasma from NO_2-exposed humans (i.e., LOX-1).	Li et al. (2011a) Section 4.3.2.9, Figure 4-3	Rats: 5,320 ppb but not 2,660 ppb NO_2
Inflammation	Limited and supportive toxicological evidence of increased transcription of some inflammatory mediators in vitro (i.e., IL-8, ICAM-1, VCAM-1) and in rats (i.e., ICAM-1, TNF-α).	Channell et al. (2012)	Human cells exposed to plasma from healthy adults: 500 ppb NO_2
		Li et al. (2011a)	Rats: 2,660 and 5,320 ppb NO_2
	Limited and inconsistent evidence in controlled human exposure studies (i.e., IL-6, IL-8, ICAM-1).	Huang et al. (2012b); Riedl et al. (2012)	Adults: 350, 500 ppb NO_2
	Inconsistent epidemiologic evidence for changes in CRP, IL-6, and TNF-RII.	Section 5.3.11.4	

Table 5-58 (Continued): Summary of evidence, which is suggestive, but not sufficient, to infer a causal relationship between short-term nitrogen dioxide (NO2) exposure and cardiovascular and related metabolic effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Other cardiovascular and related metabolic effects			
Inconclusive evidence from epidemiologic, controlled human exposure and toxicological studies	Inconsistent epidemiologic evidence for an association between NO_2, NO, or NO_X and risk of cardiac arrest and arrhythmias, cerebrovascular disease and stroke, and increased blood pressure and hypertension.	Sections 5.3.3, 5.3.4, and 5.3.6	
	Insufficient quantity of studies evaluating decompensation of heart failure and venous thrombosis and pulmonary embolism.	Stieb et al. (2009); Yang (2008) Section 5.3.5 Dales et al. (2010) Section 5.3.7	
	Lack of an association between 24-h avg NO_2 and diabetes (i.e., insulin resistance).	Kelishadi et al. (2009) Section 5.3.8	
	Inconsistent changes in HRV in controlled human exposure studies.	Huang et al. (2012b)	Healthy adults: 500 ppb NO_2
		Scaife et al. (2012) Section 5.3.11.1	Adults with pre-existing CVD: 400 ppb NO_2
	Limited epidemiologic evidence for changes in HRV and ventricular repolarization. Stronger associations observed in groups of individuals with pre-existing cardiovascular disease.	HRV: Timonen et al. (2006); Suh and Zanobetti (2010b); Zanobetti et al. (2010) Section 5.3.11.1 QT interval: Henneberger et al. (2005) Section 5.3.11.2	

CO = carbon monoxide, CRP = C-reactive protein, CVD = cardiovascular disease, EC = elemental carbon, HRV = heart rate variability, ICAM-1 = inter-cellular adhesion molecule 1, IHD = ischemic heart disease, IL = interleukin, MDA = malondialdehyde, MI = myocardial infarction, NO = nitric oxide, NO_2 = nitrogen dioxide, O_3 = ozone, PM = particulate matter, SO_2 = sulfur dioxide, UFP = ultrafine particles, VCAM-1 = vascular adhesion molecule-1.

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Tables I and II of the Preamble.

[b]Describes the key evidence and references, supporting or contradicting, that contribute most heavily to causal determination. References to earlier sections indicate where full body of evidence is described.

[c]Describes the NO_2 concentrations with which the evidence is substantiated.

5.4 Total Mortality

5.4.1 Introduction and Summary of 2008 Integrated Science Assessment for Oxides of Nitrogen

Prior to the 2008 ISA for Oxides of Nitrogen, epidemiologic studies had not been identified that examined whether an association exists between short-term NO_2 exposure and mortality. The 2008 ISA for Oxides of Nitrogen evaluated a collection of studies, including multicity studies, conducted in the U.S., Canada, and Europe, and a meta-analysis (U.S. EPA, 2008a). All of these studies reported evidence of an association between short-term NO_2 exposure and mortality with estimates ranging from 0.5 to 3.6% for a 20-ppb increase in 24-h avg or 30-ppb increase in 1-h max NO_2 concentrations. A limitation of this collection of studies was that the majority focused specifically on PM and did not conduct extensive analyses to examine the relationship between short-term NO_2 exposure and mortality.

Multicity studies conducted in the U.S. (HEI, 2003), Canada (Brook et al., 2007; Burnett et al., 2004) and Europe (Samoli et al., 2006), as well as a large study conducted in the Netherlands (Hoek, 2003), consistently reported positive associations between short-term NO_2 exposure and mortality, specifically at lag 1, with evidence that these associations remain robust in copollutant models. These results were confirmed in a meta-analysis that did not include any of the aforementioned multicity studies (Stieb et al., 2002).

Of the studies evaluated in the 2008 ISA for Oxides of Nitrogen, a limited number provided additional information (i.e., seasonal analyses, examination of cause-specific mortality, examination of effect modifiers) on the NO_2-mortality relationship. Initial evidence indicated a larger NO_2-mortality association during the warmer months (Brook et al., 2007; Burnett et al., 2004; HEI, 2003). Additionally, an examination of total and cause-specific mortality found associations similar in magnitude across mortality outcomes (total, respiratory, and cardiovascular); however, some studies reported stronger NO_2 associations for respiratory mortality (Biggeri et al., 2005; Simpson et al., 2005b). Potential effect modifiers of the NO_2-mortality relationship were examined only within the APHEA study, which found that within the European cities, geographic area and smoking prevalence modified the NO_2-mortality relationship. It is worth noting that additional multicity European studies that focused on PM (Aga et al., 2003; Katsouyanni et al., 2003) reported that cities with higher NO_2 concentrations also had higher PM risk estimates indicating that NO_2 and PM may be potential effect modifiers of each other.

In summary, the multicity studies evaluated in the 2008 ISA for Oxides of Nitrogen consistently observed positive associations between short-term NO_2 exposure and mortality. These studies indicated that associations were found to occur within the first few days after exposure and are potentially influenced by season. However, uncertainties remained in the NO_2-mortality relationship, which led to the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a) concluding that the evidence "was suggestive but not sufficient to infer a causal relationship." These uncertainties and data gaps included whether: NO_2 is acting as an indicator for another pollutant or a mix of pollutants; there is evidence for potential copollutant confounding; specific factors modify the NO_2-mortality relationship; there is seasonal heterogeneity in mortality associations; NO_2 associations are stronger with specific mortality outcomes; and the shape of the NO_2-mortality concentration-response relationship is linear.

5.4.2 Associations between Short-Term Nitrogen Dioxide Exposure and Mortality

Since the completion of the 2008 ISA for Oxides of Nitrogen, the body of epidemiologic literature that has examined the association between short-term NO_2 exposure and mortality has grown. However, similar to the collection of studies evaluated in the 2008 ISA for Oxides of Nitrogen, most of the recent studies did not focus specifically on the NO_2-mortality relationship but on other pollutants. Of the studies identified, a limited number have been conducted in the U.S., Canada, and Europe, with the majority being conducted in Asia due to the increased focus on examining the effect of air pollution on health in developing countries. Although these studies are informative in evaluation of the relationship between oxides of nitrogen and mortality, the broad implications of these studies in the context of results from studies conducted in the U.S., Canada, and Western Europe are limited. This is because studies conducted in Asia encompass cities with meteorological (Tsai et al., 2010; Wong et al., 2008), outdoor air pollution (e.g., concentrations, mixtures, and transport of pollutants), and sociodemographic (e.g., disease patterns, age structure, and socioeconomic variables) (Kan et al., 2010) characteristics that differ from cities in North America and Western Europe, potentially limiting the generalizability of results from these studies to other cities.

Overall, this section evaluates studies that examined the association between short-term NO_2 exposure and mortality and addresses the key uncertainties and data gaps in the NO_2-mortality relationship identified in the 2008 ISA for Oxides of Nitrogen: potential confounding of NO_2 associations, effect modification (i.e., sources of heterogeneity in risk estimates across cities), seasonal heterogeneity in NO_2 associations, and the NO_2-mortality C-R relationship. Other recent studies of mortality are not the focus of this evaluation because they were conducted in small single-cities, encompass a short study

duration, had insufficient sample size, and/or did not examine potential copolluant confounding. The full list of the studies can be found in Supplemental Table S5-4 (U.S. EPA, 2014i).

5.4.3 Associations between Short-term Nitrogen Dioxide Exposure and Mortality in All-Year Analyses

Multicity studies evaluated in the 2008 ISA for Oxides of Nitrogen reported consistent, positive associations between short-term NO_2 exposure and mortality in all-year analyses (U.S. EPA, 2008a). However, when focusing on specific causes of mortality, some studies reported similar risk estimates across total (nonaccidental), cardiovascular, and respiratory mortality (Samoli et al., 2006; Burnett et al., 2004), while others indicated larger respiratory mortality risk estimates compared to both total and cardiovascular mortality (Atkinson et al., 2012; Biggeri et al., 2005; Simpson et al., 2005b). Additional multicity studies focusing on COPD (Meng et al., 2013) and stroke (Chen et al., 2013) mortality further support potential differences in the NO_2-mortality association by mortality outcome. Although only a small number of multicity studies have been conducted since the completion of the 2008 ISA for Oxides of Nitrogen, these studies build upon and provide additional evidence for an association between short-term NO_2 exposure and total mortality along with potential differences by mortality outcome. Air quality characteristics and study specific details for the studies evaluated in this section are provided in Table 5-59.

Table 5-59 Air quality characteristics of studies evaluated in the 2008 Integrated Science Assessment for Oxides of Nitrogen and recently published multicity and select single-city studies.

Study	Location (Years)	Mortality Outcome(s)	Exposure Assignment	Averaging Time	Mean Concentration ppb	Upper Percentile Concentrations ppb
Biggeri et al. (2005)	8 Italian cities (1990−1999)	Total, cardiovascular, respiratory	Average of NO_2 concentrations across all monitors in each city (1−6 monitors). Monitors influenced by local traffic excluded.	24-h avg	30.1−55.0	95th: 45.8−94.0 Max: 62.6–160.7
Brook et al. (2007)	10 Canadian cities (1984−2000)	Total	Average of NO_2 concentrations across all monitors in each city.	24-h avg	NR	NR
Burnett et al. (2004)	12 Canadian cities (1981−1999)	Total, cardiovascular, respiratory	Average of NO_2 concentrations across all monitors in each city.	24-h avg	10.0−26.4	NR
HEI (2003)	58 U.S. cities[a] (1987−1994)	Total	Average of NO_2 concentrations across all monitors in each city.	24-h avg	9.2−39.4	NR
Hoek (2003)	the Netherlands (1986−1994)	Total	15 NO_2 monitors across the study area, mean concentration calculated in each region then weighted by population density in each region.	24-h avg	NR	NR
Samoli et al. (2006)	30 European cities (1990−1997)	Total, cardiovascular, respiratory	Average of NO_2 concentrations across all monitors in each city.	1-h max[b]	24.0−80.5	90th: 33.1–132.5
Simpson et al. (2005b)	4 Australian cities (1996−1999)	Total, cardiovascular, respiratory	Average of NO_2 concentrations across all monitors in each city.	1-h max	16.3−23.7	Max: 96.0–111.5
Stieb et al. (2003)	Meta-analysis, worldwide (Years NR)	Total	NA	NR	NR	NR
†Atkinson et al. (2012)	Meta-analysis, Asia (Years NR)	Total, cardiovascular, respiratory	NA	NR	NR	NR

Table 5-59 (Continued): Air quality characteristics of studies evaluated in the 2008 Integrated Science Assessment for Oxides of Nitrogen and recently published multicity and select single-city studies.

Study	Location (Years)	Mortality Outcome(s)	Exposure Assignment	Averaging Time	Mean Concentration ppb	Upper Percentile Concentrations ppb
†Bellini et al. (2007)	15 Italian cities (1996−2002)	Total, cardiovascular, respiratory	NR	24-h avg	NR	NR
†Berglind et al. (2009)	5 European cities (1992−2002)	Total	Average of NO_2 concentrations across all monitors in each city.	24-h avg	11.0−35.4	NR
†Chen et al. (2012b)	17 Chinese cities (1996−2010[c])	Total, cardiovascular, respiratory	Average of NO_2 concentrations across all monitors in each city (2−13 monitors).[e]	24-h avg	13.5−34.8	Max: 55.1−132.1
†Chen et al. (2013)	8 Chinese cities (1996−2008[d])	Stroke	Average of NO_2 concentrations across all monitors in each city (2−12 monitors).[e]	24-h avg	19.7−35.6	NR
†Chiusolo et al. (2011)	10 Italian cities[e] (2001−2005)	Total, cardiovascular, cerebrovascular, respiratory	If more than 1 monitor, average of NO_2 concentrations across all monitors in each city (1−5 monitors).	24-h avg	13.8−35.0	90th: 21.7−48.8
†Kan et al. (2010); Kan et al. (2008)	Shanghai, China (2001−2004)	Total, cardiovascular, respiratory	Average of NO_2 concentrations across 6 monitors.	24-h avg	35.4	75th: 42.1 Max: 134.9
†Faustini et al. (2013)	6 Italian cities (2001−2005)	Respiratory (out-of-hospital)	Average of NO_2 concentrations over all monitors within each city (1−5 monitors).[f]	24-h avg	24.5−35.1	NR
†Ito et al. (2011)	New York, NY (2000−2006)	Cardiovascular	Average of NO_2 concentrations across all monitors.	24-h avg	28.7	NR
†Sacks et al. (2012)	Philadelphia, PA (1992−1995)	Cardiovascular	Central site monitor.	1-h max	47.4	Max: 146.7
†Meng et al. (2013)	4 Chinese cities (1996−2008[c])	COPD	Average of NO_2 concentrations across all monitors in each city (7−8 monitors).[d]	24-h avg	30.6−35.4	NR
†Moolgavkar et al. (2013)	72 U.S. cities[g] (1987−2000)	Total	Average of NO_2 concentrations across all monitors in each city.	24-h avg	NR	NR
†Shin et al. (2012)	24 Canadian cities (1984−2004)	Cardiopulmonary	If more than 1 monitor, average of NO_2 concentrations across all monitors in each city (1−8 monitors).	24-h avg	8.7−25.0	NR

Table 5-59 (Continued): Air quality characteristics of studies evaluated in the 2008 Integrated Science Assessment for Oxides of Nitrogen and recently published multicity and select single-city studies.

Study	Location (Years)	Mortality Outcome(s)	Exposure Assignment	Averaging Time	Mean Concentration ppb	Upper Percentile Concentrations ppb
†Stieb et al. (2008)	12 Canadian cities (1981−2000)	Total	If more than 1 monitor, average of NO_2 concentrations across all monitors in each city.	3-h max	1981−1990: 24.7−42.6 1991−2000: 16.3−39.2	NR
†Wong et al. (2010); Wong et al. (2008)	4 Asian cities (1996−2004[h])	Total cardiovascular respiratory	Average of NO_2 concentrations across all monitors in each city (6−10 monitors).	24-h avg	23.2−34.6	75th: 28.5−41.2 Max: 72.6−131.9

COPD - chronic obstructive pulmonary disease, NA = not available, NO_2 = nitrogen dioxide, NR = not reported.

[a]Of the 90 cities included in the NMMAPS analysis only 58 had NO_2 data.

[b]Samoli et al. (2006) estimated 1-h max concentrations for each city by multiplying 24-h avg concentrations by 1.64.

[c]Study period varied for each city and encompassed 2 to 7 yr. Hong Kong was the only city that had air quality data prior to 2000.

[d]These monitors were "mandated to not be in the direct vicinity of traffic or of industrial sources, and not be influenced by local pollution sources, and to avoid buildings, or those housing large emitters, such as coal-, waste-, or oil-burning boilers, furnaces, and incinerators" (Chen et al., 2013; Meng et al., 2013; Chen et al., 2012b).

[e]Only 9 cities (Cagliari was excluded) were included in the formal analysis of examining potential factors that could increase the risk of mortality due to short-term NO_2 exposure.

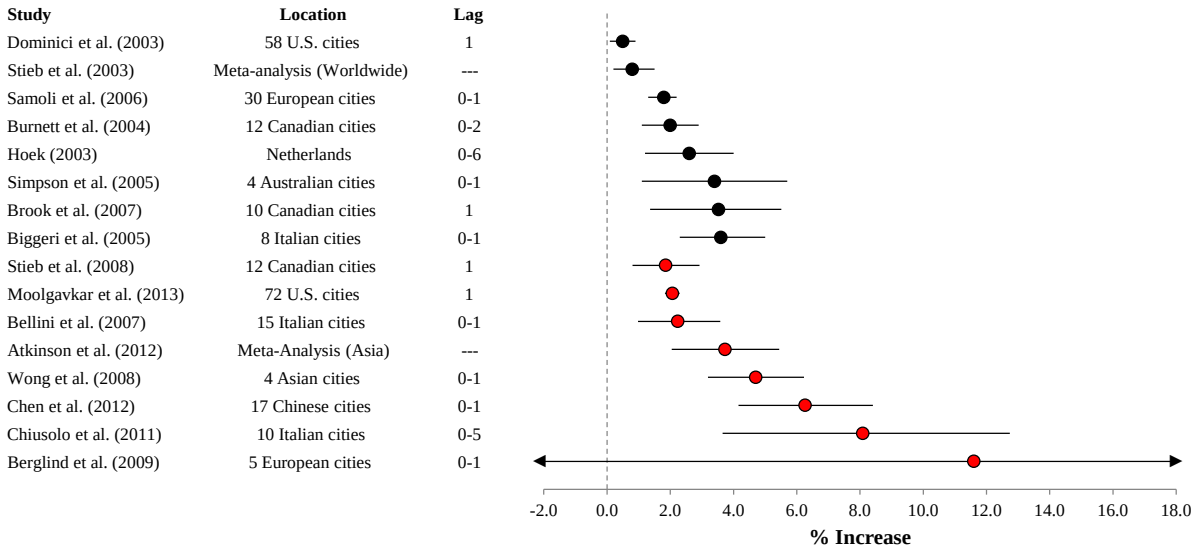
[f]Information on the monitors used in this study were obtained from Colais et al. (2012).

[g]Of the 108 cities included in the analyses using NMMAPS data only 72 had NO_2 data.

[h]The study period varied for each city, Bangkok: 1999−2003, Hong Kong: 1996−2002, and Shanghai and Wuhan: 2001−2004.

†Studies published since the 2008 ISA for Oxides of Nitrogen.

As demonstrated in Figure 5-22 and Table 5-60 multicity studies evaluated in the 2008 ISA for Oxides of Nitrogen and those recently published, consistently provide evidence of positive associations between short-term NO_2 exposure and total (non-accidental) mortality. In these multicity studies, the associations observed were in analyses that primarily examined all ages, the exceptions being Chiusolo et al. (2011) and Berglind et al. (2009), who both focused on the risk of mortality attributed to air pollution in the population ≥35 years of age. Across these studies, associations between short-term NO_2 exposure and mortality were examined primarily in the total population; however, Berglind et al. (2009) focused on a subset of the population, i.e., MI survivors. The large effect estimate for Berglind et al. (2009) could be attributed to the larger mortality rate for MI survivors: 30-day mortality rate of 14−15% and 1-year mortality rate of 22−24%, compared to populations examined in the other multicity studies (Berglind et al., 2009).



Note: Results are presented for per a 20-ppb increase in 24-h avg nitrogen dioxide concentrations or a 30-ppb increase in 1-h max nitrogen dioxide concentrations. Black = studies reviewed in the 2008 Integrated Science Assessment for Oxides of Nitrogen, Red = recent studies.

Figure 5-22 Summary of multicity studies evaluated in the 2008 Integrated Science Assessment for Oxides of Nitrogen and recently published studies that examined the association between short-term nitrogen dioxide exposure and total mortality.

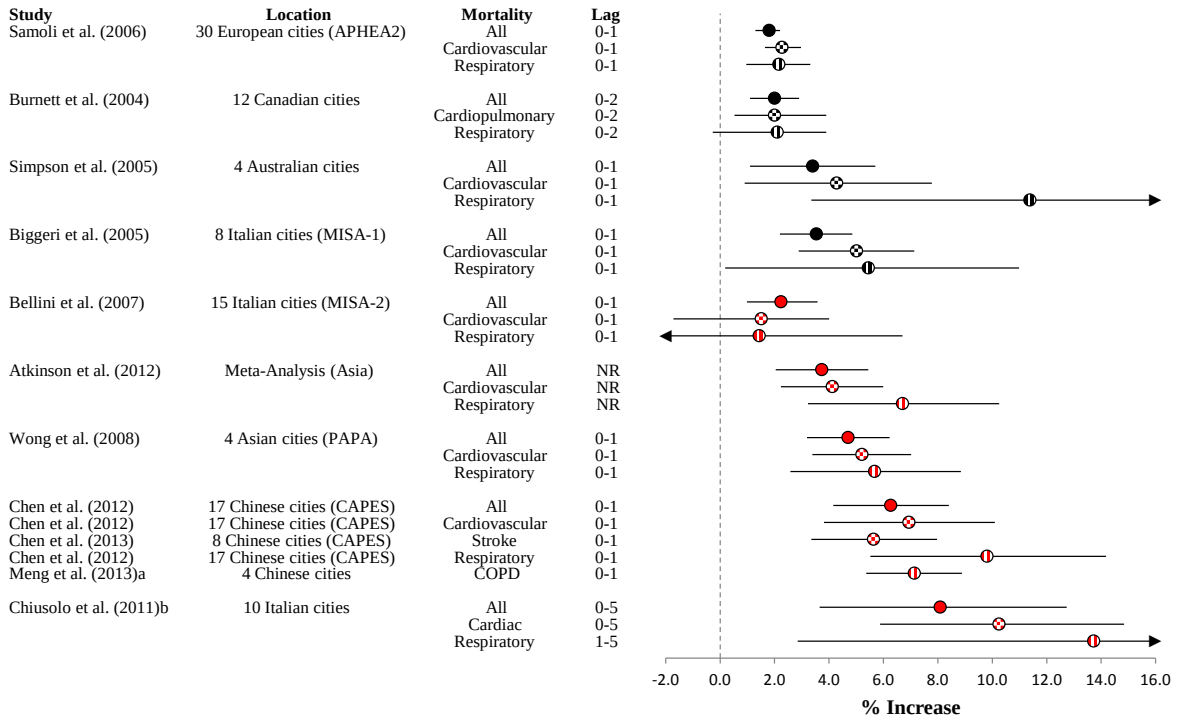
Table 5-60 Corresponding percentage increase in total mortality (95% CI) for Figure 5-22

Study	Location	Age	Lag	Averaging Time	% Increase (95% CI)
Dominici et al. (2003)	58 U.S. cities	All	1	24-h avg	0.5 (0.09, 0.90)
Stieb et al. (2003)	Meta-analysis (worldwide)	All	---	24-h avg	0.8 (0.20, 1.5)
Samoli et al. (2006)	30 European cities	All	0−1	1-h max	1.8 (1.3, 2.2)
Burnett et al. (2004)	12 Canadian cities	All	0−2	24-h avg	2.0 (1.1, 2.9)
Hoek (2003)	the Netherlands	All	0−6	24-h avg	2.6 (1.2, 4.0)
Simpson et al. (2005b)	4 Australian cities	All	0−1	1-h max	3.4 (1.1, 5.7)
Brook et al. (2007)	10 Canadian cities	All	1	24-h avg	3.5 (1.4, 5.5)
Biggeri et al. (2005)	8 Italian cities	All	0−1	1-h max	3.6 (2.3, 5.0)
†Stieb et al. (2008)	12 Canadian cities	All	1	3-h max	1.9 (0.80, 2.9)
†Moolgavkar et al. (2013)	72 U.S. cities	All	1	24-h avg	2.1 (1.8, 2.3)
†Bellini et al. (2007)	15 Italian cities	All	0−1	24-h avg	2.2 (1.0, 3.6)
†Atkinson et al. (2012)	Meta-analysis (Asia)	All	---	24-h avg	3.7 (2.1, 5.4)
†Wong et al. (2008)	4 Asian cities	All	0−1	24-h avg	4.7 (3.2, 6.2)
†Chen et al. (2012b)	17 Chinese cities	All	0−1	24-h avg	6.3 (4.2, 8.4)
†Chiusolo et al. (2011)	10 Italian cities	≥35	0−5	24-h avg	8.1 (3.7, 12.7
†Berglind et al. (2009)	5 European cities	≥35	0−1	24-h avg	11.6 (−5.9, 32.4)

CI = confidence interval.

†Studies published since the 2008 ISA for Oxides of Nitrogen.

When focusing on cause-specific mortality, recent multicity studies have reported similar patterns of associations to those evaluated in the 2008 ISA for Oxides of Nitrogen with some evidence of larger respiratory mortality risk estimates (Figure 5-23 and Table 5-61). However, in a study of 15 Italian cities, Bellini et al. (2007) observed smaller cardiovascular and respiratory mortality risk estimates compared to total mortality, which contradicts the results of Biggeri et al. (2005) of which Bellini et al. (2007) is an extension. Additionally, the total mortality results of Bellini et al. (2007) are smaller in magnitude than those observed in Biggeri et al. (2005).



Note: Black symbols = multicity studies evaluated in the 2008 Integrated Science Assessment for Oxides of Nitrogen; Red symbols = recent studies. Filled circle = total mortality; Crosshatch = cardiovascular mortality; Vertical lines = respiratory mortality. a = Although the study was not part of the CAPES study, it included four of the cities also included in CAPES; b = Study focused on individuals ≥35 years of age while the other studies focused on all ages.

Figure 5-23 Percentage increase in total, cardiovascular, and respiratory mortality from multicity studies for a 20-ppb increase in 24-hour average or 30-ppb increase in 1-hour maximum nitrogen dioxide concentrations.

Table 5-61 Corresponding percentage increase (95% CI) for Figure 5-23.

Study	Location	Age	Lag	Averaging Time	Mortality	% Increase (95% CI)
Samoli et al. (2006)	30 European cities	All	0−1	1-h max	Total Cardiovascular Respiratory	1.8 (1.3, 2.2) 2.3 (1.7, 3.0) 2.2 (1.0, 3.4)
Burnett et al. (2004)	12 Canadian cities	All	0−2	24-h avg	Total Cardiovascular Respiratory	2.0 (1.1, 2.9) 2.0 (0.5, 3.9) 2.1 (−0.3, 3.9)
Simpson et al. (2005b)	4 Australian cities	All	0−1	1-h max	Total Cardiovascular Respiratory	3.4 (1.1, 5.7) 4.3 (0.9, 7.8) 11.4 (3.4, 19.7)
Biggeri et al. (2005)	8 Italian cities	All	0−1	1-h max	Total Cardiovascular Respiratory	3.5 (2.2, 4.9) 5.0 (2.9, 7.1) 5.4 (0.2, 11.0)
†Bellini et al. (2007)	15 Italian cities	All	0−1	24-h avg	Total Cardiovascular Respiratory	2.2 (1.0, 3.6) 1.5 (−1.7, 4.0) 1.4 (−2.4, 6.7)
†Atkinson et al. (2012)	Meta-analysis (Asia)	All	---	24-h avg	Total Cardiovascular Respiratory	3.7 (2.1, 5.4) 4.1 (2.2, 6.0) 6.7 (3.2, 10.3)
†Wong et al. (2008)	4 Asian cities	All	0−1	24-h avg	Total Cardiovascular Respiratory	4.7 (3.2, 6.2) 5.2 (3.4, 7.0) 5.7 (2.6, 8.8)
†Chen et al. (2012b)	17 Chinese cities	All	0−1	24-h avg	Total Cardiovascular	6.3 (4.2, 8.4) 6.9 (3.8, 10.1)
†Chen et al. (2013)	8 Chinese cities	All	0−1	24-h avg	Stroke	5.6 (3.4, 8.0)
†Chen et al. (2012b)	17 Chinese cities	All	0−1	24-h avg	Respiratory	9.8 (5.5, 14.2)
†Meng et al. (2013)	4 Chinese cities	All	0−1	24-h avg	COPD	7.1 (5.4, 8.9)
†Chiusolo et al. (2011)	10 Italian cities	≥35	0−5	24-h avg	Total Cardiovascular	8.1 (3.7, 12.7) 10.3 (5.9, 14.8)
			1−5		Respiratory	13.7 (2.9, 25.8)

CI = confidence interval, COPD = chronic obstructive pulmonary disease.

†Studies published since the 2008 ISA for Oxides of Nitrogen.

5.4.4 Potential Confounding of the Nitrogen Dioxide-Mortality Relationship

A key uncertainty of the NO_2-mortality relationship identified in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a) was whether NO_2 acts as a surrogate of another unmeasured pollutant. As such, although the multicity studies evaluated in the 2008 ISA for Oxides of Nitrogen reported consistent evidence of an association between short-term NO_2 exposure and mortality that persisted in copollutant models, these studies often concluded that the observed mortality effects could not be attributed solely to NO_2. Copollutant analyses conducted in recent studies further attempted to identify whether NO_2 has an independent effect on mortality. Additionally, recent studies have examined whether the extent of temporal adjustment employed adequately controls for the potential confounding effects of season on the NO_2-mortality relationship.

Copollutant Confounding

In the examination of the potential confounding effects of copollutants on the NO_2-mortality relationship, it is informative to evaluate whether NO_2 risk estimates remain robust in copollutant models, specifically with traffic-related pollutants (e.g., $PM_{2.5}$, EC, CO), and whether NO_2 modifies the effect of other pollutants. Recent multicity studies examine the NO_2-mortality relationship by taking into consideration both of these aspects in different study designs and in different study locations (i.e., U.S., Canada, Europe, and Asia). However, copollutant analyses in these studies have not explicitly focused on traffic-related pollutants, complicating the overall interpretation of results regarding whether there is an independent effect of short-term NO_2 exposures on mortality.

In a study of 108 U.S. cities using data from the NMMAPS for 1987−2000 (of which 72 had NO_2 data), Moolgavkar et al. (2013) used a sub-sampling approach where a random sample of 4 cities was removed from the 108 cities over 5,000 bootstrap cycles to examine associations between short-term air pollution concentrations and mortality. This approach was used instead of the two-stage Bayesian hierarchical approach employed in the original NMMAPS analysis, which assumes that city-specific risk estimates are normally distributed around a national mean (Dominici et al., 2003). In a single-pollutant model using 100 degrees of freedom (~7 df/yr, which is consistent with NMMAPS) to control for temporal trends, Moolgavkar et al. (2013) reported a 2.1% (95% CI: 1.8, 2.3) increase in total (nonaccidental) mortality at lag 1 day for a 20-ppb increase in 24-h avg NO_2 concentrations. The single-pollutant result is larger in magnitude than that observed in (Dominici et al., 2003), which only included 58 cities in the NO_2 analysis

(Figure 5-22). In a copollutant analysis with PM_{10}, the NO_2-mortality risk estimate was relatively unchanged (1.9% [95% CI: 1.3, 2.4]), and similar to the copollutant results in (Dominici et al., 2003).

Stieb et al. (2008) reported results consistent with Moolgavkar et al. (2013) in a study that focused on the development of a new air quality health index in Canada. Focusing on lag day 1 and models using 10 df per year, Stieb et al. (2008) examined whether copollutants confounded the single-pollutant results in both copollutant and multipollutant models with CO, O_3, PM_{10}, $PM_{2.5}$, and SO_2. However, the study did not clearly identify which results pertained to which model. As stated previously in this ISA, it is important to note that multipollutant models are difficult to interpret due to the multicollinearity often observed between pollutants and as a result are not used to inform upon whether there is evidence of copollutant confounding. In models using all available data and limited to days with PM data the results of the copollutant and multipollutant analyses conducted by Stieb et al. (2008) indicate that the NO_2-mortality relationship remain relatively unchanged when adjusted for other pollutants, including some traffic-related pollutants (quantitative results not presented).

Additional studies conducted in Europe and Asia also provide evidence indicating that NO_2-mortality associations remain robust in copollutant models; however, these studies have also not focused on traffic-related pollutants. Chiusolo et al. (2011) conducted a multicity study of 10 Italian cities using a time-stratified, case-crossover approach as part of the Italian Epi Air multicenter study "Air Pollution and Health: Epidemiological Surveillance and Primary Prevention." The authors reported consistent, positive associations for total and cause-specific mortality (i.e., cardiac, cerebrovascular, and respiratory), ranging from an 8.1 to 13.7% increase for a 20-ppb increase in 24-hour NO_2 concentrations using an unconstrained distributed lag of 0−5 days (lag 1−5 days was used for respiratory mortality). In copollutant analyses, NO_2 risk estimates remained robust in models with PM in all-year analyses and with O_3 in analyses restricted to the summer season (i.e., April−September) (Table 5-62).

Table 5-62 Percentage increase in total and cause-specific mortality for a 20-ppb increase in 24-hour average NO_2 concentrations in single- and co-pollutant models with PM_{10} in all-year analyses or O_3 in summer season analyses.

Mortality	Season	Model	% Increase (95% CI)
All natural	All-year	NO_2 (lag 0−5)	8.1 (3.7, 12.7)
		With PM_{10} (lag 0−5)	7.5 (1.9, 13.5)
	April−September	NO_2 (lag 0−5)	17.8 (12.3, 23.6)
		With O_3 (lag 0−5)	18.2 (13.1, 23.6)
Cardiac	All-year	NO_2 (lag 0−5)	10.3 (5.9, 14.8)
		With PM_{10} (lag 0−5)	10.1 (4.0, 16.4)
	April−September	NO_2 (lag 0−5)	19.2 (11.4, 27.4)
		With O_3 (lag 0−5)	18.8 (10.7, 27.5)
Cerebrovascular	All-year	NO_2 (lag 0−5)	9.1 (−0.5, 19.7)
		With PM_{10} (lag 0−5)	9.9 (−2.6, 24.1)
	April−September	NO_2 (lag 0−5)	33.0 (19.2, 48.3)
		With O_3 (lag 0−5)	30.2 (13.9, 48.8)
Respiratory	All-year	NO_2 (lag 1−5)	13.7 (2.9, 25.8)
		With PM_{10} (lag 0−5)	13.4 (2.9, 24.8)
	April−September	NO_2 (lag 1−5)	41.3 (16.2, 71.7)
		With O_3 (lag 0−5)	43.4 (14.6, 79.5)

Note: Concentrations converted from µg/m^3 to ppb using the conversion factor of 0.532, assuming standard temperature (25°C) and pressure (1 atm).

CI = confidence interval, NO_2 = nitrogen dioxide, O_3 = ozone, PM = particulate matter.

Source: Reproduced with permission from Environmental Health Perspectives (Chiusolo et al., 2011).

The Public Health and Air Pollution in Asia (PAPA) study as well as the CAPES collectively found that the NO_2-mortality association remains robust in copollutant models with other criteria air pollutants in analyses conducted in Asian cities. The PAPA study examined the effect of air pollution on mortality in four cities, one in Thailand (i.e., Bangkok) and three in China (i.e., Hong Kong, Shanghai, and Wuhan) (Wong et al., 2010; Wong et al., 2008). In these study locations, PM_{10} and SO_2 concentrations are much higher than those reported in the U.S.; however, NO_2 and O_3 concentrations are

fairly similar (Wong et al., 2010; Wong et al., 2008). Copollutant analyses were only conducted in the individual cities; a combined four-city analysis was not conducted. In models using lag 0−1 days NO_2 concentrations in the Chinese cities, NO_2 mortality risk estimates were relatively unchanged in copollutant models (quantitative results not presented). However, in Bangkok, the NO_2-mortality risk estimate was attenuated in models with PM_{10}.

The results from the Chinese cities in the PAPA study are consistent with those found in CAPES (Chen et al., 2012b). In a two-stage Bayesian hierarchical model, where the first stage followed the PAPA protocol, Chen et al. (2012b) reported a 6.3% increase (95% CI: 4.2, 8.4) in total mortality, 6.9% increase (95% CI: 3.8, 10.1) for cardiovascular mortality, and 9.8% increase (95% CI: 5.5, 14.2) for respiratory mortality for a 20-ppb increase in 24-h avg NO_2 concentrations at lag 0−1 days. Although NO_2 was moderately correlated with both PM_{10} and SO_2, 0.66 and 0.65, respectively, NO_2-mortality associations, although attenuated, remained positive across total, cardiovascular, and respiratory mortality with the percentage increase in mortality ranging from 4.6−6.7% in copollutant models with PM_{10} and 5.2−7.0% in models with SO_2 for a 20-ppb increase in 24-h avg NO_2 concentrations.

In addition to examining whether copollutants confound the NO_2-mortality relationship, studies also conducted analyses to examine if there was any indication that NO_2 modifies the PM-mortality relationship. The Air Pollution and Health: A European and North American Approach study, although it focused specifically on examining the PM_{10}-mortality relationship, also conducted an analysis to identify whether NO_2 modifies the PM_{10}-mortality relationship. In both the European and U.S. data sets, as mean NO_2 concentrations and the NO_2/PM_{10} ratio increased, there was evidence that the risk of PM_{10} mortality increased. These results are consistent with Katsouyanni et al. (2003) and Katsouyanni et al. (2001), who reported higher PM risk estimates in cities with higher NO_2 concentrations, suggesting that NO_2 and PM may be effect modifiers of each other.

Temporal Confounding

Recent studies have also examined whether the NO_2-mortality relationship is subject to temporal confounding. These studies have focused on examining the effect of increasing the number of df employed per year to control for temporal trends on NO_2-mortality risk estimates. Using the entire data set, which encompassed the years 1981−2000, Stieb et al. (2008) examined the effect of using an alternative number of df to adjust for seasonal cycles on NO_2-mortality risk estimates. In analyses of single-day lags from 0 to 2 days in single-pollutant models, the authors reported comparable risk estimates for each individual lag day when using 6, 8, 10, 12, and 14 df per year. Similar to Stieb et al.

(2008), the PAPA study also examined the impact of alternative approaches to controlling for temporal trends on mortality risk estimates. In models using 4, 6, 8, 10, or 12 df per year, Wong et al. (2010) also reported relatively similar results across the df per year specified, with some evidence for a slight attenuation of the NO_2-mortality association in Wuhan, China as the df per year increased.

Unlike Stieb et al. (2008) and Wong et al. (2010), who conducted a systematic analysis of the influence of increasing the df per year to control for temporal trends on the NO_2-mortality relationship, Moolgavkar et al. (2013) only compared models that used 50 df (~3.5 df per year) or 100 df (~7 df per year) in the statistical model. However, similar to both Stieb et al. (2008) and Wong et al. (2010), Moolgavkar et al. (2013) reported similar results regardless of the number of df used, 2.0% (95% CI: 1.8, 2.3) for a 20-ppb increase in 24-h avg NO_2 concentrations at lag 1 day in the 50 df model and 2.1% (95% CI: 1.8, 2.3) in the 100 df model.

5.4.5 Modification of the Nitrogen Dioxide-Mortality Relationship

To date, a limited number of studies have examined potential effect measure modifiers of the NO_2-mortality relationship. In the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a), Samoli et al. (2006) provided evidence of regional heterogeneity in NO_2-mortality associations and higher NO_2-mortality risk estimates in cities with a lower prevalence of smoking as part of the APHEA-2 study. Recent multicity studies conducted in Italy (Chiusolo et al., 2011), Chile (Cakmak et al., 2011b), and Asia (Chen et al., 2012b) conducted extensive analyses of potential effect measure modifiers of the NO_2-mortality relationship and identified specific factors that may characterize populations potentially at increased risk of NO_2-related mortality (see Chapter 7). These studies presented evidence indicating that older adults (≥65 years of age), females, individuals with pre-existing cardiovascular or respiratory diseases, and individuals of lower SES, specifically lower income and educational attainment, are at greater risk. It should be noted that demographic as well as socioeconomic differences between countries may complicate the interpretation of results across these studies, and subsequently the ability to make generalizations across locations regarding the factors that may modify the NO_2-mortality association.

5.4.6 Potential Seasonal Differences in the Nitrogen Dioxide-Mortality Relationship

Studies evaluated in the 2008 ISA for Oxides of Nitrogen indicated seasonal differences in the NO_2-mortality relationship with evidence of larger associations in the warm or summer season. Recent multicity studies conducted in Canada (Shin et al., 2012; Stieb et al., 2008) and Italy (Chiusolo et al., 2011; Bellini et al., 2007) further support these previous findings but also raise additional questions in light of the seasonal patterns in NO_2 concentrations observed in the U.S. and Canada (i.e., higher concentrations in the winter months compared with the summer months) and the higher personal-ambient relationship in the summer compared with the winter (Section 2.5.4).

In the 12 Canadian city study, Stieb et al. (2008) reported that NO_2-mortality risk estimates were larger in the warm season (April−September) compared with the cool season (October−March) (quantitative results not presented). These results are consistent with those reported by Shin et al. (2012) in a study that examined year-to-year changes in the association between short-term NO_2 exposure and mortality (i.e., cardiopulmonary and non-cardiopulmonary) across 24 Canadian cities during 1984−2004. In seasonal analyses, NO_2 associations with cardiopulmonary mortality at lag 0−2 days were observed to be stronger in the warm season (April−September) compared with the cold season (October−March). Shin et al. (2012) suggest that the larger NO_2 mortality effects in the warm season could be due to the role of NO_2 in the atmospheric reactions that form O_3, and subsequently suggests that the relationship between NO_2 and O_3 does not allow for a clear assessment of the independent effects of NO_2. However, in Canada, as well as the U.S., NO_2 concentrations are higher in the cold season compared to the warm season. Additionally, NO_2 and O_3 are not well correlated during the summer (r ranging from 0.0 to 0.40), which makes it less likely O_3 is a confounder of the NO_2-mortality relationship (Section 3.4.4.1).

To date, U.S.-based multicity studies have not examined whether the seasonal patterns of NO_2-mortality associations observed in Canadian multicity studies are similar in the U.S. However, a few single-city U.S.-based studies that focused on cardiovascular mortality inform upon whether there is evidence of seasonal differences in NO_2-total mortality associations (Sacks et al., 2012; Ito et al., 2011). In a study conducted in New York City that examined the association between short-term exposure to air pollution and cardiovascular mortality, Ito et al. (2011) reported similar effect estimates in all-year (1.8% [95% CI: 0.17, 3.3] for a 20-ppb increase in 24-h avg avg NO_2 concentrations at lag 1 d day) and seasonal (warm: 1.8% [95% CI: −0.4, 3.9]; cold: 2.3% [95% CI: 0.0, 4.7]) analyses. It should be noted that the study did not conduct copollutant analyses and the NO_2-mortality pattern of associations was similar to that observed for $PM_{2.5}$ and EC.

Sacks et al. (2012) also examined potential seasonal differences in the NO_2-cardiovascular mortality association in a study conducted in Philadelphia, PA that examined the influence of various approaches to control for seasonality and the potential confounding effects of weather on the air pollution-cardiovascular mortality relationship. Across models, the authors found that either: NO_2-mortality associations were similar between warm and cold seasons; or that associations were slightly larger in magnitude during the warm season. These results suggest that the modeling approach employed may influence the NO_2-mortality associations observed, specifically with regard to whether there is evidence of seasonal differences in associations, but the various approaches did not influence the direction of the observed association.

Multicity studies conducted in Italy provide evidence consistent with that observed in the Canadian multicity studies. In the MISA-2 study, Bellini et al. (2007) reported larger NO_2-mortality risk estimates in the summer (April−September) compared with the winter (October−March) for total (6.4 vs. 0.9% for a 20-ppb increase in 24-h avg NO_2 concentrations at lag 0−1 days), respiratory (9.1 vs. −0.04%), and cardiovascular (7.3 vs. −0.2%) mortality. In an analysis of 10 Italian cities, Chiusolo et al. (2011) supports the results of Bellini et al. (2007) by indicating larger NO_2-mortality risk estimates in the warm season compared with all-year (Table 5-62) for total (nonaccidental) mortality and cause-specific mortality (i.e., cardiac, cerebrovascular, and respiratory).

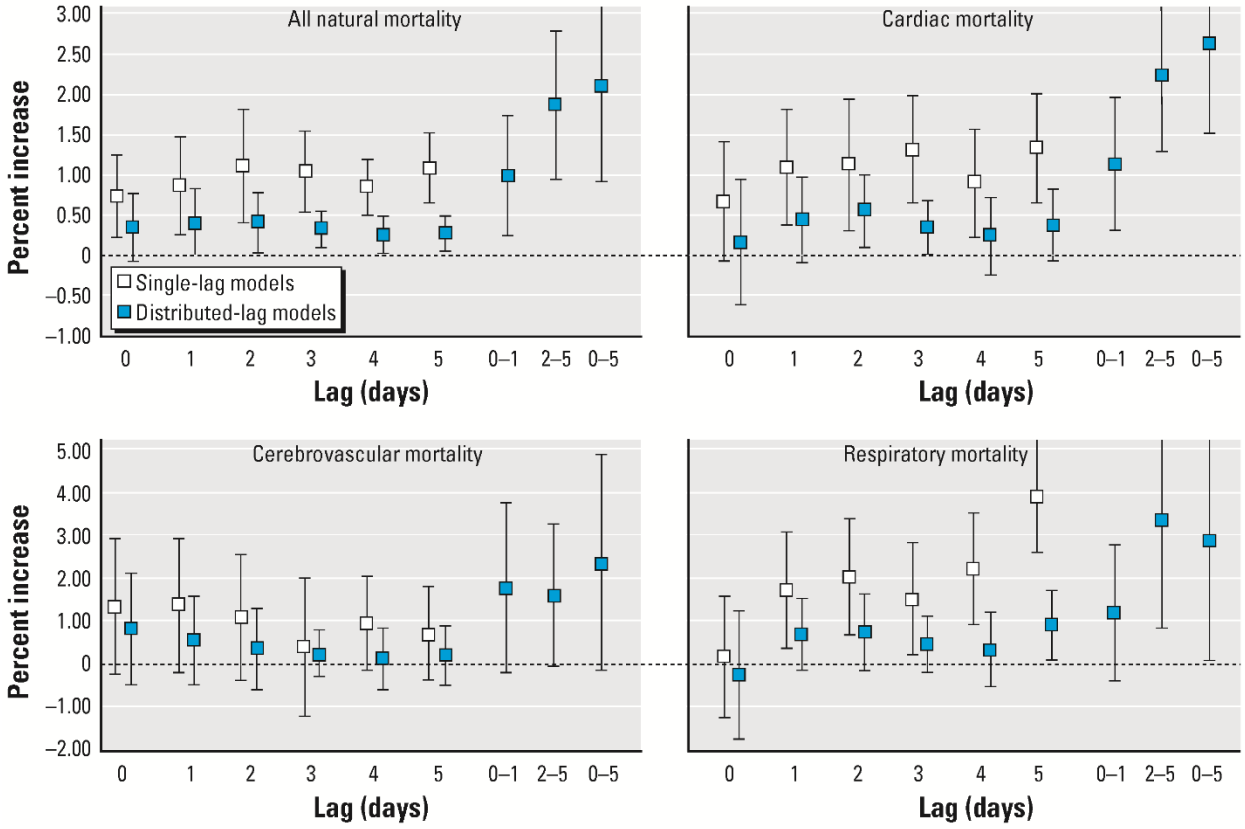
The evidence for increased NO_2-mortality associations in the warm season, as presented in the Canadian and Italian multicity studies (Shin et al., 2012; Stieb et al., 2008; Brook et al., 2007; Burnett et al., 2004), differs from the seasonal patterns observed in a study conducted in Shanghai as part of the PAPA study (Kan et al., 2010; Kan et al., 2008). The authors reported evidence of increased NO_2-mortality risk estimates in the cold season compared with the warm for total (nonaccidental) mortality (cold: 4.7 vs. warm: 1.7% for a 20-ppb increase in 24-h avg NO_2 at lag 0−1 days), cardiovascular (cold: 4.8 vs. warm: 1.1%), and respiratory mortality (cold: 10.4 vs. warm: −5.1%). Across all of the gaseous pollutants examined, mortality risk estimates were double the size or larger in the cool season, whereas PM_{10} mortality risk estimates were similar across seasons except for respiratory mortality (larger in the cool season). The authors speculate these seasonal differences could be due to seasonal exposure differences specific to Shanghai (i.e., limited time spent outdoors and increased air conditioning use in the warm season because of high temperature and humidity and heavy rain, versus more time spent outdoors and open windows in the cool season) (Kan et al., 2010; Kan et al., 2008). The results of (Kan et al., 2010; Kan et al., 2008) highlight the complexity of clearly identifying seasonal patterns in NO_2-mortality associations across locations with drastically different seasonal weather patterns.

5.4.7 Nitrogen Dioxide-Mortality Concentration-Response Relationship and Related Issues

Lag Structure of Associations

The 2008 ISA for Oxides of Nitrogen found consistent evidence across studies indicating that NO_2-mortality effects occur within the first few days after exposure, with multiple studies demonstrating the largest effect occurring the day after exposure (i.e., lag 1 day) (U.S. EPA, 2008a). Recent multicity studies have conducted additional analyses examining multiday lags, which further inform the lag structure of associations between short-term NO_2 exposure and mortality.

In the analysis of 10 Italian cities, Chiusolo et al. (2011) examined the lag structure of associations between mortality and short-term NO_2 exposure through both single-day and multiday lag analyses. Multiday analyses consisted of a priori defined lags (i.e., 0−1, 2−5, and 0−5 days) examined using an unconstrained distributed lag model. In addition to examining single-day lags of 0 to 5 days, the authors also explored the pattern of associations observed over each individual day using a constrained polynomial distributed lag model. It is important to note that the individual lag days of a constrained distributed lag model are not directly interpretable; however, this analysis allowed Chiusolo et al. (2011) to visually display the potential latency of the NO_2 effect on mortality. Collectively, the single- and multi-day lag analyses support an immediate effect of NO_2 on mortality but also provide evidence for a prolonged effect extending out to 5 days for all mortality outcomes (Figure 5-24).

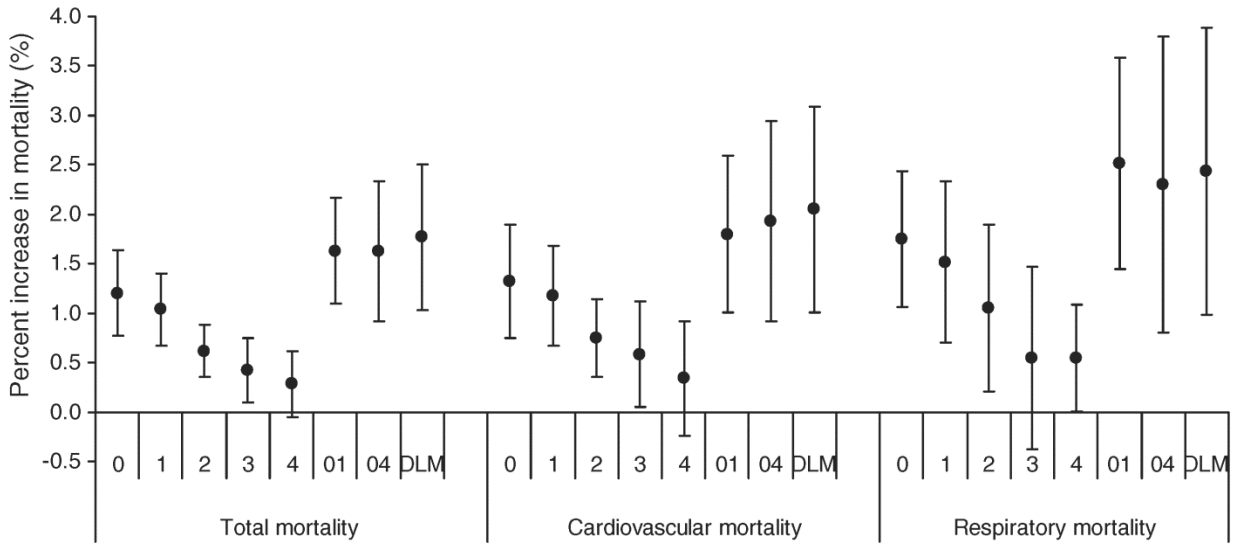


Source: Reproduced with permission from Environmental Health Perspectives (Chiusolo et al., 2011).

Figure 5-24 Percentage increase in total and cause-specific mortality due to short-term nitrogen dioxide exposure at single day lags, individual lag days of a constrained polynomial distributed lag model, and multiday lags of an unconstrained distributed lag model.

Chen et al. (2012b) also conducted an extensive analysis of the lag structure of associations for the NO_2-mortality relationship as part of CAPES. Multiday lags were examined by averaging multiple single lag days and using a constrained polynomial distributed lag model of 0−4 days. Chen et al. (2012b) reported the largest effect at single day lags of 0 and 1 and the average of lags 0−1 days indicating an immediate effect of NO_2 on mortality (Figure 5-25). However, the similar or larger magnitude 0−4 day avg and distributed lag model results provide some evidence for a delayed NO_2 effect on total, cardiovascular, and respiratory mortality, which is consistent with the results of Chiusolo et al. (2011) (Figure 5-24). These results were further supported by studies of cause-specific mortality. Chen et al. (2013) as part of CAPES, in a subset of eight Chinese cities, reported the largest magnitude of an NO_2 effect on stroke mortality at lag

0−1 days, but the association remained positive and statistically significant in an analysis of lag 0−4 days (Section 5.3.8). In an analysis of COPD mortality in four Chinese cities, Meng et al. (2013) also provided evidence of associations larger in magnitude for multiday averages, suggestive of a prolonged effect, with the largest association at lag 0−4 and slightly smaller associations for a lag of 0−1 days (Section 5.2.8). These results are consistent with Faustini et al. (2013) in a study of out-of-hospital respiratory mortality in six Italian cities that found upon examining both single- and multi-day lags the strongest associations with NO_2 were for lags of 2−5 and 0−5 days.



Percentage increase (mean and 95% CI) of daily mortality associated with a 10 μg/m^3 (5.3 ppb) increase of NO_2 concentrations, using different lag structures in the CAPES cities. Multiday average lag 01 corresponds to 2-day moving average, and lag 04 corresponds to 5-day moving average of NO_2 concentration of the current and previous 4 days. DLM: polynomial distributed lag model, representing the cumulative effects of NO_2.

Source: Reprinted with permission of Elsevier Ltd. (Chen et al., 2012b).

Figure 5-25 Percentage increase in total and cause-specific mortality due to short-term nitrogen dioxide exposure in single- and multi-day lag models.

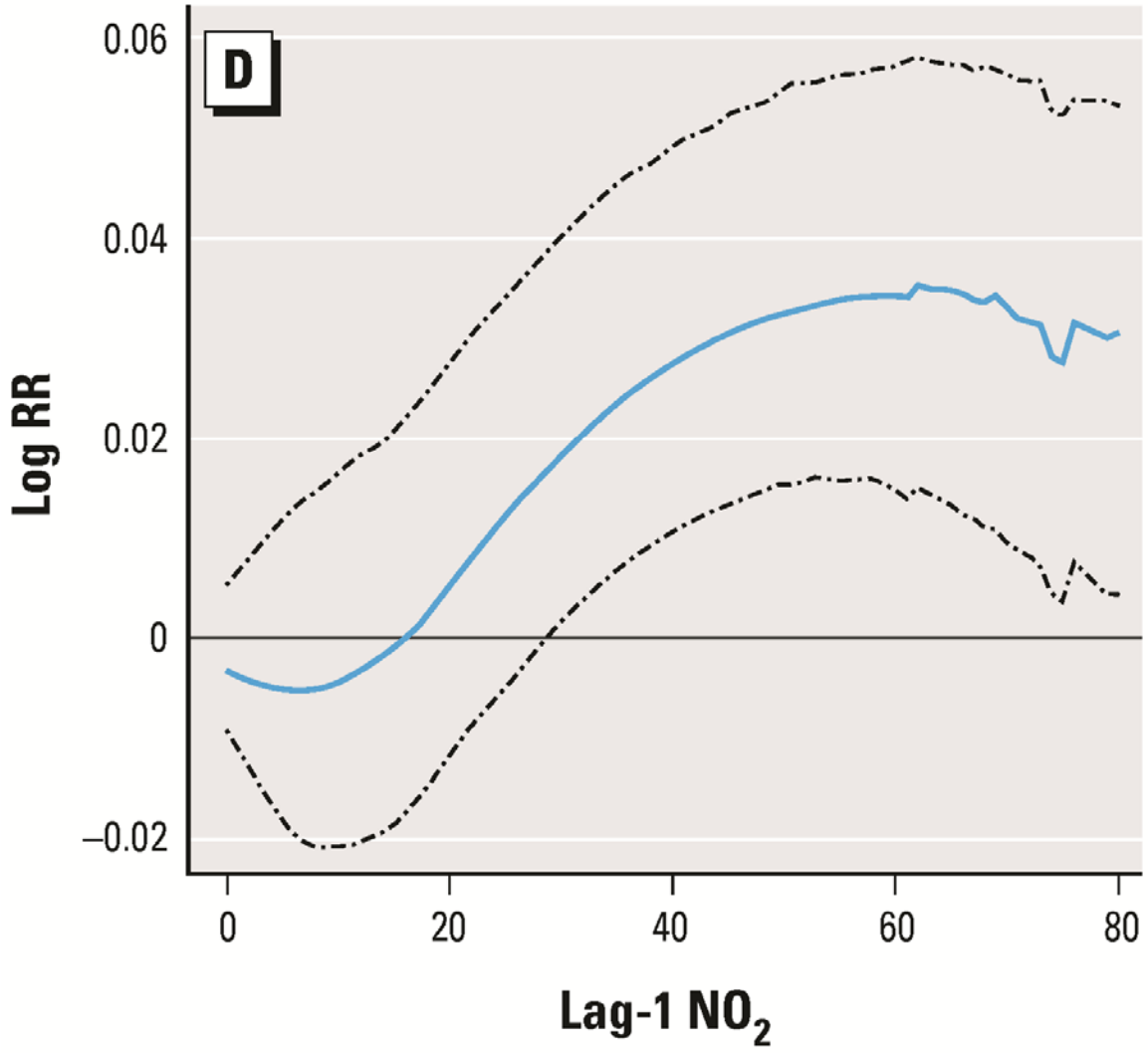
Additional studies that examined associations between NO_2 and mortality at single-day lags or multiday averages provide evidence that is consistent with those studies evaluated in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a), which demonstrated strong associations between NO_2 and mortality at lag 1. In the analysis of 12 Canadian cities, Stieb et al. (2008) found the strongest association between short-term NO_2 exposure and mortality at lag 1 when examining single-day lags of 0−2 days. Wong et al. (2008) and

Wong et al. (2010) examined single and multiday lags in each individual city in the PAPA study. In the three Chinese cities, similar to Stieb et al. (2008), the authors reported evidence of immediate effects of NO_2 on mortality; with the strongest association occurring for a 0−1 day lag. However, in Bangkok, the lag structure of associations was different and more in line with those observed in Chiusolo et al. (2011) and Chen et al. (2012b), with the strongest association occurring at a lag of 0−4 days.

Concentration-Response Relationship

The studies evaluated in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a) that examined the association between short-term NO_2 exposure and mortality did not conduct formal analyses of the C-R relationship. Recent studies published since the completion of the 2008 ISA for Oxides of Nitrogen have examined the NO_2-mortality C-R relationship in both multi- and single-city analyses, focusing on the shape of the C-R curve and whether a threshold exists.

Using a subsampling approach, Moolgavkar et al. (2013) examined the shape of the C-R relationship between short-term air pollution exposures and mortality in the NMMAPS data set by applying a nonlinear function (i.e., natural splines with 6 df) to each pollutant. This analysis provides support for a linear relationship between short-term NO_2 exposures and mortality (Figure 5-26). Although Moolgavkar et al. (2013) state that the C-R relationship for NO_2 "suggest(s) non-linearity and threshold like behavior" the widening of the confidence intervals at the tails of the distribution prevents a clear interpretation of the shape of the curve where the data density is low. It should be noted that the confidence intervals approach zero at the low end of the NO_2 distribution due to the way the model is structured.



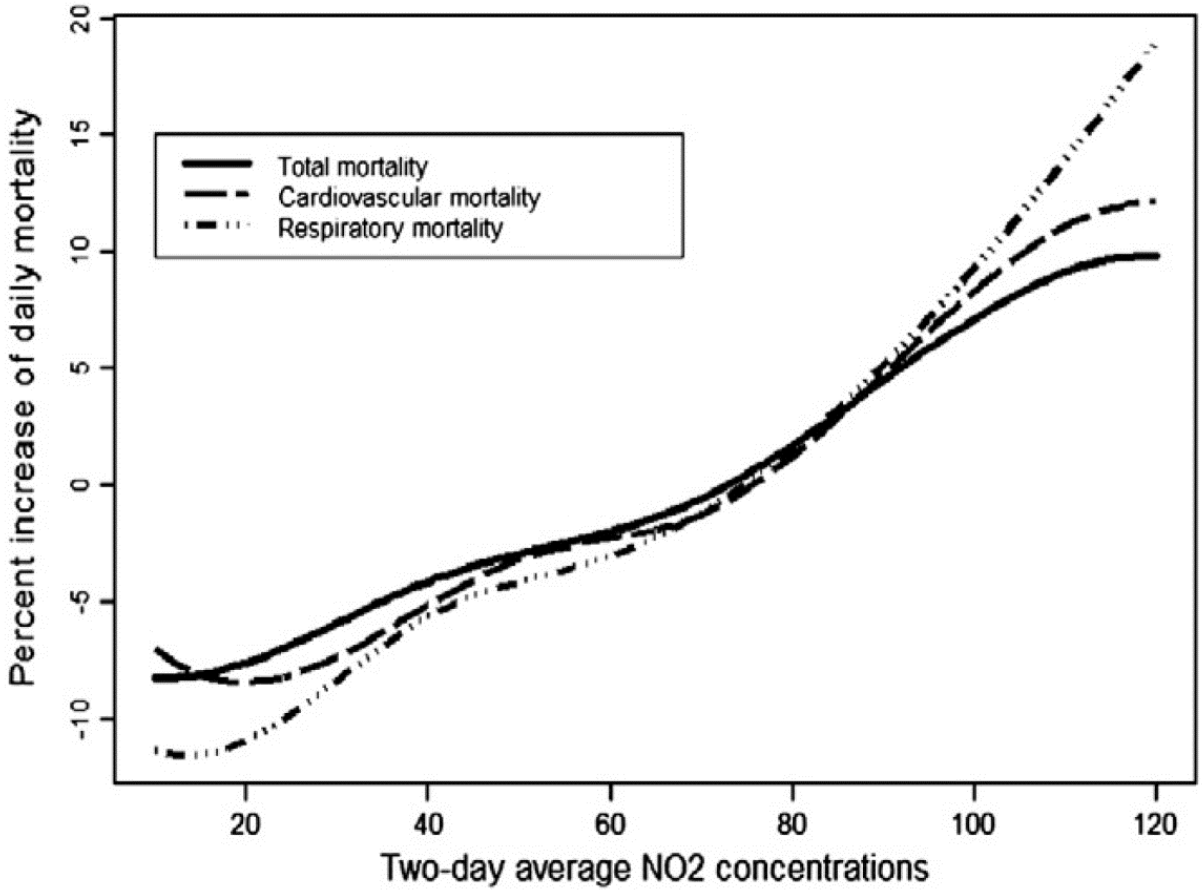
Source: Reproduced with permission from *Environmental Health Perspectives* (Moolgavkar et al., 2013).
Note: RR = relative risk.

Figure 5-26 Flexible ambient concentration-response relationship between short-term nitrogen dioxide (NO_2, in ppb) exposure and mortality at lag day 1. Pointwise means and 95% CIs adjusted for size of the bootstrap sample.

The evidence for a linear C-R relationship between short-term NO_2 exposure and mortality was further supported by Stieb et al. (2008) in a pooled analysis of 12 Canadian cities. The authors examined three functional forms (i.e., linear, quadratic, and cubic polynomial) and assessed the model fit using the sum of the Akaike Information

Criterion. Stieb et al. (2008) indicated that the linear function was the best fit of the NO_2-mortality relationship (quantitative results not presented).

Multicity studies conducted in Asia examined the NO_2-mortality C-R relationship through either a combined analysis using data from all cities or by examining the C-R relationship in individual cities. Chen et al. (2012b) examined the shape of the NO_2-mortality C-R curve across all cities as part of CAPES for total, cardiovascular, and respiratory mortality using 24-h avg NO_2 concentrations at lag 0−1 days. To limit the influence of extreme NO_2 concentrations on the shape of the C-R curve, concentrations greater than 120 $\mu g/m^3$ (62.4 ppb), which represented only 3% of the data, were excluded. The authors used a cubic spline with two knots at different concentrations for each of the mortality outcomes [40 $\mu g/m^3$ (20.8 ppb) and 70 $\mu g/m^3$ (36.4 ppb) for total mortality, 50 $\mu g/m^3$ (26.0 ppb) and 70 $\mu g/m^3$ (36.4 ppb) for cardiovascular mortality, and 40 $\mu g/m^3$ (20.8 ppb) and 60 $\mu g/m^3$ (31.2 ppb) for respiratory mortality]. Chen et al. (2012b) found evidence of a linear relationship between short-term NO_2 exposure and total and cause-specific mortality (Figure 5-27), which was confirmed by the lack of a statistically significant difference in the deviance between the spline and linear fit models. These results are further supported by examinations of the C-R relationship for the cause-specific mortality outcomes of stroke [(Chen et al., 2013); Section 5.3.10] and COPD [Meng et al. (2013); Section 5.2.8], which also provided evidence of a linear relationship.

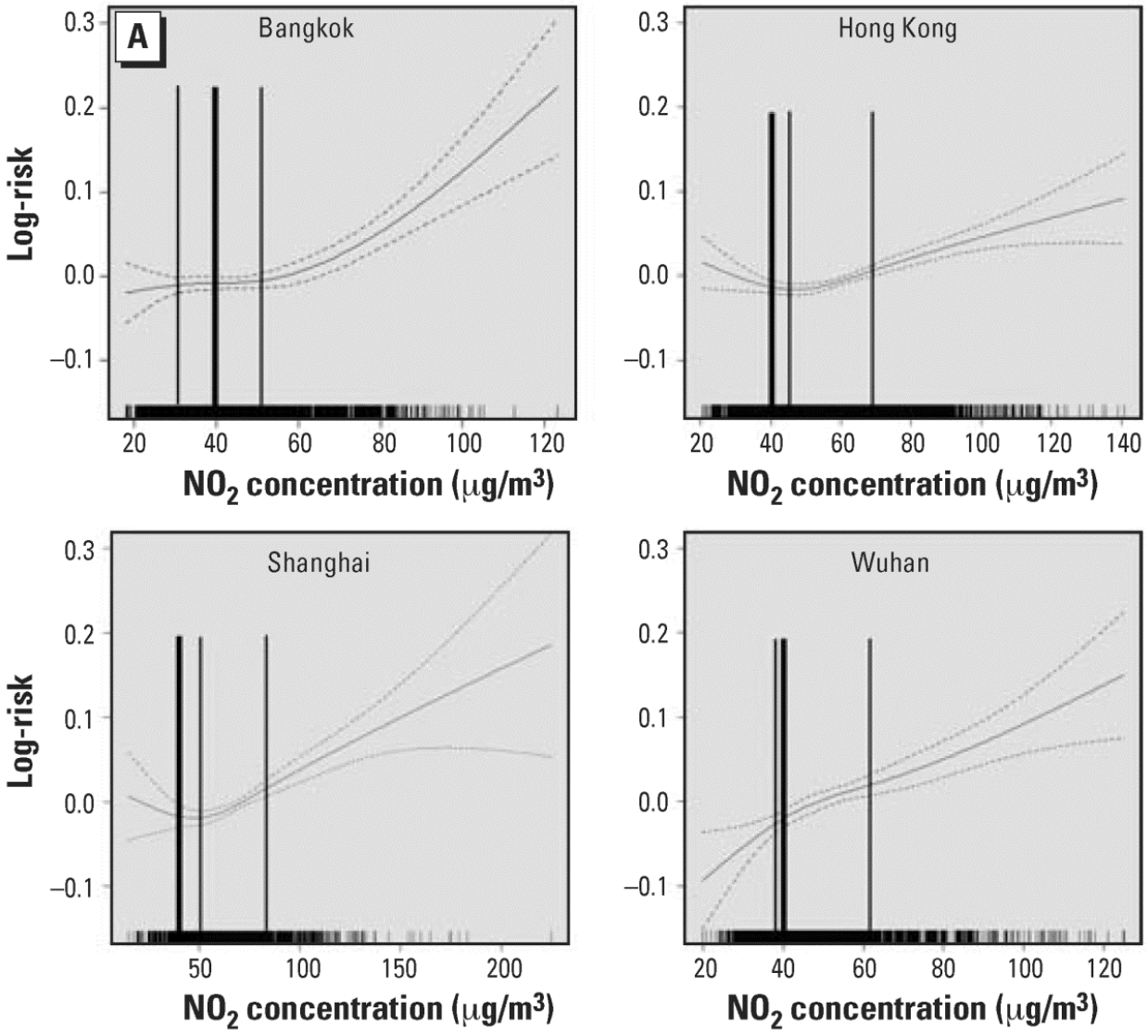


Source: Reprinted with permission of Elsevier Ltd. (Chen et al., 2012b).
Note: NO_2 concentrations on the *x*-axis are in the unit of μg/m^3.

Figure 5-27 CAPES concentration-response curve for the association between total and cause-specific mortality and 24-hour average nitrogen dioxide (NO_2) concentrations at lag 0−1 days.

The four-city PAPA study (Wong et al., 2010; Wong et al., 2008) also examined the NO_2-mortality C-R relationship but only focused on the shape of the C-R curve in each individual city. The C-R curve for the NO_2-mortality relationship was assessed by applying a natural spline smoother with 3 df to NO_2 concentrations. To examine whether the NO_2-mortality relationship deviates from linearity, the deviance between the smoothed (nonlinear) pollutant model and the unsmoothed (linear) pollutant model was examined. The C-R curves in the three Chinese cities further support the results from Stieb et al. (2008) and Chen et al. (2012b) by indicating a linear relationship between short-term NO_2 concentrations and mortality (Figure 5-28). Specifically, the evidence for linearity was strongest between the 25th and 75th percentiles of the NO_2 concentrations

in each city with some uncertainty in the shape of the C-R curve at lower concentrations where the data density is low, generally below the 25th percentile. The results of the analysis for Bangkok, which provides evidence for nonlinearity, are consistent with what has been observed in examinations of city-specific C-R curves for other air pollutants (e.g., PM and O_3). That is, the heterogeneity in city-specific risk estimates can translate into heterogeneity in the shape of the C-R curve, which has often been hypothesized to be due to city-specific exposure characteristics and demographics. The results from the Bangkok analysis highlight the difficulty in interpreting a combined C-R curve across cities, when there is evidence for city-to-city differences in the association between short-term NO_2 exposure and mortality.



Note: Thin vertical lines represent interquartile range of NO_2 concentrations in each city. The thick line was included by Wong et al. (2008) to depict where the World Health Organization 1-year averaging time standard for NO_2 of 40 μg/m^3 (20.8 ppb) could be found along the distribution of NO_2 concentrations in each city.

Source: Reproduced with permission from Environmental Health Perspectives (Wong et al., 2008).

Figure 5-28 Concentration-response curve for association between total mortality and 24-hour average nitrogen dioxide (NO_2) concentrations at lag 0−1 days in the four cities of the Public Health and Air Pollution in Asia study.

5.4.8 Summary and Causal Determination

Recent multicity studies evaluated since the completion of the 2008 ISA for Oxides of Nitrogen continue to provide consistent evidence of positive associations between short-term NO_2 exposures and total mortality. Although the body of evidence is larger, key uncertainties and data gaps still remain, which contribute to the conclusion that the evidence for short-term NO_2 exposures and total mortality is suggestive, but not sufficient to infer a causal relationship. This conclusion is consistent with that reached in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a). Recent multicity studies evaluated have further informed key uncertainties and data gaps in the NO_2-mortality relationship identified in the 2008 ISA for Oxides of Nitrogen including confounding, modification of the NO_2-mortality relationship, potential seasonal differences in NO_2-mortality associations, and the shape of the NO_2-mortality C-R relationship. However, questions remain regarding whether NO_2 is independently associated with mortality, specifically due to the lack of copollutant model analyses that focus on traffic-related pollutants. This section describes the evaluation of evidence for total mortality with respect to the causal determination for short-term NO_2 exposure, using the framework described in Table II of the Preamble. The key evidence, as it relates to the causal framework, is summarized in Table 5-63.

Collectively, the evidence from recent multicity studies of short-term NO_2 exposures and mortality consistently demonstrate the NO_2-mortality association is robust in copollutant models with PM_{10}, O_3, and SO_2. However, NO_2 is often highly correlated with other traffic-related pollutants complicating the ability to disentangle the independent effects of NO_2 from those of other measured or unmeasured pollutants associated with traffic (Section 1.4.3) (Figure 3-6), adding uncertainty to the interpretation of the association between NO_2 and total mortality. In addition, studies that focused on PM and examined whether NO_2 modified the PM-mortality relationship reported that PM risk estimates increased as NO_2 concentrations increased or the ratio of NO_2/PM increased. These results suggest that NO_2 and PM may be effect modifiers of each other. This is consistent with the conclusions of the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a). In addition to copollutant analyses, recent studies examined the influence of the extent of temporal adjustment on NO_2-mortality risk estimates and reported similar results across a range of degrees of freedom per year.

An examination of factors that may contribute to increased risk of NO_2-related mortality, as discussed in Chapter 7, found evidence indicating that older adults (≥65 years of age), females, individuals with pre-existing cardiovascular or respiratory diseases, and individuals of lower SES, specifically lower income and educational attainment, are at greater risk. Studies that examined whether there are seasonal differences in the

NO_2-mortality relationship found greater effects in the warm or summer months in multicity studies conducted in Canada and Europe. However, these results are contradicted by a study conducted in Asia where larger effects were observed in the cold season. These between-study differences in seasonal associations are more than likely a reflection of the different seasonal weather patterns observed between countries (Kan et al., 2010; Kan et al., 2008).

Those studies that examined the lag structure of associations for the NO_2-mortality relationship observed that there continues to be evidence of an immediate effect (i.e., lag 0 to 1 day), which is consistent with studies evaluated in the 2008 ISA for Oxides of Nitrogen. Recent studies also provided evidence for a prolonged effect on mortality in distributed lag models with lags ranging from 0−4 to 0−5 days (Chen et al., 2012b; Chiusolo et al., 2011). Multicity studies have examined the shape of the C-R relationship and whether a threshold exists in both a multi- and single-city setting. These studies have used different statistical approaches and consistently demonstrated a linear relationship with no evidence of a threshold within the range of NO_2 concentrations currently found in the U.S. However, consistent with observations from C-R analyses conducted for other criteria pollutants [e.g., PM (U.S. EPA, 2009) and O_3 (U.S. EPA, 2013a)], an examination of the C-R relationship in individual cities, specifically in China, has demonstrated heterogeneity in the shape of the curve across cities (Wong et al., 2010; Wong et al., 2008).

Overall, recent epidemiologic studies build upon and support the conclusions of the 2008 ISA for Oxides of Nitrogen for total mortality. However, the biological mechanism that could lead to mortality as a result of short-term NO_2 exposures has not been clearly characterized. This is evident when evaluating the underlying health effects (i.e., cardiovascular effects in Section 5.3 and respiratory effects in Section 5.2) that could lead to cardiovascular (~35% of total mortality) and respiratory (~9% of total mortality) mortality, the components of total mortality most thoroughly evaluated (Hoyert and Xu, 2012). An evaluation of epidemiologic studies that examined the relationship between short-term NO_2 exposure and cardiovascular effects found consistent evidence for myocardial infarction and inconclusive epidemiologic and experimental evidence for other cardiovascular endpoints. However, important uncertainties remain especially regarding disentangling whether there is an independent effect of NO_2 on cardiovascular effects, which is the same uncertainty in total mortality studies. Overall this evidence provides limited coherence and biological plausibility for NO_2-related cardiovascular mortality. For respiratory effects, there is causal evidence for NO_2-related asthma exacerbation supported by toxicological and controlled human exposure studies demonstrating increased airway responsiveness (Section 5.2.2.1) in response to short-term NO_2 exposures as well as epidemiologic studies reporting respiratory-related

morbidity including hospital admissions and ED visits, specifically for asthma (Section 5.2.2.4). However, the biological mechanism that explains the continuum of effects that could lead to respiratory-related mortality also remains unclear. Additionally, it is important to note studies that examine the association between short-term NO_2 exposures and mortality rely on central site monitors, which may contribute to exposure measurement error and underestimate associations observed (Section 3.4.5.1). In conclusion, the consistent positive associations observed across various multicity studies is limited by the uncertainty due to whether NO_2 is independently associated with total mortality as well as the uncertainty in the biological mechanism that could lead to NO_2-induced mortality. Collectively, this body of evidence is suggestive, but not sufficient to infer a causal relationship between short-term NO_2 exposure and total mortality.

Table 5-63 Summary of evidence, which is suggestive, but not sufficient to infer, a causal relationship between short-term nitrogen dioxide (NO_2) exposure and total mortality.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Consistent epidemiologic evidence from multiple, high-quality studies at relevant NO_2 concentrations	Increases in mortality in multicity studies conducted in the U.S., Canada, Europe, and Asia.	Section 5.4.3 Table 5-60	Mean 24-h avg: 9.2-55.0 ppb Mean 1-h max: 16.3-80.5 ppb Mean 3-h max: 16.3-42.6 ppb. Table 5-59
Uncertainty regarding potential confounding by traffic-related copollutants	Although NO_2 associations were relatively unchanged in copollutant models with PM_{10}, SO_2, and O_3; NO_2 is often highly correlated with other traffic-related pollutants (e.g., $PM_{2.5}$, EC, and CO) complicating the interpretation of whether NO_2 is independently associated with total mortality.	(Moolgavkar et al. (2013); Chen et al. (2012b); Chiusolo et al. (2011); Wong et al. (2010); Stieb et al. (2008); Wong et al. (2008)) Section 3.4.5, Figure 3-6; Section 5.4.4	
	NO_2 and PM may be effect modifiers of each other.	(Katsouyanni et al. (2009); Katsouyanni et al. (2003); Katsouyanni et al. (2001))	
Uncertainty regarding exposure measurement error	Studies that examine the association between short-term NO_2 exposures and mortality rely on central site monitors.	Sections 3.4.5.1 and 3.5	

Table 5-63 (Continued): Summary of evidence, which is suggestive, but not sufficient to infer, a causal relationship between short-term nitrogen dioxide (NO2) exposure and total mortality.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Uncertainty due to limited coherence and biological plausibility with cardiovascular and respiratory morbidity evidence	Consistent epidemiologic evidence for myocardial infarction. Inconclusive epidemiologic and experimental evidence for other cardiovascular endpoints. Uncertainties with respect to the independent effect of NO_2 on cardiovascular effects contributing to limited coherence and biological plausibility for NO_2-related cardiovascular mortality, which comprises ~35% of total mortality[d].	Section 5.3.12 Table 5-58	
	Consistent evidence for asthma exacerbation from experimental studies demonstrating increased airway responsiveness and epidemiologic studies demonstrating asthma-related morbidity. Uncertainty as to the biological mechanism that explains the continuum of effects leading to NO_2-related respiratory mortality, which comprises ~8% of total mortality[d].	Section 5.2.9 Table 5-45	

CO = carbon monoxide, EC = elemental carbon, NO_2 = nitrogen dioxide, O_3 = ozone, PM = particulate matter, SO_2 = sulfur dioxide.

[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, supporting or contradicting, contributing most heavily to causal determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where full body of evidence is described.

[c]Describes the NO_2 concentrations with which the evidence is substantiated (for experimental studies, below 5,000 ppb).

[d]Statistics taken from American Heart Association (2011)

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CHAPTER 6 INTEGRATED HEALTH EFFECTS OF LONG-TERM EXPOSURE TO OXIDES OF NITROGEN

6.1 Scope and Issues Considered in Health Effects Assessment

6.1.1 Scope of Chapter

As in the preceding chapter for short-term exposure, with consideration of exposure measurement error, effects of other correlated pollutants, and mode of action information to support biological plausibility, this chapter summarizes, integrates, and evaluates the evidence for a broad spectrum of health effects associated with long-term exposure (i.e., more than 1 month to years) to oxides of nitrogen. This chapter comprises evaluations of the epidemiologic and toxicological evidence for the effects of long-term exposure to oxides of nitrogen on health outcomes related to respiratory effects (Section 6.2), cardiovascular and related metabolic effects (Section 6.3), reproductive and developmental effects (Section 6.4), and mortality (Section 6.5). Chapter 6 concludes with a discussion of the evidence for the cancer effects of oxides of nitrogen (Section 6.6). To characterize the weight of evidence in a cohesive manner, results from both short-term (i.e., up to 1 month) and long-term exposure studies specific to reproductive and developmental effects are included in this chapter. These results are identified according to exposure duration in the text and tables throughout Section 6.4.

Individual sections for broad health categories (e.g., respiratory effects) begin with a summary of conclusions from the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) followed by an evaluation of recent (i.e., published since the completion of the 2008 ISA for Oxides of Nitrogen) studies that builds upon evidence from previous reviews. Within each of these sections, results are organized into smaller outcome groups (e.g., asthma development) that are made up of a continuum of subclinical to clinical effects. The discussion of individual effects is then organized by specific scientific discipline (i.e., epidemiology, toxicology). This organization permits a clear description of the extent of coherence and biological plausibility for the effects of oxides of nitrogen on a group of related outcomes, and in turn, a transparent characterization of the weight of evidence in drawing conclusions.

Sections for each of the broad health categories (e.g., respiratory effects, cardiovascular, and related metabolic effects) conclude with an integrated assessment of evidence and conclusions regarding causality. A determination of causality has been made for a broad

health category (e.g., respiratory effects) or smaller group of related outcomes (e.g., birth outcomes) by evaluating the evidence for each category or group independently with the causal framework (described in the Preamble to this ISA). A unique situation arises in the evaluation of mortality. Findings for cause-specific mortality (i.e., respiratory, cardiovascular) were used to assess the continuum of effects and inform the causal determinations for respiratory and cardiovascular effects. A separate causal determination was made for total mortality (Section 6.5), based primarily on the evidence for nonaccidental causes of mortality combined but also informed by the extent to which evidence for the spectrum of cardiovascular and respiratory effects provides biological plausibility for NO_2-related total mortality. Judgments regarding causality were made by evaluating the evidence for the full range of exposures to oxides of nitrogen or ambient concentrations in animal toxicological and epidemiologic studies defined in this ISA to be relevant to ambient exposure (i.e., concentrations up to 5,000 ppb as described in Section 1.2. Experimental studies that examined higher concentrations were evaluated particularly to inform mode of action.

6.1.2 Evidence Evaluation and Integration to Form Causal Determinations

As for relationships of health effects with short-term exposure, judgments regarding causality were made by evaluating evidence for the consistency of findings across multiple studies, the coherence of findings across related endpoints and across disciplines, and the extent to which chance, confounding (i.e., bias due to a correlation with NO_2 exposures or ambient concentrations and relationship with the outcome), and other biases could be ruled out with reasonable confidence. This evaluation involved the integration among various lines of evidence and consideration of the quality of individual studies (detailed in Section 5.1.2 and Table 5-1).

Epidemiologic studies of long-term NO_X or NO_2 exposure generally rely on between-subject differences in exposure between subjects. These differences may be by location of residence (spatial differences) or time periods that vary in long-term ambient NO_X or NO_2 concentrations, for example. For the assessment of potential confounding, long-term exposure epidemiologic studies were evaluated for the extent to which they considered other factors associated with health outcomes and were spatially correlated with NO_X or NO_2 exposure that varied between subjects. These potential confounding factors can include socioeconomic status (SES), diet, smoking or exposure to environmental tobacco smoke, medication use, and copollutant exposures (Table 5-1). Epidemiologic studies varied in the extent to which they considered potential confounding. Because no single study considered all potential confounding factors and not all potential confounding factors were examined in the collective body of evidence,

residual confounding by unmeasured factors is possible. Residual confounding is also possible by poorly measured factors. The evidence was examined based on factors well documented in the literature to be associated with NO_2 exposure and health outcomes. The limitations of multivariable models, including copollutant models, to examine potential confounding were considered in drawing inferences about the independent effects of NO_2 (Section 5.1.2.2). Specific to copollutant confounding, the magnitude of correlations between NO_2 and copollutants is considered. The potential for differential measurement error for NO_2 and copollutants is also considered.

This ISA presents epidemiologic effect estimates for associations with health outcomes scaled to the same increment of oxides of nitrogen to increase comparability among studies that report effect estimates scaled to various changes in concentrations of oxides of nitrogen (e.g., interquartile range of concentrations for the study period or an arbitrary unit such as 10 ppb). For long-term exposure metrics, effect estimates are scaled to a 10-ppb increase in NO_2 or NO and a 20-ppb increase in NO_X. These increments were derived by calculating the U.S. nationwide percentile distributions for annual average concentrations (Table 2-2) and then calculating the approximate difference between the median (a typical pollution year) and the 95th percentile (a more polluted year) of annual average concentrations among monitors in the State and Local Air Monitoring Stations network. Long-term averages of ambient oxides of nitrogen are lower in concentration than short-term averages, less variable across time, and do not differ widely among averages of multiple months, annual averages, or multiyear averages [see Table S6-1; (U.S. EPA, 2014)]. Thus, all long-term exposure metrics were scaled to the same increment. Effect estimates that were reported in terms of $\mu g/m^3$ are converted to ppb and standardized for NO_2 and NO but not NO_X. Because the proportions of NO_2 and NO are unknown for the various NO_X metrics, concentrations cannot be converted from $\mu g/m^3$ to ppb. And, data are not available to calculate the percentiles of NO_X concentrations in $\mu g/m^3$ at a national scale for the U.S. or other countries. Therefore, the ISA presents effect estimates based on $\mu g/m^3$ of NO_X as they are reported in individual studies.

To form causal determinations, evidence was integrated across a spectrum of related endpoints, including cause-specific mortality, and across disciplines to assess the extent to which chance, confounding, and other biases could be ruled out with reasonable confidence. Animal toxicological studies can provide direct evidence for health effects related to NO_2 exposures. Coherence between toxicological and epidemiologic findings can address uncertainties such as whether epidemiologic associations with health outcomes reflect an independent effect of ambient NO_2 exposure or are potentially confounded by other factors. Experimental studies also can provide biological plausibility by describing key events within the modes of action for health effects. Thus, integration of evidence was used to inform uncertainties for any particular outcome or discipline due

to factors such as publication bias, selection bias, exposure measurement error, or confounding by copollutant exposures. The subsequent sections assess study quality and strength of inference and integrate evidence across multiple lines of evidence to evaluate relationships between oxides of nitrogen and various health effects.

6.2 Respiratory Effects

6.2.1 Introduction

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) examined the epidemiologic and toxicological evidence for the relationship between long-term exposure to NO_2 and respiratory effects and concluded that the evidence was suggestive, but not sufficient, to infer a causal relationship. The key supporting evidence comprised associations in epidemiologic studies of higher NO_2 exposure with decrements in lung function and partially irreversible decrements in lung development in children. However, several sources of uncertainty were acknowledged. For example, results from the Southern California Children's Health Study (CHS) indicated that decrements in lung development in children were associated with higher ambient NO_2 concentrations (Gauderman et al., 2004), but similar associations were also found for traffic-related pollutants such as $PM_{2.5}$ and EC, proximity to traffic (<500 m), as well as acid vapor, O_3, and PM_{10}. Generally, the high correlation among long-term averages of traffic-related pollutants made it difficult to discern the independent effects of NO_2. Further, although animal toxicological studies demonstrated that long-term exposure to NO_2 resulted in permanent morphologic changes to the lung, particularly in the centriacinar region and bronchiolar epithelium, they provided little evidence for effects on key events within the mode of action for NO_2-related decreases in lung function or development. Additional uncertainty was related to the inconsistent cross-sectional evidence for associations between long-term exposure to NO_2 and increases in asthma prevalence and incidence. For example, two cohort studies, the CHS in Southern California (Gauderman et al., 2005) and a birth cohort study in the Netherlands (Brauer et al., 2007) observed positive associations, while other studies did not find consistent associations between long-term NO_2 exposure and asthma. Epidemiologic studies conducted in both the U.S. and Europe also reported inconsistent results regarding an association between long-term exposure to NO_2 and respiratory symptoms.

This section presents the current body of evidence examining the relationship between long-term exposure to NO_2 and respiratory effects. A large body of recent studies has evaluated the development of asthma and bronchitis and reduced lung function and

development in children and the incidence of asthma and bronchitis in adults. Longitudinal studies of the incidence of asthma in children, the largest and strongest evidence base, is presented first. Development of allergic disease, chronic disease severity, lung function and development, respiratory infections, chronic obstructive pulmonary disease (COPD), and respiratory morphology are discussed thereafter. No recent animal toxicological studies evaluating respiratory effects of long-term NO_2 exposure have been published since the release of the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008), but previous studies are evaluated to inform the biological plausibility for the array of respiratory effects examined.

Emphasis is placed on the longitudinal cohort studies, which compared to cross-sectional studies can better characterize the temporality between exposure and incidence of a health effect. For the onset of asthma, the prospective designs take into account the difference between the first occurrence of asthma and the exacerbation of asthma by defining asthma incidence as diagnosis of asthma by a physician in the time since the previous follow-up period. For other health effects, such as respiratory symptom occurrence or pulmonary function changes, aspects of the study design or statistical methods must consider potential effects of short-term exposure.

NO_2 exposure assessment methods are discussed to describe the utility of various methods in representing the variability in NO_2 concentrations in the study areas and, in turn, the strength of inference about relationships with respiratory effects. The potential for confounding by traffic-related copollutants is discussed also. Correlations between NO_2 and traffic-related pollutants [e.g., CO, EC/BC, and $PM_{2.5}$ among others] are presented in the summary tables and text. Copollutant regression modeling results are discussed in the text when they are available. Long-term exposure to indoor concentrations of NO_2 has been examined in relation to some respiratory effects and is discussed to describe the extent of coherence with the effects observed with ambient exposure to NO_2. NO_2 exposure estimates examined in the epidemiologic studies in this section are generally annual averages unless stated otherwise.

6.2.2 Development of Asthma or Chronic Bronchitis

Asthma is a chronic disease characterized by chronic inflammation, development of airway hyperresponsiveness (AHR), and in some cases, airway remodeling. In characterizing the evidence for a relationship between long-term NO_2 exposure and asthma development, this section evaluates asthma incidence in children in longitudinal cohort studies. Cross-sectional and prevalence studies were reviewed and are discussed to inform discussion of potential copollutant confounding and other policy-relevant issues

[see Annex tables, Tables AX6 3-15, AX6 3-16, and AX6 3-17 of the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) for descriptions of previous studies]. This section also characterizes evidence for airway responsiveness, allergic sensitization, and pulmonary inflammation, which are key events in the mode of action for a relationship between NO_2 exposure and asthma development (Figure 4-2). A few studies examined a composite index of asthma and chronic bronchitis or chronic bronchitis alone.

6.2.2.1 Asthma or Chronic Bronchitis in Children

Since the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008), recent prospective and retrospective longitudinal cohort studies provide a strong evidence base that generally demonstrates a positive relationship between asthma incidence in children and long-term NO_2 exposure. Details from these key longitudinal studies are presented in Table 6-1 and Figure 6-1. The evidence base includes studies from North America, Europe, and Asia that use varied designs and analyses. Several exposure methods were used that were designed to provide individual exposure estimates. A uniform health-effect indicator, physician-diagnosed asthma, was used. The studies followed children from birth to ages 7 to 12 years of age or from ages 8 to 18 years of age.

Table 6-1 Prospective cohort studies of long-term exposure to nitrogen dioxide (NO_2) or sum of NO_2 and nitric oxide (NO_X) and asthma incidence in children.

Study[a]	Exposure Assessment	Pollutant Correlation	Statistical Methods	Comments	Results (95% CI)[b]
Vancouver high asthma risk birth cohort					
Carlsten et al. (2011c) n = 184 children at 7 yr of age Related publications: Carlsten et al. (2011a) Carlsten et al. (2011b) Henderson et al. (2007) Cohort with high risk of asthma: parental report of at least 1 first-degree relative with asthma or 2 first-degree relatives with other allergic disease (atopic dermatitis, seasonal or perennial allergic rhinitis, or food allergy).	LUR used to estimate annual concentrations at the birth residential address of each subject for NO_2, $PM_{2.5}$, BC, NO as previously developed, described and validated (Henderson et al., 2007). Resolution 10 m. Annual means from 16 monitors show a strong 1:1 relationship with the mean concentrations for sites used in LUR models. Slope = 0.89 (R^2 = 0.98). NO_2 normally distributed with a mean of 16.2 (SD of 5.6) ppb. The R^2 values for NO_2 models (114 sites) range from 0.56 to 0.60 are consistent across models built with different traffic variables. Mean error estimates based on leave one out cross validation had SD of about 15% [0.0 (2.75)].	Pearson R: NO-NO_2 = 0.8 NO_2-$PM_{2.5}$ = 0.7 NO-$PM_{2.5}$ = 0.5 BC-NO_2 = 0.5 BC-NO = 0.3	Multiple logistic regression analysis adjusted for maternal education, history of asthma (in mother, father or siblings), atopic status at age 1 yr.	Follow-up at 7 yr of age, represented 63% of the cohort entering the study at its onset. The key characteristics of children who returned for assessment did not differ from those in the original cohort. Air pollution estimates for 1995 generated from 2003 annual averages adjusted for temporal trends.	OR among all children: 2.9 (0.8, 10.9) Association observed with $PM_{2.5}$ with wide CI; no association observed for BC. OR in 13 children with both allergist diagnosis of asthma and bronchial hyperactivity: 1.3 (0.9, 2.2) per 10-ppb NO

Table 6-1 (Continued): Prospective cohort studies of long-term exposure to nitrogen dioxide (NO_2) or sum of NO_2 and nitric oxide (NO_X) and asthma incidence in children.

Study[a]	Exposure Assessment	Pollutant Correlation	Statistical Methods	Comments	Results (95% CI)[b]
Children, Allergy, Milieu, Stockholm, Epidemiology Survey (BAMSE)					
Gruzieva et al. (2013) N = 3,633 Birth cohort followed up to 12 yr of age. Enrolled between 1994 and 1996. Related publications: Gruzieva et al. (2012) Nordling et al. (2008) Wickman et al. (2002)	Dispersion models used to estimate NO_X for all addresses in the yr 1994 to 2008 representing when the first child was born until the end of the 12-yr follow-up. Model is regularly validated against measurements at air quality-monitoring stations. The correlation between measured NO_2 concentrations and calculated traffic-related NO_2 from dispersion modeled NO_X for 487 addresses in the study had an *r* value of 0.74. Used time and activity patterns and different places of exposure to estimate exposure.	*r* = 0.96 between NO_X and PM_{10} concentrations during the first yr of life.	Multinomial regression/GEE adjusted for municipality, SES, yr the house was built, and mother or father with doctor diagnosis of asthma and asthma medication.	Associations were stronger for the oldest children and for nonallergic asthma. Participation at the 1st yr was 96%, at the 2nd yr 94%, at 4 yr 91%, at 8 yr 84%, and at 12 yr 82%. The distribution of risk factors was similar in the study population and the original cohort.	OR for NO_X during the first yr of life and development of incident asthma at 12 yr of age. 1.87 (1.0, 3.44) per 46.8 µg/m^3 NO_X

Table 6-1 (Continued): Prospective cohort studies of long-term exposure to nitrogen dioxide (NO_2) or sum of NO_2 and nitric oxide (NO_X) and asthma incidence in children.

Study[a]	Exposure Assessment	Pollutant Correlation	Statistical Methods	Comments	Results (95% CI)[b]
Chiba prefecture, Japan cohort					
Shima et al. (2002) N = 1,910 children in 8 communities at age 6 yr. 1st graders between 1989 and 1992 were followed to 6th grade.	The average annual concentrations of air pollutants for the 10-yr period from 1988 to 1997 at ambient air monitoring stations approximately 1 to 2 km from study schools. Almost all the childrens' homes and schools were about 1 km from the sites.	NR	Logistic regression model adjusted for sex, history of allergic diseases, respiratory diseases prior to age 2 yr, parental history of allergic diseases, maternal smoking habits, type of heater used in winter in the home, and construction elements of the house.	The follow up included 1,910 children (66.9% of the original cohort) at 6 yr. The percentage of children who were not followed was slightly higher in urban than in rural communities because urban subjects changed residence more frequently. Questionnaire responses were unavailable for 944 children in the follow-up period, primarily because of changed residence 3 yr prior to entering 1st grade, which provides an exposure estimate related to the child's location.	OR: 1.71 (1.04, 2.79)

Table 6-1 (Continued): Prospective cohort studies of long-term exposure to nitrogen dioxide (NO_2) or sum of NO_2 and nitric oxide (NO_X) and asthma incidence in children.

Study[a]	Exposure Assessment	Pollutant Correlation	Statistical Methods	Comments	Results (95% CI)[b]
Taiwan Children Health Study (TCHS)					
Lee et al. (2012b) N = 3,160 from 14 communities Ages 12−14 yr at baseline in 2007. Mean follow-up 2 yr.	Annual avg NO_2 calculated from 14 monitoring stations between 2007 and 2009. Almost all childrens' homes and schools were 1 km from the sites. Two NO_2 strata: Low: < median 17.5 ppb High: > median 17.5 ppb Median annual avg NO_2 22.1 ppb in high communities 14.0 ppb in low communities	NR	Poisson regression models adjusted for prenatal maternal smoking, family history of asthma, family history of atopy, and community.	At the 2-yr follow-up period, 96.9% of the children completed the questionnaire and pulmonary function tests.	NO_2 observed to modify the protective effect of high lung function on risk of asthma incidence. RR for asthma per interquartile range increase in percentage predicted FVC: Low NO_2 communities: 0.82 (0.72, 0.93) High NO_2 communities: 1.00 (0.88, 1.14)

Table 6-1 (Continued): Prospective cohort studies of long-term exposure to nitrogen dioxide (NO_2) or sum of NO_2 and nitric oxide (NO_X) and asthma incidence in children.

Study[a]	Exposure Assessment	Pollutant Correlation	Statistical Methods	Comments	Results (95% CI)[b]
British Columbia birth cohort					
Clark et al. (2010) N = 2,801 Mean age at follow-up: 48 mo (SD: 7). All 1999 and 2000 births in southwest British Columbia eligible. Related publication: (Henderson et al., 2007).	Central site monitors, LUR, and point source derived IDW summation of emissions. All estimated for the postal code level. (92% was at resolution of a city block or block face). For LUR, model R^2 is 0.53 for NO_2. In the sampling yr (2003), measurements at LUR sites exhibit a strong 1:1 relationship with annual means at central monitoring sites. Slopes are 1.03 (R^2 = 0.96) and 0.89 (R^2 = 0.98) for NO and NO_2, respectively (Henderson et al., 2007). For IDW, exposures assigned using the 3 closest monitors within 50 km weighted by their distance to the postal code of interest.	Correlations among pollutants were generally high. Quantitative results reported only for O_3. r = −0.7 to −0.9.	Conditional logistic regression adjusted for native status, breast-feeding, maternal smoking, income quartile, maternal age, birth weight, and gestational length.	The potential limitation of the young age of the children when wheezing is more common was addressed by restricting asthma cases to children with a hospital admission or at least two outpatient diagnoses of asthma which indicate severe ongoing symptoms. LUR and IDW results for NO_2 were similar. Results for $PM_{2.5}$ were smaller for both LUR and IDW than for NO_2.	OR for LUR: 1.26 (1.08, 1.48) OR for IDW: 1.24 (1.14, 1.34) Asthma diagnosis associated with early life exposure to CO, PM_{10}, SO_2, BC and proximity to point sources. Traffic-related pollutants were associated with the highest risks.

Table 6-1 (Continued): Prospective cohort studies of long-term exposure to nitrogen dioxide (NO_2) or sum of NO_2 and nitric oxide (NO_X) and asthma incidence in children.

Study[a]	Exposure Assessment	Pollutant Correlation	Statistical Methods	Comments	Results (95% CI)[b]
Childrens Health Study (CHS), Southern California communities					
Jerrett et al. (2008) N = 217 children Ages 10−18 yr Enrolled in 1993 or 1996 from 11 communities. Asthma assessed over 8 yr of follow-up.	Palmes tubes outside home, 2 weeks summer and winter to provide annual and seasonal levels. Over the 11 communities, mean (SD) annual avg NO_2 ranged from 9.6 (2.5) to 51.3 (4.4) ppb.	No quantitative data. Correlations between residential NO_2 and various measures of traffic proximity or modeled pollutant concentrations reported to be moderate to high.	Random-effects Cox proportional hazards models adjusted for median household income, proportion of respondents with low education, percentage of males unemployed, percentage living in poverty, temperature, and humidity.	Within-community effects indicative of long-term local traffic sources were similar to effects of community average NO_2 across communities, suggesting that both regional and local pollution contributed to associations with asthma. The range of NO_2 within communities was smaller than that between communities.	HR: 1.51 (1.12, 2.05)
McConnell et al. (2010) N = 120 Ages 4.8 to 9.0 yr New cohort established 2002−2003 in 13 communities. 3 yr of follow-up Related publications: Wu et al. (2005) Peters et al. (1999) Benson (1984)	Community central site pollutant measurements and line source dispersion model for residential and school NO_X. The overall within-community variability of personal exposures using time-activity categories across communities was highest for NO_2, followed by EC, PM_{10}, $PM_{2.5}$, and CO (Wu et al., 2005).	NR In 2000, correlations for measured NO_2 or residential NO_2 with freeway- and nonfreeway-related NO_2 from the dispersion model were r = 0.56 and r = 0.34, respectively, indicating that the measured and modeled metrics may have some level of independence (Gauderman et al., 2005).	Multilevel Cox proportional hazards model adjusted for sociodemographic characteristics, exposure to cigarette and wildfire smoke, health insurance, housing characteristics, history of allergy, and parental asthma.	After a 3-yr follow-up period, 74% of the baseline cohort remained. Follow-up was lower among Hispanic children and children with lower SES than non-Hispanic white children and children with higher SES. However, the NO_2 association was similar after adjusting for these factors. Risk was higher in children with high parental stress compared to low parental stress (Shankardass et al., 2009).	HR for central site NO_2: 1.39 (1.07, 1.80) HR for modeled NO_X from freeways: 1.67 (1.32, 2.12) near homes 1.88 (1.10, 3.19) near schools. OR for $PM_{2.5}$ central site (range 13.9 to 17.4 μg/m^3): 1.66 (0.91, 3.05)

Table 6-1 (Continued): Prospective cohort studies of long-term exposure to nitrogen dioxide (NO_2) or sum of NO_2 and nitric oxide (NO_X) and asthma incidence in children.

Study[a]	Exposure Assessment	Pollutant Correlation	Statistical Methods	Comments	Results (95% CI)[b]
Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study, the Netherlands					
Gehring et al. (2010) N = 3,863 Follow-up from birth to age 8 yr. Related publications: Wijga et al. (2014) Eeftens et al. (2011) Hoek et al. (2008) Brauer et al. (2007) Brauer et al. (2003) Brauer et al. (2002)	LUR to estimate annual concentrations for birth address of each child. NO_2 measurements conducted in 2007 agreed well with NO_2 measurements taken in 1999−2000 at the same locations (R^2 = 0.86). LUR models from 1999−2000 and 2007 explained 85 and 86% of observed spatial variance, respectively. Developed from 40 sites. 16 urban/suburban, 12 regional, 12 traffic. 5−12% of population lived near major roads. Buffer >50 m. NO_2 annual mean: (10−90%) 13.5 ppb (7.8−18.5 ppb).	Copollutants were highly correlated. NO_2-$PM_{2.5}$: r = 0.93 NO_2-soot: r = 0.96 $PM_{2.5}$-soot: r = 0.97	Generalized estimating equations adjusted for sex, study arm, use of mite-impermeable mattress covers, allergies of mother and father, maternal and paternal education, maternal smoking during pregnancy, breastfeeding, presence of a gas stove in the child's home, presence of older siblings, and any smoking at home.	Participation was high at all ages, starting with 94.4% in the first yr and 82% in the eighth yr. Characteristics between the original cohort and studied groups are similar.	Adjusted OR of 1.36 (1.09, 1.67) without adjustment for study region. Similar results were observed for $PM_{2.5}$ and soot. Adjusted OR of 1.32 (0.93, 1.85) with adjustment for study region.

Table 6-1 (Continued): Prospective cohort studies of long-term exposure to nitrogen dioxide (NO_2) or sum of NO_2 and nitric oxide (NO_X) and asthma incidence in children.

Study[a]	Exposure Assessment	Pollutant Correlation	Statistical Methods	Comments	Results (95% CI)[b]
Gene and Environment Prospective Study in Italy (GASPII)					
Ranzi et al. (2014) N = 672 Birth cohort enrolled from 2 large obstetric hospitals in Rome from June 2003 to October 2004. Related publications: Cesaroni et al. (2012)	NO_2 assessed for each residential address during the follow-up period using LUR. NO_2 measured simultaneously at 78 locations in winter, spring, and fall. Annual average concentrations calculated. Mean (SD) NO_2 across 78 sites: 23.7 (5.85) ppb	O_3 inversely correlated on the spatial scale with NO_2. Spearman *r*: −0.34.	Logistic regression adjusted for sex, age, breastfeeding at 3 mo, day care attendance, presence of any pets in the home, siblings, maternal and paternal smoking, maternal smoking during pregnancy, maternal and paternal education, presence of molds or dampness at home, familial asthma or allergies.	Information on subsequent health outcomes and additional variables was obtained by questionnaires at 6 mo, 15 mo, 4 yr, and 7 yr for 694, 664, 581 and 497 children, respectively. Participation was at 70% at the 7-yr follow-up.	OR for time-weighted average NO_2: 1.17 (0.63, 2.20) Adjusted for O_3: 1.11 (0.54, 2.25)
Genes-environment & admixture in Latino Americans and the study of African Americans, asthma, genes, & environments					
Nishimura et al. (2013) N = 4,320 Ages 8−21 yr Multicity study: Chicago, IL; Bronx, NY; Houston, TX; San Francisco Bay Area, CA and Puerto Rico.	Average NO_2 over the first 3 yr of life were calculated by averaging NO_2 using IDW from the 4 closest monitors within 50 km of the residence. Mean (SD) NO_2 across cities: 9.9 (2.9) to 32.1 (5.7).	NR	Logistic regression models adjusted for age, sex, ethnicity, and composite SES. Sensitivity analysis conducted with additional covariates: maternal gestational smoking, ETS in the household between 0 and 2 yr old, and maternal language of preference.	Region-specific results suggest that risk of asthma due to air pollution may not be uniform throughout the nation and could depend on local characteristics, such as varying proportions of different racial/ethnic groups and differing pollution sources and/or weather patterns.	OR for NO_2 during the first yr of life, all cities combined: 1.37 (1.08, 1.72) OR per 1 μg/m^3 $PM_{2.5}$: 1.03 (0.90, 1.18)

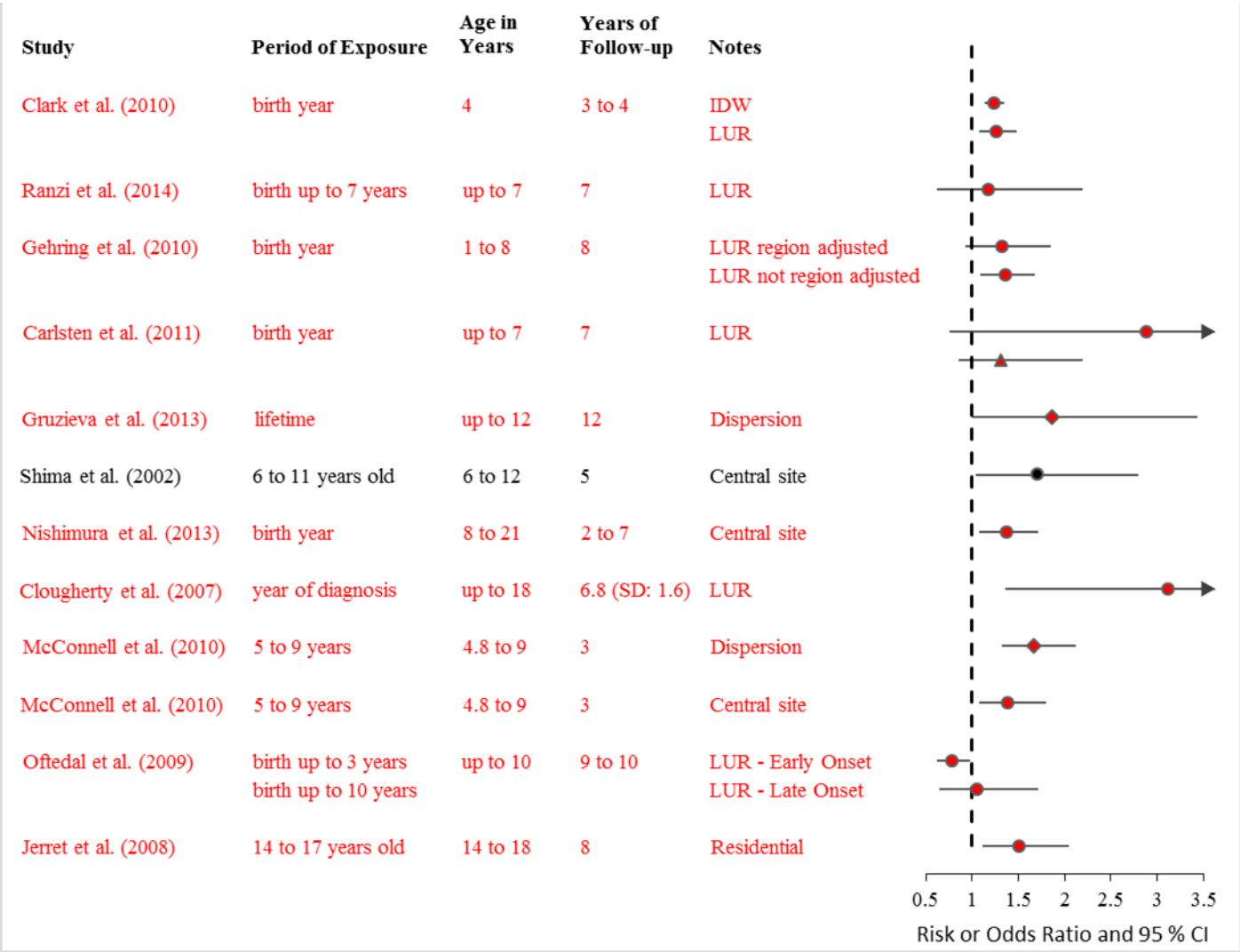
Table 6-1 (Continued): Prospective cohort studies of long-term exposure to nitrogen dioxide (NO_2) or sum of NO_2 and nitric oxide (NO_X) and asthma incidence in children.

Study[a]	Exposure Assessment	Pollutant Correlation	Statistical Methods	Comments	Results (95% CI)[b]
Maternal-infant smoking study of East Boston					
Clougherty et al. (2007) N = 413 full cohort/255 lifetime residents. Enrolled at birth between 1987 and 1993. Asthma ascertained in 1997 at mean age of 6.8 yr.	LUR model (R^2 = 0.83). More variability in NO_2 was explained by spatial (R^2 = 0.53) than temporal variables (R^2 = 0.29).	NR	Regression model adjusted for maternal asthma, education, and smoking before and after pregnancy, child's sex and age.	Observed an association between NO_2 and asthma solely among urban children exposed to violence.	OR for NO_2 in the yr of diagnosis: 3.12 (1.36, 7.15) in group with high exposure to violence.
Oslo Norway birth cohort					
Oftedal et al. (2009a) N = 2,329 Follow-up from birth in 1992−1993 to age 9−10 yr. Related publications: Oftedal et al. (2009b) Laupsa and Slordal (2003) Walker et al. (1999) Gronskei et al. (1993)	NO_2 estimated by dispersion model and assigned at updated individual addresses during lifetime. Dispersion model estimates of long-term NO_2 averages were well correlated with measurements from 10 monitoring stations in Oslo. r = 0.76.	PM_{10} and $PM_{2.5}$ highly correlated with NO_2 (r = 0.79−0.91).	Cox proportional hazard regression and logistic regression adjusted for sex, parental atopy, maternal smoking in pregnancy, paternal education, and maternal marital status at the child's birth.	Several long-term exposure periods examined: exposure in first yr of life, average exposure from birth to asthma onset, and previous yr's exposure before completing the questionnaire.	RR for NO_2 in first yr of life and asthma onset at any age: 0.87 (0.76, 1.00) Average NO_2 onset not associated with asthma onset before or after age 4 yr. Increment of NO_2 for RR not reported.

BAMSE = Children, Allergy, Milieu, Stockholm, Epidemiology Survey; BC = black carbon; CHS = Children's Health Study; CI = confidence interval; CO = carbon monoxide; EC = elemental carbon; ETS = environmental tobacco smoke; FVC = forced vital capacity; GASPII = Gene and Environmental Prospective Study in Italy; GEE = generalized estimating equations; HR = hazard ratio; IDW = inverse distance weighting; LUR = land-use regression; NO = nitric oxide; NO_2 = nitrogen dioxide; NO_X = sum of NO and NO_2; OR = odds ratio; PIAMA = Prevention and Incidence of Asthma and Mite Allergy; $PM_{2.5}$ = 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; RR = risk ratio(s), relative risk; SD = standard deviation; SES = socioeconomic status; TCHS = Taiwan Children Health Study.

[a]Studies are presented in the order of appearance in the text.

[b]Results are presented for a 10 ppb change in NO_2 unless otherwise specified.



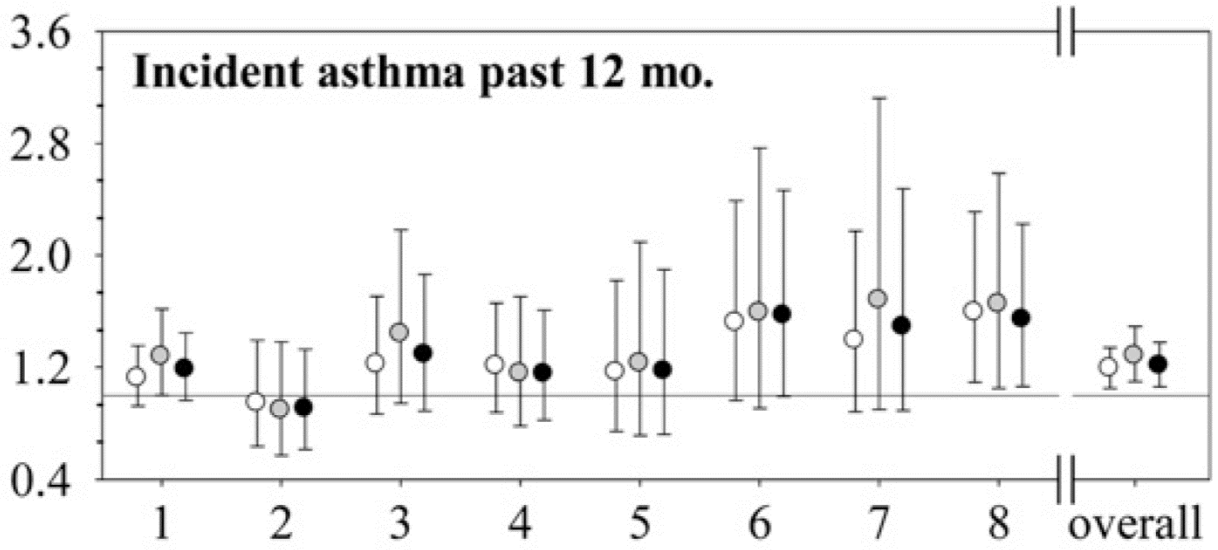
Note: Studies in red are recent studies. Studies in black were included in the 2008 ISA for Oxides of Nitrogen. All effect estimates in this plot are standardized to 10 ppb, with the exception of Gruzieva et al. (2013) and Oftedal et al. (2009a). See Table 6-1 for further study details and quantitative results. Circles = NO_2; Triangles = NO; Diamond = NO_X.

Figure 6-1 Associations of long-term exposure to nitrogen dioxide (NO_2), nitric oxide (NO), and the sum of NO and NO_2 (NO_X) with asthma incidence from prospective studies of children.

Transient wheezing is common in infants and often resolves as the child ages (Martinez et al., 1995), and thus the reliability of asthma diagnosis in infants is a factor to consider. As a child progresses in age, the reliability of diagnosis of asthma would be expected to strengthen. Consistent with this hypothesis, associations of NO_2 and asthma incidence in children are greater in magnitude at later age evaluation and longer follow-up time. In these studies, the methods used to determine the incident of asthma are similar across

studies. Children were required to be disease free at the start of the study. In the majority of studies, asthma incidence was assessed using an annual respiratory questionnaire that asked parents whether a doctor has ever diagnosed the child as having asthma, without having fulfilled the definition of asthma at any previous time of follow-up. Several studies assessed asthma incidence in a different manner but also demonstrated association with NO_2. For example, Carlsten et al. (2011a) used a pediatric allergist to assess asthma in the children when they were 7 years old. Gruzieva et al. (2013) defined asthma incidence as children at 12 years of age having at least 4 episodes of wheeze in the last 12 months, or at least one episode in combination with prescription of inhaled corticosteroids, which would have been provided by a physician making a diagnosis of asthma. Krämer et al. (2009) examined the incidence of doctor-diagnosed asthmatic/spastic/obstructive bronchitis/asthma, which is not uniform with the other studies evaluating asthma incidence, and thus not included here. The use of questionnaires to determine asthma incidence is a best practice (Burr, 1992; Ferris, 1978) and adds to the strength of inference from the available studies.

Associations between long-term NO_2 and asthma incidence are observed consistently across studies that are diverse in the age of assessment of asthma, period of NO_2 exposure examined, and duration of follow-up (Table 6-1 and Figure 6-1). Within the birth cohorts, results are reported at age 7 through age 12 years reflecting the length of follow-up time. Additionally, various periods of exposure were examined, including the first year of life, the year previous to asthma diagnosis, and cumulative exposure. The cohorts that start at age 6 years and go up to 17 years examined a similar array of exposure periods. In the birth cohorts examined by Gehring et al. (2010) and Gruzieva et al. (2013), results are presented for multiple ages in the follow-up period. In Gehring et al. (2010), age-specific associations, estimated from models with air pollution-age interaction terms, indicate small differences in the associations of NO_2 with asthma and related symptoms with age (Figure 6-2). However, larger odds ratios (ORs) were observed at ages 6−8 years, which is consistent with the view that as a child progresses in age, the reliability of the diagnosis of asthma would be expected to strengthen. Gruzieva et al. (2013) observed an association for NO_X estimated for the first year of life by dispersion models with asthma incidence at age 12 years but not at earlier ages, consistent with lower reliability of asthma diagnosis in young children and the extended follow-up to obtain newer cases. No association was observed with two other exposure period evaluated: (1) NO_X average since the date of the previous follow-up or (2) during the preceding 12 months. Both were periods of lower exposure.



Note: Results are presented as adjusted except study region odds ratios (ORs) with 95% confidence intervals. Because the study region is an important determinant of air pollution concentrations in the LUR models that were used to estimate exposures, the adjustment for region may be an over adjustment. ORs were calculated for an interquartile range increase in air pollution levels of 5.5 ppb for NO_2; blank circle = NO_2; Gray circle = $PM_{2.5}$; and Black circle = soot.

Source: Reprinted with permission of American Thoracic Society, Gehring et al. (2010).

Figure 6-2 Adjusted overall and age-specific association between annual average levels of air pollution at the birth address and asthma during the first 8 years of life.

A pooled analysis of six birth cohort studies (5,115 children) examined incident physician-diagnosed asthma from birth to 7−8 years of age [confirmed by pediatric allergist in two cohorts; (Macintyre et al., 2014a)]. Individual estimates of annual average NO_2 assigned to each child's birth address using land-use regression (LUR) was associated with asthma with an OR of 1.48% (95% CI: 1.06, 2.06) per 10-ppb increase in NO_2. Recent meta-analyses (Anderson et al., 2013; Gasana et al., 2012; Gowers et al., 2012; Takenoue et al., 2012; Bråbäck and Forsberg, 2009) also report positive associations between long-term NO_2 exposure and asthma. Some of these meta-analyses mixed children and adults, and some included both cross-sectional and prospective studies. In Japan, Hasunuma et al. (2014) evaluated the health-improving effect of anti-air pollution measures from 1997 to 2009. Analysis showed that a reduction in the ambient NO_2 concentrations was associated with a reduction in the prevalence of asthma.

In limited analysis, Shima et al. (2002) observed a linear concentration-response relationship for NO_2-related asthma across communities. Whereas, Carlsten et al. (2011c) observed higher risk estimates in the second and third tertiles of NO_2; the very wide and

overlapping CIs do not strongly demonstrate a linear relationship. These studies did not conduct analysis to evaluate whether there is a threshold effect.

The set of studies examining asthma among children used a variety of exposure assessment techniques, including LUR models, inverse distance weighting (IDW), Palmes tubes outside subjects' homes, dispersion models with or without central site monitoring data, and central site monitoring data (Table 6-1). Information in Section 3.4.5.2 aids interpreting these methods with regard to potential exposure measurement error. Many asthma incidence studies used exposure assessment techniques intended to characterize the exposure for subjects in a representative manner. The limitations and strengths of these methods that help inform the strength of inference about the relationship between NO_2 exposure and asthma incidence are discussed next. Recent prospective birth cohort studies that estimated residential ambient NO_2 concentrations using LUR provide strong support for a relationship with asthma development (Figure 6-1). Misrepresenting the differences between subjects in NO_2 exposure due to the high variability often observed in ambient NO_2 concentrations can lead to bias in health effects association (Section 3.4.5.2). The studies of LUR are noteworthy in that the NO_2 exposure estimates were spatially aligned to subjects' residences, and many provided validation that LUR estimates well represented the spatial variation in ambient NO_2 concentrations in the study area (Table 6-1). Similar to NO_2 estimated by LUR, asthma incidence was associated with NO_2 exposure estimated from passive sampling outside the subjects' homes and from monitoring sites located 1 to 2 km from the home and/or the school.

Unlike other LUR studies of asthma, Clark et al. (2010) assigned NO_2 exposure for the first year of life at the postal code level. A similar effect was estimated for IDW for the first year of life. Because the spatial resolution was at the postal code level, and not the residential level, there may be more uncertainty associated with the exposure assessment (Section 3.4.5.2). Similarly, the use of IDW may also introduce uncertainty due to poor accounting for localized sources between modeling sites (Section 3.2.1.1). In Toronto, individual level residential and school NO_2 estimated by IDW and LUR were similarly associated with asthma prevalence in a retrospective cross-sectional analysis (Dell et al., 2014). Thus, the representativeness of IDW estimates to the spatial pattern of NO_2 concentrations may vary across locations.

Jerrett et al. (2008) modeled the effects of the within- and between-community variation in NO_2 in 11 of the 13 CHS communities. This approach allowed examination of the independent contributions of local NO_2 and regional NO_2 to the associations with asthma. Both within-community variation and between-community variation in NO_2 were associated with the development of asthma, providing evidence that both regional and

local pollution contributed to the observed associations. One strength of this analysis' exposure assessment is that monitors were placed outside study participants' residences to capture a good representation of the spatial distribution of the true NO_2 exposures (Section 3.4.5.2). Moreover, the 2-week measurement periods across cities were synchronized within 4 hours, and the measurement methodology was identical for each city. However, the Palmes tubes used to measure NO_2 outside of the residences are subject to positive biases that could negatively bias the effect estimate (Section 3.2.2).

Dispersion models have known limitations for accurately estimating within-community conditions, including oversimplification of the NO_X reaction model and inaccurate representation of the meteorological conditions, which can add uncertainty to the effect estimate (Section 3.2.1.2). As with IDW, exposure measurement error associated with dispersion model estimates of NO_2 or NO_X may vary by location. Oftedal et al. (2009a) indicated that dispersion model estimates of NO_2 correlated well with central site concentrations (r = 0.76). Specific to NO_X, estimates may not represent NO_2 exposure equally among subjects. The very high correlations that have been observed between NO_X and other traffic-related pollutants such as CO, EC, and $PM_{2.5}$ (r > 0.94) estimated from dispersion models add uncertainty in attributing associations to NO_X specifically.

Higher long-term exposure to ambient NO_2 is consistently associated with the development of asthma in children as examined in several longitudinal studies. Such associations were found with NO_2 assessed from LUR models that were demonstrated to well represent the variability in NO_2 in study locations and measurements made outside subjects' homes (Table 6-1). These exposure estimates for subjects' residences provide a strong basis for inferring associations of NO_2 with asthma incidence. Associations also were observed with NO_2 assessed from central site monitors. Such measurements have well-known limitations in capturing the spatial heterogeneity in ambient NO_2 concentrations within an area; however, some studies limited monitors to those within 1−2 km from children's schools.

Another factor influencing inference about the effects of NO_2 on asthma development is the ability to disentangle the independent effects of NO_2 from the effects of other pollutants in the ambient mixture, particularly those related to traffic. The potential for copollutant confounding in the studies of asthma was informed by examining the correlations with NO_2 and results of copollutant models. The available correlations between NO_2 and other pollutants for these long-term prospective studies are found in Table 6-1. The level of detail varies from study to study; in some cases, the correlations are not reported or a statement of moderate to high correlation is reported without quantitative results. Specifically, in this evidence base, no data were reported for the correlations between NO_2 and ultrafine particles (UFP) or CO. For BC, the data show a

correlation of 0.5 in one study. For $PM_{2.5}$, correlations range from about 0.7 to 0.93. The strong correlations often observed between NO_2 and $PM_{2.5}$ make it difficult to interpret the results for NO_2 in the context of the traffic-related pollutant mixture, and introduces uncertainty to the NO_2 results.

Further, no prospective study evaluated copollutant models for asthma incidence among children. Hwang et al. (2005), in a cross-sectional study of 32,672 school children in Taiwan, observed that the association for NO_X measured at monitoring stations within 1 km of the schools with physician-diagnosed asthma remained relatively unchanged after including either sulfur dioxide (SO_2), PM_{10}, or O_3 in a copollutant model. Importantly, this study did not analyze NO_2 or potential confounding by traffic-related copollutants. The strong correlations between NO_2 and $PM_{2.5}$, the limited or no data on correlations with other traffic-related pollutants, and the lack of examination of potential confounding by traffic-related copollutants introduces uncertainty in distinguishing an independent effect of NO_2 based on just epidemiologic results.

In the groups of studies relating NO_2 exposure to asthma incidence in children, $PM_{2.5}$ was evaluated in several of the studies presented in Table 6-1. McConnell et al. (2010) assigning exposure from a central site observed a smaller effect for $PM_{2.5}$ than for NO_2 with broader CIs; they did not report a correlation between NO_2 and $PM_{2.5}$. Carlsten et al. (2011c) reported a correlation between NO_2 and $PM_{2.5}$ of $r = 0.7$; and NO_2 and BC of $r = 0.5$. Using LUR exposure estimates, they observed a stronger odds ratio but wider CI for $PM_{2.5}$ than NO_2, and no risk for BC. Nishimura et al. (2013) did not report correlations between NO_2 and copollutants and observed for IDW exposure estimates a smaller odds ratio for $PM_{2.5}$ than NO_2, for which the odds ratio was larger and the CI was broader. Gehring et al. (2010) reported a correlation between NO_2 and $PM_{2.5}$ of 0.93 and, using LUR estimates, observed similar ORs for NO_2 and $PM_{2.5}$. Clark et al. (2010) reported high correlations between NO_2 and the other pollutants but did not provide quantitative data. Based on exposure assessment by LUR and IDW at the postal code level, Clark et al. (2010) observed $PM_{2.5}$ effect estimates that were smaller than those for NO_2 with broader CIs. However, for BC estimated by LUR, the odds ratio was larger than that for NO_2. Thus, stronger effect estimates with smaller CIs were generally observed for NO_2 than for $PM_{2.5}$.

Early-life influences have been implicated in the potential development of asthma as discussed in recent reviews (Kim et al., 2013; Kudo et al., 2013). While the first year of life is considered to be an important period for factors affecting asthma development, other periods also play a role especially when considering the multifaceted aspect and the natural history and pathophysiology of asthma. Studies examining the association between long-term exposure to NO_2 and asthma in children vary in exposure period

evaluated, age of asthma diagnosis, and length of follow-up time. Several involve birth cohorts followed to an age of 8 to 12 years. Across studies, no single critical time window of NO_2 exposure was identified. Associations were observed for NO_2 in the birth year (Carlsten et al., 2011c) and lifetime average NO_2 (Ranzi et al., 2014). Other studies found larger magnitudes of association with NO_2 exposure in the first 3 years of life (Nishimura et al., 2013) or year of diagnosis (Clougherty et al., 2007). Often, the various early life exposure periods that are evaluated are highly correlated with one another, making it difficult to interpret the results or identify a single exposure window of concern. Exposure measurement error also may vary across time periods.

6.2.2.2 Asthma or Chronic Bronchitis in Adults

Since the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008), recent longitudinal cohort studies have examined asthma/chronic bronchitis in adults in relation to long-term exposure to NO_2 and observed positive associations. In the European Community Respiratory Health Survey (ECRHS) cohort, this relationship in adults was examined using various health effects definitions and exposure assessment approaches.

The relationship between home outdoor NO_2 and chronic bronchitis defined as productive (phlegm) chronic cough more than 3 months each year was analyzed in the ECRHS cohort (Sunyer et al., 2006). The follow-up time period was 8.9 years. Individual level, indoor kitchen and outdoor (at the kitchen window) residential NO_2 was measured using Palmes tubes during a 14-day period in 1,634 households of subjects who did not change residences during the follow-up. This was repeated in 659 households (45%) 6 months later. A linear concentration-response relationship with NO_2 was observed only in females. NO_2 was associated with chronic bronchitis after adjustment for traffic intensity at the residence.

In a meta-analysis, Cai et al. (2014) cross-sectionally assessed the associations of outdoor air pollution on the prevalence of chronic bronchitis symptoms in adults in five cohort studies participating in the European Study of Cohorts for Air Pollution Effects (ESCAPE) project. Annual average NO_2, NO_X, as well as PM_{10}, $PM_{2.5}$, $PM_{absorbance}$, PM_{coarse} between 2008−2011 were assigned to home addresses by LUR. Symptoms examined were chronic bronchitis (cough and phlegm for ≥3 months of the year for ≥2 years), chronic cough (with/without phlegm) and chronic phlegm (with/without cough). Overall, there were no associations with any air pollutant or traffic exposure.

New onset asthma was related to NO_2 exposure in the ECRHS adult cohort as ascertained by a positive response to the question “Have you ever had asthma?” (Jacquemin et al., 2009b) and a continuous asthma score (Jacquemin et al., 2009a). Asthma incidence was

defined as reporting asthma in the follow-up (1999 to 2001) but not at baseline (1991 to 1993). Subjects' home addresses were geocoded and linked to outdoor NO_2 estimates developed from NO_X emissions. For "ever having asthma," the adjusted OR was 1.96 (95% CI: 1.04, 3.70) per 10 ppb increase in NO_2. The OR for asthma incidence based on the ratio of the mean asthma score was 1.48 (95% CI: 1.18, 1.85) for each increase of 10 ppb of NO_2.

In a preliminary examination of a smaller group of the ECRHS cohort, the prospective Respiratory Health in Northern Europe cohort study, Modig et al. (2009) used dispersion models and two definitions of asthma among 3,824 adults aged 20−44 years at inclusion: (1) the cumulative number of onset cases of asthma and (2) incident cases of asthma. Asthma was defined as no asthma attacks during the last 12 months and no current use of asthma medication in the first survey plus having asthma or ever being diagnosed with asthma at follow-up. NO_2 concentrations estimated at the home were associated with risk of developing asthma. The OR was 2.04 (95% CI: 1.14, 3.65) for the cumulative number of onset cases of asthma and 2.25 (95% CI: 1.00, 5.07) for the incident definition of cases per 10-ppb increase in NO_2 concentration. The OR for asthma increased across NO_2 tertiles, indicating a concentration-dependent relationship. With the first tertile as the reference, the OR was higher for the third tertile (OR_{onset} 1.58 [95% CI: 0.96, 2.6]; $OR_{incident}$ 2.06 [95% CI: 0.98, 4.32]) than for the second tertile (OR_{onset} 1.17 [95% CI: 0.70, 1.94]; $OR_{incident}$ 1.77 [95% CI: 0.86, 3.64]).

In 13 European cities, Castro-Giner et al. (2009) prospectively examined asthma incidence and prevalence in the large (2,577 subjects at follow-up) adult population-based ECRHS cohort. In the longitudinal analysis, for the 120 subjects who developed asthma during the follow-up period, NO_2 was associated with new-onset asthma with an OR of 2.20 (95% CI: 1.17, 4.10) per 10-ppb increase. For asthma prevalence, an association was indicated among subjects who changed homes rather than subjects who lived in the same home during follow-up (movers OR: 2.53 [95% CI: 1.16, 5.56]; nonmovers OR: 1.04 [95% CI: 0.66, 1.64]). However, for new-onset asthma, evidence for association was stronger among nonmovers than movers (nonmovers OR: 2.39 [95% CI: 1.10, 5.22]; movers OR: 2.09 [95% CI: 0.70, 6.12]).

In summary, among adults, long-term NO_2 exposure generally is associated with asthma incidence and chronic bronchitis. The longitudinal design of the studies adds strength to the interpretation of results as do the various approaches to defining asthma. However, the strength of inference is limited because findings are based on one cohort. While the studies aimed to produce more individual measures of exposure at the residence, most exposures in these studies were assessed by dispersion models.

6.2.2.3 Subclinical Effects Underlying Development of Asthma or Chronic Bronchitis

Animal toxicological studies demonstrate that long-term NO_2 exposure enhances both responsiveness of airways and the development of allergic responses. Animal toxicological studies and epidemiological studies of long-term exposure show increases in pulmonary inflammation and oxidative stress, providing evidence of NO_2-induced airway injury and suggesting a mechanistic basis for the development of asthma in relation to NO_2 exposure.

Airway Responsiveness

Animal toxicological studies have demonstrated that NO_2 exposure enhances responsiveness of airways to nonspecific and specific challenges. A subchronic-duration study demonstrated concentration-dependent increases in airway responsiveness to histamine in NO_2-exposed guinea pigs (Kobayashi and Miura, 1995). In this study, one experiment demonstrated AHR after 6 weeks of exposure to 4,000 ppb, but not 60 or 500 ppb NO_2. In another experiment, AHR was observed in guinea pigs exposed to 4,000 ppb NO_2 for 6 weeks and to 2,000 ppb for 6 and 12 weeks and to 1,000 ppb for 12 weeks. Specific airways resistance in the absence of a challenge agent was increased in guinea pigs exposed to 2,000 and 4,000 ppb NO_2 for 12 weeks, which indicates the development of airways obstruction. Another subchronic-duration exposure study found delayed bronchial responses, measured as increased respiration rate, in guinea pigs sensitized and challenged with *C. albicans* and exposed to NO_2 [4,760 ppb, 4 h/day, 5 days/week, 6 weeks (Kitabatake et al., 1995)]. However, NO_2 exposure (4,000 ppb, 2 h/day, 3 months) failed to alter airway responsiveness to a nonspecific challenge in rabbits sensitized at birth with house dust mite antigen (Douglas et al., 1995). Overall, results are consistent with those demonstrated in rodent models with short-term exposures to NO_2 (Section 4.3.2.5) and are supported by effects demonstrated on key events underlying these responses, including inflammation, allergic sensitization, and airway remodeling (Section 4.3.2).

Development of Allergic Responses

Toxicological studies provide some experimental evidence that is coherent with the development of allergic responses seen in some of the epidemiologic studies (Section 6.2.4). One subchronic-duration toxicological study showed that exposure to 4,000 ppb NO_2 for 12 weeks led to enhanced immunoglobulin E (IgE)-mediated release of histamine from mast cells isolated from guinea pigs (Fujimaki and Nohara, 1994). This

response was not found in mast cells from rats similarly exposed in the same study. Furthermore, two short-term studies provide evidence that exposure to NO_2 leads to T-derived lymphocyte helper 2 (Th2) skewing and/or allergic sensitization in healthy adults and naïve animal models, as discussed in Sections 4.3.2.6 and 5.2.7.4 (Pathmanathan et al., 2003; Ohashi et al., 1994). Findings of increased histamine release from mast cells, increased nasal eosinophils, and increased Th2 cytokines seen in humans and animal models exposed to NO_2 provide support for the epidemiologic evidence relating NO_2 exposure to asthma development and the findings in some of the epidemiologic studies for the association of NO_2 exposure with the development of allergic responses.

Pulmonary Inflammation and Oxidative Stress

Inflammation and oxidative stress are key events in the mode of action for development of asthma (Figure 4-2). Long-term NO_2 exposure has been shown to induce pulmonary inflammation or oxidative stress in toxicological and epidemiologic studies, but results are not entirely consistent. Similarly, there is some evidence for a relationship of short-term exposure to NO_2 with pulmonary inflammation and oxidative stress (Section 5.2.7.4) to describe a potential pathophysiologic basis for development of asthma in response to repeated NO_2 exposures.

Epidemiologic Evidence in Children

In the CHS cohort of 1,211 schoolchildren from eight Southern California communities, annual average NO_2 was associated with a longitudinal increase in exhaled nitric oxide (eNO; using a flow rate of 50 mL/sec) in 2006−2007 and 2007−2008 (Berhane et al., 2014). This association was observed with adjustment for short-term NO_2 assessed from central monitoring sites and was independent of asthma status. Based on prior findings in CHS (Bastain et al., 2011) that elevated eNO is associated with increased risk of new onset asthma, an effect of long-term exposure to NO_2 on increases in eNO over time is consistent with a role for NO_2 in asthma pathogenesis.

Using LUR models to estimate annual average NO_2 exposure, Liu et al. (2014a) observed null associations with eNO among all children (N = 1,985, ages 10 years, ESCAPE) and those without asthma (n = 1,793) in both the single and copollutant (PM_{10}) models. NO_2 was positively associated with eNO in the 192 children with asthma.

Using a cross-sectional prevalence design, Dales et al. (2008) examined the relationship of eNO and NO_2 in a cohort of 2,402 healthy school children. NO_2 was estimated for each child's residential postal code. Quantitative results were not reported; the study authors only indicate that NO_2 showed positive but statistically nonsignificant

associations with eNO. An eNO-roadway density association persisted after adjustment for air pollutant concentrations (NO_2, SO_2, and $PM_{2.5}$) within the previous 24 and 48 hours of the eNO measure, indicating that the association was unlikely to be confounded by an unmeasured short-term exposure effect.

The short-term evidence base provides support for the development of asthma in relation to NO_2 exposure. Evidence for short-term NO_2-associated increases in oxidative stress and pulmonary inflammation, particularly allergic inflammation (Section 5.2.7.4), informs key events within the modes of action for AHR and asthma development (Section 4.3.5 and Figure 4-2).

Toxicological Evidence

Similar to studies of short-term NO_2 exposure (Section 5.2.4.2), some animal toxicological studies of long-term exposure show increases in pulmonary inflammation and oxidative stress. Compared with short-term exposure studies, long-term studies provide more evidence of NO_2-induced pulmonary injury. Details from these studies, all of which were reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008), are presented in Table 6-2.

Many studies investigating NO_2-induced injury and oxidative stress in the airway measured changes in lipids, which are necessary for both lung function and defense. Sagai et al. (1982) and Ichinose et al. (1983) reported that rats exposed to 40 or 120 ppb NO_2 for 9 or 18 months had increased ethane exhalation and that exposure to 40 ppb for 9 months resulted in increased lipid peroxidation. Arner and Rhoades (1973) showed that rats exposed to 2,900 ppb NO_2 for 9 months had decreased lipid content leading to increased surface tension of the lung surfactant and altered lung mechanics.

Histopathological assessment of lung tissue showed that long-term exposure to NO_2 resulted in alveolar macrophage (AM) accumulation and areas of hyperinflation (Gregory et al., 1983). Kumae and Arakawa (2006) exposed rats to 200, 500, or 2,000 ppb NO_2 from birth or the weanling period (5 weeks old) and assayed bronchoalveolar lavage fluid (BALF) at 8 and 12 weeks of age. Lymphocytes increased at 8 weeks with exposure to 500 ppb NO_2 in the embryonic group, and macrophages and neutrophils were increased at 12 weeks with exposure to 500 ppb NO_2. No changes in differential cell counts were observed in the weanling group at 8 weeks of age, but at 12 weeks of age, lymphocytes were increased with exposures above 500 ppb and neutrophils were increased at 2,000 ppb. The embryonic group also had increased tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) at 8 weeks but not at 12 weeks, while in the weanling group, IFN-γ was increased only at 12 weeks.

Table 6-2 Animal toxicological studies of the respiratory effects of long-term nitrogen dioxide (NO_2) exposure.

Study	Species (Strain); Age; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Arner and Rhoades (1973)	Rats (Long Evans); M	2,900 ppb 5 days/weeks for 9 mo	Histopathologic evaluation and morphometry
Aranyi et al. (1976)	Mice	500 ppb continuously, 2,000 ppb continuously, 100 ppb continuously with daily 3-h peaks of 1,000 ppb, or 500 ppb with daily 1-h peaks of 2,000 ppb for 4, 12, 21, 24, 28, or 33 weeks	Morphometry
Ayaz and Csallany (1978)	Mice (C57BL/6J); F; n = 120	500 ppb or 1,000 ppb continuously for 17 mo	Morphometry
Blair et al. (1969)	Mice; n = 4/group	500 ppb for 6, 18, or 24 h/day, 7 days/week for 3−12 mo	Histopathologic evaluation
Chang et al. (1986)	Rat (F344); 1-day or 6 weeks; M; n = 8/group	(1) 500 ppb continuously with two, daily 1-h spikes of 1,500 ppb, 5 days/week for 6 weeks, (2) 2,000 ppb continuously for 7 days/week for 6 weeks; Two 1-h spikes daily to 6,000 ppb (6-week rats only)	Histopathologic evaluation and lung morphometry
Crapo et al. (1984)	Rat (CD, Fischer 344); 6 week; M	2,000 ppb for 23 h/day; two daily 30-min spikes of 6,000 ppb	Morphometric analysis of proximal alveolar and distal alveolar regions
Ehrlich and Henry (1968)	Mice (Swiss albino); F; n = ≥30/group, n = 4−8/group	(1) 500 ppb continuously, (2) 500 ppb for 6 h/day, (3) 500 ppb for 18 h/day; (1−3) for 1 to 12 mo; challenged with *Klebsiella pneumoniae* after exposure	Mortality, hematology, serum LDH, body weight, bacterial clearance
Fujimaki and Nohara (1994)	Rats (Wistar); 8 weeks; M; n = 10/group Guinea pigs (Hartley); 8 weeks; n = 10/group	1,000, 2,000, or 4,000 ppb continuously for 12 weeks	Mast cell counts and histamine release
Furiosi et al. (1973)	Monkey (*Macaca speciosa*), rat (Sprague-Dawley); maturing (monkey), weanling (rat); M/F (monkey), M (rat); n = 4−5/group (monkey), n = 15−25/group (rat)	(1) 2,000 ppb NO_2 continuously, (2) 330 µg/m^3 NaCl continuously, (3) 2,000 ppb NO_2 + 330 µg/m^3 NaCl continuously; (1−3) for 14 mo	Histopathologic evaluation, hematology

Table 6-2 (Continued): Animal toxicological studies of the respiratory effects of long-term nitrogen dioxide (NO_2) exposure.

Study	Species (Strain); Age; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Greene and Schneider (1978)	Baboons; 3 to 4 yr; M/F; n = 6	2,000 ppb 8 h/day, 5 days/week for 6 mo	Immunologic and histopathologic evaluation
Gregory et al. (1983)	Rat (Fischer 344); 14−16 weeks; n = 4−6/group	(1) 1,000 ppb, (2) 5,000 ppb, (3) 1,000 ppb with two daily, 1.5-h spikes of 5,000 ppb; (1−3) 7 h/day for 5 days/week for up to 15 weeks	Histopathological evaluation, BAL fluid analysis (LDH, ALKP, glutathione peroxidase), antioxidant enzymes in lung homogenates
Hayashi et al. (1987)	Rat (Wistar); M; n = 18−160/group	500 ppb or 5,000 ppb continuously for up to 19 mo	Morphological changes, histology
Henry et al. (1970)	Squirrel monkeys; M; n = 37	5,000 ppb continuously for 2 mo; challenge with *Klebsiella pneumoniae* or influenza after exposure	Infection resistance, mortality, peripheral blood markers, and respiratory function
Ichinose et al. (1983)	Rats (JCL, Wistar); 8 and 13 weeks; M	(1) 10,000 ppb continuously for 2 weeks; (2) 400, 1,200, or 4,000 ppb continuously for 1, 2, 4, 8, 12, or 16 weeks; (3) 40, 400, or 4,000 ppb continuously for 9, 18, or 27 mo	Histopathologic evaluation and morphometry
Kumae and Arakawa (2006)	Rats (Brown-Norway); prenatal exposure; F; n = 201	200, 500, or 2,000 ppb pre- and post-natal for up to 12 post-natal weeks	Immunologic evaluation (alveolar macrophage activity)
Kubota et al. (1987)	Rat (JCL Wistar); 2 mo; M; n = 3−4/group	40, 400, or 4,000 ppb continuously for 9, 18, and 27 mo	Serological examination and lung morphometry
Lafuma et al. (1987)	Hamster (Golden Syrian); M; n = 7−9/group	2,000 ppb NO_2 for 8 h/day for 5 days/week for 2 mo	Lung histopathology and morphometry, lung mechanics, serum elastase activity, and protease inhibitor capacity
Mercer et al. (1995)	Rats (Fischer 344); 7 weeks; M; n = 5/group	500 ppb continuously with 2 daily, 1-h peaks of 1,500 ppb for 9 weeks	Histopathologic evaluation and morphometry
Miller et al. (1987)	Mice (CD-1); 4−6 weeks; F; n = 18−21/treatment group	(1) 200 ppb, (2) 200 ppb daily continuously for 7 days/week with 2 daily, 1-h peaks of 780 ppb 5 days/week; (1−2) 16, 32, or 52 weeks	Histopathologic evaluation, pulmonary function, and antibacterial host defenses

Table 6-2 (Continued): Animal toxicological studies of the respiratory effects of long-term nitrogen dioxide (NO_2) exposure.

Study	Species (Strain); Age; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Sagai et al. (1982)	Rats (JCL, Wistar); 8 weeks; M; n = 6−12/group	10,000 ppb continuously for 2 weeks	Antioxidant levels, enzyme activity, lipid peroxidation
Sagai et al. (1984)	Rats (JCL Wistar); 8 weeks; M; n = 4−6/group	40, 400, or 4,000 ppb continuously for 9, 18, or 27 mo	Histopathologic evaluation and morphometry
Sherwin and Richters (1982)	Mice (Swiss Webster); young adults; M; n = 30/group	340 ppb for 6 h/day for 5 days/week for 6 weeks	Type II pneumocytes in the lungs and alveolar wall area
Stevens et al. (1988)	Rat (Fischer 344); young adult, neonate; M; n = 1 or 6/group	500, 1,000, or 2,000 ppb continuously with two daily, 1-h spikes at 1,500, 3,000, or 6,000 ppb for 5 days/week for 6 weeks	Pulmonary function
Tepper et al. (1993)	Rats (Fischer 344); 60 days; M; n = 11−16/group	500 ppb continuously 7 days/week with two daily, 2-h spikes of 1,500 ppb, 5 days/week for up to 78 weeks	Pulmonary function and lung disease
Wagner et al. (1965)	Dog (Mongrels); M; n = 6−10/group Rabbit; M; n = 4−8/group Guinea pig (English); M; 15−31/group Rat (Sherman); M; n = 20−40/group Mice (HLA, C57Bl/6J, CAF/Jax); M; n = 60−110/group	1,000 or 5,000 ppb continuously for 10−18 months	Pulmonary function and histopathology

ALKP = alkaline phosphatase; BAL = bronchoalveolar lavage; LDH = lactate dehydrogenase; NaCl = sodium chloride; NO_2 = nitrogen dioxide.

Oxidative stress resulting from NO_2 exposure has been further characterized in a number of studies, and the varying effects of NO_2 on antioxidant levels and enzyme activity were presented in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008). After NO_2 exposure, studies have reported both increased and decreased activity of enzymes involved in the glutathione cycle (Sagai et al., 1984; Gregory et al., 1983; Ayaz and Csallany, 1978). Sagai et al. (1984) reported increased nonprotein sulfhydryl levels and glutathione S-transferase (GST) activity in adult male rats after 9 and 18 months of exposure to 400 ppb NO_2 and decreased glutathione peroxidase (GPx) activity, while glucose-6-phosphate dehydrogenase activity increased after exposure to 4,000 ppb NO_2. There were no changes in the activity of 6-phosphogluconate dehydrogenase, superoxide

dismutase (SOD), or disulfide reductase after exposure to 400 ppb NO_2. Gregory et al. (1983) reported increased glutathione peroxidase activity in BALF after 6 weeks of exposure to 5,000 ppb NO_2; however, at 15 weeks, enzyme activity returned to control levels although slight changes in pathology were reported. Ayaz and Csallany (1978) showed that continuous exposure to 1,000 ppb NO_2 for 17 months decreased GPx activity in Vitamin E-deficient mice, while Vitamin E-supplemented mice had increased glutathione peroxidase activity.

These studies demonstrate that long-term NO_2 exposure modifies oxidant balance in the airway and can initiate inflammation; however, the observations from these studies at concentrations relevant to ambient exposures do not consistently show this to be the case across species. Antioxidant enzymes are involved in response to NO_2 exposure, but this response is variable and transient. Overall, these findings are similar to the effects reported from short-term exposures (Section 5.2.2.5).

6.2.2.4 Summary of Development of Asthma or Chronic Bronchitis

The recent evidence base adds several longitudinal studies, including prospective studies, that consistently find a positive association of various NO_2 exposure measures with asthma incidence in children at several ages. The exposure estimates include residential measurements and individual, residential ambient NO_2 concentrations estimated by LUR. Other studies use NO_2 measurements at sites 1−2 km from the subjects' school or home. Asthma incidence also was associated with neighborhood-level ambient NO_2 concentrations estimated by IDW or NO_2 or NO_X estimated for residential locations by dispersion models, which are associated with greater uncertainty in representing the spatial variability in ambient NO_2 concentrations. In adults, positive associations are also observed; however, this evidence base is limited primarily to one adult cohort in Europe, and exposure measures are from dispersion models. Overall, there is a consistency of association that is observed across exposure assessment methods and ages of children examined from 10 to 18 years. None of the studies examined whether there was evidence for an association of NO_2 with health effects independent from other traffic-related pollutants, such as UFP, CO, BC/EC, and $PM_{2.5}$.

Toxicological and controlled human exposure studies reduce some of the uncertainty in the epidemiologic evidence by providing biological plausibility for a relationship between long-term NO_2 exposure and asthma development. In the pathophysiology of asthma, recurrent pulmonary inflammation, allergic sensitization, and subsequent development of AHR play important roles (Section 4.3.5, Figure 4-2).

Long-term-exposure toxicological studies demonstrate NO_2-induced AHR, and experimental studies of repeated short-term exposures provide evidence for NO_2-induced development of allergic responses in healthy adults and animal models as well as increases in neutrophils in healthy adults. In one study of guinea pigs, NO_2-induced (1,000−4,000 ppb) increases in AHR was accompanied by an increase in specific airways resistance, suggesting that airway remodeling may contribute to the development of AHR [(Kobayashi and Miura, 1995); Section 4.3.2.5]. Mechanistic studies indicate that inflammatory mediators and structural changes occurring due to airway remodeling can alter the contractility of airway smooth muscle. Epidemiologic evidence points to associations between short-term increases in ambient NO_2 concentrations and increases in pulmonary inflammation in healthy children and adults (Section 5.2.2.5). An epidemiologic study also indicates an association of long-term NO_2 exposure with longitudinal changes in pulmonary inflammation in healthy children that were independent of short-term changes in NO_2 concentrations. There also is some evidence for pulmonary oxidative stress induced by short-term NO_2 exposure in healthy adults (Section 5.2.7.4) and long-term exposure in rodents (Section 6.2.2.3), although results overall are not consistent. The positive relationship between NO_2 exposures and asthma in longitudinal epidemiological studies and the small body of evidence indicating NO_2 effects on inflammation, allergic sensitization, and AHR, which are key events in the mode of action for the development of asthma, support an independent role for NO_2 exposure in development of asthma.

6.2.3 Severity of Asthma, Chronic Bronchitis, and Chronic Obstructive Pulmonary Disease: Respiratory Symptoms and Hospital Admissions

In the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008), there was limited evidence, consisting of a single prospective cohort study and several cross-sectional studies, to support an association between long-term exposure to NO_2 and respiratory symptoms. Evidence was inconsistent, with uncertainties in the cross-sectional studies related to the temporality of exposure and occurrence of symptoms. Key recent prospective cohort studies evaluating the relationship between respiratory symptoms in children and long-term exposure to NO_2 are summarized in Table 6-3. Studies that evaluated indoor NO_2 concentrations are discussed first, followed by studies related to outdoor NO_2 concentrations. Cross-sectional studies generally are consistent with longitudinal studies (Annesi-Maesano et al., 2012; Ghosh et al., 2012b; Dong et al., 2011; Mi et al., 2006; Pattenden et al., 2006; Nicolai et al., 2003; Brauer et al., 2002; Gehring et al., 2002; Zemp et al., 1999) and were reviewed and are discussed in other sections as appropriate and summarized in the Annex Table AX6.3-17 of the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008).

6.2.3.1 Indoor Nitrogen Dioxide and Respiratory Symptoms in Children and Adults

Effects of indoor NO_2 may not be confounded by all of the same copollutants as outdoor NO_2, although potential confounding by other indoor pollutants, such as emissions from heating sources could occur. For long-term NO_2 exposures, the recent indoor prospective study of school-aged children (Belanger et al., 2013) and the adult indoor prospective study (Hansel et al., 2013) provide evidence that supports a relationship between long-term NO_2 exposure and respiratory symptoms in children with asthma and former smokers with COPD.

Belanger et al. (2013) observed positive associations of asthma severity score, wheeze, nighttime symptoms, and rescue medication use with indoor residential NO_2 where the mean monitoring length was 33 [standard deviation (SD): 7] days. Figure 6-3 illustrates the concentration-response relationships between indoor NO_2 and asthma-related effects using a constrained, natural spline function of $\ln(NO_2)$ and 95% confidence limits as well as threshold functions for each outcome. In adjusted models with quintiles of NO_2 exposure, concentrations >14.3 ppb compared with the reference level (≤6 ppb, designated as the threshold value) was associated with increased risk of a one-level increase in asthma severity score (OR: 1.43 [95% CI: 1.08, 1.88]). These same exposures were also associated with increased risks of wheeze (OR: 1.53 [95% CI: 1.16, 2.02]), night symptoms (OR: 1.59 [95% CI: 1.24, 2.01]), and rescue medication use (OR: 1.74 [95% CI: 1.34, 2.26]). Every fivefold increase in NO_2 exposure >6 ppb was associated with an increase in asthma severity score (OR: 1.37 [95% CI: 1.01, 1.89]) and asthma morbidity measured by wheeze (OR: 1.49 [95% CI: 1.09, 2.03]), night symptoms (OR: 1.52 [95% CI: 1.16, 2.00]), and rescue medication use (OR: 1.78 [95% CI: 1.33, 2.38]).

Recent infant studies are consistent with earlier results (Samet et al., 1993) that showed no association between 2-week avg exposure to NO_2 and the incidence and duration of respiratory illness. Raaschou-Nielsen et al. (2010b) and Sonnenschein-Van der Voort et al. (2012) found no associations between indoor NO_2 exposure and wheezing in infants.

Table 6-3 Prospective studies of long-term nitrogen dioxide exposure and respiratory symptoms in children.

Study[a]	Exposure Assessment	Pollutant Correlation	Statistical Methods	Comments	Results 95% CI[b]
Longitudinal New England Indoor Children's Asthma Study					
Belanger et al. (2013) N = 1,642 Ages 5−10 yr Followed for 1 yr. Asthma severity score from 2006 through 2009. Symptoms: wheeze, nighttime symptoms, rescue medication use and an asthma severity score [which consist of symptoms and medication use based on the Global Initiative for Asthma (NHLBI, 2002)].	Palmes tubes in bedrooms and dayroom for 4 weeks for 4 seasons.	NR	Hierarchical ordered logistic regression adjusted for age, sex, atopy, season of monitoring, race/ethnicity, mother's education, smoking in the home, and all five variables for combined specific sensitization and exposure to indoor allergens. Analyses were based on repeated measures of both NO_2 and asthma outcomes.	Included maintenance medication use as a covariate in models because the use of maintenance medication is also associated with SES.	OR per fivefold increase in NO_2 exposure above 6 ppb Asthma severity score: 1.37 (1.01, 1.89) Wheeze: 1.49 (1.09, 2.03) Night symptoms: 1.52 (1.16, 2.00) Rescue medication use: 1.78 (1.33, 2.38)

Table 6-3 (Continued): Prospective studies of long-term nitrogen dioxide exposure and respiratory symptoms in children.

Study[a]	Exposure Assessment	Pollutant Correlation	Statistical Methods	Comments	Results 95% CI[b]
Childrens Health Study (CHS) Southern California Communities					
McConnell et al. (2003) N = 475 Fourth graders (aged 9−10) and seventh graders (aged 12−13) with a history of asthma or bronchitic symptoms at study entry who completed two or more follow-up questionnaires any time during the yr 1996 to 1999. Yearly follow-up for 4 yr. The overall participation rate in the surveyed classrooms was high (82%).	Annual average NO_2 calculated from monitoring sites established in each of the 12 communities. 4-yr mean (SD; 1996−1999) NO_2 concentrations across communities: 19.4 (11.3) ppb.	Within-community correlations differed in that NO_2 could be distinguished from most other major pollutants except OC and inorganic acid. O_3: 0.59 PM_{10}: 0.20 $PM_{2.5}$: 0.54 $PM_{10-2.5}$: −0.22 I ACID: 0.65 O ACID: 0.48 EC: 0.54 OC: 0.67	Three-stage regression adjusted for age, maternal smoking history, child's sex maternal and child's race; within-community estimates were adjusted for between-community effects of the pollutant, and vice versa.	Copollutant model results: Within communities NO_2 with $PM_{2.5}$: 1.05 NS NO_2 with I ACID: 1.09[c] NO_2 with O ACID: 1.07[d] NO_2 with EC: 1.05[d] NO_2 with OC: 1.04 NS NO_2 with O_3: 1.06 NS NO_2 with PM_{10}: 1.07[d] NO_2 with $PM_{10-2.5}$: 1.08[c] Between communities NO_2 with $PM_{2.5}$: 1.01 NS NO_2 with I ACID: 1.02 NS NO_2 with O ACID: 1.02 NS NO_2 with EC: 1.01 NS NO_2 with OC: 1.01 NS NO_2 with O_3: 1.02[d] NO_2 with PM_{10}: 1.01 NS NO_2 with PM_{10-25}.: 1.02[d]	OR within communities 1.97 (1.22, 3.18) OR between communities 1.22(1.00, 1.49)

Table 6-3 (Continued): Prospective studies of long-term nitrogen dioxide exposure and respiratory symptoms in children.

Study[a]	Exposure Assessment	Pollutant Correlation	Statistical Methods	Comments	Results 95% CI[b]
Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study, the Netherlands					
Gehring et al. (2010) N = 3,863 children in birth cohort aged 1 to 8 yr	LUR models used to provide annual NO_2 concentrations for birth address of each participant.	NO_2-$PM_{2.5}$: 0.93 NO_2-soot: 0.96	Generalized estimating equations adjusted for sex, study arm (intervention or natural history), use of mite-impermeable mattress covers, allergies of mother and father, maternal and paternal education, maternal prenatal smoking, breastfeeding, presence of a gas stove in the child's home, presence of older siblings, smoking, signs of dampness and pets in the child's home, day-care attendance, and Dutch nationality.	No associations were found with atopic eczema, allergic sensitization, and bronchial hyperresponsiveness. No copollutant analyses were conducted.	OR for asthma symptoms: 1.17 (0.98, 1.39) without adjustment for study region. OR for wheeze: 1.27 (1.07, 1.50) without adjustment for study region.

Table 6-3 (Continued): Prospective studies of long-term nitrogen dioxide exposure and respiratory symptoms in children.

Study[a]	Exposure Assessment	Pollutant Correlation	Statistical Methods	Comments	Results 95% CI[b]
Children, Allergy, Milieu, Stockholm, Epidemiology Survey (BAMSE)					
Gruzieva et al. (2013) N = 3,633 Followed from birth (1994−1996) to age 12 yr. Related publications: Melén et al. (2008) Nordling et al. (2008)	Dispersion models used to calculate NO_X for all addresses in the yr 1994 to 2008, representing when the first child was born until the end of the 12-yr follow-up.	NO_X-PM_{10} for first yr of life: 0.96	Multinomial regression/GEE adjusted for municipality, SES, yr the house was built, and heredity.		OR for wheeze at 12 yr of age, 3 or more episodes: 1.35 (0.79, 2.29) per 20 ppb NO_X. No association of NO_X after the first yr of life with asthma symptoms.

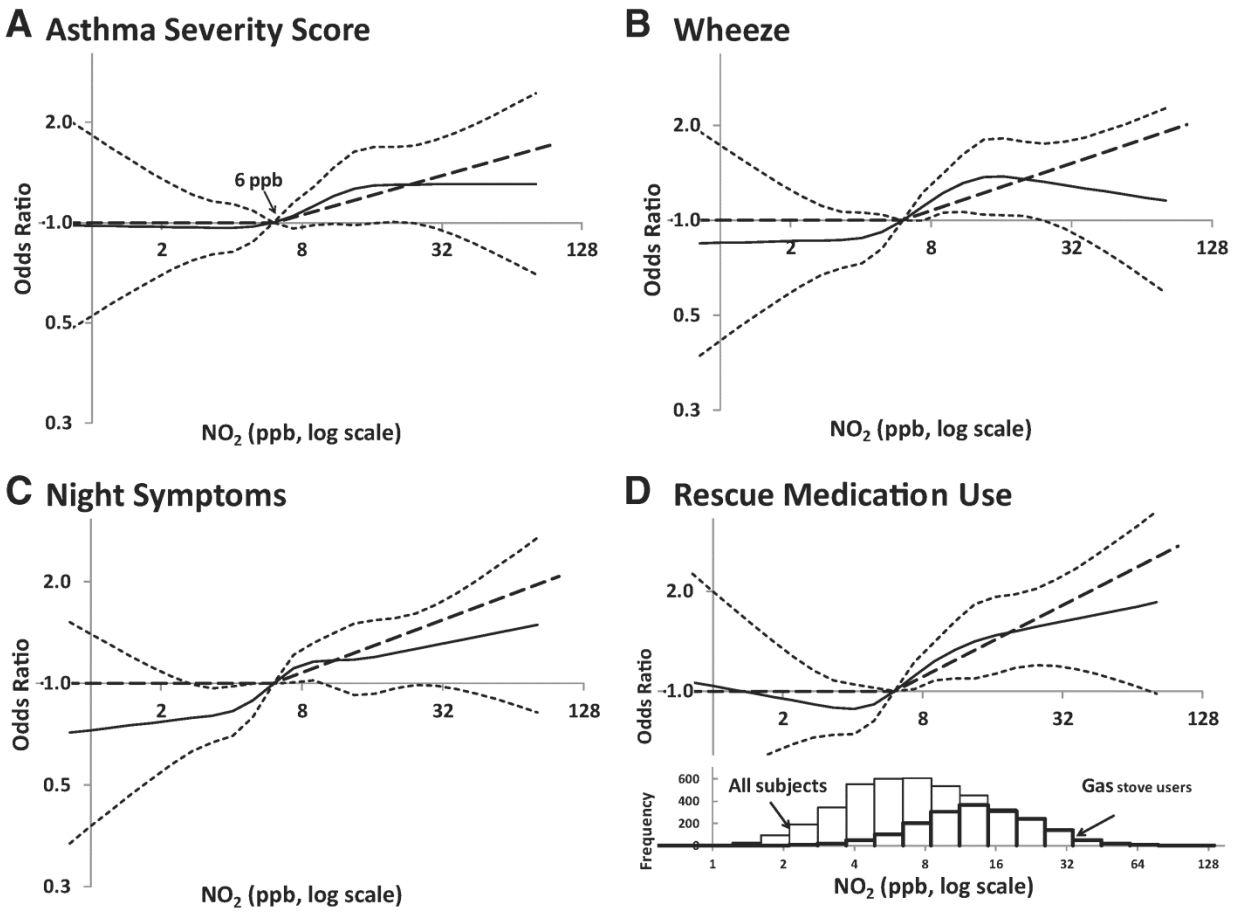
BAMSE = Children, Allergy, Milieu, Stockholm, Epidemiology Survey; CHS = Children's Health Study; CI = confidence interval; EC = elemental carbon; GEE = generalized estimating equations; I ACID = inorganic acid; LUR = land-use regression; NO_2 = nitrogen dioxide; NO_X = sum of NO and NO_2; NS = not statistically significant; O ACID = organic acid; OC = organic carbon; OR = odds ratio; PIAMA = Prevention and Incidence of Asthma and Mite Allergy; $PM_{2.5}$ = 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; $PM_{10-2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 10 µm and greater than a nominal 2.5 µm; SD = standard deviation; SES = socioeconomic status.

[a]Studies are presented in the order of appearance in the text.

[b]Results are presented per 10-ppb increase in NO_2 unless otherwise specified.

[c]$p < 0.05$.

[d]$p < 0.01$.



Note: Also shown is a histogram of NO_2 concentrations measured in subjects' homes (lower portion of panel D) for all observations (thin border) and observations taken in homes of gas stove users (bold border). Indoor NO_2 was modeled as a continuous variable of log-transformed concentrations.

Source: Reprinted with permission of Wolters Kluwer Health, Belanger et al. (2013).

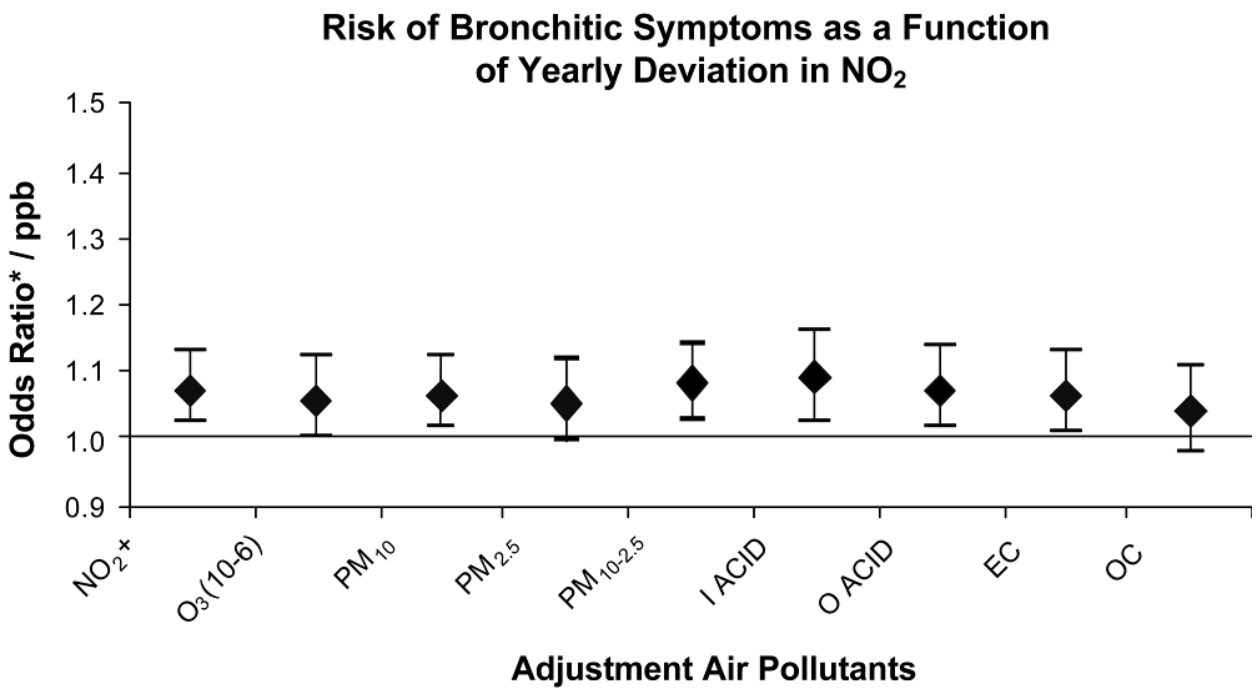
Figure 6-3 Concentration-response relationships between respiratory effects and indoor nitrogen dioxide (NO_2) illustrated with constrained, natural spline functions (solid lines) with 95% confidence limits (small dashed lines) and threshold function (bold dashed line) from hierarchical ordered logistic regression models.

Hansel et al. (2013) investigated indoor NO_2 and $PM_{2.5}$ concentrations in relation to respiratory effects among former smokers with COPD in Baltimore, MD. Air sampling was performed for 1 week at baseline, 3 months, and 6 months in the participant's bedroom and the main living area, which was identified as an additional room where the

participant reported spending the most time. Clinic visits occurred at baseline, 3 months, and 6 months. No interaction was indicated between $PM_{2.5}$ and NO_2, and increasing NO_2 concentrations in the main living area were independently associated with increased dyspnea and increased rescue medication use with adjustment for $PM_{2.5}$. Higher bedroom NO_2 concentrations were associated with increased risk of nocturnal awakenings (OR: 1.59 [95% CI: 1.05, 2.42] per 10-ppb increase) and severe exacerbations (OR: 1.65 [95% CI: 1.02, 2.64]). NO_2 concentrations were not associated with lung function. There was indication of outdoor NO_2 concentrations contributing to indoor NO_2. Among the 26 subjects who lived within 4.8 km of a central monitoring site, outdoor NO_2 concentrations explained 25% of the variance in indoor NO_2 concentrations.

6.2.3.2 Outdoor Nitrogen Dioxide and Respiratory Symptoms in Children

A number of studies (Table 6-3) have observed an association between various respiratory symptoms in children and long-term exposure to outdoor NO_2. McConnell et al. (2003) examined children with asthma for bronchitic symptoms, including daily cough for 3 months in a row, congestion or phlegm 3 months in a row, or bronchitis. Thus, while these symptoms may have started with acute exacerbation of asthma, they were likely to represent chronic indolent symptoms. In copollutant models, the effects of yearly variation in NO_2, ascertained from a central monitoring site in each community, were only modestly reduced by adjusting for another traffic-related copollutant such as $PM_{2.5}$, EC, or organic carbon (OC; Figure 6-4 and Table 6-3).



Source: McConnell et al. (2003). Reprinted with permission of the American Thoracic Society. Copyright © 2014 American Thoracic Society. American Journal of Respiratory and Critical Care Medicine, 168(7): 790−797. Official Journal of the American Thoracic Society.

Figure 6-4 Odds ratios for within-community bronchitis symptoms associated with nitrogen dioxide (NO_2), adjusted for other pollutants in copollutant models for the 12 communities of the Children's Health Study.

Gehring et al. (2010) examined a composite of asthma symptoms (one or more attacks of wheeze, shortness of breath, prescription of inhalation steroids), wheeze (transient, late onset, persistent), sneezing, hay fever, atopic eczema, and prevalence of asthma and observed positive associations with LUR modeled NO_2 exposures. Gruzieva et al. (2013) examined wheeze, categorized as either one or more episodes or three or more episodes in the past year and observed an association with NO_X concentrations from a dispersion model. Aguilera et al. (2013) observed an association between NO_2 exposure and increased risk of upper and lower respiratory tract infections in infants.

Cross-sectional studies provide information that informs the potential for copollutant confounding of associations found with various NO_2 measures. Hwang and Lee (2010) observed positive associations between NO_2 and bronchitic symptoms in children with asthma in copollutant models with $PM_{2.5}$ generally similar to what was observed for single pollutant models (ORs for NO_2 adjusted for $PM_{2.5}$: 2.25 [95% CI: 1.17, 4.33] for

bronchitis; 1.60 [95% CI: 0.76, 3.34] for chronic phlegm; 1.30 [95% CI: 0.53, 3.12] for chronic cough; 2.21 [95% CI: 1.23, 3.97] for bronchitic symptoms per 10-ppb increase in NO_2).

Outdoor Nitrogen Dioxide and Respiratory Symptoms in Adults with Asthma

Studies examining the relationship between long-term NO_2 exposure and respiratory symptoms in adults include prospective studies of asthma incidence in adults discussed in Section 6.2.2.2. Most studies assessed NO_2 exposure from dispersion models. Jacquemin et al. (2009b) report that all the associations between NO_2 and asthma symptoms at ECHRS II were positive. The strongest was for waking "with a feeling of tightness in the last 12 months." Symptoms in the last 12 months at ECRHS II among people without asthma at baseline were also associated with NO_2.

Zemp et al. (1999) report, in a cross-sectional study, the Swiss Study on Air Pollution and Lung Disease in Adults (SAPALDIA), an association between NO_2 and prevalence of respiratory symptoms in adults. Bentayeb et al. (2010) report cross-sectional associations to be weakly positive for cough and phlegm in adults (≥65 years old, in Bordeaux, France) in relation to NO_2 exposure.

Outdoor Nitrogen Dioxide and Asthma Hospital Admissions in Adults

Recent studies represent the first evaluation of the association between long-term NO_2 exposure and hospital admissions for asthma. The relationship between long-term pollutant exposures on the risk for asthma hospital admissions [International Classification of Diseases (ICD)-10: J45−46] in people aged 50−65 years at baseline was evaluated in the Danish Diet, Cancer and Health cohort study (Andersen et al., 2012a). Associations between NO_2 concentration estimated by the Danish Air geographic information system (GIS) dispersion modelling system and hospital admission were found in the full cohort [hazard ratio (HR) per-10 ppb NO_2: 1.44 [95% CI: 1.14, 1.84]). NO_2 was estimated to have a similar effect on the first asthma hospital admission (HR: 1.36 [95% CI: 1.03, 1.80]), but people with a previous asthma hospital admission were at greater risk for re-admission (HR: 3.05 [95% CI: 1.57, 5.90]). NO_2 was associated with a much larger risk of asthma hospital admission among people with previous admission for COPD (HR: 2.34 [95% CI: 1.25, 4.40]). Some of the observed effects could possibly be ascribed to the short-term effects of increases in air pollution on the days prior to asthma admission. The 35-yr avg, 15-yr avg, and 1-yr avg NO_2 at follow-up were highly correlated (r = 0.88, 0.92) and were more strongly associated with asthma hospital admission than was 1-yr avg NO_2 at baseline. The authors indicated that they could not

discern whether the results reflected the importance of more recent exposures or the better performance of the dispersion model in more recent years. The NO_2 exposure estimates may be less certain for earlier time periods because of uncertainty in emission factors and traffic counts that are used as inputs to the dispersion model.

An ecological time-series study, (Delamater et al., 2012) and a cross-sectional study, (Meng et al., 2010) provide inconsistent results in regards to asthma-related emergency department (ED) visits or hospital admissions. Meng et al. (2010) examined subjects ages 1 to 65+ years who reported physician-diagnosed asthma (N = 1,502) in the San Joaquin Valley, CA from among participants of the 2001 California Health Interview Survey. Subjects were assigned annual average concentrations for NO_2 based on residential ZIP code and the closest air monitoring station within 8 km, but data on duration of residence were not available. No associations were found between average annual concentrations of NO_2 and the odds of asthma-related ED visits or hospital admissions. No quantitative results were shown for NO_2.

6.2.3.3 Summary of Severity of Asthma, Chronic Bronchitis, and Chronic Obstructive Pulmonary Disease

Longitudinal studies conducted in children observed associations between long-term ambient NO_2 exposure metrics and an array of respiratory symptoms in school-age children. Results in infants were inconsistent, but transient symptoms are common in infants, but these symptoms may not have strong implications for developing respiratory disease. For children, ambient NO_2 exposure was assessed with outdoor residential measurements, LUR that estimated exposure at subjects' homes, and central site measurements. The McConnell et al. (2003) study is unique in that it is the only prospective study examining bronchitic symptoms in children with asthma. The study authors report stronger associations for NO_2 variation within communities (within-community associations cannot be confounded by any time-fixed personal covariates) than for NO_2 variation between communities.

Further supporting a relationship with NO_2, indoor NO_2 was associated with asthma symptoms and medication use in children with asthma (Belanger et al., 2013) and respiratory symptoms in former smokers with COPD (Hansel et al., 2013). The effect estimates for indoor NO_2 were generally larger than those reported in the studies of outdoor NO_2, and Belanger et al. (2013) provided evidence for a concentration-dependent increase in NO_2-related symptoms. These indoor NO_2 exposures may be part of a different mix of air pollutants than in the ambient air and support an independent effect of NO_2.

An uncertainty in the evidence base is the potential influence of short-term NO_2 exposure. While many studies aimed to characterize chronic symptoms, they did not examine whether associations were independent of short-term NO_2 exposure. Another uncertainty is the potential confounding by other traffic-related pollutants. The available correlations between NO_2 and other pollutants for these respiratory-symptom, long-term prospective studies are found in Table 6-3. Specifically, no data for CO are available. The data show a correlation of 0.54 for ambient EC and 0.96 for soot. For $PM_{2.5}$, the correlations range from about 0.54 to 0.93 (Gehring et al., 2010; McConnell et al., 2003). In limited analysis, NO_2 associations with symptoms persisted with adjustment for EC or $PM_{2.5}$ as measured at central sites and was somewhat attenuated with adjustment for OC. The collective evidence from this group of prospective studies is supportive of a relationship of long-term exposure to NO_2 and increased respiratory symptoms using various indicators in children with asthma, but evidence identifying an independent association of long-term NO_2 exposure is limited.

6.2.4 Development of Allergic Disease

6.2.4.1 Epidemiologic Studies of Children or Adults

Recent cross-sectional studies report results for various aspects of allergic responses and long-term exposure to NO_2. Allergic sensitization indicators included measures of IgE, allergic rhinitis, skin prick test, and reporting respiratory allergy/hay fever. Various age groups were examined, including children less than 6 years old, children aged about 10 years, and adults. As described in Section 6.2.2.3, the few available experimental studies provide support for an effect of long-term or repeated short-term NO_2 exposure on development of allergic responses.

In a nationally representative sample of the U.S. population, Weir et al. (2013) linked annual average concentrations of NO_2 to allergen-specific IgE data for participants 6 years old and older in the 2005−2006 National Health and Nutrition Examination Survey using both monitor-based (within 32.2 km) air pollution estimates and the Community Multiscale Air Quality model (36 km) and observed that increased concentrations of NO_2 were associated with positive IgE to any allergen, inhalant, and indoor allergens.

In the German Infant Nutritional Intervention (GINI) and Lifestyle-Related Factors on the Immune System and the Development of Allergies in Childhood (LISA) cohorts, analysis of individual-based exposure to NO_2 derived from LUR and allergic disease outcomes during the first 6 years of life (Morgenstern et al., 2008) indicated associations

with eczema. Some associations with allergen-specific IgE and hay fever were positive but imprecise with wide 95% CIs. Previous analyses of these cohorts did not indicate associations with runny nose and sneezing at age 2 years (Morgenstern et al., 2007; Gehring et al., 2002). A longitudinal study of the LISA and GINI cohorts (Fuertes et al., 2013) found no evidence that NO_2 exposure in the birth year increases the prevalence of allergic rhinitis or increases risk of aeroallergen sensitization as determined by allergen-specific IgE in children examined at age 10 years. Air pollution concentrations decreased in the study areas during this time.

Annesi-Maesano et al. (2007) related individual data on allergy from 5,338 schoolchildren (ages 10.4 ± 0.7 years) attending 108 randomly chosen schools in six French cities to the concentration of NO_2 measured in school yards with passive diffusion samplers and at fixed-site monitoring stations. In examining associations of a 5-day avg NO_2 concentration with lifetime prevalence of allergic conditions, the authors used the short-term exposure metric to represent long-term exposure NO_2. NO_2 was positively associated with flexural dermatitis and skin prick test to indoor allergens but not with allergic rhinitis or atopic dermatitis. In a large cross-sectional study of school children in Taiwan, Hwang et al. (2006) observed that a 10-ppb increase in NO_2 was associated with a higher prevalence of allergic rhinitis, with an OR of 1.11 (95% CI: 1.08, 1.15). Parker et al. (2009) evaluated the association between ambient pollution monitoring data and childhood respiratory allergies in the U.S. using the 1999−2005 National Health Interview Survey of approximately 70,000 children and observed no associations between NO_2 and the reporting of respiratory allergy/hay fever.

As part of the same study of schools in six French cites, Annesi-Maesano et al. (2012) evaluated the relationship between indoor air quality in schools and the previous-year allergic and respiratory health of schoolchildren (mean age 10.4 years). For each pollutant, a 5-day mean concentration in the classroom was computed and categorized into tertiles, independent of the city (low <9.7 ppb, medium >9.7 to <12.9 ppb, high >12.9 ppb NO_2). Between-school and within-school variability of the measured indoor pollutants were estimated using linear mixed models for longitudinal data. Among children with atopy (N = 1,719), high NO_2 was related to previous-year allergic asthma but not allergic rhinitis.

Nordling et al. (2008) reported that exposure to dispersion-modeled NO_X from traffic during the first year of life was associated with sensitization (measured as specific IgE) to inhalant allergens, especially pollen (OR: 1.24 [95% CI: 1.04, 1.49] per 10-ppb increase in NO_2). The relationship between the development of allergic sensitization in children during the first 8 years of life and long-term exposure to NO_X was evaluated in a prospective analysis of the Children, Allergy, Milieu, Stockholm, Epidemiology Survey

(BAMSE) cohort (Gruzieva et al., 2012). There was no overall risk of sensitization at 4 years of age associated with NO_X assessed from a dispersion model.

In a cross-sectional analysis of 30,139 Chinese children aged 3 to 12 years, Dong et al. (2011) evaluated the relationship between 3-yr avg of NO_2 and allergy rhinitis. Among children without allergic predisposition (N = 26,004), several positive associations with NO_2 were observed, mainly in males. Among children with an allergic predisposition, associations were detected in males and females.

Pujades-Rodriguez et al. (2009) examined a cohort of 2,644 adults aged 18−70 years living in Nottingham, U.K. to evaluate the relationship between NO_2 exposure and allergy-related effects. In cross-sectional analyses, they found generally null associations between NO_2 concentration and skin test positivity, total IgE, and questionnaire-reported eczema or hay fever. Total IgE levels were not related to NO_2 concentrations in 369 adults with asthma in five French centers as part of the Epidemiological Study on the Genetics and Environment of Asthma (Rage et al., 2009) but were related to O_3 concentrations.

6.2.4.2 Summary of Development of Allergic Responses

As described in Section 6.2.2.3, a few available experimental studies demonstrate the effects of repeated short- or long-term NO_2 exposure on development of an allergic phenotype in healthy adults and animal models. These findings not only suggest the possibility that chronic or recurrent exposure to NO_2 may lead to the development of asthma but also support a role for NO_2 exposure in the development of allergic conditions.

Long-term NO_2 exposure has been linked to various indicators of allergic sensitization in a few cross-sectional studies of children, but not consistently for outcomes such as allergic rhinitis or hay fever. In children 6 years and younger and for adults, the various allergic indicators are not related to NO_2 exposure. For the age group of children about 10 years old, NO_2 was related to allergic sensitization as assessed by allergen-specific IgE or skin prick test in cross-sectional studies but not the longitudinal study. NO_2 metrics aimed at characterizing individual exposures, such as 5-day measurements in school yards and residential estimates from LUR, produced inconsistent results.

6.2.5 Lung Function and Lung Development

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) characterized long-term prospective studies as showing a relationship between NO_2 concentrations and decrements in lung function and reduction in lung development in children. A key uncertainty associated with these studies was the high correlation of NO_2 concentrations with other ambient pollutants. Recent prospective cohort studies add to the evidence base that evaluates the relationship between supervised lung function tests and long-term NO_2 exposure.

6.2.5.1 Lung Function and Development in Children

The key longitudinal prospective studies summarized in Table 6-4 and Figure 6-7 continue to show a relationship between long-term NO_2 exposure and decrements in lung function, especially as children reach later ages. Lung function continues to increase through early adulthood with growth and development, then declines with aging (Stanojevic et al., 2008; Zeman and Bennett, 2006; Thurlbeck, 1982). Thus, the relationship between long-term NO_2 exposure and decreased lung function over time in school-age children into early adulthood is an indicator of decreased lung development.

The CHS has examined three separate cohorts for pollutant effects on lung function and development [1993 cohort in Gauderman et al. (2004), 1993 and 1996 cohorts in Breton et al. (2011) and Gauderman et al. (2007), and 2002 cohort in Urman et al. (2014)]. The results of Breton et al. (2011) are consistent with earlier results from Gauderman et al. (2004). Both Gauderman et al. (2007) and Urman et al. (2014) assessed copollutant models that included another regional or near-roadway pollutant, and Urman et al. (2014) examined the joint effect of NO_X and $PM_{2.5}$.

Table 6-4 Prospective studies of long-term nitogen dioxide exposure and lung function and lung development in children.

Study[a]	Exposure Assessment	Pollutant Correlation	Statistical Methods	Comments	Results (95% CI)[b] FEV_1 and FVC (mL) MMEF, PEF, and FEF (mL/sec)
Children's Health Study (CHS) California Communities					
Gauderman et al. (2004) N = 1,759 Ages 10−18 yr from 1993 cohort 8-yr follow-up.	Central monitoring station in each of 12 study communities, beginning in 1994. Average hourly concentrations of NO_2 used to compute annual averages. Then, calculated long-term mean pollutant concentrations (from 1994 through 2000). NO_2 range across communities: 34.6 ppb	NO_2-acid vapor: 0.87 NO_2-PM_{10}: 0.67 NO_2-$PM_{2.5}$: 0.79 NO_2-EC: 0.94 NO_2-OC: 0.64	2 stage linear regression adjusted for log values for height, BMI, BMI squared, race, Hispanic ethnic background, doctor-diagnosed asthma, any tobacco smoking by the child in the preceding yr, exposure to environmental tobacco smoke, and exercise; or respiratory tract illness on the day of the test and indicator variables for the field technician and the spirometer.	Children who moved away from their recruitment community were classified as lost to follow-up and not tested further. The number of children available for follow-up was 1,414 in 1995, 1,252 in 1997, 1,031 in 1999, and 747 in 2001, reflecting the attrition of approximately 10% of subjects per yr. Model fit was not better in copollutant than in single-pollutant models. No quantitative data shown. Adjustment for 3-day avg NO_2 before each child's lung function test did not alter association for long-term NO_2.	FVC: −27.5 (−54.7, −0.2) FEV_1: −29.3 (−47.5, −11.1) MMEF: −61.0 (−109.1, −12.8)

Table 6-4 (Continued): Prospective studies of long-term nitogen dioxide exposure and lung function and lung development in children.

Study[a]	Exposure Assessment	Pollutant Correlation	Statistical Methods	Comments	Results (95% CI)[b] FEV_1 and FVC (mL) MMEF, PEF, and FEF (mL/sec)
Gauderman et al. (2007) N = 3,677 children Mean age 10 yr (SD 0.44) 12 CHS communities	Central community monitoring sites for NO_2. Range of mean NO_2 across communities: 34.6 ppb Identified several indicators of residential exposure to traffic from large roads.	NR	Regression analysis. Examined models containing both local traffic exposure and regional air pollutants. Adjusted for height, height squared, BMI, BMI squared, present asthma status, exercise or respiratory illness on the day of the test, any tobacco smoking by the child in the previous yr, and indicator variables for field technician.	NO_2 and distance to freeways were independently associated with decrements in lung development. Compared with living >1,500 away from a freeway, living within 500 m of a freeway was associated with a mean percentage-predicted FEV_1 of 97.0% (94.6, 99.4) and MMEF of 93.4% (89.1, 97.7).	Change in FEV_1 over 8-yr period: −32 mL/sec 95% CI not reported.
Breton et al. (2011) 2 cohorts of fourth-grade children 1993 cohort 1: n = 1,759 1996 cohort 2: n = 2,004 Mean age at baseline: 10.0 yr. Monitored for 8 yr, through 12th grade.	Central monitoring stations in each of the original 12 study communities from 1994 to the present. Average hourly concentrations of NO_2 used to compute annual averages. NO_2 range across communities: 33.9 ppb	NO_2-$PM_{2.5}$ = 0.79 NO_2-O_3 = −0.11	Hierarchical mixed effects with adjustment for height, height squared, BMI, BMI squared, current asthma status, exercise or respiratory illness on the day of the test, any tobacco smoking by the child in the last yr, glutathione S-transferase mu 1 genotype, and indicator variables for the field technician.	Main purpose was to determine whether sequence variation in genes in the glutathione synthesis pathway alters susceptibility to air pollution effects on lung function. Haplotype "0100000" was associated with a 39.6 mL, 29.1 mL, and 51.0 mL/sec reduction in 8-yr growth of FEV_1, FVC, and MMEF, respectively.	FEV_1: −29.83 (−49.96, −9.70) FVC: −29.84 (−54.73, −4.95) MMEF: −54.38 (−90.80, −17.96)

Table 6-4 (Continued): Prospective studies of long-term nitogen dioxide exposure and lung function and lung development in children.

Study[a]	Exposure Assessment	Pollutant Correlation	Statistical Methods	Comments	Results (95% CI)[b] FEV_1 and FVC (mL) MMEF, PEF, and FEF (mL/sec)
Urman et al. (2014) N = 1,811 children (82% of the active cohort) from 8 CHS communities Cohort established in 2002.	LUR developed from 900 monitoring sites in CHS communities (Franklin et al., 2012). Near roadway NO_2, NO, and NO_X assessed based on (1) residential distance to the nearest freeway or major road and (2) estimated near-roadway contributions to residential NO_2, NO, and NO_X. Cross-validation R^2 for NO_2 = 0.69, 0.72 Regional O_3, NO_2, PM_{10}, $PM_{2.5}$ assessed from central sites. Community mean centered distribution for NO_2: 6.4 ppb	For regional pollutant concentrations: NO_2-PM_{10}: 0.06 NO_2-$PM_{2.5}$: 0.60 For near-roadway NO, NO_2, and NO_X (within communities): >0.90	Linear regression models (with fixed effects for each study community) for near-roadway estimates. Mixed model (with random intercept for community) for regional pollutants and joint effects with near-roadway NO_2, NO, and NO_X. Adjusted for log of height and its squared value, BMI, BMI squared, sex, age, sex × age interaction, race, Hispanic ethnicity, respiratory illness at time of test, and field technician and study community.	Lung function deficits of 2−3% were associated with regional PM_{10}, $PM_{2.5}$, and O_3 across the range of exposure between communities. NO_2 was associated with FEV_1 but not FVC. Associations with regional pollution and near-roadway NO_X were independent in models adjusted for each. The effects of near-roadway NO_X were not modified by regional pollutant concentrations.	Per 20 ppb increase in near-roadway NO_X: FVC: −1.74% (−2.93, −0.55%) FEV_1: −1.23% (−2.44, −0.01%) Associations were observed in all communities and were similar for NO_2 and NO. Residential proximity to a freeway was associated with a reduction in FVC.

Table 6-4 (Continued): Prospective studies of long-term nitogen dioxide exposure and lung function and lung development in children.

Study[a]	Exposure Assessment	Pollutant Correlation	Statistical Methods	Comments	Results (95% CI)[b] FEV_1 and FVC (mL) MMEF, PEF, and FEF (mL/sec)
Children, Allergy, Milieu, Stockholm, Epidemiology Survey (BAMSE)					
Schultz et al. (2012) N = 1,924 children followed from birth until 8 yr of age. Related Publications: Nordling et al. (2008)	Long-term NO_X exposure estimated using dispersion model and emission inventories. Time-weighted average exposures for various time windows estimated based on lifetime residential, day care, and school addresses. Short-term exposure assessed from central sites.	NR	Linear regression adjusted for municipality, sex, age, height and heredity for asthma and/or allergy. Additional adjustments for temperature, relative humidity, ozone, and PM_{10} levels during 3−7 days before each child's pulmonary function test showed little effect on the estimates of the long-term effects of air pollution.	The odds ratios associated with 80 and 85% of predicted FEV_1 were 2.1 (95% CI: 0.6, 8.1) and 3.4 (95% CI: 1.6, 7.4), respectively, for first yr exposure to NO_X. Specific adjustment for short-term NO_X was not discussed.	Per 47 µg/m^3 increase in NO_X in first yr of life: −34.9 mL (−80.1, 10.4) in FEV_1 Group sensitized against any common inhalant or food allergens, and those with asthma at 8 yr: −98.9 mL (−169.4, −28.4) No clear association seen with NO_X after infancy.

Table 6-4 (Continued): Prospective studies of long-term nitogen dioxide exposure and lung function and lung development in children.

Study[a]	Exposure Assessment	Pollutant Correlation	Statistical Methods	Comments	Results (95% CI)[b] FEV_1 and FVC (mL) MMEF, PEF, and FEF (mL/sec)
PIAMA					
Eenhuizen et al. (2013) N = 880 children age 4 yr	Long-term average air pollution concentrations of NO_2, $PM_{2.5}$, and soot at the residential address at birth were assessed using LUR models. Mean NO_2 10.4 ppb Daily average air pollution concentrations on the day of interrupter resistance (Rint) measurement obtained from central sites.	For birth address: NO_2-$PM_{2.5}$: 0.93 NO_2-soot: 0.96 NO_2 on test day and long-term NO_2: 0.55 NO_2 on the day before the test and long-term NO_2: 0.57	Multiple linear regression adjusted for sex, age at examination (days), height, weight, maternal prenatal smoking, any smoking in the child's home, use of gas for cooking, parental allergy, dampness in the home, education of the parents, season, temperature, and humidity on the day of the Rint measurement.	First report of an association in 4-yr old children. Rint at age 4 yr predicted asthma and wheeze at age 8 yr. Long-term average $PM_{2.5}$ and soot associated with Rint. Adjustment for individual level confounders, season and weather on the test day reduced air pollution effect estimates only slightly. A monotonic increase of Rint with increasing NO_2 concentration was seen, with no threshold identified.	Change in Rint: 0.05 (0.001, 0.11) kPa*s/L

Table 6-4 (Continued): Prospective studies of long-term nitogen dioxide exposure and lung function and lung development in children.

Study[a]	Exposure Assessment	Pollutant Correlation	Statistical Methods	Comments	Results (95% CI)[b] FEV_1 and FVC (mL) MMEF, PEF, and FEF (mL/sec)
Manchester Asthma and Allergy Study (MAAS)					
Mölter et al. (2013) Mölter et al. (2010a) Mölter et al. (2010b) Mölter et al. (2012) N = 1,185 Birth cohort recruited between 1995 and 1997 and evaluated at ages 3. 5. 8 and 11 yr.	Microenvironmental Exposure Model that estimates personal exposure by incorporating children's time-activity patterns and LUR modeled concentrations. The modeled estimates agreed well with measured NO_2 concentrations. Mean (SD) NO_2: 14.76 (6.6) ppb	PM_{10} and NO_2 moderately to strongly correlated in all exposure time windows. Pearson r = 0.59 to 0.89.	Generalized estimating equations. Potential confounding variables evaluated included sex, age, ethnicity, older siblings, sensitization, asthma or current wheeze, family history of asthma, parental smoking, parental atopy, daycare attendance during the first 2 yr of life, hospitalization during the first 2 yr of life, presence of a gas cooker in the home, presence of a dog or cat in the home, visible signs of dampness or mold in the home, body height, body weight, BMI, maternal age at birth, gestational age, duration of breast feeding, Tanner stage (age 11 only), and socioeconomic status (paternal income).		Change in percentage predicted FEV_1 from age 5−11 yr: −15.60 (−26.13, −5.26). Based on the average predicted FEV_1 in cohort of 1.65 L, change equals total decrease in FEV_1 of 263 mL. Change in post-bronchodilator FEV_1 from age 5−11 yr: −22 (−37, −8.0). Equivalent to total 413 mL decrease.

Table 6-4 (Continued): Prospective studies of long-term nitogen dioxide exposure and lung function and lung development in children.

Study[a]	Exposure Assessment	Pollutant Correlation	Statistical Methods	Comments	Results (95% CI)[b] FEV_1 and FVC (mL) MMEF, PEF, and FEF (mL/sec)
Mexico City School Children Cohort					
Rojas-Martinez et al. (2007a) Rojas-Martinez et al. (2007b) N = 3,170 children Age 8 yr at baseline, from 31 schools Examined April 1996 through May 1999.	NO_2 assigned from closest air-quality monitoring station within 2 km of school. NO_2 mean (SD) across communities: 27.2 (10.9) to 42.6 (13.2)	24-h avg NO_2 and 8-h avg O_3: 0.166 24-h avg NO_2 and 24-h avg PM_{10}: 0.250	General linear mixed models adjusted for age, BMI, height, height by age, weekday time spent in outdoor activities, and environmental tobacco smoke.	NO_2, O_3, and PM_{10} were associated with decrements in lung development after adjusting for short-term averages (day before lung function measurement) for the pollutants.	Girls FVC: −40 (−46, −34) FEV_1: −27 (−33, −22) $FEF_{25-75\%}$: 7 (−8, 18) Boys FVC: −38 (−44, −31) FEV_1: −22 (−28, −16) $FEF_{25-75\%}$: 3 (−10, 16)

Table 6-4 (Continued): Prospective studies of long-term nitogen dioxide exposure and lung function and lung development in children.

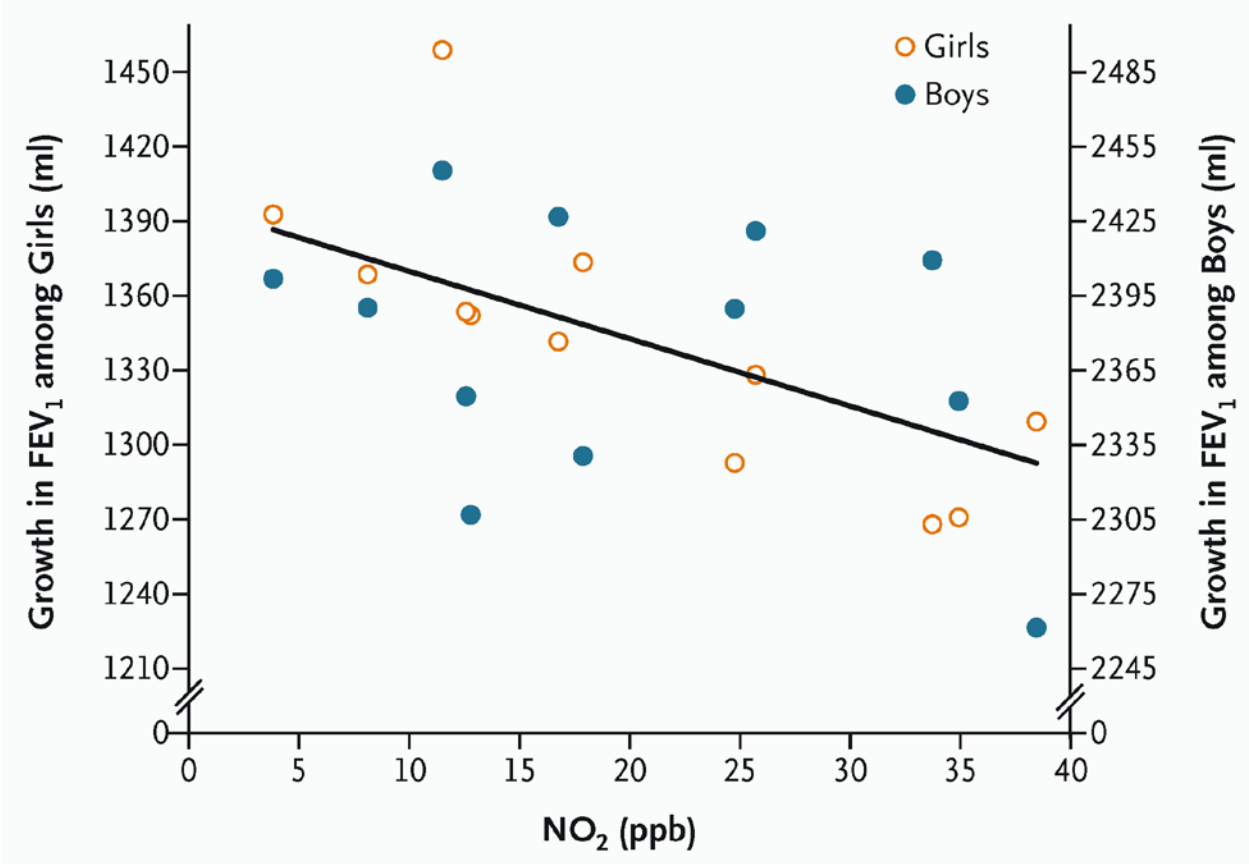
Study[a]	Exposure Assessment	Pollutant Correlation	Statistical Methods	Comments	Results (95% CI)[b] FEV_1 and FVC (mL) MMEF, PEF, and FEF (mL/sec)
Oslo Birth Cohort					
Oftedal et al. (2008) N = 2,307 children ages 9 and 10 yr Children lived in Oslo, Norway, since birth. Spirometry conducted 2001−2002. Related Publications: Slørdal et al. (2003)	NO_2 estimated for 1 km^2 of residence with dispersion model based on emissions, meteorology, topography, and central site measurements. Modeled values represented ambient concentrations reasonably well. Mean NO_2: 16 ppb in the first yr 11.86 ppb for lifetime exposure	NO_2 and PM *r* = 0.83−0.95	Multiple linear regression adjusted for sex, height, age, BMI, birth weight, temperature Lags 1−3 days before the lung function test, current asthma, indicator for participation in the Oslo Birth Cohort study, maternal smoking in early lifetime, parental ethnicity, education, and smoking.	Associations with NO_2 were stronger in girls. In models that included both short- and long-term NO_2 exposures, only the association with long-term NO_2 remained.	NO_2 in first yr of life among all children: FEV_1: −6.0 (−18.0, 6.2) FVC: −1.4 (−14.6, 11.8) PEF: −57.9 (−92.5, −22.3) $FEF_{50\%}$: −37.3 (−71.2, −3.5)

BAMSE = Children, Allergy, Milieu, Stockholm, Epidemiology Survey; BMI = body mass index; CHS = Children's Health Study; CI = confidence interval; EC = elemental carbon; FEF = forced expiratory flow; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; kPa*s/L = kilopascals per second per liter; LUR = land-use regression; MAAS = Manchester Asthma and Allergy Study; MMEF = maximum (or maximal) midexpiratory flow; NO = nitric oxide; NO_2 = nitrogen dioxide; NO_X = sum of NO and NO_2; O_3 = ozone; OC = organic carbon; PEF = peak expiratory flow; PIAMA = Prevention and Incidence of Asthma and Mite Allergy; PM = particulate matter; $PM_{2.5}$ = 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;SD = standard deviation.

Note: $FEF_{50\%}$ = forced expiratory flow at 50% of forced vital capacity. $FEF_{25-75\%}$ = forced expiratory flow between 25 and 75% of forced vital capacity.

[a]Studies are presented in the order of appearance in the text.

[b]Results are presented for a 10 ppb change in NO_2 and 20 ppb change in NO_X unless otherwise specified.



Source: Reprinted with permission of the Massachusetts Medical Society, Gauderman et al. (2004).

Figure 6-5 Community-specific average growth in forced expiratory volume in 1 second (FEV_1; mL) among girls and boys during the 8-year period from 1993 to 2001, plotted against average nitrogen dioxide (NO_2) concentrations from 1994 through 2000.

Urman et al. (2014) examined lung function in 1,811 cohort children (82% of the active cohort) from eight communities in the CHS cohort established in 2002−2003 for near-roadway and regional air pollution exposure effects. Since the beginning of this study, regional pollutant levels have been measured continuously at central monitoring locations in the study communities. LUR models were developed from 900 monitoring sites in the CHS communities (Franklin et al., 2012). Distance to roadway and central site air pollution were also analyzed. LUR models also included NO_X estimated from a dispersion model to estimate near-roadway NO_X. For forced expiratory volume in 1 second (FEV_1), there was little change in the association of near-roadway NO_X after adjusting for a copollutant (i.e., O_3, $PM_{2.5}$ or PM_{10}). Central site NO_2 remained associated with FEV_1 after adjustment for near-roadway NO_X. Near-roadway NO_2 also was

associated with decrements in lung function. Near-roadway NO_X was associated with lung function decrements in children with and without asthma, suggesting that traffic-related pollution may potentially affect all children. The association for within-community near-roadway NO_X was somewhat less than that for between-community regional NO_X, although the strength of inferences from quantitative comparisons of effect estimates for regional and near-roadway pollution are limited.

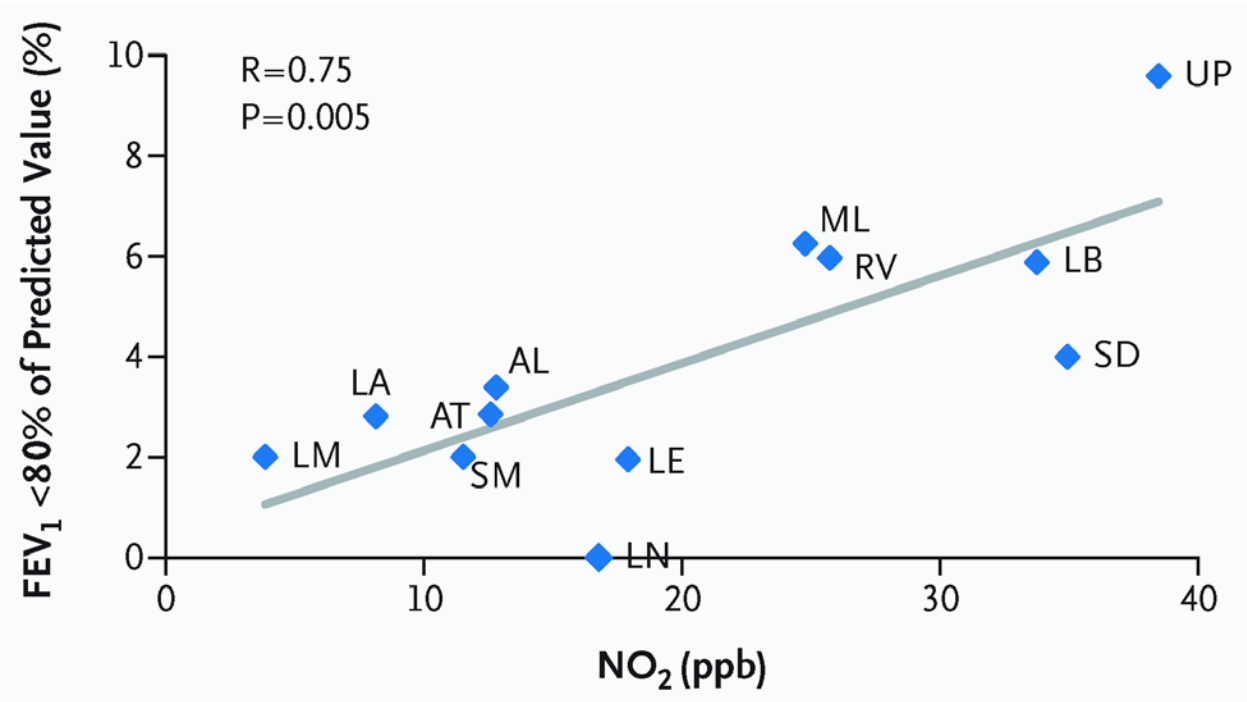
Gauderman et al. (2007) reported results of an 8-year follow-up on 3,677 children who participated in the CHS. The FEV_1 reduction was −31.5 mL (95% CI not reported) for an increase in NO_2 of 10 ppb. Children living <500 m from a freeway (N = 440) had deficits in lung development over the 8-year follow-up compared to children who lived at least 1,500 m from a freeway. When examined in the same model, both distance to freeway and NO_2 measured at community central sites were associated with decrements in lung development. Acid vapor, EC, PM_{10}, and $PM_{2.5}$, but not O_3, were associated with reduced lung development. There was no evidence that the association for NO_2 differed according to distance to freeway or vice versa. Throughout the 8-year follow-up, around an 11% loss of study participants per year was observed.

Gauderman et al. (2004) examined the 1993 CHS cohort of 1,759 children aged 10 to 18 years and states that although the average increase in FEV_1 over time was larger in boys than in girls, the associations of lung development with NO_2 measured at community air monitoring sites did not differ between the sexes, as shown in Figure 6-5. As depicted by the regression line in Figure 6-5, for both sexes combined, the average difference in FEV_1 growth over the 8-year period between the communities with the lowest and highest 8-yr avg NO_2 concentration (34.6 ppb difference) was −101.4 mL (95% CI: −164.5, −38.4).

Gauderman et al. (2004) further indicated that NO_2 exposure over the 8-year follow-up was associated with clinically relevant decrements in attained lung function at the age of 18 years (Figure 6-6). Clinical importance was defined as FEV_1 less than 80% of the predicted value for height, body mass index (BMI), sex, race/ethnicity, and asthma status. Across the 12 communities, higher NO_2 was associated with an increase in the percentage of children with FEV_1 less than 80% predicted.

A recent study examined the relationship between long-term exposure to air pollution and lung function in 1,924 school-age children in the Swedish birth cohort BAMSE (Schultz et al., 2012). NO_X exposure during the first year of life was associated with a deficit in FEV_1. The odds ratios of having a deficit of 80% and 85% of predicted FEV_1 were 1.69 (95% CI: 0.67, 4.2), and 2.6 (95% CI: 1.4, 4.5), respectively, for a 20 ppb increase in NO_X. No impact of short-term air pollution exposure on the estimates of the long-term

effects of air pollution was observed in analyses of PM_{10}; similar analyses were not conducted for NO_X.



Note: The correlation coefficient (*R*) and *P* value are shown for each comparison. AL = Alpine, AT = Atascadero, LE = Lake Elsinore, LA = Lake Arrowhead, LN = Lancaster, LM = Lompoc, LB = Long Beach, ML = Mira Loma, RV = Riverside, SD = San Dimas, SM = Santa Maria, and UP = Upland. NO_2 = nitrogen dioxide.

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Figure 6-6 Community-specific proportion of 18-year-olds with a forced expiratory volume in 1 second (FEV_1) below 80% of the predicted value, plotted against the average concentrations of nitrogen dioxide (NO_2) from 1994 through 2000.

Because of the difficulties of lung function examinations in young children, such as those 4 years old or younger, limited data are available. Eenhuizen et al. (2013) assessed the relationship between long- and short-term exposure to traffic-related air pollution and interrupter resistance (Rint), an indicator of airway resistance, in 4-year-old children participating in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) Dutch birth cohort study. Of the original invited 1,808 children, a total of 880 children were in the final analysis. The children with valid Rint data did not have different characteristics than the population recruited for the study. Long-term average concentrations of NO_2, $PM_{2.5}$, and soot at the residential address at birth were assessed

using LUR models as discussed in Section 6.2.2.1, and daily average air pollution concentrations on the day of clinical examination were obtained. Positive associations were observed between long-term average NO_2 concentrations and Rint. Such findings are supported by the study showing NO_2-induced increased airway resistance in guinea pigs (Section 6.2.2.3). They also support a relationship between NO_2 and asthma, given that Rint at age 4 years was a predictor of asthma and wheeze at age 8 years. A monotonic increase in Rint with increasing NO_2 concentration, with no suggestion of a threshold was observed. Short-term exposure was not associated with interrupter resistance. NO_2 concentrations on the test day and the day before the test were moderately correlated with long-term concentrations. This is the first report of an association in 4-year-old children. Because of the high correlation between modelled $PM_{2.5}$, NO_2 and soot (quantitative data not reported), the study could not disentangle an independent association for any of the examined pollutants.

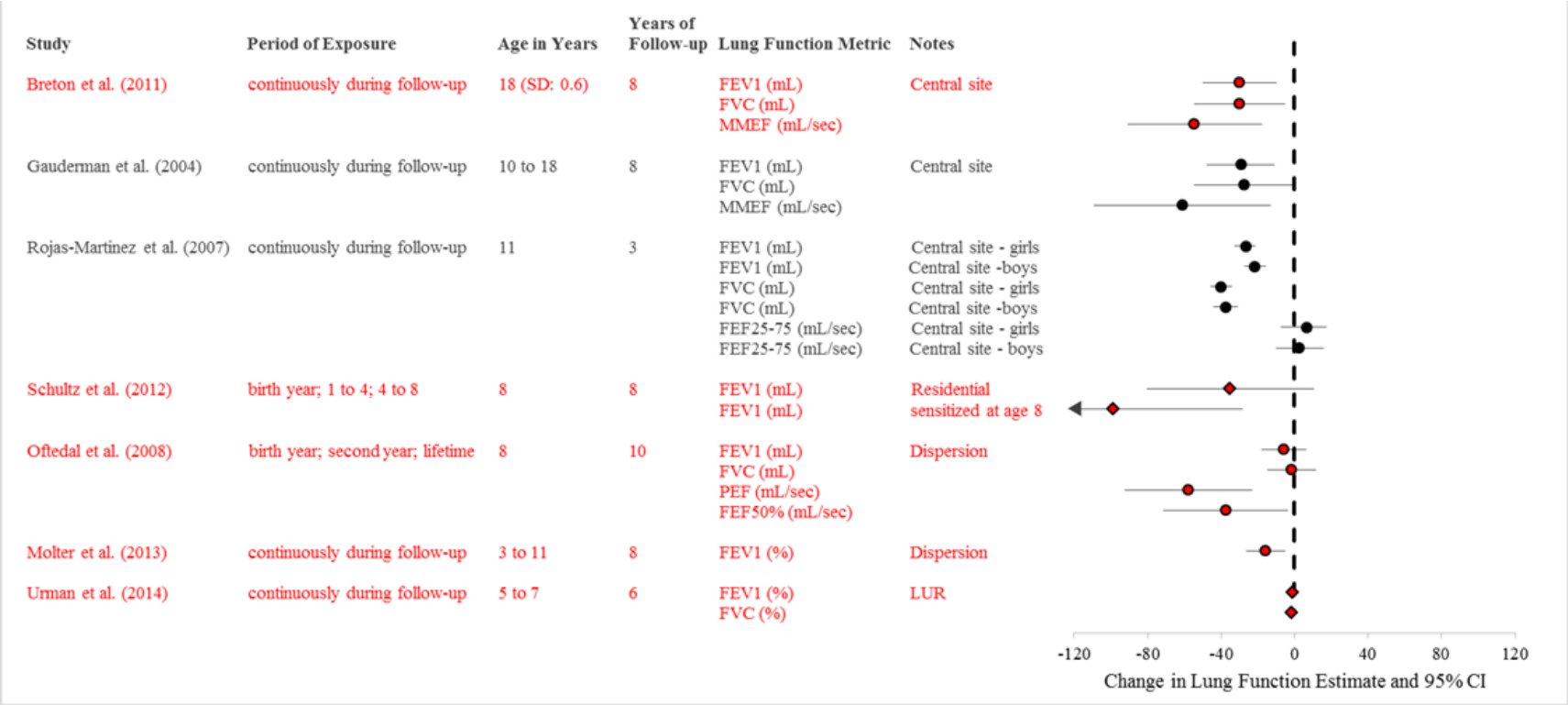
The long-term effects of PM_{10} and NO_2 exposure on specific airway resistance (sRaw) and FEV_1 before and after bronchodilator treatment was examined within the Manchester Asthma and Allergy Study (MAAS) birth cohort [N = 1,185; (Mölter et al., 2013)]. At age 11 years, the cohort size was 813. The authors utilized an LUR model that incorporated children's time-activity patterns to produce total exposure estimates with spatial resolution at the individual level rather than community level (the Microenvironmental Exposure Model). The model was validated and there was good agreement between modeled and measured total personal NO_2 concentrations for short-term averaging times (Mölter et al., 2012). Lifetime exposure to NO_2 was associated with less growth in FEV_1 (percentage predicted) over time, both before (16.0% [95% CI: −26.0, −0.5] for a 10-ppb increase in NO_2) and after bronchodilator treatment (23% [95% CI: −37.0, −9.0]).

As part of ESCAPE, Gehring et al. (2013) analyzed data from birth cohort studies conducted in Germany, Sweden, the Netherlands, and the U.K. that measured lung function at 6 to 8 years of age (N = 5,921). The five birth cohorts [BAMSE, MAAS, German Infant Nutritional Intervention covers urban Munich, Germany, and its surrounding areas (GINI SOUTH, GINI/LISA), and PIAMA] were discussed in Section 6.2.2.1. Annual average exposures to NO_2, NO_X, $PM_{2.5}$, PM_{10}, PM coarse, and PM absorbance at the birth address and current address were estimated by LUR models, except for the BAMSE cohort, for which a dispersion model was used. Associations of lung function with estimated air pollution concentrations and traffic indicators were examined for each cohort using linear regression analysis, and then combined by random effects meta-analysis. Across the five cohorts, annual mean (SD) for NO_2 ranged from 7.44 (2.87) to 12.6 (1.91) ppb. Long-term associations were adjusted for short-term changes in pollutants measured at central sites. NO_2 and NO_X estimated for the current

address were associated with decrements in both FEV_1 and forced vital capacity (FVC) as were $PM_{2.5}$ and $PM_{2.5}$ absorbance. NO_2 and $PM_{2.5}$ at the current address also were associated with peak expiratory flow (PEF). For the five cohorts combined, NO_2 at the birth address was associated with a smaller decrease in lung function than was NO_2 for the current address. Short-term (7-day avg) exposure to NO_2 and PM_{10} also were associated with lung function decrements. Two traffic measures (i.e., traffic intensity nearest street and traffic load on major roads within a 100-m buffer) were associated with deficits in lung function, although the effect estimates had wide confidence intervals, indicating imprecise associations. Annual average concentrations of NO_2, NO_X, $PM_{2.5}$, and PM_{10} at the current address were associated with clinically relevant lung function decrements (FEV_1 < 85% predicted). In copollutant models with NO_2 and $PM_{2.5}$, effect estimates for both pollutants were reduced, but the relative impact on NO_2 and $PM_{2.5}$ differed among lung function indices. The association for NO_2 was reduced more than that for $PM_{2.5}$, for FEV_1, and PEF. In contrast, the association for $PM_{2.5}$ was reduced more for FVC than for NO_2. These findings add to the notion that exposure to NO_2 may result in reduction in lung function in school children, but uncertainties related to the potential for confounding by traffic-related copollutants do not conclusively support independent effects.

In Mexico City, Mexico, Rojas-Martinez et al. (2007b) and Rojas-Martinez et al. (2007a) evaluated lung development in a prospective cohort of children aged 8 years at baseline. Long-term pollutant exposures were assigned from the closest central monitoring site located within 2 km of schools. An unspecified number of children were lost to follow-up during the study, mainly because they moved to another area of the city or to another city altogether. Information was obtained from a total of 3,170 children. A 10-ppb increase in NO_2 was associated with an annual deficit in FEV_1 of 27 (95% CI: 22, 33) mL in girls and 22 (95% CI: 16, 28) mL in boys. The negative association for NO_2 persisted in copollutant models with O_3 or PM_{10}. A deficit in lung development was observed for O_3, PM_{10}, and NO_2 after adjusting for the short-term associations of these pollutants (previous-day concentrations).

A cohort study in Oslo, Norway, examined associations of short- and long-term NO_2 and other pollutant exposures on PEF and forced expiratory flow at 25% of forced vital capacity and 50% of forced vital capacity in 2,307 children ages 9−10 years (Oftedal et al., 2008). In models that included both short- and long-term NO_2 exposures estimated from dispersion models, only the association with long-term NO_2 remained. Adjusting for a contextual socioeconomic factor diminished the association with NO_2.



Note: Studies in red are recent studies. Studies in black were included in the 2008 ISA for Oxides of Nitrogen. All mean changes in this plot are standardized to a 10-ppb increase in NO_2 and a 20-ppb increase in NO_X concentration. Effect estimates from studies measuring NO_X in $\mu g/m^3$ (Schultz et al., 2012) have not been standardized. Circles = NO_2; Diamonds = NO_X.

Figure 6-7 Associations of nitrogen dioxide (NO_2) or the sum of nitric oxide and NO_2 (NO_X) with lung function indices from prospective studies.

6.2.5.2 Lung Function in Adults

Prospective studies evaluating long-term NO_2 exposure and pulmonary function in adults are discussed. The limited number of cross-sectional studies (Forbes et al., 2009b; Sekine et al., 2004) have results that are consistent with those from prospective studies.

Götschi et al. (2008) examined the relationship between air pollution and lung function in adults in the ECRHS cohort. FEV_1 and FVC were assessed at baseline and after 9 years of follow-up from 21 European centers (followed-up sample N = 5,610). Quantitative results were not reported; NO_2 was reported only to show no statistically significant association with average lung function. This is in contrast to the results from Ackermann-Liebrich et al. (1997) (SAPALDIA) and Schikowski et al. (2005) [Study on the Influence of Air Pollution on Lung, Inflammation, and Aging (SALIA)], which examined far more homogenous populations than the population assessed in the ECRHS.

A recent study (Boogaard et al., 2013) evaluated the impact on pulmonary function of a reduction in outdoor pollution concentrations resulting from the policy implementation of forbidding old heavy duty vehicles in all inner cities and other related policies. At 12 locations in the Netherlands, air pollution concentrations were measured on the street where participants lived within 500 m of subjects' homes. Respiratory health was measured in 2008 and 2010, during which air pollution concentrations decreased. The study population included both children and adults. Eighty-four percent were above 30 years of age at baseline. The participation rate in the study was around 10%. Over the two time periods, 585 subjects were re-evaluated for spirometry. Reductions in concentrations of NO_2 and NO_X as well as soot, copper (Cu), and Fe were associated with increases in FVC. Airway resistance decreased with a decline in PM_{10} and $PM_{2.5}$, although these associations were somewhat less consistent. No associations were found with eNO. Results were driven largely by the small group of residents living at the one urban street where traffic flow as well as air pollution were drastically reduced.

In a Nottingham, U.K. cohort of adults aged 18−70 years, lung function changes were evaluated in a cross-sectional analysis of 2,599 subjects at baseline and a longitudinal analysis of 1,329 subjects followed up 9 years later (Pujades-Rodriguez et al., 2009). There were no substantial cross-sectional associations between home proximity to the roadside and NO_2 concentration with lung function or any other outcome. Also, neither exposure was associated with a decline in FEV_1 over time. Insufficient contrast in NO_2 exposure (interquartile range: 18.1−19.1 ppb) may be a factor in the inability to detect any associations for NO_2 in this study population.

6.2.5.3 Toxicological Studies of Lung Function

Studies included in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) showed inconsistent evidence of changes in lung function in animals after long-term exposure to concentrations of NO_2 relevant to ambient exposure. No recent studies are available. Arner and Rhoades (1973) published early studies that exposed rats to 2,900 ppb NO_2 continuously for 5 days/week for 9 months and reported changes in lipid composition in the airway that could be related to observed functional consequences, including decreased lung volume and compliance and increased surface tension, although these changes have not been consistently observed in animal studies.

Tepper et al. (1993) exposed rats to a background concentration of 500 ppb NO_2 for 16 h/day followed by a 6 hour peak of 1,500 ppb and 2 hours of downtime for up to 78 weeks. Frequency of breath was significantly slower in these animals and was paralleled by a trend toward increased tidal volume, expiratory resistance, and inspiratory and expiratory time, although changes were not statistically significant. Mercer et al. (1995) and Miller et al. (1987) published studies with similar exposures in rats and mice, respectively, and also reported that NO_2 exposure did not alter lung function, although mice tended to have slightly decreased vital capacity from 16 to 52 weeks of exposure.

Inconsistent effects were also described in studies of long-term NO_2 exposure in the range of 6−7 weeks. Stevens et al. (1988) exposed 1-day and 7-week old rats to 500, 1,000, and 2,000 ppb NO_2 continuously with two daily peaks at three times the baseline concentration (1,500, 3,000, and 6,000 ppb) for 1−7 weeks and observed different results among age groups. Rats exposed from 1 day of age had increased lung compliance after 3 weeks of exposure that returned to control levels by 6 weeks (1,000 ppb with 3,000 ppb peaks). In rats exposed from 7 weeks of age, compliance was decreased after 6 weeks of exposure at 1,000 and 2,000 ppb NO_2. In an 8-week study, Lafuma et al. (1987), reported increased lung volumes in animals exposed to 2,000 ppb (8 h/day, 5 days/week), but vital capacity and compliance were not affected.

6.2.5.4 Summary of Lung Function

In children, recent findings from longitudinal studies provide further support that early-life NO_2 exposure is associated with long-lasting impact on the lung development. Additionally, cross-sectional studies (Gao et al., 2013; Svendsen et al., 2012; Lee et al., 2011b; Rosenlund et al., 2009b; Tager et al., 2005; Sekine et al., 2004; Moseler et al., 1994) report associations between NO_2 exposure and decrements in lung function in children. A meta-analysis across five birth cohorts in Europe using LUR exposure

estimates reported results consistent with the rest of the evidence base. Investigation in adults is limited, but some longitudinal studies indicate associations. Associations are observed in young children aged 4 years. Effects were mainly on FEV_1, which reflects the mechanical properties of the airways and not as much on FVC, representing lung size. Two longitudinal studies examined associations with short-term NO_2 and report that the short-term exposures do not impact the association between long-term NO_2 exposure and lung function. Recent studies use exposure methods such as LUR that provide more individual exposure estimates than central sites do. Results have been observed in various locations in studies using varied exposure assessment methods, lung function measurements, and time of follow-up with children. A linear concentration-response relationship was observed in one study. In limited analysis of copollutant models, NO_2 associations persisted with adjustment for O_3, and the NO_X association persisted with adjustment for $PM_{2.5}$. In copollutant models with $PM_{2.5}$, NO_2 remained associated with FVC but not FEV_1 or PEF.

The limited analysis of potential confounding by traffic-related copollutants, inconsistent results with $PM_{2.5}$ adjustment, and high copollutant correlations often observed produces uncertainty as to whether NO_2 has an independent effect on lung function. Animal studies do not address this uncertainty in the epidemiologic evidence as they demonstrate inconsistent effects of long-term NO_2 exposure on lung function. However, age may be an important factor that influences the effect of NO_2 on lung function that has not been adequately addressed by the existing body of toxicological evidence.

6.2.6 Changes in Lung Morphology

While no recent studies are available, the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) reported that animal toxicological studies demonstrate morphological changes to the respiratory tract resulting from exposure to NO_2. Details from the available studies are presented in Table 6-2. Studies examined long-term exposures to NO_2 to determine effects on lung structure and morphology and report variations in response to concentrations below 5,000 ppb. Wagner et al. (1965) exposed dogs, rabbits, guinea pigs, rats, hamsters, and mice to 1,000, 5,000, or 25,000 ppb NO_2 for up to 18 months and found enlarged air space and edema and areas of mild to moderately thickened septae with chronic inflammatory cells. However, some of these observations were also made in control animals and were not considered to be significant in any species. Importantly, this study demonstrated differences in sensitivity to NO_2 across species. Furiosi et al. (1973) exposed monkeys and rats to 2,000 ppb NO_2 continuously for 14 months and also found species-specific responses; monkeys experienced hypertrophy of the bronchiolar epithelium that was most notable in the respiratory bronchioles in addition to

development of a cuboidal phenotype in the squamous proximal bronchiolar epithelium. In rats, these effects were more occasional under identical exposure conditions.

The majority of other morphologic studies employed rodent models to evaluate effects of NO_2 exposure. Chang et al. (1986) compared responses in mature and juvenile rats to an urban exposure pattern of NO_2 for 6 weeks (500 ppb continuously with two daily peaks at 1,500 ppb). Mature rats were more sensitive to NO_2 exposure and exhibited increased surface density of the alveolar basement membrane and decreased air space in the proximal alveolar regions, accompanied by an increase in lung volume attributable to Type II cell hyperplasia and increases in fibroblasts, alveolar macrophages, and extracellular matrix. In the juvenile rats, effects of exposure were limited to thinning of Type II cells that were spread over more surface area compared to controls. Mercer et al. (1995) found more subtle effects in rats with this exposure; lungs did not appear to have differences in alveolar septal thickness, parenchymal cell populations, or cellular size and surface area after 9 weeks of exposure. Although the frequency of fenestrae was increased in the alveolar epithelium, there were no changes found in the extracellular matrix or interstitial cells. Crapo et al. (1984) conducted a 6-week study in rats with a similar exposure pattern at higher concentrations (2,000 ppb NO_2 for 23 h/day with two 30-minute peaks of 6,000 ppb) and reported hypertrophy and hyperproliferation of the alveolar epithelium. In another study, rats were exposed to a similar urban exposure pattern in addition to a single high concentration for up to 15 weeks; these animals had subpleural alveolar macrophage accumulation and areas of focal hyperinflation, though the mean linear intercept (MLI), a measure of free distance in the air space, was not changed (Gregory et al., 1983). Conversely, Lafuma et al. (1987) reported that hamsters exposed to 2,000 ppb NO_2 for 8 h/day, 5 days/week for 8 weeks had increased MLI and decreased internal surface area, but no lesions were found in the bronchiole or bronchiolar epithelium, alveolar ducts, or alveolar epithelium.

Kubota et al. (1987) conducted a 27-month study in rats that included pathological assessments of the airways after continuous exposure to 40, 400, or 4,000 ppb NO_2. At the highest exposure, rats had increased bronchial epithelial proliferation after 9 and 18 months, and by 27 months, proliferation and edema resulted in fibrosis. Exposure to 400 ppb produced similar morphological changes in the bronchial epithelium that was not apparent until 27 months. Exposure to 40 ppb NO_2 did not result in morphological changes that could be identified by microscopic techniques. Studies conducted at similar concentrations and durations reported analogous effects. Blair et al. (1969) and Hayashi et al. (1987) exposed mice and rats, respectively to 500 ppb for up to 19 months. Blair et al. (1969) described an increase in alveolar size after 3 months of exposure with loss of cilia in respiratory bronchioles, which persisted at 12 months. After 4 months of exposure, Hayashi et al. (1987) reported Type II cell hypertrophy and interstitial edema

leading to thickened alveolar septa at 6 months and fibrous pleural thickening at 9 months. Similarly, exposure to 500 ppb for 7 months resulted in interstitial edema and Type II cell hyperplasia in rats, and additional injury at 1,000 ppb included loss of cilia in the terminal bronchioles (Yamamoto and Takahashi, 1984). Type II cell hyperplasia was also documented by Sherwin and Richters (1982) as well as an increase in the MLI. These studies demonstrate that long-term exposure to relatively high ambient concentrations of NO_2 can result in subtle changes in lung morphology including Type II cell hyperplasia, loss of cilia in the bronchiolar region, and enlarged airspace.

6.2.7 Respiratory Infection

Toxicological studies, as reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008), demonstrated NO_2-induced mortality from infection in experiment animals as well as changes in host defense mechanisms. Epidemiologic investigation of the relationship between long-term NO_2 exposure and respiratory infection is limited, particularly in prospective studies.

6.2.7.1 Epidemiologic Studies of Respiratory Infection

Respiratory infection and symptoms of infection in infants and young children up to 3 years of age were examined in recent studies (Aguilera et al., 2013; Sunyer et al., 2004). In a multicenter prospective cohort study, Sunyer et al. (2004) observed no associations between 2-week indoor NO_2 exposure and lower respiratory tract infections during the first year of life. Aguilera et al. (2013) observed an association between increased NO_2 exposure estimated by LUR and increased risk of upper and lower respiratory tract infections in infants.

In a population-based case-control study in Hamilton, Ontario, Canada, Neupane et al. (2010) examined the relationship between ambient NO_2, $PM_{2.5}$, and SO_2 and hospital admission for community-acquired pneumonia in 345 hospitalized patients aged 65 years or more. Control participants (n = 494) aged 65 years or more were randomly selected by telephone calls from the same community as cases from July 2003 to April 2005. Air pollutants in this study were assessed as exposures over the previous 12 months to test the chronic effect rather than an acute effect. Three methods were used to estimate the annual average NO_2 concentrations: daily ambient data, LUR models, and IDW. Participants had to present to the emergency room with at least two signs and symptoms for pneumonia and have a new opacity on a chest radiograph interpreted by a radiologist as being compatible with pneumonia. NO_2 and $PM_{2.5}$ were associated with hospital

admission for community-acquired pneumonia, but SO_2 was not. NO_2 exposures estimated by all three methods were associated with pneumonia (ORs per 10 ppb increase: 3.20 [95% CI: 1.37, 7.45] for IDW; 1.97 [95% CI: 1.21, 3.19] for bicubic spline; 1.93 [95% CI: 1.00, 3.74] for LUR). There was no mention of adjustment for short-term exposure effects, and it is not clear what the relative impacts on respiratory infections are for short-term versus long-term exposure. While there are associations observed between short-term NO_2 exposure and hospital admissions for pneumonia (Table 5-30), quantitative comparisons with long-term NO_2 exposure effect estimates may not be informative given the differences in exposure assessment methods and distribution of NO_2 concentrations.

The association between parent report of physician-diagnosed pneumonia, croup, and otitis media during early childhood and annual average concentrations of NO_2, NO_X, $PM_{2.5}$, $PM_{2.5}$ absorbance, PM_{10}, and particulate matter with a nominal aerodynamic diameter less than or equal to 10 μm and greater than a nominal 2.5 μm ($PM_{2.5–10}$; coarse PM) was examined in 10 European birth cohorts: BAMSE (Sweden), Gene and Environmental Prospective Study in Italy [GASPII (Italy)], Gene and Environmental Prospective Study in Italy plus environmental and genetic influences (GINIplus), Lifestyle-Related factors on the Immune System and the Development of Allergies in Childhood plus the influence of traffic emissions and genetics [LISAplus (Germany)], MAAS (U.K.), PIAMA (the Netherlands), and four Infancia y Medio Ambiente cohorts [Spain; (MacIntyre et al., 2014b)]. Exposures were estimated using LUR models and assigned to children based on their residential address at birth. Identical protocols were used to develop LUR models for each study area as part of the ESCAPE project. There was a complete outcome (at least one), exposure (a minimum of NO_2 and NO_X), and potential confounder information for 16,059 children across all 10 cohorts. For pneumonia, the meta-analysis produced a combined adjusted OR of 1.64 (95% CI: 1.02, 1.65) per 10-ppb increase in NO_2. NO_2 was associated with otitis media but not croup. The air pollution data used to build the LUR models were measured in 2008−2011, but children in the study cohorts were born as early as 1994. To address this temporal mismatch, a sensitivity analyses was conducted using routine monitoring data to back-extrapolate LUR estimates and produced results generally consistent with the main findings. Correlations between $PM_{2.5}$ and NO_2 ranged between 0.42 and 0.80, and correlations between $PM_{2.5}$ absorbance and NO_2 ranged between 0.40 and 0.93. For pneumonia, the ORs (95% CI) for NO_2 in copollutant models with $PM_{2.5}$ and $PM_{2.5}$ absorbance were respectively: 1.32 (0.72, 2.42) and 1.36 (0.57, 3.28), showing attenuation and large increase in width of 95% CIs from the single pollutant model for NO_2.

6.2.7.2 Toxicological Studies of Respiratory Infection

Long-term NO_2 exposure has been shown to increase susceptibility of experimental animals to infection. Henry et al. (1970) published a study showing that squirrel monkeys exposed to 5,000 ppb NO_2 for a period of 2 months and then exposed to *Klebsiella pneumoniae* or Influenza had increased markers of infection, white blood cell counts and erythrocyte sedimentation rate (ESR), 3 days post-infection. Furthermore, 2 of the 7 monkeys exposed to NO_2 died at 3 and 10 days post-infection. When influenza virus was given 24 hours prior to NO_2 exposure and after NO_2 exposure, tidal volume and respiratory rate increased and the ESR increased. One of the three exposed monkeys died 5 days post-infection. Ehrlich and Henry (1968) and Ehrlich (1980) also studied the effects of NO_2 on *Klebsiella pneumoniae* infection in mice. Exposures were either continuous or intermittent (6 or 18 h/day) at a concentration of 500 ppb NO_2 and bacterial challenge was administered at 1, 3, 6, 9, and 12 months. Continuous exposure to NO_2 for 3 months or longer resulted in increased mortality rates after infection, whereas intermittent exposures led to increased mortality at 6, 9, and 12 months. Likewise, Miller et al. (1987) showed increased mortality in mice exposed to a base of 200 ppb NO_2 with two daily 1-hour peaks of 800 ppb and subsequent challenge with *Streptococcus zooepidemicus* at 16, 32, and 52 weeks.

6.2.7.3 Subclinical Effects Underlying Respiratory Infection

Impaired host defense mechanisms can increase susceptibility to bacterial and viral infection, and toxicological studies have demonstrated that experimental animals exposed to concentrations of NO_2 relevant to ambient exposure for periods greater than 6 weeks have modulated lung host defense including altered characteristics of AMs. Details from these studies, which were also reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008), are presented in Table 6-2.

Alveolar macrophages play a critical role in removing pathogens from the airways and impaired function can increase susceptibility to infection and injury. Aranyi et al. (1976) found that AM morphology was abnormal after 21 weeks of continuous exposure to 2,000 ppb NO_2 and/or a base of 500 ppb NO_2 with 3-hour peaks of 2,000 ppb, although exposures at lower concentrations had no effects on AM morphology. Chang et al. (1986) showed that exposure to 500 ppb NO_2 continuously with 1,500 ppb 1-hour peaks twice daily for 6 weeks increased the number of macrophages in the alveoli and their cellular volume. Gregory et al. (1983) reported similar findings and observed AM accumulation in lung sections by light microscopy after exposure to 5,000 ppb NO_2 or a base of 1,000 ppb NO_2 with 5,000 ppb spikes twice each day for 15 weeks.

Greene and Schneider (1978) investigated the effects of NO_2 exposure on the function of AMs isolated from antigen-sensitized baboons exposed to 2,000 ppb NO_2 for 8 h/day, 5 days/week for 6 months and found that AMs had diminished response to migration inhibitory factor obtained from antigen-stimulated lymphocytes. However, sample size in this study was small: 3 exposed to NO_2 and antigen, 1 exposed to NO_2 alone, 1 exposed to antigen alone, and 1 air control. Other studies have not reported on this endpoint.

In addition to AMs, mast cells play an important role in host defense and inflammatory processes. Fujimaki and Nohara (1994) investigated the effects of a 12-week continuous exposure to 1,000, 2,000, and 4,000 ppb NO_2 in both rats and guinea pigs. Although the number of mast cells in the airway increased after exposure to 2,000 and 4,000 ppb, these changes were not statistically significant. Histamine, released by mast cells, was reduced in rats at 2,000 ppb NO_2 and increased in guinea pigs at 4,000 ppb. This observation suggests species differences in response to NO_2 exposure.

6.2.7.4 Summary of Respiratory Infection

In the small body of epidemiologic studies, long-term NO_2 exposure estimated for subjects' homes by LUR was associated with respiratory infections in school children and pneumonia hospital admissions in adults ages 65 years or older. Results were inconsistent in infants. Particularly for hospital admissions, it is not clear whether the association observed for long-term NO_2 exposure is independent of an association with short-term exposure. As examined in school children, associations for long-term NO_2 exposure were positive with adjustment for $PM_{2.5}$ or $PM_{2.5}$ absorbance, but the 95% CIs were very wide. Thus, an independent association for NO_2 is not clearly indicated. A small body of toxicological studies provide support for an independent effect of NO_2 exposure on respiratory infections, showing that mice and monkeys exposed to NO_2 concentrations in the range of 200 to 5,000 ppb for periods greater than 6 weeks have increased infection-induced mortality and altered AM morphology and function.

6.2.8 Chronic Obstructive Pulmonary Disease

Recent epidemiologic studies have examined associations between long-term NO_2 exposure and effects related to COPD, including the study of indoor NO_2 and respiratory symptoms in adults with COPD (Hansel et al., 2013) described in Section 6.2.3.1. There are few studies examining COPD development, and results are inconsistent. In a prospective cohort study, Andersen et al. (2011) estimated outdoor annual average NO_2 and NO_X since 1971 by a validated LUR model for residential locations and calculated

time-weighted averages for 15-, 25- and 35-year periods (Raaschou-Nielsen et al., 2000). No other pollutants were considered. COPD hospital admissions were ascertained from 1976, and incidence of COPD was defined as first hospital admission between 1993−1997 and June 2006. COPD incidence was associated with the 35- and 25-year mean concentration of NO_2 (HR: 1.28; [95% CI: 1.07, 1.54] and 1.22 [95% CI: 1.03, 1.45] per 10-ppb increase) and 35-year mean concentration of NO_X (1.16 [95% CI: 1.04, 1.31] per 20-ppb increase). Weaker positive associations were observed with 25-year mean NO_X, 15-year mean NO_2 and NO_X, and baseline residence traffic proxies (major road within 50 m, traffic load within 200 m). The associations with NO_2 were stronger than those with NO_X. The association was stronger in people with diabetes and asthma compared to the rest of the cohort, but no difference in association was observed by smoking or occupational exposure. COPD incidence was most strongly associated with 35-yr avg NO_2, suggesting that long-duration, possibly lifetime exposure may be associated with development of COPD.

Gan et al. (2013) evaluated a population-based cohort in Canada that included a 5-year exposure period and a 4-year follow-up period. All residents aged 45−85 years who resided in Metropolitan Vancouver, Canada, during the exposure period and did not have known COPD at baseline were included in this study (N = 467,994). Five-year average residential exposures to NO_2 and NO as well as BC, $PM_{2.5}$, and wood-smoke were estimated using LUR models, incorporating changes in exposure over time due to changes in residences. COPD incidence was ascertained from a hospital admissions database and defined as admission during the follow-up period. Mortality data were also studied and are discussed in Section 6.2. The Spearman correlations for NO_2 with BC and $PM_{2.5}$ were respectively 0.39 and 0.47. The association of 5-year NO_2 with COPD hospital admission was null. The exposure period examined in this study was shorter than that in Andersen et al. (2011) (i.e., 25−35 years).

In the ECRHS cohort, the association of NO_X with prevalence of COPD and related symptoms was investigated by two methods for assessing exposure to power plant-specific emissions of NO_X and SO_2 (Amster et al., 2014). NO_X exposures (8-yr avg) related to power plant emissions were estimated for subjects' residences (N = 2,244) based on kriging ambient concentrations from 20 central site monitors downwind of the power plant (source approach), and peak emission events (event approach) were defined as 30-minute concentrations that exceeded 125 ppb NO_2. Neither source-based nor event-based power plant NO_X emissions was associated with COPD prevalence. Respiratory symptoms were associated with source-based NO_X but not event-based NO_X.

In a cross-sectional study, Wood et al. (2009) examined the association of outdoor air pollution with respiratory phenotype (PiZZ type) in alpha 1-antitrypsin deficiency

(α-ATD) from the U.K. α-ATD registry. This deficiency leads to exacerbated responses to inflammatory stimuli. In total, 304 PiZZ subjects underwent full lung-function testing and quantitative high-resolution computed tomography to identify the presence and severity of COPD emphysema. Annual average NO_2 was estimated for subjects' homes with dispersion models. NO_2 was associated with improved gas transfer and less severe emphysema. Similar associations were observed with SO_2 and particles. In contrast, O_3 was associated with worse gas transfer and more severe emphysema, albeit accounting for only a small proportion of the lung function variability. NO_2 was negatively correlated with O_3, which might explain NO_2 associations with gas transfer and emphysema severity. The dispersion model also may not well represent long-term NO_2 exposures.

6.2.9 Summary and Causal Determination

There is likely to be a causal relationship between long-term NO_2 exposure and respiratory effects, based strongly on evidence integrated across disciplines for a relationship with asthma development. There is continued epidemiologic evidence for effects on decrements in lung function and partially irreversible decrements in lung development in children. Other, more limited lines of evidence include NO_2-related increases in respiratory symptoms in children with asthma, chronic bronchitis/asthma incidence in adults, COPD hospital admissions, and respiratory infection.

The conclusion of a likely to be causal relationship represents a change from the "suggestive, but not sufficient, to infer a causal relationship" determined in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008). The main difference in the evidence base in this review compared to the 2008 ISA is recent epidemiologic evidence from several longitudinal studies that indicate associations between asthma incidence in children and long-term NO_2 exposures estimated for outside children's homes. In contrast, the 2008 ISA for Oxides of Nitrogen reported inconsistent findings from a limited number of cross-sectional studies that examined asthma prevalence. An additional uncertainty identified in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) was the potential for NO_2 to serve as an indicator for another combustion-related pollutant or mixture. Because of the high correlations among traffic-related pollutants and limited examination of copollutant confounding, the independent effects of long-term NO_2 exposure could not be clearly discerned at the time of the last review. While this uncertainty continues to apply to the epidemiologic evidence across the respiratory effects examined, coherence of epidemiologic evidence for asthma with the limited previous toxicological evidence for both AHR and development of allergic responses, which are key events in the mode of action for asthma development, provides support for an independent effect of long-term

exposure to NO_2 on development of asthma. The key evidence supporting the likely to a causal relationship is detailed in Table 6-5 using the framework described in Table II of the Preamble to this ISA.

6.2.9.1 Evidence on Development of Asthma

Multiple longitudinal, prospective and retrospective studies demonstrate associations between higher ambient NO_2 concentrations measured in the first year of life, in the year of diagnosis, or over a lifetime and asthma incidence in children. Results are consistent across locations based on various study designs and cohorts (Table 6-1). Consistency across studies in the use of questionnaires to ascertain parent report of physician-diagnosed asthma, a best practice (Burr, 1992; Ferris, 1978), adds to the strength of inference about associations with NO_2.

A pooled analysis across six birth cohorts relating NO_2 with ever asthma (OR: 1.48 [95% CI: 1.06, 2.04] per 10 ppb increase) (Macintyre et al., 2014a) is consistent with results from individual studies. Individual studies relied upon various methods to estimate exposure, and several characterize the exposure at the subject's residence using LUR models that were demonstrated to well represent the spatial variability in the study areas. Further, associations were observed across a range of ambient NO_2 concentrations [(Carlsten et al., 2011c; Gehring et al., 2010; Jerrett et al., 2008; Clougherty et al., 2007); Table 6-6] and resolutions or buffers used in the LUR models [e.g., 10 m (Carlsten et al., 2011c) to 500 m (Clougherty et al., 2007). In limited analysis of the concentration-response relationship, results did not consistently indicate a linear relationship in the range of ambient NO_2 concentrations examined (Carlsten et al., 2011c; Shima et al., 2002). These studies did not conduct analyses to evaluate whether there is a threshold for effects. Limited supporting evidence for chronic bronchitis incidence in adults is provided in the ECRHS cohort, which prospectively evaluated individual NO_2 concentrations outdoor at the home using Palmes tubes (Sunyer et al., 2007). For asthma incidence in adults, recent studies in the ECRHS cohort report relationships with NO_2 estimated from dispersion models.

Epidemiologic studies of asthma development in children have not clearly characterized potential confounding by other traffic-related pollutants or mixtures pollutants [e.g., CO, BC/EC, volatile organic compounds (VOCs)]. In the longitudinal studies of asthma incidence, correlations with $PM_{2.5}$ and BC were often high (e.g., $r = 0.7–0.96$), and no studies of asthma incidence evaluated copollutant models to address copollutant confounding, making it difficult to evaluate the independent effect of NO_2. Across studies that examined both NO_2 and $PM_{2.5}$, positive associations were observed between $PM_{2.5}$

concentrations and asthma development, although the effects are smaller in magnitude compared to those for NO_2 (Nishimura et al., 2013; Clark et al., 2010; McConnell et al., 2010). Correlations between NO_2 and $PM_{2.5}$ were not reported in these three studies. One cross-sectional study (Hwang and Lee, 2010), examined copollutant models but not with traffic-related copollutants.

The uncertainty in the epidemiologic evidence base is partly reduced by the biological plausibility provided by findings from experimental studies that demonstrate NO_2-induced effects on key events that are specific to the mode of action for development of asthma (Figure 4-2). Though not observed in all studies, AHR and airway remodeling was reported following 6−12 weeks of exposure of guinea pigs to NO_2 [1,000−4,000 ppb; (Kobayashi and Miura, 1995)]. Experimental studies also indicate that short-term exposure repeated over several days and long-term NO_2 exposure can induce Th2 skewing/allergic sensitization by showing increased Th2 cytokines, airway eosinophils, and IgE-mediated responses (Sections 4.3.5, 6.2.2.3). Findings for short-term NO_2 exposure support asthma development by describing a potential role for repeated exposures to lead to recurrent episodes of inflammation and allergic responses. Epidemiologic evidence for NO_2-related allergic responses is inconsistent but reported in a few studies (Section 6.2.4).

Recurrent pulmonary inflammation and oxidative stress are key early events in the pathophysiology of asthma (Figure 4-2). While the effects of long-term NO_2 exposure on oxidative stress in toxicological studies are variable and transient (Section 6.2.2.3), there is evidence supporting a relationship between short-term NO_2 exposure and increased pulmonary inflammation. Evidence from controlled human exposure studies indicate NO_2-induced increases in neutrophils in healthy adults, and epidemiologic evidence also points to associations between ambient NO_2 concentrations and increases in pulmonary inflammation in healthy children and adults (Section 5.2.2.5). While epidemiologic evidence overall is inconsistent in showing associations of long-term NO_2 exposure with pulmonary inflammation (Section 6.2.2.3), a recent longitudinal study observed that annual average NO_2 was associated with increases in eNO over time in children without asthma (Berhane et al., 2014). Such findings support a relationship between long-term NO_2 exposure and asthma development because the association was independent of short-term change in NO_2 concentrations, and elevated eNO was associated with increased risk of new onset asthma in the cohort. This limited evidence base for NO_2-related development of AHR and allergic responses and increases in pulmonary inflammation combined with the consistent evidence for NO_2-related development of asthma in children describe a coherent and biologically plausible sequence of events by which long-term NO_2 exposure could lead to asthma development.

6.2.9.2 Evidence on Lung Function

Another line of evidence indicating a relationship between long-term NO_2 exposure and respiratory effects includes multiple, longitudinal epidemiologic studies observing associations between long-term NO_2 exposure and decrements in lung function and partially irreversible decrements in lung development in children. Expanding on evidence reviewed in the 2008 ISA (Figure 6-5), recent studies consistently demonstrate associations with individual-level NO_2 exposure estimates based on time-activity patterns and/or LUR (Urman et al., 2014; Eenhuizen et al., 2013; Mölter et al., 2013). Associations also are observed with NO_2 assessed from central sites. Some studies found an NO_2 concentration-dependent decrement in lung development (Rojas-Martinez et al., 2007a; Gauderman et al., 2004) based on comparisons among communities or a multipollutant model (with O_3 and PM_{10}) which has uncertain reliability.

Potential confounding of long-term NO_2-related decrements in lung function and lung development by traffic-related copollutants has not been evaluated, although an association was observed with adjustment for O_3 or PM_{10}. Toxicological studies do not clearly support epidemiologic findings. NO_2-induced changes in lung function were inconsistently demonstrated in animal models [(Tepper et al., 1993; Stevens et al., 1988; Lafuma et al., 1987); Section 6.2.5.3]. Long-term NO_2 exposure was observed to alter lung morphology in adult experimental animals but not juvenile animals (Section 6.2.6), but the changes observed do not appear to contribute to altered lung function or the effects observed in epidemiologic studies.

6.2.9.3 Evidence on Respiratory Symptoms

Several longitudinal studies consistently demonstrate increases in respiratory symptoms in children with asthma in relation to increased ambient NO_2 concentrations (Section 6.2.3, Table 6-3). Associations were observed with NO_2 estimated from central sites and NO_2 estimated for children's homes using LUR. Studies did not examine whether associations of long-term NO_2 were independent of short-term exposure; however, McConnell et al. (2003) assessed chronic symptoms as a daily cough for 3 months or congestion/phlegm for 3 months. Limited information from longitudinal studies of indoor NO_2 support an association with respiratory symptoms in children with asthma and adults with COPD (Belanger et al., 2013; Hansel et al., 2013). Findings for indoor NO_2 exposure provide support for an independent relationship between NO_2 and respiratory effects because NO_2 may exist as part of a different air pollutant mixture indoors than in the ambient air (Section 5.2.9.6). In limited analysis of copollutant models, associations of NO_2 with respiratory symptoms in children persisted with

adjustment for the traffic-related copollutants $PM_{2.5}$, EC, or OC. Potentially limiting inference from these results, pollutants were measured from central sites, and correlations with NO_2 were high in some cases (0.75 for $PM_{2.5}$, 0.92 for EC).

6.2.9.4 Evidence on Respiratory Infection

In the limited body of epidemiologic studies, findings do not consistently indicate associations between long-term NO_2 exposure and respiratory infection. Findings in infants are inconsistent, and associations with pneumonia hospital admissions in adults could be due to short-term exposure. An evaluation of 10 European births demonstrated associations of residential estimates of NO_2 exposure with parent report of physician-diagnosed pneumonia and otitis media (MacIntyre et al., 2014b). Adjustment for $PM_{2.5}$ or $PM_{2.5}$ absorbance produced associations for NO_2 with wide 95% CIs, limiting inferences about independent NO_2 associations (Section 6.2.7.1). The strongest evidence for effects of long-term NO_2 exposure (500−2,000 ppb for 1 month up to 1 year) in toxicological studies indicates increased respiratory infection (Section 6.2.7.2). NO_2 exposure has been observed to increase mortality in rodents and squirrel monkeys following bacterial challenge (Miller et al., 1987; Henry et al., 1970), and findings for alterations to alveolar macrophage function and morphology describe key events in the underlying mode of action (Gregory et al., 1983; Aranyi et al., 1976).

6.2.9.5 Analysis of Potential Confounding by Traffic-Related Copollutants

Potential confounding of long-term NO_2 associations with respiratory effects by traffic-related copollutants has been examined to a limited extent, particularly in longitudinal analyses of asthma incidence. In longitudinal cohorts of children, copollutant models were analyzed for chronic bronchitic symptoms in the CHS (McConnell et al., 2003) and lung function and respiratory infection in ESCAPE (MacIntyre et al., 2014b; Gehring et al., 2013). Copollutants evaluated include EC, OC, $PM_{2.5}$ absorbance, and $PM_{2.5}$. The results ranged from persistence of the NO_2 effect or only modest reduction of NO_2 effect to attenuation of NO_2 effect. In some cases, correlations with NO_2 were moderate (r = 0.37−0.46 for $PM_{2.5}$, 0.52 for $PM_{2.5}$ absorbance, and 0.58 for OC). However, high correlations often were reported (r = 0.72−0.80 for $PM_{2.5}$ or 0.75−0.92 for $PM_{2.5}$ absorbance or EC). Although some studies support an independent association for NO_2, inconsistencies in the evidence base and limited analysis of the array of potential confounding traffic-related copollutants, leave uncertainty in the epidemiologic studies in

disentangling the independent effect of NO_2 from other traffic-related pollutants or mixtures.

6.2.9.6 Conclusion

Taken together, recent epidemiologic studies and previous experimental studies provide evidence that there is likely to be a causal relationship between long-term NO_2 exposure and respiratory effects (Table 6-5). The strongest evidence is provided by the consistent findings for associations between NO_2 exposure and increases in asthma incidence and decrements in pulmonary function in children, particularly for NO_2 exposures estimated for children's homes. Experimental evidence indicating AHR induced by long-term NO_2 exposure, and development of an allergic phenotype with repeated short-term and long-term NO_2 exposure provides a pathophysiological basis for the effects of long-term NO_2 exposure on the development of asthma and reduces previous uncertainty related to the independent effect of NO_2. However, because the experimental evidence is limited, particularly for long-term exposure, there remains some uncertainty regarding an independent effect of long-term NO_2 exposure on asthma. Indoor studies are limited in number but support associations of respiratory symptoms with indoor NO_2, which may exist as part of a different pollutant mixture than in the ambient air. In addition, other studies that characterized the concentration-response for the relationship between NO_2 and an array of respiratory effects ranging from respiratory symptoms and airway resistance in children to chronic bronchitis and asthma in adults, generally observed a linear relationship. Copollutant models with traffic-related copollutants such as $PM_{2.5}$ and $PM_{2.5}$ absorbance provide mixed evidence for respiratory symptoms and lung function decrements to inform an independent relationship for NO_2. Potential confounding by other traffic-related copollutants is unexamined, and largely unavailable for studies of asthma in children. Overall, the consistent epidemiologic and consistent but limited experimental evidence for development of asthma is sufficient to conclude that there is likely to be a causal relationship between long-term NO_2 exposure and respiratory effects.

Table 6-5 Summary of key evidence for a likely to be a causal relationship between long-term nitrogen dioxide (NO_2) exposure and respiratory effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Asthma Development			
Consistent epidemiologic evidence from multiple, high quality studies with relevant NO_2 concentrations	Consistent evidence for increases in asthma incidence in diverse cohorts of children in U.S., Europe, Canada, and Asia. Asthma ascertainment by parental report of doctor diagnosis.	Carlsten et al. (2011c), Clougherty et al. (2007), Gehring et al. (2010), Jerrett et al. (2008), Shima et al. (2002) Weak evidence: Ranzi et al. (2014) Section 6.2.2.1, Table 6-1, Figure 6-1	Means across studies of LUR: 13.5, 17.3, 27.5 ppb Upper percentiles: 75th: 15.1, 15.4 ppb Max: 69.4 ppb Range in mean residential NO_2 measurements across communities: 9.6 to 51.3 ppb Range in mean central site NO_2 across communities: 7.3−31.4 ppb
	Supporting evidence for asthma incidence or chronic bronchitis in the ECHRS cohort of adults.	Jacquemin et al. (2009b), Modig et al. (2009), Sunyer et al. (2006) Section 6.2.2.2	
Consistent evidence for NO_2 metrics with lower potential for exposure measurement error	In children, asthma associated with residential NO_2 estimated using well validated LUR models or by monitoring.	Carlsten et al. (2011c), Clougherty et al. (2007), Gehring et al. (2010), Jerrett et al. (2008) Section 6.2.2.1	
Uncertainty regarding potential confounding by traffic-related copollutants	When reported, correlations with $PM_{2.5}$ and EC often were high (r = 0.7−0.96). No copollutant models analyzed. Associations found with adjustment for SES, family history of asthma, smoking exposure, housing characteristics, and presence of gas stove.	Table 6-1	

Table 6-5 (Continued): Summary of key evidence for a likely to be a causal relationship between long-term nitrogen dioxide (NO2) exposure and respiratory effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Coherence with respiratory effects of short-term NO_2 exposure	Limited epidemiologic evidence for increases in pulmonary inflammation in healthy children and adults with exposures assessed in subjects' locations and associations adjusted for BC/EC, OC, PNC, or $PM_{2.5}$.	Strak et al. (2012), Steenhof et al. (2013), Lin et al. (2011) Section 5.2.7.4	Max for 5-h avg: 96 ppb Means for 24-h avg across seasons: 24.3−45.3 ppb
	Evidence from controlled human exposure studies for increased airway responsiveness in healthy adults.	Section 5.2.7.1	1,000−2,000 ppb for 3 h but not below
Limited and supporting toxicological evidence at relevant NO_2 exposures	Increased AHR in guinea pigs with long-term or short-term NO_2 exposure.	Kobayashi and Miura (1995), Kobayashi and Shinozaki (1990)	1,000−4,000 ppb for 6−12 weeks 4,000 ppb for 7 days
Some Evidence for Key Events in Mode of Action			
Allergic responses	Increased IgE-mediated histamine release in mast cells from rodents.	Fujimaki and Nohara (1994)	4,000 ppb for 12 weeks
	Experimental findings for development of Th2 phenotype with short-term NO_2.	Pathmanathan et al. (2003), Ohashi et al. (1994)	2,000 ppb over 4 consecutive days; 3,000 ppb for 2 weeks
	Inconsistent epidemiologic evidence for allergic diseases or responses in children with long-term exposure.	Section 6.2.4.1	
Airway remodeling	Increased airway resistance with airway hyperresponsiveness in guinea pigs.	Kobayashi and Miura (1995)	1,000−4,000 ppb for 6−12 weeks

Table 6-5 (Continued): Summary of key evidence for a likely to be a causal relationship between long-term nitrogen dioxide (NO2) exposure and respiratory effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Inflammation	Increases in lymphocytes, PMNs, in rats with long-term exposure. Increases in PMNs in healthy adults with repeated short-term exposure.	Kumae and Arakawa (2006), Blomberg et al. (1999)	500 or 2,000 ppb for 5 weeks in rats 2,000 ppb for 4 h/day for 4 days
	Longitudinal changes in eNO in children independent of asthma status.	Berhane et al. (2014)	
	Inconsistent associations in other studies with exposure assessment by LUR and central site.	Liu et al. (2014a)	Residential by LUR: Mean 11.3 95th: 15.3 ppb
Oxidative stress	Varying and transient effects on antioxidant levels and enzyme activity.	Ayaz and Csallany (1978), Gregory et al. (1983), Sagai et al. (1984)	400, 1,000, 5,000 ppb for 6 weeks to 18 mo

Table 6-5 (Continued): Summary of key evidence for a likely to be a causal relationship between long-term nitrogen dioxide (NO2) exposure and respiratory effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Severity of Asthma			
Consistent epidemiologic evidence but uncertainty regarding NO_2 independent effects	Consistent evidence for increases in respiratory symptoms in children with asthma. Exposure assessment by central site measurements and LUR.	McConnell et al. (2003), Gehring et al. (2010) Figure 6-5, Table 6-3.	Residential NO_2 by LUR: Mean: 13.5 ppb 10th−90th percentile: 7.8−18.5 ppb Central site: Mean, Max 4-yr avg for 12 communities: 19.4, 38.0 ppb
	Associations with respiratory symptoms remain robust with adjustment for a traffic-related copollutant: $PM_{2.5}$, EC, or OC. But, analysis is limited and based on central site exposure assessment.	McConnell et al. (2003), Hwang and Lee (2010) Table 6-3	
	In limited analysis, associations with respiratory symptoms remain robust with adjustment for O_3, SO_2, $PM_{10-2.5}$, or PM_{10}.		
	Evidence for associations between indoor NO_2 and respiratory symptoms in children with asthma ages 5−10 yr; inconsistent evidence in younger children and infants.	Belanger et al. (2013)	Mean daily indoor NO_2: 10.6 ppb 75th: 12.5 ppb

Table 6-5 (Continued): Summary of key evidence for a likely to be a causal relationship between long-term nitrogen dioxide (NO2) exposure and respiratory effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Lung Function and Development			
Consistent epidemiologic evidence from multiple, high-quality studies but uncertainty regarding NO_2 independent effects	Epidemiologic evidence for decrements in lung function and partially irreversible decrements in lung development in children.	Gauderman et al. (2004), Rojas-Martinez et al. (2007a), Mölter et al. (2013), Gehring et al. (2013), Urman et al. (2014), Eenhuizen et al. (2013)	NO_2 by LUR: Means across communities: 7.4−12.6 ppb Overall study mean: 13.5 ppb, 75th: 15.4 ppb Central site NO_2 mean across communities: 27.2−42.6 ppb
	In limited analysis, associations are inconsistent with adjustment for $PM_{2.5}$ but robust with adjustment for PM_{10} or O_3. Residential NO_2-$PM_{2.5}$ correlations vary across cohorts. Pearson r = 0.31−0.76.	Gehring et al. (2013), Rojas-Martinez et al. (2007b)	
Uncertain relevance of toxicological evidence	Changes in lung morphology including increases in edema, hypertrophy of lung epithelium, fibrotic changes in adult not juvenile animals. Uncertain relevance to epidemiologic findings.	Kubota et al. (1987), Hayashi et al. (1987)	500 ppb for 19 mo, 4,000 ppb for 9−27 mo
Respiratory Infection			
Consistent toxicological evidence	Increased mortality of mice and monkeys with NO_2 exposure and challenge with bacterial or viral infection.	Henry et al. (1970), Ehrlich and Henry (1968), Ehrlich (1980), Miller et al. (1987)	500 ppb for 3 mo, 5,000 ppb for 2 mo, 200 ppb base plus daily spike of 800 ppb for 16−52 weeks
Limited and inconsistent epidemiologic evidence	Associations found with physician-diagnosed pneumonia, otitis media, and croup in multicounty European cohort study but not consistently in other studies.	Macintyre et al. (2014a)	Range in mean across 10 birth cohorts: 7.5−23.7 ppb
Limited evidence for key events in mode of action	Increased macrophage infiltration to lung tissue or increased lymphocytes in BAL fluid of experimental animals.	Gregory et al. (1983)	5,000 ppb for 15 weeks

Table 6-5 (Continued): Summary of key evidence for a likely to be a causal relationship between long-term nitrogen dioxide (NO2) exposure and respiratory effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
COPD			
Limited and inconsistent epidemiologic evidence	Inconsistent evidence for hospital admissions for COPD in adults. Unclear whether independent of short-term exposure effects.	Andersen et al. (2011), Gan et al. (2013)	

BALF = bronchoalveolar lavage fluid; BC = black carbon; COPD = chronic obstructive pulmonary disease; EC = elemental carbon; eNO = exhaled nitric oxide; IgE = immunoglobulin E; LUR = land-use regression; NO_2 = nitrogen dioxide; O_3 = ozone; OC = organic carbon; $PM_{2.5}$ = 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; $PM_{10-2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 10 µm and greater than a nominal 2.5 µm; PMN = polymorphonuclear cell(s), polymorphonuclear leukocyte; PNC = particle number concentration; SES = socioeconomic status; SO_2 = sulfur dioxide; Th2 = T-derived lymphocyte helper 2.

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Tables I and II of the Preamble.

[b]Describes the key evidence and references contributing most heavily, but not necessarily exclusively, to causal determination. Where applicable, uncertainties and inconsistencies are described. References to earlier sections indicate where full body of evidence is described.

[c]Describes the NO_2 concentrations with which the evidence is substantiated (for experimental studies, below 5,000 ppb).

6.3 Cardiovascular and Related Metabolic Effects

6.3.1 Introduction

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) concluded that "the available epidemiologic and toxicological evidence was inadequate to infer the presence or absence of a causal relationship" between cardiovascular effects and long-term NO_2 exposure. This section updates the previous review with the inclusion of recent studies on the cardiovascular and related cardiometabolic effects of NO_2 and NO_X exposure in humans, animals, and cells. Data from individual studies can be found in summary tables at the end of each section and an integrated summary of the evidence is presented in Section 6.3.9.

At the completion of the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) one epidemiologic study of the association of cardiovascular disease (CVD) with long-term exposure to NO_2 was available for inclusion in the document. Miller et al. (2007) studied 65,893 post-menopausal women (50−79 years old) without previous CVD from 36 U.S. metropolitan areas. Exposures to air pollution were estimated by assigning the annual

(2000) mean air pollutant concentration measured at the monitor nearest to the subject's five-digit residential ZIP Code centroid. In single-pollutant models, $PM_{2.5}$ showed the strongest associations with the CVD events [myocardial infarction (MI), revascularization, angina, congestive heart failure, coronary heart disease (CHD) death], followed by SO_2. The association of NO_2 with overall CVD events was 1.04 (95% CI: 0.96, 1.12) per 10-ppb increase and NO_2 not associated with CVD events when the data set was restricted to those with nonmissing exposure data. The available animal toxicological evidence was limited to studies of changes in HR, vagal response, and alterations in specific hematological parameters [e.g., hematocrit, hemoglobin, ethrythrocytes; (U.S. EPA, 2008, 1993)].

Large, prospective studies with consideration of potential confounding and other sources of bias are emphasized in this section (see Table 5-1 for study evaluation guidelines). The exposure assessment method was also an important consideration in the evaluation of long-term exposure and cardiovascular and related cardiometabolic health effects, given the spatial variability typically observed in ambient NO_2 concentrations (Section 2.5.3). Exposure assessment was evaluated drawing upon discussions in Section 3.2 and Section 3.4.5. In general, LUR model predictions have been found to correlate well with outdoor NO_2 concentration measurements (Section 3.2.1.1). A select number of recent studies have employed exposure assessment methods such as LUR to represent the spatial variability of NO_2. Statistics indicating the correlation between predicted and measured NO_2 concentrations are presented where available.

Several recent epidemiologic studies report positive associations of NO_2 and NO_X exposure with heart disease, diabetes, stroke, and hypertension. The body of evidence is strongest for heart disease and diabetes and includes several large, longitudinal studies with consideration of multiple potential confounding factors including age, sex, BMI, smoking, and pre-existing conditions (Tables 6-6 and 6-7). Some of these studies employed validated exposure assessment methods such as LUR, which were demonstrated to capture the spatial variability of NO_2 concentration. The extent to which the studies inform the independent effect of NO_2 exposure through their consideration of correlated copollutants (i.e., CO, BC, $PM_{2.5}$) and noise is discussed in the section. A small number of experimental animal studies examining the effect of NO_2 on oxidative stress and the progression of vascular disease provided limited support for the biological plausibility of the effects observed in the epidemiologic studies.

6.3.2 Heart Disease

Several studies published since the 2008 ISA for Oxides of Nitrogen examine the association of long-term NO_X exposure and heart disease. Although the evidence from the epidemiologic studies is not entirely consistent, several prospective studies and/or studies with exposure assessment strategies designed to capture the spatial variability of NO_2 report positive associations. Most studies adjust for a wide array of potential confounders such as age, sex, BMI, smoking, and pre-existing conditions (Table 6-6) but uncertainty remains regarding the extent to which findings can be explained by correlated copollutant exposures and noise.

Table 6-6 Epidemiologic studies of the association of long-term exposure to nitrogen dioxide (NO_2) or the sum of nitric oxide and NO_2 (NO_X) with heart disease.

Study	CohortLocation Study Period	Mean (ppb)	Exposure Assessment	Effect Estimates (95% CI)
Cesaroni et al. (2014)	ESCAPE Project, 11 Cohorts, 5 countries in Europe 2008−2012	NO_2: Range of means across cohorts: 4.2 (3.2−5.8) to 31.9 (22.3−40.9)	Annual avg NO_2, NO_X, LUR, 40 monitoring sites, linked to geocoded addresses.	Coronary events NO_2 HR: 1.06 (0.96, 1.16) per 10-ppb increase NO_2 NO_X HR: 1.01 (0.98, 1.05) per 20 µg/m^3 NO_X* Covariate adjustment: marital status, education, occupation, smoking status duration and intensity, and SES. Copollutant adjustment: none
Gan et al. (2011)	Population based cohort in Vancouver, Canada) 1999−2002 N = 452,735	NO_2: Mean: 16.3 IQR: 4.5 NO: Mean: 26.1 IQR: 10.8	LUR, 5-yr avg concentration (NO_2 and NO, 1995−1998) and 4 yr avg concentration (1999−2002; 10-m spatial resolution). Concentrations assigned to postal code centroids (typically ~1 city block in urban areas and larger in less populated areas).	CHD hospitalization (ICD-9 410−414) RR (NO_2): 0.93 (0.89, 0.98) RR (NO): 0.96 (0.92, 1.00) per 10 ppb NO_2 and NO Covariates: age, sex, pre-existing diabetes, COPD, hypertension, and SES. Copollutant adjustment: none

Table 6-6 (Continued): Epidemiologic studies of the association of long-term exposure to nitrogen dioxide (NO_2) or the sum of nitric oxide and NO_2 (NO_X) with heart disease.

Study	CohortLocation Study Period	Mean (ppb)	Exposure Assessment	Effect Estimates (95% CI)
Beckerman et al. (2012)	Cohort of pulmonary patients in Toronto, Canada 1992−1999 N = 2,414	NO_2: Median: 22.9 IQR: 4.0	LUR, avg of fall 2002 and spring 2004 NO_2 concentration, assigned at the postal code centroid (typically 1 block, or single building and larger in less populated areas).	IHD prevalence (ICD-9 412−414)—old MI, angina or other IHD RR: 1.24 (1.01, 1.53) per 10 ppb Covariate adjustment: gender, age, pack-yr smoking, BMI, deprivation index, and diabetes. RR 1.17 (1.01, 1.36) per 10 ppb after adjustment for covariates above plus O_3 and $PM_{2.5}$ (in same model).
Rosenlund et al. (2009a)	SHEEP Study in Stockholm, Sweden 1985−1996 N = 24,347 cases, 276,926 controls	5th−95th: 15.9 cases Median (cases): 6.9 Median (controls): 6.3	5-yr avg NO_2 concentration assessed by dispersion modeling of traffic-related emissions. 25-m resolution inner city, 100-m urban, 500-m regional/countryside. Concentrations assigned to residential address.	First nonfatal MI OR: 0.96 (0.93, 1.00) per 10 ppb Covariates: age, sex, calendar yr, and SES.
Hart et al. (2013)	NHS 11 States in the U.S. 1990−2008 N = 121,700	NR	Dispersion model to predict annual avg (2000) NO_2 concentration, assigned to residential address. Main results were for traffic proximity.	Incident MI HR: 1.22 (0.99, 1.50) per 1-ppb increase in NO_2 between addresses Covariate adjustment: BMI, physical activity, healthy diet score, alcohol, hypercholesterolemia, high blood pressure, diabetes, family history of MI, smoking status, mental health status, father's occupation, marital status, husband's education, education level, employment, and median income/home value. Copollutants adjustment: none

Table 6-6 (Continued): Epidemiologic studies of the association of long-term exposure to nitrogen dioxide (NO_2) or the sum of nitric oxide and NO_2 (NO_X) with heart disease.

Study	CohortLocation Study Period	Mean (ppb)	Exposure Assessment	Effect Estimates (95% CI)
Lipsett et al. (2011)	CTS Cohort in CA. June 1996−Dec 2005 N = 124,614	NO_2: Mean (5th−95th): 33.59 IQR: 10.29 NO_X: Mean: 95.6 IQR: 58.31	Gridded pollutant surface (250-m spatial resolution) developed using IDW fixed site monitor concentrations (1995−2005) linked to geocoded residential address. Defined representative range of 3−5 km (neighborhood and regional monitors, respectively) for NO_X and NO_2 to account for spatial variability of pollutant.	MI incidence NO_X: HR 1.01 (0.91, 1.11) per 20 ppb NO_X NO_2: HR 1.06 (0.88, 1.27) per 10 ppb NO_2 Covariate adjustment: age, race, smoking second-hand smoke, BMI, lifetime physical activity, nutritional factors, alcohol, marital status, menopausal status, hormone therapy, hypertension medication and aspirin, and family history of MI/stroke. Copollutant adjustment: none
Atkinson et al. (2013)	National GP Patient Cohort in the U.K. 2003 N = 836,557	Mean (SD): 12.0 IQR: 5.7 ppb	Annual average NO_2 concentration (2002) derived from dispersion models identifying all known emissions sources (1 by 1 km resolution), linked to residential post-codes. Concentrations linked to post-code centroids that typically include 13 residential addresses.	MI incidence HR: 0.97 (0.90, 1.04) Arrhythmia incidence HR: 0.98 (0.91, 1.04) Heart failure incidence HR: 1.11 (1.02, 1.21) per 10 ppb Covariates: age, sex, smoking, BMI, diabetes, hypertension, and index of multiple deprivation. Copollutant adjustment: none
de Kluizenaar et al. (2013)	Eindhoven, Netherlands 1991−2003 N = 18,213	NO_2 5th−95th: 7.5	Dispersion model 1 × 1 km resolution, linked to residential address.	
Dong et al. (2013a)	33 communities in 11 districts of 3 cities in Liaoning Province, China 2006−2008 N = 24,845	NO_2: Mean: 18.7 Median: 17.5 IQR: 4.8	District-specific 3-yr avg NO_2 concentrations for communities within 1 km of an air monitoring station (selected to maximize intra- and inter-city gradients).	Self-reported CVD OR: 1.04 (0.60, 1.82) per 10-ppb increase Copollutant adjustment: none

Table 6-6 (Continued): Epidemiologic studies of the association of long-term exposure to nitrogen dioxide (NO_2) or the sum of nitric oxide and NO_2 (NO_X) with heart disease.

Study	CohortLocation Study Period	Mean (ppb)	Exposure Assessment	Effect Estimates (95% CI)
Miller et al. (2007)	WHI Cohort in 36 U.S. cities 1994−1998	NR	Annual avg (2000): nearest monitor to residence ZIP code centroid (overall effect based on intra- and inter-city gradients).	Incident CVD events HR: 1.04 (0.96, 1.12) per 10 ppb Covariates: age, ethnicity, education, household income, smoking, diabetes, hypertension, systolic blood pressure, BMI, and hypercholesterolemia. Copollutant adjustment: none

BMI = body mass index; CBVD = cerebrovascular disease; CHD = coronary heart disease; CI = confidence interval; COPD = chronic obstructive pulmonary diesease; CTS = California Teachers Study; CVD = cardiovascular disease; ESCAPE = European Study of Cohorts for Air Pollution Effects; GP = general practice; HR = hazard ratio; ICD = international Classification of Diseases; IDW = inverse distance weighting; IHD = ischemic heart disease; IQR = interquartile range; LUR = land-use regression; MI = myocardial infarction; NHS = Nurses Health Study; NO = nitric oxide; NO_2 = nitrogen dioxide; NO_X = sum of NO and NO_2; NR = no quantitative results reported; O_3 = ozone; OR = odds ratio; $PM_{2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 2.5 μm; RR = risk ratio(s), relative risk; SES = socioeconomic status; SHEEP = Stockholm Heart Epidemiology Program; WHI = Women's Health Initiative.

*NO_X results that are originally reported in $\mu g/m^3$ are not standardized if the molecular weight needed to convert to ppb is not reported.

Cesaroni et al. (2014) reported an increased risk of incident coronary events of 1.06 (95% CI: 0.96, 1.16) per 10-ppb increase in NO_2. This large study of 11 cohorts from 5 countries used LUR to assign exposure at each participant's residence. Authors reported good performance of exposure models based on their comparison of predicted estimates and concentrations measured at 40 sites ($R^2 \geq 0.61$). A study of pulmonary patients in Toronto, Canada (Beckerman et al., 2012) reported an increased risk of 1.17 (95% CI 1.01, 1.36) per 10-ppb increase in NO_2 with ischemic heart disease (IHD) prevalence after adjustment for individual covariates as well as simultaneous adjustment for O_3 and $PM_{2.5}$. In this study, LUR was also used to estimate NO_2 concentrations, which were assigned at the post-code centroid level (typically one block area or specific building in this study area). In another prospectively designed study, Gan et al. (2011) examined the association of long-term exposure to BC, $PM_{2.5}$, NO_2, and NO with CHD hospitalization and mortality among participants (45−85 year-olds) residing in Vancouver, Canada enrolled in the universal health insurance system. In this study, LUR was used to predict NO_2 concentrations at a resolution of 10 m. These predicted concentrations were adjusted using factors derived from regulatory monitoring data and then linked to each participant's postal code of residence (typically one block or specific building in this study area). After adjustment for potential confounders, NO_2 and NO were inversely associated with CHD hospitalization (HR: 0.93 [95%CI: 0.89, 0.98] and

HR: 0.96 [95% CI: 0.92, 1.00] per 10 ppb); however, positive associations of NO_2 and NO with CHD mortality were observed (Section 6.5.2).

Several other studies characterized NO_2 exposure using IDW estimates of concentration from central site monitors or dispersion models that captured a range of spatial resolutions. Uncertainties associated with these models are described in detail in Sections 3.2.2 and 3.2.3. Briefly, estimates derived from IDW monitor concentrations may not capture the true variability in NO_2 concentration from local sources if the monitor coverage is not adequately dense, thus underestimating concentrations. Biases in dispersion model output can occur in either direction and depend on the complexity of the topography, meteorology, and sources that are modelled.

Lipsett et al. (2011) determined the association of incident MI with long-term exposure to NO_2, NO_X, other gases (CO, O_3, SO_2) and PM in a prospective study. These authors followed a cohort of California public school teachers aged 20−80 years old (N = 124,614). Each participant's geocoded residential address was linked to a pollutant surface with a spatial resolution of 250 m, which was determined by IDW interpolation of pollutant concentrations measured at fixed site monitors within a representative range of 3−5 km. Those living outside the radial range for which the monitor was intended to provide representative data were excluded from the analysis. The authors observed a positive association between NO_2 and incident MI (HR: 1.06 [95% CI: 0.88, 1.27] per 10 ppb). In a study of women enrolled in the Nurses Health Study (NHS), Hart et al. (2013) reported an increased risk of incident MI associated with living consistently near sources of traffic. Although the exposure assessment for the main analyses in this study was based on distance to roadway, the authors used a dispersion model to predict the change in NO_2 concentration among those who moved from one address to another. They observed an increased risk of incident MI in association with current NO_2 compared with NO_2 concentration at the previous address (Table 6-6).

Rosenlund et al. (2009a) conducted a case-control study of first MI using the Swedish registry of hospital discharges and deaths for Stockholm County and randomly selected population-based controls. Predicted 5-yr avg NO_2 concentrations were determined and linked to each participant's geocoded address using dispersion models. The resolution of the predicted concentrations corresponded to 500 m in the countryside, 100 m in urban areas, and 25 m in the inner city. Five-year average NO_2 concentration was associated with fatal MI (OR: 1.14 [95% CI: 1.09, 1.19] per 10 ppb) but not with nonfatal MI (OR: 0.96 [95% CI: 0.93, 1.00] per 10 ppb). CO and PM_{10} were also associated with fatal cases of MI in this population. Atkinson et al. (2013) examined the association of incident cardiovascular disease with NO_2. These authors studied patients (aged 40−89 years) registered with 205 general practices across the U.K. Predicted annual average NO_2

concentrations within 1 km by 1 km grids, estimated using dispersion models, were assigned to participants based on their residential postal code. Cardiovascular disease outcomes included in the analysis were MI, arrhythmias, and heart failures. Authors reported a positive association between NO_2 and heart failure in fully adjusted models (HR: 1.11 [95% CI: 1.02, 1.21] per 10 ppb). Incident MI and arrhythmia were not associated with NO_2 concentration in this analysis. A similar pattern of findings were observed for the associations between PM and these outcomes (associations with CHD and MI were null while the association of PM_{10} with heart failure was increased).

de Kluizenaar et al. (2013) assigned NO_2 exposure to participants' residential addresses, based on dispersion modelling with a 1 × 1 km resolution, reported an association of NO_2 with CHD and cerebrovascular disease (CBVD) hospitalizations that was robust to adjustment for individual level covariates and noise (HR 1.18 [95% CI: 0.9, 1.48] per 10 ppb increase in NO_2). As discussed above, the only study available for inclusion in the previous assessment reported a null association between NO_2 and incident CVD events (Miller et al., 2007) comparing annual average concentration assigned at the ZIP code centroid level to study participants across 36 U.S. cities. Dong et al. (2013a) reported a small, imprecise increase in the prevalence of self-reported CVD comparing 3-yr avg concentrations for communities within 1 km of an air monitoring station across 3 cities in Liaoning, China (OR: 1.04 [95%CI: 0.60, 1.82] per 10 ppb increase NO_2).

Overall, several epidemiologic studies, including some large studies with prospective designs, adjustment for known risk factors for cardiovascular disease such as age, sex, BMI, and smoking, and use of exposure assessment methods designed to achieve high spatial resolution (Cesaroni et al., 2014; Beckerman et al., 2012), provide evidence that long-term exposure to NO_2 is associated with the risk of heart disease. Although positive associations between MI and CHD were not observed consistently across studies, some studies reporting null or inverse associations with CHD or MI morbidity reported increased risk of mortality from these causes (Gan et al., 2011; Rosenlund et al., 2009a)and a positive association with heart failure was reported by Atkinson et al. (2013). The few studies that accounted for confounding by $PM_{2.5}$ (Beckerman et al., 2012) or noise (de Kluizenaar et al., 2013) provide limited evidence that estimates are robust to adjustment for these factors. In general these studies were not designed to distinguish the independent effect of NO_2 from the effects of other traffic-related pollutants (e.g., BC, EC, CO), noise, or stress.

6.3.3 Diabetes

There were no epidemiologic studies examining the association of NO_2 exposure with diabetes or insulin deficiency in the 2008 ISA for Oxides of Nitrogen. Recent large prospective studies using exposure assessment methods designed to achieve high spatial resolution, provide some evidence of an association (Table 6-7). However, studies overall have not distinguished an independent effect of NO_2 on diabetes.

Coogan et al. (2012) examined the association of long-term NO_X exposure with incident diabetes among black women residing in Los Angeles, CA. An LUR model was applied to estimate exposure at each participant's residential address and cross validation of the model performance produced an R^2 value of approximately 92%. An increased risk of 1.25 (95% CI: 1.07, 1.46) per interquartile range (IQR) increase (12.4 $\mu g/m^3$) in NO_X after adjustment for a wide array of potential confounders including traffic-related noise exposure. Negligible attenuation in the effect estimate for NO_X was reported after adjustment for $PM_{2.5}$. The correlation between NO_X and $PM_{2.5}$ concentrations ($PM_{2.5}$ estimated using kriging), was low (r = 0.27) but correlations between NO_X and NO_2 or other traffic-related copollutants were not reported. An increased risk of type II diabetes in association with LUR estimates of NO_2 was reported among older adult women living in the Ruhr district of West Germany (HR: 1.55 [95% CI: 1.20, 2.00] per 10 ppb increase in NO_2; (Kramer et al., 2010)). In this study, nondiabetic women (age 54−55) were followed over 16 years (1990−2006), and alternate NO_2 exposure assessment methods (mean monitor concentration and emission inventory-based methods) were compared. Relative risks determined by these alternative methods were smaller and less precise compared to those obtained using validated LUR models, which were reported to explain 92% of the variance in NO_2. Although diabetes status was self-reported in this study, a validation study comparing self-reported diabetes from the questionnaire to answers obtained during a clinical exam interview indicated 99% concordance. In an analysis of a subgroup (n = 363) of these women, Teichert et al. (2013) observed positive associations of NO_2 and NO_X exposure (estimated for the period 10−20 years prior to the baseline exam) with impaired glucose metabolism (IGM). Risk estimates were robust to adjustment for an array of biomarkers of subclinical inflammation.

In a large study of prevalent diabetes ascertained by self-report among randomly selected adults (ages 18−65 years), which also used LUR, Eze et al. (2014) reported a positive association (OR: 1.43 [95% CI: 1.08, 1.90] per 10 ppb increase in NO_2). Noise was included among the array of potential confounders for which the final model was adjusted. The association of NO_2 with diabetes was attenuated after adjustment for PM_{10} in a copollutant model (OR: 1.07 [95% CI: 0.72, 1.61] per 10 ppb increase in NO_2). In another study of diabetes prevalence using LUR, Brook et al. (2008) reported an

increased risk in the prevalence of diabetes mellitus (Types I and II) of 1.48 (95% CI: 1.00, 2.19) per 10 ppb increase in NO_2 among female respiratory patients in two Canadian cities. NO_2 exposure was not associated with diabetes in male patients, however. Prevalent diabetes was not associated with NO_2 exposure estimated by LUR in a semirural population in the Netherlands (Dijkema et al., 2011).

A large prospective study examined the association of NO_2 exposure with diabetes incidence among participants of the Danish Diet, Cancer, and Health Cohort (Andersen et al., 2012c). A validated dispersion model ($r \geq 0.75$ for correlation between measured and predicted 1/2-yr avg NO_2 concentration; (Hertel et al., 2014)) was used to assign mean NO_2 concentration since 1971 based on residential address history. No association between NO_2 exposure and diabetes was observed in fully adjusted models (HR: 1.00 [95% CI: 0.89, 1.12] per 10 ppb increase in NO_2); however, after restricting the analyses to confirmed cases of diabetes, a weak positive association was observed (HR: 1.04 [95% CI: 1.00, 1.08] per 10 ppb increase in NO_2). Long-term exposure to traffic noise was not associated with a higher risk of diabetes in this population (Sørensen et al., 2013). Another study designed to evaluate the association of long-term exposure to aircraft noise with diabetes found that, although associations with metabolic outcomes such as waist circumference were observed, no association of Type II diabetes or BMI with noise was present. (Eriksson et al., 2014).

The association of NO_2 concentration with insulin resistance in children was examined in one study. In a study of Homeostatic Model Assessment (HOMA) of insulin resistance, a metric derived from blood glucose and serum insulin measurements among 10 year old children (n = 397), Thiering et al. (2013) reported that NO_2 was associated with a 28% increase in insulin resistance (95% CI: 6.7, 51.7%) per 10 ppb NO_2.

Generally consistent associations of NO_2 (Teichert et al., 2013; Andersen et al., 2012c; Kramer et al., 2010) as well as NO_X (Coogan et al., 2012) with diabetes or impaired insulin metabolism are reported in prospective studies using LUR to assign exposure. Associations of NO_2 with prevalent diabetes among females and respiratory patients are reported in some (Eze et al., 2014; Brook et al., 2008) but not all studies (Dijkema et al., 2011). Findings regarding the potential for noise exposure to confound observed associations of NO_2 and NO_X with diabetes are limited. Overall, studies have not distinguished an independent effect of NO_2 from other traffic related exposures on diabetes.

Table 6-7 Epidemiologic studies of the association of long-term exposure to nitrogen dioxide (NO_2) or the sum of nitric oxide and NO_2 (NO_X) with cardiometabolic disorders.

Study	Cohort Location Study Period	Mean (ppb)	Exposure Assessment	Effect Estimates (95% CI)
Coogan et al. (2012)	BWHS Cohort in Los Angeles, CA 1995−2005 N = 3,236	NO_X (μg/m^3): Mean: 43.3 Median: 41.6 IQR: 12.4	LUR to estimate exposure at participant's residences (summer and winter measurements were taken at 183 sites in Los Angeles to estimate an annual mean).	Diabetes [per IQR (12.4 μg/m^3) increase in NO_X]: IRR: 1.25 (1.07,1.46) Covariate adjustment: age, BMI, yr of education, income, number of people in the household, smoking, drinks per week, h/week of physical activity, neighborhood socioeconomic status score, and family history of diabetes. IRR: 1.24 (1.05, 1.45), adjusted for above covariates plus $PM_{2.5}$
Kramer et al. (2010)	SALIA Cohort in Ruhr district, West Germany 1990−2006 N = 1,755 Older adult women 54−55 yr at enrollment	NO_2 (monitors): Median: 22.15 IQR: 13.23 NO_2 (estimated using—emissions inventories): Median: 6.37 IQR: 10.09 LUR: Median: 18.33 IQR: 7.97	4 methods: 5 yr (1986−1998) mean monitor concentrations (8 × 8 km grid monitor coverage) to capture broad scale variability in NO_2. Emission inventories used to determine exposure to traffic pollution (1-km grid). LUR to estimate the NO_2 exposure at participant's residence. Distance from residence to roadway <100 km.	Diabetes HR (per 10 ppb NO_2) Monitor concentration: HR: 1.25 (1.01, 1.53) Emission Inventory: HR: 1.15 (1.04,1.27) LUR: HR: 1.55 (1.20, 2.00) Covariate adjustment: age, BMI, heating with fossil fuels, workplace exposure to dust/fumes, extreme temperature, smoking, and education. Copollutant adjustment: none
Teichert et al. (2013)	SALIA Cohort in Ruhr district, West Germany 2003−2009 Subgroup, N = 363 Older adult women 54−55 yr at enrollment	NO_X (μg/m^3): Population with IGM Mean: 74.1 SD: 31.2 Population without IGM Mean: 69.3 SD: 30.0 NO_2 (ppb): Population with IGM Mean: 21.09 SD: 5.79 Population without IGM Mean: 20.08 SD: 4.09	3 methods: 5-yr (2003−2007) mean monitor concentration nearest to residence (8 × 8 km grid monitor coverage) to capture broad scale variability in NO_2. LUR to estimate the NO_2 exposure at participant's residences (40 sites). Back extrapolation using the ratio method to estimate concentrations 10−20 yr prior to disease.	IGM: OR: 1.41 (1.01,1.97) per IQR NO_X 43.16 μg/m^3 IGM: OR: 1.63 (1.06, 2.51) per 10 ppb NO_2 Covariate adjustment: age, BMI, smoking status, passive smoking, education, exposure to indoor mold, and season of blood sampling. Copollutant adjustment: none

Table 6-7 (Continued): Epidemiologic studies of the association of long-term exposure to nitrogen dioxide (NO2) or the sum of nitric oxide and NO2 (NOX) with cardiometabolic disorders.

Study	Cohort Location Study Period	Mean (ppb)	Exposure Assessment	Effect Estimates (95% CI)
Brook et al. (2008)	Patients who attended 2 respiratory clinics in Hamilton and Toronto, Canada 2002−2004 N = 7,634	NO_2: Hamilton Female Median: 15.3 IQR: 3 Male Median: 15.2 IQR: 3.2 Toronto Female Median: 22.9 IQR: 3.9 Male Median: 23 IQR: 20.8	LUR to estimate participants' exposures. Measurements collected at ~250 sites selected using a location-allocation model.	Odds ratio for diabetes mellitus (per 10 ppb NO_2): Female OR: 1.48 (1.00, 2.19) Male OR: 0.90 (0.60,1.37) Both sexes combined OR: 1.16 (0.82, 1.65) Covariate adjustment: age, BMI, and neighborhood income. Copollutant adjustment: none
Thiering et al. (2013)	GINIplus and LISAplus Cohorts in Germany Oct 2008−Nov 2009 n = 397	NO_2: Mean: 11.53 SD: 2.82	Exposure estimates at each participant's residence were calculated using an LUR. Annual average concentration extrapolated from NO_2 measurements taken during three 2 week periods (warm, cold, and intermediate seasons) at 40 sites.	Percentage difference in insulin resistance per 10 ppb NO_2: 28.06 (6.7, 51.7) Covariate adjustment: sex, birth weight, study center, parental education, study, study design, puberty status, age, BMI, and exposure to smoke. Copollutant adjustment: none
Eze et al. (2014)	SAPALDIA Cohort in Switzerland 1991−2002 N = 6,372	NO_2: Mean: 15.03 IQR: 6.06	LUR to estimate 10-yr avg NO_2 concentrations using dispersion model (200 × 200 m resolution). Model incorporated LUR components to help prevent underestimation at background sites. Annual NO_2 trends were combined with residential histories to estimate long-term exposure.	Percentage increase in diabetes prevalence (per 10 ppb NO_2): OR: 1.43 (1.08,1.90) Covariate adjustments: age, sex, educational level, neighborhood SEI, lifestyle, BMI, noise, hypertension, hs-CRP, and dyslipidemia. OR: 1.07 (0.72, 1.61), adjusted for above covariates plus PM_{10}

Table 6-7 (Continued): Epidemiologic studies of the association of long-term exposure to nitrogen dioxide (NO2) or the sum of nitric oxide and NO2 (NOX) with cardiometabolic disorders.

Study	Cohort Location Study Period	Mean (ppb)	Exposure Assessment	Effect Estimates (95% CI)
Dijkema et al. (2011)	Wesfriesland, Netherlands (semirural) 2007 N = 8,018	NO_2 range: Q1: 4.7−7.5 Q2: 7.5−8.1 Q3: 8.1−8.8 Q4: 8.8−19.1	LUR to estimate NO_2 concentration at residential address at the time of recruitment.	OR (prevalence of Type II diabetes) Q1: referent Q2: 1.03 (0.82, 1.31) Q3: 1.25 (0.99, 1.56) Q4: 0.8, (0.63, 1.02) Covariate adjustment: average monthly income, age, and sex.
Andersen et al. (2012c)	Diet, Cancer, and Health Cohort in Copenhagen or Aarhus, Denmark Jan.1995−June 2006 N = 57,053	NO_2: 1971−end of follow-up Median: 7.7 IQR: 2.6 1991−end of follow-up Median: 8.13 IQR: 2.97	Source dispersion model that sums local air pollution from street traffic based on traffic location and density. AirGIS used to predict the NO_X, NO_2, and NO concentrations at each participant's residence.	Diabetes HR (per 10 ppb NO_2) 1971−end of follow-up HR all diabetes: 1.00 (0.89,1.12) HR confirmed diabetes*: 1.16 (1.00, 1.35) 1991−end of follow-up HR all diabetes: 1.04 (0.93, 1.17) HR confirmed diabetes*: 1.16 (1.04, 1.3) Covariate adjustment: sex, BMI, waist-to-hip ratio, smoking status, smoking duration, smoking intensity, environmental tobacco smoke, educational level, physical/sports activity in leisure time, alcohol consumption, fruit consumption, fat consumption, and calendar yr. Copollutant adjustment: none Note: confirmed diabetes defined as exclusion of cases included solely because of glucose blood test.

BMI = body mass index; BWHS = Black Women's Health Study; CI = confidence interval; GINIplus = German Infant Nutritional Intervention plus environmental and genetic influences.; HOMA = homeostatic model assessment; HR = hazard ratio; hs-CRP = high sensitivity C-reactive protein; IGM = impaired glucose metabolism; IQR = interquartile range; IRR = incidence rate ratios; LISAplus = Lifestyle-Related Factors on the Immune System and the Development of Allergies in Childhood plus the influence of traffic emissions and genetics: LUR = land-use regression model; NO = nitric oxide; NO_2 = nitrogen dioxide; NO_X = sum of NO and NO_2; OR = odds ratio; $PM_{2.5}$ = 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; Q1 = first quartile; Q2 = second quartile; Q3 = third quartile; Q4 = fourth quartile; SALIA = Study on the Influence of Air Pollution on Lung, Inflammation, and Aging; SAPALDIA = Swiss study on Air Pollution and Lung Disease in adults; SD = standard deviation; SEI = socio-economic index.

6.3.4 Cerebrovascular Disease and Stroke

Several studies published since the 2008 ISA for Oxides of Nitrogen examine the association of long-term NO_2 exposure and stroke (Table 6-8). Evidence from

epidemiologic studies is not consistent and there is uncertainty regarding the extent to which findings can be explained by noise or copollutant exposures.

A hospital-based case-control study in Edmonton, Canada reported a positive association of NO_2 exposure with ischemic stroke (OR: 1.06 [95% CI: 0.88, 1.27] per 10 ppb increase) and a stronger positive association with hemorrhagic stroke (OR: 1.14 [95% CI 0.85, 1.55]) but not with transient ischemic attack (TIA) (OR: 0.90 [95% CI: 0.74, 1.10] (Johnson et al., 2013). This was the only study of stroke to use LUR to estimate NO_2 concentration at the participants' residences. Findings were similar in an ecological analysis of annual incidence of stroke also conducted in Edmonton, Canada. Positive, imprecise associations with hemorrhagic and nonhemorrhagic stroke incidence were observed with IDW weighted average NO_2 concentration assigned based on residential ZIP code (Johnson et al., 2010). Associations of stroke with CO and traffic density were also observed in this study.

Andersen et al. (2012b) conducted a study of long-term traffic-related NO_2 exposure and incident stroke using data from a large cohort study of residents of Copenhagen, Denmark enrolled in the Danish Diet, Cancer, and Health Study. The Danish GIS-based air pollution and human exposure dispersion modelling system was used to predict NO_2 concentrations for geocoded residential address histories up to approximately 35 years in duration. Authors report an increase in ischemic stroke incidence (HR 1.19 [95% CI: 0.88, 1.61]) but not hemorrhagic stroke incidence (HR: 0.80 [95% CI: 0.52, 1.22). In an analysis of the same data set that adjusted for traffic-related road noise as a potential confounder, Sørensen et al. (2014) reported a substantially attenuated risk estimate between NO_2 concentration at the time of diagnosis and ischemic stroke [incidence rate ratios (IRR: 1.04 [95% CI: 0.85, 1.26]). The association for the combined effect of the highest tertile of noise and the highest tertile of NO_2 was increased (IRR: 1.28 [95% CI: 1.09, 1.52]) and the association with fatal strokes persisted after adjustment for noise, however. A study of IHD and cerebrovascular diseases combined reported that associations with NO_2 were robust to adjustment for noise and other individual-level covariates (HR: 1.18 [95% CI 0.9, 1.48] per 10 ppb increase) (de Kluizenaar et al., 2013).

Lipsett et al. (2011) analyzed the association of incident stroke with long-term exposure to NO_2, NO_X, other gases (CO, O_3, SO_2), and PM. These authors analyzed data from a cohort of California public school teachers and assigned exposure by linking IDW pollution concentrations from monitors within a representative range of 3−5 km to participants' geocoded addresses. An association with incident stroke that was close to the null value (HR: 1.02 [95%CI: 0.90, 1.15] per 10-ppb increase in NO_2) was observed. Estimates for the association of other pollutants (PM_{10}, $PM_{2.5}$, SO_2, and O_3) with incident stroke were increased.

Atkinson et al. (2013) examined the association of incident cardiovascular disease with NO_2. These authors studied patients (aged 40−89 years) registered with 205 general practices across the U.K. Predicted annual average NO_2 concentrations within 1 km by 1 km grids, estimated using dispersion models, were assigned to participants based on their residential postal code. Incident stroke was not associated with NO_2 concentration in this analysis. An increase in NO_2 concentration for communities within 1 km of an air monitoring station was associated with self-reported stroke prevalence in a multicity study in China (OR 1.27 [95% CI: 0.92, 1.74] per 10 ppb increase) (Dong et al., 2013a). Oudin et al. (2011) reported no association between long-term NO_X exposure and ischemic stroke in a population-based registry-based case-control study conducted in Scania, Sweden. Exposure was characterized using dispersion models to estimate outdoor NO_X concentrations within 500 m by 500 m grids and linking those predicted concentrations to geocoded residential addresses. Although no association of NO_X exposure with stroke was observed, modification of the association of diabetes and stroke by long-term NO_X exposure was reported in this study.

Although several studies report an increased risk between NO_2 exposure and stroke and/or cerebrovascular disease, estimates are generally imprecise (de Kluizenaar et al., 2013; Dong et al., 2013a; Johnson et al., 2013; Andersen et al., 2012b; Johnson et al., 2010). Some studies reported weak or null associations (Atkinson et al., 2013; Lipsett et al., 2011). The positive associations observed for stroke were not consistent across stroke subtype. Johnson et al. (2013) observed a larger increased risk for hemorrhagic compared to ischemic stroke in a LUR study while Andersen et al. (2012b) observed an increase for ischemic not hemorrhagic stroke in the Danish Diet, Cancer, and Health Study. The association with ischemic stroke observed by Andersen et al. (2012b) was diminished after further adjustment for noise although an interaction between highest tertile of NO_2 and highest tertile of noise was observed (Sørensen et al., 2014). Evidence from epidemiologic studies is not consistent, only one study using LUR to capture the fine scale variability of NO_2 concentrations was available, and there is uncertainty regarding the extent to which findings can be explained by noise or copollutant exposures.

Table 6-8 Epidemiologic studies of the association of long-term exposure to nitrogen dioxide (NO_2) or the sum of nitric oxide and NO_2 (NO_X) with cerebrovascular disease or stroke.

Study	Cohort Location Study Period	Mean (ppb)	Exposure Assessment	Effect Estimates (95% CI)
Johnson et al. (2013)	Edmonton, Canada Jan 2007−Dec 2009 N = 4,696 cases, 37,723 controls	NO_2: Mean Cases: 15.4 Controls: 15.2	LUR model of NO_2 concentrations matched to residential postal code (spatial resolution <50 m).	Stroke hospitalizations: All stroke: 1.02 (0.88, 1.18) IS: 1.06 (0.88, 1.27) TIA: 0.90 (0.74, 1.10) HS: 1.14 (0.85, 1.55) per 10 ppb NO_2 Covariate adjustment: age, sex, and SES. Copollutant adjustment: none
Johnson et al. (2010)	Edmonton, Canada Jan 2003−Dec 2007	NO_2: Mean: 15.7 IQR: 2.2	IDW average monitor NO_2 concentration assigned at postal code centroid level.	Ecological analysis of stroke incidence rates: HS ED visits Q1 RR 1.0 (reference) Q2 RR: 0.88 (0.68, 1.14) Q3 RR: 1.03 (0.79, 1.20) Q4 RR: 1.13 (0.90, 1.43) Q5 RR: 1.14 (0.92, 1.52) non-HS ED visits Q1 RR 1.0 (reference) Q2 RR: 1.0 (0.85, 1.18) Q3 RR: 1.05 (0.92, 1.20) Q4 RR: 1.02 (0.87, 1.18) Q5 RR: 1.08 (0.91, 1.27) * Results for all stroke and TIA also presented. Covariate adjustment: age, sex, and household income. Copollutant adjustment: none
Sørensen et al. (2014)	Danish Diet, Cancer, and Health Cohort 1993/1997− June 2006 N = 57,053	NO_2: Median: 8.1 10th: 6.3 90th: 12.4 IQR: 3.0	Predicted traffic-related NO_2 concentration using dispersion models, linked to geocoded residential address.	Noise-adjusted IS incidence NO_2: IRR 1.04 (0.85, 1.26) per 10 ppb NO_2 (at the time of diagnosis) NO_X*: IRR: 0.97 (0.92, 1.03) per 20 $\mu g/m^3$ NO_X Covariate adjustment: age, sex, education, municipality, SES, smoking status and intensity, intake of fruits, vegetables, alcohol, and coffee, physical activity, BMI, and calendar y. Copollutant adjustment: none

Table 6-8 (Continued): Epidemiologic studies of the association of long-term exposure to nitrogen dioxide (NO_2) or the sum of nitric oxide and NO_2 (NO_X) with cerebrovascular disease or stroke.

Study	Cohort Location Study Period	Mean (ppb)	Exposure Assessment	Effect Estimates (95% CI)
Andersen et al. (2012b)	Danish Diet, Cancer, and Health Cohort 1993/1997− June 2006 N = 57,053	NO_2: Median: 8.1 10th: 6.3 90th: 12.4 IQR: 3.0	Predicted traffic-related NO_2 concentration using AirGIS (validated dispersion model), linked to geocoded residential address.	IS incidence NO_2: HR 1.19 (0.88, 1.61) HS Incidence NO_2: HR: 0.80 (0.52, 1.22) Covariate adjustment: smoking status, duration, intensity; ETS, gender, BMI, education, sports activity, alcohol consumption, fruit consumption, fat consumption, hypertension, and hypercholesterolemia. Copollutant adjustment: none
de Kluizenaar et al. (2013)	Eindhoven, Netherlands 1991−2003 N = 18,213	NO_2 5th−95th: 7.5 ppb	Dispersion model, 1 × 1 km resolution, linked to residential address.	Hospital admissions for IHD or CBVD HR: 1.16 (0.9, 1.43) Noise-adjusted HR: 1.18 (0.9, 1.48) Covariate adjustment: age, sex BMI, smoking, education, exercise, marital status, alcohol use, work situation, and financial difficulty. Copollutant adjustment: none
Lipsett et al. (2011)	CTS Cohort in California, U.S. June 1996−Dec 2005 N = 133,479	NO_2: Mean: 33.59 IQR: 10.29 NO_X: Mean: 95.6 IQR: 58.31	Geocoded residential address linked to gridded pollutant surface (250-m spatial resolution) developed using IDW fixed site monitor concentrations (1995−2005). Defined representative range of 3−5 km (neighborhood and regional monitors respectively) for NO_X and NO_2 to account for spatial variability of pollutant.	Stroke incidence NO_X: HR 1.02 (0.96, 1.09) NO_2: HR 1.02 (0.90, 1.15) per 10 ppb NO_2 and 20 ppb NO_X Covariate adjustment: age, race, smoking, second-hand smoke, BMI, lifetime physical activity, nutritional factors, alcohol, marital status, menopausal status, hormone therapy, hypertension, medication and aspirin, and family history of MI/stroke. Copollutant adjustment: none
Atkinson et al. (2013)	National GP Patient Cohort in the U.K. 2003 N = 836,557	Mean (SD): 12.0 IQR: 5.7 ppb	Annual average NO_2 concentration for 2002 at a 1 by 1 km resolution derived from dispersion models identifying all known emissions sources and linked to residential post-codes.	Stroke incidence HR: 0.98 (0.91, 1.06) Covariates: age, sex, smoking BMI, diabetes, hypertension, and index of multiple deprivation. Copollutant adjustment: none

Table 6-8 (Continued): Epidemiologic studies of the association of long-term exposure to nitrogen dioxide (NO_2) or the sum of nitric oxide and NO_2 (NO_X) with cerebrovascular disease or stroke.

Study	Cohort Location Study Period	Mean (ppb)	Exposure Assessment	Effect Estimates (95% CI)
Dong et al. (2013a)	33 communities in 11 districts of 3 cities in Liaoning Province, China 2006−2008 N=24,845	NO_2: Mean: 18.7 Median 17.5 IQR 4.8	District-specific 3-yr avg NO_2 concentrations for communities within 1 km of an air monitoring station (selected to maximize intra- and inter-city gradients).	Self-reported stroke: OR: 1.27 (0.92, 1.74) per 10-ppb increase *Sex-specific results also presented. Covariate adjustment: age, gender, education, occupation, family income, BMI, hypertension, family history of stroke, family history of CVD, smoking status, drinking, diet, and exercise. Copollutant adjustment: none

BMI = body mass index; CI = confidence interval; CTS = California Teachers Study; CBVD = cerebrovascular disease; CVD = cardiovascular disease; ED = emergency department; ETS = environmental tobacco smoke; GP = general practice; HR = hazard ratio; HS = hemorrhagic stroke; IDW = inverse distance weighting; IHD = ischemic heart disease; IQR = interquartile range; IS = ischemic stroke; LUR = land-use regression; MI = myocardial infarction; NO_2 = nitrogen dioxide; NO_X = sum of NO and NO_2; non-HS = non-hemhorragic stroke; OR = odds ratio; Q1 = first quantile; Q2 = second quantile: Q3 = third quantile; Q4 = fourth quantile; Q5 = fifth quantile; RR = risk ratio(s), relative risk; SD = standard deviation; SES = socioeconomic status; TIA = transient ischemic attack.

*NO_X results that are originally reported in $\mu g/m^3$ are not standardized if the molecular weight needed to convert to ppb is not reported.

6.3.5 Hypertension

There were no studies of the effect of long-term NO_2 or NO_X exposure on hypertension in the 2008 ISA for Oxides of Nitrogen. Several recent studies of both children and adults add to the evidence base (Table 6-9). Overall, findings from both studies of adults and children were inconsistent. Further, the independent effect of NO_2 was not distinguished from the effect of noise and other traffic pollutants in the epidemiologic studies reporting positive associations.

Table 6-9 Epidemiologic studies of the association of long-term exposure to nitrogen dioxide (NO_2) or the sum of nitric oxide and NO_2 (NO_X) with hypertension and blood pressure.

Study	Cohort Location Study Period	Mean/ Median (ppb)	Exposure Assessment	Effect Estimates (95% CI)
Studies of Adults				
Coogan et al. (2012)	BWHS Cohort in Los Angeles, CA 2006 N = 3,236	NO_X: Mean: 43.3 µg/m^3 Median: 41.6 µg/m^3 IQR: 12.4 µg/m^3	LUR incorporating traffic, land use, population, and physical geography was used to estimate exposure at the participants' residences. Summer and winter field measurements taken at 183 sites in Los Angeles and averaged to estimate an annual mean.	Hypertension (per IQR increase in NO_X)* IRR = 1.14 (1.03, 1.25) Copollutant adjustment: none IRR = 1.11 (1.00, 1.23) Copollutant adjustment: $PM_{2.5}$ Covariate adjustment: age, BMI, yr of education, income, number of people in the household, smoking, drinks per week, h/week of physical activity, neighborhood socioeconomic status score, and noise.
Foraster et al. (2014)	REGICOR Cohort in Girona, Spain 2007−2009 N = 3,836	NO_2: Median: 14.1 IQR: 6.22	LUR to estimate NO_2 concentrations at participant's geocoded address. Primary model inputs were air sampler height and traffic-related variables.	Blood pressure change (mmHg per 10 ppb NO_2) Participants taking BP-lowering medications Systolic: 2.24 (−2.58, 7.06) Participants not taking BP-lowering medications Systolic: 2.52 (0.26, 4.80) Covariates: age, age squared, sex, living alone, education, diabetes, BMI, nighttime railway noise, smoking, alcohol consumption, deprivation, daily NO_2, temperature, and nighttime railway, and traffic noise. Copollutant adjustment: none

Table 6-9 (Continued): Epidemiologic studies of the association of long-term exposure to nitrogen dioxide (NO2) or the sum of nitric oxide and NO2 (NOX) with hypertension and blood pressure.

Study	Cohort Location Study Period	Mean/ Median (ppb)	Exposure Assessment	Effect Estimates (95% CI)
Sørensen et al. (2012)	Diet, Cancer, and Health Cohort in Copenhagen or Aarhus, Denmark 2000−2002 N = 45,271	NO_X: ($\mu g/m^3$) 1-yr avg baseline Median: 20.2 IQR: 72.5 Follow-up Median: 20.0 IQR: 71.1 5-yr avg baseline Median: 19.6 IQR: 73.2 Follow-up Median: 19.3 IQR: 71.4	Air pollution modeled using source dispersion model that sums local air pollution from street traffic based on traffic location and density. The AirGIS modeling system was used to predict the NO_X, NO_2, and NO concentrations at each participant's residence.	Blood pressure change (mmHg in response to doubling NO_X) 1-yr period Systolic: −0.53 (−0.88, −0.19) 5-yr period Systolic: −0.84 (−0.84, −0.16) 5-yr period hypertension OR: 0.96 (0.91,1.00) Covariates: traffic noise, short term NO_X, temperature, relative humidity, age, sex, calendar yr, center of enrollment, length of school attendance, body mass index, smoking status, alcohol intake, intake of fruit and vegetables, sport during leisure time, and season. Copollutant adjustment: none
Dong et al. (2013b)	33 communities in 11 districts of 3 cities in Liaoning Province, China 2006−2008 N = 24,845	NO_2: Mean: 18.7 Median: 17.5 IQR: 4.8	District-specific 3-y avg NO_2 concentrations for communities within 1 km of an air monitoring station (selected to maximize intra- and inter-city gradients).	Blood pressure change (mmHg per 10 ppb NO_2) Diastolic: 0.46 (−0.10, 1.02) Systolic: 0.48 (−0.44,0.088) Estimated change in the prevalence of hypertension OR: 1.20 (1, 1.43) Covariate adjustment: smoking status, duration, intensity; ETS, gender, BMI, education, sports activity, alcohol consumption, fruit consumption, fat consumption, hypertension, and hypercholesterolemia. Copollutant adjustment: none

Table 6-9 (Continued): Epidemiologic studies of the association of long-term exposure to nitrogen dioxide (NO2) or the sum of nitric oxide and NO2 (NOX) with hypertension and blood pressure.

Study	Cohort Location Study Period	Mean/ Median (ppb)	Exposure Assessment	Effect Estimates (95% CI)
Studies of Children				
Liu et al. (2014b)	GINIplus and LISAplus Cohorts in Germany October 2008−November 2009 N = 2,368	NO_2: Mean: 12.4 Median: 18.85 IQR: 3.42	Exposure estimates at each participant's residence were calculated using an LUR. An annual average was extrapolated from NO_2 measurements taken during three 2-week periods (warm, cold, and intermediate seasons) at 40 sites.	Blood pressure change (mmHg per 10 ppb NO_2) Systolic: 0.32 (−1.32, 1.96) Diastolic: −0.18 (−1.40, 1.05) Covariate adjustment: Cohort study, area, gender, age of child, BMI, physical activity, maternal smoking during pregnancy, parental education level, parental history of hypertension, 7-day level of air pollutants, and 7-day temperature. Copollutant adjustment: None Systolic: −0.56 (−3.30, 2.19) Diastolic: −2.25 (−4.59, 0.088) N = 605 Covariate Adjustment: Additionally adjusted for road-traffic noise. Copollutant adjustment: none
Bilenko et al. (2013)	PIAMA Birth Cohort in the Netherlands February 2009−February 2010 N = 1,432	NO_2: Long-term Median: 11.6 IQR: 4.1 Short-term Median: 8.8 IQR: 7.1	Long term: Exposure estimates at each participant's residence were calculated using an LUR. An annual average was extrapolated from NO_2 measurements taken during three 2-week periods (warm, cold, and intermediate seasons) at 80 sites. Note: short-term exposure also estimated.	Blood pressure change (mmHg per 10 ppb NO_2) Long-term Diastolic: 0.80 (−0.44, 2.05) Systolic: −0.073(−0.17,0.16) Short-term Diastolic: 0.24 (−0.77, 1) Systolic: 0.11 (−0.44, 0.92) Covariate adjustment: age, sex height, BMI, cuff size, gestational age at birth, birthweight, weight gain during the first yr of life, breast feeding, maternal smoking during pregnancy, parental smoking in the child's home, physical activity, puberty development scale, maternal education, maternal hypertension during pregnancy, pneumonia, and/or otitis media during the first 2 yrs of life, ambient temperature, and room temperature. Copollutant adjustment: none

Table 6-9 (Continued): Epidemiologic studies of the association of long-term exposure to nitrogen dioxide (NO2) or the sum of nitric oxide and NO2 (NOX) with hypertension and blood pressure.

Study	Cohort Location Study Period	Mean/ Median (ppb)	Exposure Assessment	Effect Estimates (95% CI)
Clark et al. (2012)	UK RANCH Study 2001−2003 N = 719 children (9−10 yr), N = 11 schools		Combined emission-dispersion and regression model to assign annual avg NO_2 concentration at 20 × 20 m resolution at each school.	No associations reported between systolic or diastolic blood pressure. Covariate adjustment: age, gender, employment status, crowding, home ownership, mother's educational level, long-standing illness, language spoken at home, parental support for school work, classroom window glazing type, and noise. Copollutant adjustment: none

BMI = body mass index; BWHS = Black Women's Health Study; CI = confidence interval; ETS = environmental tobacco smoke; GINIplus = German Infant Nutritional Intervention plus environmental and genetic influences; IQR = interquartile range; IRR = incidence rate ratios; LISAplus = Lifestyle-Related factors on the Immune System and the Development of Allergies in Childhood plus the influence of traffic emissions and genetics; NO = nitric oxide; NO_2 = nitrogen dioxide; NO_X = sum of NO and NO_2; RANCH = Road Traffic and Aircraft Noise Exposure and Children's Cognition and Health; LUR = land-use regression model; OR = odds ratio; PIAMA = Prevention and Incidence of Asthma and Mite Allergy; $PM_{2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 2.5 μm; REGICOR = Registre Gironí del Cor.

*NO_X results that are originally reported in $\mu g/m^3$ are not standardized if the molecular weight needed to convert to ppb is not reported.

Coogan et al. (2012) examined the association of long-term NO_X exposure with incident hypertension among black women residing in Los Angeles, CA. An LUR model was used to estimate exposure at each participant's residential address, and cross validation of the model performance produced an R^2 value of approximately 92%. These authors reported an increased risk of 1.14 (95% CI: 1.03, 1.25%) per IQR (12.4 $\mu g/m^3$) increase in NO_X after adjustment for a wide array of potential confounders including traffic-related noise exposure. Slight attenuation in the effect estimate for NO_X was reported after adjustment for $PM_{2.5}$. Although the correlation between NO_X and $PM_{2.5}$ ($PM_{2.5}$ concentration was estimated using kriging) was low ($r = 0.27$), correlations between NO_X and other traffic-related copollutants were not reported.

In a cross-sectional study, Foraster et al. (2014) reported that NO_2 was associated with an increase in systolic blood pressure that was attenuated to varying degrees depending on the method adjustment for medication use but not with hypertension. In this study, LUR ($R^2 = 0.63$) was used to estimate NO_2 exposure among participants (35−83 years) of a large population-based cohort study. Both short-term exposure to NO_2 and noise were adjusted for in the analysis, in addition to an array of other potential confounders. Associations with systolic blood pressure were stronger among those with cardiovascular disease, those living alone, and those living in areas with high traffic load and traffic

noise. In a large study involving Danish adults (50−64 year old), Sørensen et al. (2012) reported an inverse association between NO_2 and both systolic and diastolic blood pressure at baseline while largely null findings were report for self-reported incident hypertension. A validated dispersion model was used in this study to predict annual and 5-yr avg NO_2 concentration and to assign exposure based on residential address history [$r \geq 0.9$ for correlation between measured and predicted 1/2-yr avg NO_2 concentration; (Sørensen et al., 2012)] Dong et al. (2013b) reported imprecise associations of average NO_2 concentration from monitoring stations located within 1 km of the community where study participants resided, with prevalent hypertension and increased blood pressure. Stronger associations of hypertension with PM_{10}, SO_2, and O_3 were reported in this study.

Some additional studies examined the association of NO_2 with blood pressure in children. Liu et al. (2014b) reported an increase in diastolic blood pressure that was diminished after adjustment for traffic-related noise exposure. Bilenko et al. (2013), however, reported an association between NO_2 and diastolic blood pressure that was robust to adjustment for noise. Both studies used LUR models to assign NO_2 exposure at each participant's residential address. A study designed to evaluate the effect of aircraft noise on cognition among school children (9−10 years old) reported no association between NO_2 and blood pressure after adjustment for noise (Clark et al., 2012).

Overall, findings from studies of adults and studies of children report weak, inconsistent results for the association between NO_2 and hypertension and increased blood pressure, although one prospective study using LUR to estimate exposure and adjusting for a cardiovascular disease risk factors reported an association of NO_X with hypertension (Coogan et al., 2012). Uncertainties remain regarding the independent effect of NO_2 on hypertension and blood pressure and specifically whether confounding by correlated traffic-related pollutants or noise can explain the positive associations observed.

6.3.6 Markers of Cardiovascular Disease

Some epidemiologic and toxicological studies published since previous assessments have investigated the effects of long-term NO_2 exposure on risk factors and markers of cardiovascular disease risk, such as arterial stiffness, subclinical atherosclerosis, circulating lipids, and heart rate variability (HRV). Previous information was limited. The 1993 Air Quality Criteria Document (AQCD) for Oxides of Nitrogen (U.S. EPA, 1993) reported a significant reduction in HR in rats exposed to 1,200 and 4,000 ppb NO_2 for 1 month but not after lower concentrations or longer durations of exposure (Suzuki et al., 1981). There were no changes in vagal responses in rats exposed to 400 ppb NO_2 for 4 weeks (Tsubone and Suzuki, 1984).

Several recent cross-sectional analyses of long-term exposure to NO_2 evaluated vascular markers of cardiovascular disease. Rivera et al. (2013) used LUR to estimate NO_2 concentrations. Increases in carotid intima-media thickness (cIMT) observed in crude models were attenuated in fully adjusted models while a positive association between NO_2 and high ankle brachial index (ABI >1.3) remained. LUR was also used to assess vascular damage in health young adults (Lenters et al., 2010). Increases in pulse wave velocity, augmentation index, but not cIMT in association with NO_2 exposure were observed in this study. In a study that used IDW methods for spatial interpolation and linked to participants' residential addresses, exposure to NO_2 during childhood lifestages was not associated with an increase in cIMT among young adults (Breton et al., 2012).

The effects of NO_2 in relation to autonomic function in a random selection of Swiss cohort study participants have also been examined. In this study, Felber Dietrich et al. (2008) linked measures of HRV to annual NO_2 concentration at each participant's residential address using dispersion model predictions supplemented with land use and meteorological data. Annual average NO_2 concentration was associated with decreased standard deviation of beat-to-beat (NN) intervals, an index of total HRV, nighttime low-frequency component of HRV (LF), and LF/high frequency component of HRV (HF) ratio in women. No associations with other parameters of HRV were observed in these data.

A recent experimental animal study by Seilkop et al. (2012) reported changes in markers that are characteristic of vascular disease development and progression (see Table 6-10 for toxicological study details). Mice were exposed for 50 days to various multipollutant atmospheres (diesel or gasoline exhaust, wood smoke, or simulated "downwind" coal emissions) comprising varying concentrations of NO_2 (0−3,670 ppb). A data mining technique known as Multiple Additive Regression Trees analysis was employed to identify associations between the 45 different exposure component categories, including NO_2, and various effects [markers of oxidative stress (discussed in Section 5.3.4) and cardiovascular disease stability and progression (endothelin-1 (ET-1), matrix metalloproteinase (MMP)-3, MMP-7, MMP-9, tissue inhibitor of metalloproteinase-2 (TIMP-2))]. The results demonstrated NO_2 was among one of the strongest predictors of responses. More specifically, NO_2 ranked among the top three predictors for ET-1 and TIMP-2; however, the study design did not allow for the independent effects of NO_2 to be evaluated.

Table 6-10 Study details for toxicological studies examining cardiovascular effects from long-term nitrogen dioxide (NO_2) exposure.

Study	Species (Strain); Age; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
de Burbure et al. (2007)	Rats (Wistar); 8 weeks; M; n = 8/group	High (6 µg/day) or low (1.3 µg/day) selenium; (1) 1,000 ppb, 28 days, 6 h/day, 5 days/week (Se^+/Se^-); (2) 10,000 ppb, 28 days, 6 h/day, 5 days/week; (3) 5,000 ppb, 5 days, 6 h/day; (4) 50,000 ppb, 30 min	GPx in plasma and RBC lysate; SOD activity in RBC lysate; GST activity in RBC lysate; TBARS in plasma. Endpoints examined immediately and 48 h after exposure.
Fenters et al. (1973)	Squirrel monkeys; adult; M; n = 4	1,000 ppb NO_2, continuously for 16 mo; challenged with influenza virus	Hemoglobin and hematocrit levels were measured throughout the study.
Furiosi et al. (1973)	Monkeys (*Macaca speciosa*); adult; M/F; n = 4 Rats (Sprague-Dawley); 4 weeks; M; n = 8	(1) 2,000 ppb NO_2, continuously for 14 mo	Erythrocyte, hematocrit, and hemoglobin levels were measured throughout the study.
Seilkop et al. (2012)	Mice (ApoE−/−); 10 weeks; M; n = 8−10	NO_2 (along with 700 other components) Fed a high-fat diet; 260, 745, and 3,670 ppb (along with dilutions of 1/3 and 1/10); 6 h/day, 7 days/week for 50 days	ET-1, VEGF, MMP-3, MMP-7, MMP-9, TIMP-2, HO-1, TBARS in proximal aorta 18-h after exposure.
Suzuki et al. (1981)	Rats; NR; NR; n = 6	400, 1,200, and 4,000 ppb NO_2; 1, 2, and 3 mo	HR and hemoglobin levels measured after 1, 2, and 3 mo exposures.
Takano et al. (2004)	Rats (OLETF and LETO); 4 weeks; M; n = 10−14	160, 800, or 4,000 ppb NO_2; continuously for 32 weeks	BW, Triglyceride, HDL, total cholesterol, HDL/total cholesterol, sugar measured 8 weeks following exposure.
Tsubone and Suzuki (1984)	Rats (Wistar); 9−13 weeks; M; n = 6	400 and 4,000 ppb NO_2; continuously for 1 and 4 weeks, respectively; Immediately after exposure, animals were injected with 5 µg/kg BW phenyl diguanide	HR was measured 10 sec after injection.
Wagner et al. (1965)	Dogs; adult; M; n = 6−10/group	1,000 or 5,000 ppb NO_2; continuously for 18 mo	Hemoglobin and hematocrit levels were measured quarterly throughout exposure.

BW = body weight; ET-1 = endothelin-1; GPx = glutathione peroxidase; GST = glutathione s-transferase; HDL = high density lipoprotein; HO-1 = heme oxygenase-1; HR = hazard ratio; LETO = Long-Evans Tokushima; NO_2 = nitrogen dioxide; NR = no quantitative results reported; OLETF = Otsuka Long-Evans Tokushima Fatty; RBC = red blood cell; SOD = superoxide dismutase; TBARS = thiobarbituric acid reactive substances; TIMP-2 = tissue inhibitor of metalloproteinase-2; VEGF = vascular endothelial growth factor

In another study, Takano et al. (2004) reported that obese rats (Otsuka Long-Evans Tokushima Fatty) had elevated levels of triglycerides and decreased high-density lipoprotein (HDL) and HDL/total cholesterol levels after long-term exposure to 160 ppb NO_2 compared to clean air. HDL levels were also decreased after 800 ppb NO_2 exposure in the obese strain and in the nonobese rats (Long-Evans Tokushima). The authors suggested that obese animals were at greater risk of dyslipidemia following NO_2 exposure.

Overall, a limited number of epidemiologic and toxicological studies have evaluated long-term NO_2 exposure on markers of cardiovascular disease. There is some evidence for increased arterial stiffness, increased markers for cardiovascular disease stability and progression, dyslipidemia, decreased HRV, and reduced HR; however, these effects have only been reported in one study each. Findings from several studies of cIMT are inconsistent. Further, the independent effect of NO_2 is not consistently distinguished in the available body of epidemiologic and toxicological evidence.

6.3.7 Inflammation and Oxidative Stress

Inflammation and oxidative stress have been shown to play a role in the progression of chronic cardiometabolic disorders including heart disease and diabetes. Although studies of inflammation and oxidative stress were not generally available for inclusion in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008), a number of null findings related to changes in hematological parameters were reported. Hematocrit and hemoglobin levels were unchanged in squirrel monkeys (Fenters et al., 1973), rats (Suzuki et al., 1981), or dogs exposed to ≤5,000 ppb NO_2 (Wagner et al., 1965). However, Furiosi et al. (1973) reported polycythemia due to reduced mean corpuscular volume and an increased trend in the ratio of neutrophil to lymphocytes in the blood of NO_2-exposed monkeys and similar increases in erythrocyte counts in NO_2-exposed rats.

A limited number of studies published since the 2008 ISA for Oxides of Nitrogen have evaluated markers of inflammation and oxidative stress. Forbes et al. (2009a) examined the association of predicted annual average NO_2 concentrations with C-reactive protein (CRP) and fibrinogen among an English population. Multilevel linear regression models were used to determine pooled estimates across three cross-sectional surveys conducted during different years. Each participant's postal code of residence was linked to a predicted annual average NO_2 concentration derived from dispersion models. NO_2 was not associated with increased CRP or fibrinogen in these data nor were PM_{10}, SO_2, or O_3. A study conducted among men and women (45−70 year-olds) in Stockholm reported an association of 30-yr avg traffic-related NO_2 concentrations estimated using dispersion

models with increases in interleukin-6 (IL-6) and CRP but not with TNF-α, fibrinogen, or PAI-1 (Panasevich et al., 2009). Associations between several metrics of SO_2 exposure and increased IL-6 and CRP were observed in this study. In another analysis from this study, long-term exposure to NO_2 interacted with IL-6 and TNF polymorphisms on an additive scale with regard to increased MI risk (Panasevich et al., 2013). In a study of COPD patients, annual average NO_2 concentration was associated with increases in interleukin-8 (IL-8) but not with the other markers studied including CRP, TNF-α IL-6, fibrinogen, and hepatocyte growth factor (Dadvand et al., 2014b).

de Burbure et al. (2007) examined oxidative stress markers in rats on a low selenium (Se-L) or supplemented selenium (Se-S) diet exposed to 1,000 ppb NO_2 for 28 days. Blood Se levels decreased significantly in both groups immediately after the 28-day exposure and continued to decrease in the Se-S diet rats following a 48-hour recovery period. GPx, of which Se is an integral component, also decreased immediately and 48 hours after exposure only in the plasma of Se-S diet rats. However, GPx levels increased in red blood cells (RBC) of Se-L diet rats immediately after the 28-day exposure and increased in both groups 48 hours later. RBC SOD activity increased in both groups immediately after the exposure and decreased in Se-L diet rats 48 hours later. GST was increased for both groups immediately after the 28-day exposure and continued to increase after the 48-hour recovery period, potentially compensating for the increase in thiobarbituric acid reactive substances (TBARS) immediately after exposure.

As discussed in Section 6.3.6, Seilkop et al. (2012), examined the effects of NO_2 exposure, in a multipollutant context, on markers of oxidative stress [heme oxygenase-1 (HO-1) expression and TBARs, indicator of lipid peroxidation] in ApoE−/− mice fed a high-fat diet. Mice were exposed to various atmospheres (diesel or gasoline exhaust, wood smoke, or simulated "downwind" coal emissions) with varying concentrations of NO_2 (0−745 ppb) for 50 days. Associations between the oxidative stress indicators and the 45 different exposure component categories were determined using a data mining technique known as Multiple Additive Regression Trees analysis. The results demonstrated NO_2 was among one of the strongest predictors of response for TBARS but not HO-1.

Overall, a limited number of epidemiologic and toxicological studies have evaluated long-term NO_2 exposure on inflammation and oxidative stress with some, but not all, studies reporting positive associations. In general, findings of the epidemiologic studies are mixed, and the animal toxicological studies do not consistently separate the effect of NO_2 from other copollutants.

6.3.8 Cardiovascular Mortality

Results of studies of long-term exposure to NO_2 and cardiovascular diseases are coherent with findings reporting associations of long-term NO_2 exposure with total and cardiovascular mortality. Consistent, positive associations with total mortality, as well as deaths due to cardiovascular disease have been observed in cohort studies conducted in the U.S. and Europe (Section 6.5.2, Figure 6-9, and Table 6-16). Specifically, the strongest evidence comes from a number of recent studies that have observed positive associations between exposure to NO_2 and NO_X and IHD mortality (Cesaroni et al., 2013; Chen et al., 2013; Lipsett et al., 2011; Yorifuji et al., 2010), mortality due to coronary heart disease (Gan et al., 2011; Rosenlund et al., 2008), and circulatory mortality (Yorifuji et al., 2010; Jerrett et al., 2009). Coherence is also provided for the effect of long-term exposure and cardiovascular effects by the evidence from studies of short-term cardiovascular mortality and morbidity (Section 5.3).

6.3.9 Summary and Causal Determination

Overall, the evidence is suggestive, but not sufficient, to infer a causal relationship between long-term exposure to NO_2 and cardiovascular and related cardiometabolic effects. This conclusion is based on the consideration of recent epidemiologic studies reporting associations of NO_2 with heart disease, diabetes, stroke, and hypertension. While well-conducted studies of NO_X are also available, these studies are less informative regarding the independent effect of NO_2 exposure on the cardiovascular system. Although associations with these cardiovascular and related cardiometabolic outcomes were not entirely consistent across studies, several studies reporting positive associations were prospective in design and did not report evidence that findings were likely to be biased by selective participation or missing data. Additionally, these studies adjusted for a wide array of cardiovascular risk factors and used exposure assessment methods that captured the fine scale variability in NO_2 concentration. Uncertainty remains, however, regarding the independent effect of NO_2 relative to other traffic-related exposures and noise. Some support is provided by a limited body of evidence demonstrating biological plausibility, as well as consistent associations between long-term NO_2 exposure and cardiovascular mortality. This current conclusion represents a change from the conclusion drawn in the 2008 ISA for Oxides of Nitrogen, which stated that the evidence was inadequate to infer the presence or absence of a causal relationship. The evidence for cardiovascular effects with respect to the causal determination for long-term NO_2 exposure is detailed below using the framework described in Table II of the Preamble to this ISA. The key evidence as it relates to the causal determination is summarized in Table 6-11.

Briefly, the 2008 ISA for Oxides of Nitrogen concluded that the available evidence was inadequate to infer the presence or absence of a causal relationship between long-term NO_2 exposure and cardiovascular disease. Although Miller et al. (2007) reported a positive association between long-term NO_2 exposure and cardiovascular events among post-menopausal women, an independent effect of NO_2 was not distinguished in this study. Several studies evaluating hematological parameters reported mixed results that included no changes in hematocrit or hemoglobin and increased erythrocyte count.

Evidence from recent, large and well-conducted prospective epidemiologic studies generally supports the association of long-term exposure to NO_2 with heart disease and diabetes. The strongest evidence for heart disease comes from a large multicohort prospective study using LUR to predict NO_2 concentrations on a finely resolved spatial scale (Cesaroni et al., 2014) with supporting evidence from cross-sectional study of CHD hospitalizations also using LUR (Beckerman et al., 2012). Consistent findings from multiple epidemiologic studies of cardiovascular mortality support these morbidity findings (Section 6.5.2). Studies using dispersion models or IDW for exposure assessments were less consistent, with some reporting positive associations (Lipsett et al., 2011) and others reporting null associations with morbidity outcomes (Atkinson et al., 2013; Rosenlund et al., 2009a). As noted in Section 3.2.3, IDW and dispersion modeling may not adequately capture the spatial variability in NO_2 concentrations, resulting in biased exposure estimates. Several large prospective studies also using LUR report increased risk of diabetes, impaired glucose metabolism, and increased insulin resistance with NO_X and NO_2 exposure (Coogan et al., 2012; Kramer et al., 2010) (Teichert et al., 2013; Thiering et al., 2013). The evidence for the association of long-term NO_2 exposure with stroke and hypertension is less consistent.

Studies in human populations offer limited support that long-term NO_2 exposures may be associated with increased high ABI (Rivera et al., 2013), arterial stiffness (Lenters et al., 2010) and markers of inflammation [CRP and IL-6; (Panasevich et al., 2009)]. Epidemiologic studies of the effect of NO_2 on cIMT are inconsistent. Toxicological studies provide limited evidence that NO_2 may be independently associated with the effects observed in epidemiologic studies by demonstrating dyslipidemia and oxidative stress in animals after long-term exposure (de Burbure et al., 2007; Takano et al., 2004). Evidence for cardiovascular effects is provided by both controlled human exposure and animal toxicological studies that report increased markers of oxidative stress (Channell et al., 2012; Li et al., 2011) and inflammation (Huang et al., 2012; Riedl et al., 2012) after short-term NO_2 exposure, however.

Although several epidemiologic studies report positive associations of NO_2 or NO_X exposure with heart disease and diabetes, confounding by correlated traffic-related

pollutants and noise remains an uncertainty. Animal toxicological studies provide only limited support for the biological plausibility of associations observed in the epidemiologic studies. In addition, the annual average or 5-yr avg residential exposures were typically considered surrogates for long-term exposure, and residential stability was assumed (or sometimes required for eligibility). Further, most studies did not disentangle the effects of long-term from short-term exposure. These general limitations introduce some uncertainty with regard to the specific patterns of exposure associated with the observed effects. Overall, the evidence from some epidemiologic studies of cardiovascular and cardiometabolic effects is suggestive, but not sufficient, to infer a causal relationship between long-term NO_2 exposure and cardiovascular and related cardiovascular effects.

Table 6-11 Summary of evidence, which is suggestive, but not sufficient, to infer a causal relationship between long-term nitrogen dioxide (NO_2) exposure and cardiovascular and related metabolic effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations (ppb) Associated with Effects[c]
CHD and MI			
Evidence from epidemiologic studies generally supportive but not entirely consistent	Findings from a large multicohort prospective study using LUR provides evidence that NO_2 is associated with coronary events.	Cesaroni et al. (2014)	Range of means across cohorts: 4.2−31.9
	Supporting evidence from cross-sectional epidemiologic study of CHD hospitalizations and study of MI incidence using IDW.	Beckerman et al. (2012)	Median: 22.9
		Lipsett et al. (2011)	Mean (5th−95th): 33.59
	Inverse or null associations of NO_2 with CHD or MI reported in some epidemiologic studies.	Atkinson et al. (2013)	Mean: 12.0
		Rosenlund et al. (2009a)	Median; 6.9 (cases), 6.3 (controls)
		Gan et al. (2011)	Mean: 16.3
Consistent associations from multiple, high-quality epidemiologic studies of cardiovascular mortality support morbidity findings	Strongest evidence of mortality from IHD, CHD, and circulatory diseases, including supporting evidence of positive mortality associations from studies noted above that report weak or null associations with cardiovascular morbidity outcomes.	Rosenlund et al. (2009a); Gan et al. (2011) Section 6.5.2	See above
Uncertainty remains regarding potential confounding by traffic-related pollutants and noise	Overall, studies did not consistently adjust for $PM_{2.5}$, BC, EC, CO, or noise.	Section 6.3.32	

Table 6-11 (Continued): Summary of evidence, which is suggestive, but not sufficient, to infer a causal relationship between long-term nitrogen dioxide (NO2) exposure and cardiovascular and related metabolic effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations (ppb) Associated with Effects[c]
Diabetes			
Evidence from epidemiologic studies generally consistent and supportive	Large prospective studies using LUR report increased risk of diabetes incidence, impaired glucose metabolism, and increased insulin resistance with NO_X and NO_2 exposure.	Coogan et al. (2012)	Mean (NO_X): 43.3
		Kramer et al. (2010)	Median: 18.33
		Teichert et al. (2013)	Mean: ~21 (among those w IGM); Mean: ~20 (among those w/o IGM)
		Thiering et al. (2013)	Mean: 11.53
	Supporting evidence that NO_2 exposure is associated with prevalent diabetes.	Eze et al. (2014)	Mean: 15.03
		Brook et al. (2008)—females only	Mean: ~15 (Hamilton); Mean: ~23 (Toronto)
	Association observed among confirmed cases of diabetes but not overall.	Andersen et al. (2012c)	Median: ~8
Uncertainty remains regarding potential confounding by traffic pollutants	Consistent but limited evidence that associations are robust to adjustment for noise.	Eze et al. (2014); Sørensen et al. (2013); Eriksson et al. (2014)	See above
	Overall, studies did not consistently adjust for $PM_{2.5}$, BC, EC, CO, or noise.	Section 6.3.4	
	Associations observed with NO_X may not inform the independent effect of NO_2.	Coogan et al. (2012)	

Table 6-11 (Continued): Summary of evidence, which is suggestive, but not sufficient, to infer a causal relationship between long-term nitrogen dioxide (NO2) exposure and cardiovascular and related metabolic effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations (ppb) Associated with Effects[c]
Cerebrovascular Disease and Stroke			
Inconsistent evidence from epidemiologic studies	Some studies of variable quality report increased, typically imprecise, risks of stroke and/or cerebrovascular disease with NO_2. Inconsistency across stroke subtype.	Johnson et al. (2013),	Mean: 15.4 (cases), 15.2 (controls)
		Johnson et al. (2010),	Mean: 15.7
		Andersen et al. (2012b),	Median: 8.1
		de Kluizenaar et al. (2013)	NR
		Dong et al. (2013a)	Mean: 18.7
	Other studies reported weak or null associations.	Atkinson et al. (2013),	Mean: 12.0
		Lipsett et al. (2011)	Mean: 33.59
Confounding bias cannot be ruled out	Studies not consistently robust to adjustment for noise.	Sørensen et al. (2014)	See above
		de Kluizenaar et al. (2013)	
	Uncertainty regarding potential confounding by traffic pollutants.	Section 6.3.54	

Table 6-11 (Continued): Summary of evidence, which is suggestive, but not sufficient, to infer a causal relationship between long-term nitrogen dioxide (NO2) exposure and cardiovascular and related metabolic effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations (ppb) Associated with Effects[c]
Hypertension			
Overall, studies of hypertension and increased blood pressure are inconsistent	Large prospective study using LUR to estimate NO_X exposure reports association with hypertension.	Coogan et al. (2012)	Mean (NO_X): 43.3
	Cross-sectional association between NO_2 and increased blood pressure but not hypertension.	Foraster et al. (2014)	Median: 14.1
	Large prospective study using LUR does not report positive associations with hypertension and/or increased blood pressure.	Sørensen et al. (2012)	Median: 20.2
Uncertainty regarding potential confounding by traffic related pollutants and noise	Overall, studies did not consistently adjust for $PM_{2.5}$, BC, EC, CO, or noise.	Section 5.3.6	
	Association observed with NO_X may not inform the independent effect of NO_2.	Coogan et al. (2012)	
Biological Plausibility and Coherence for Cardiovascular and Related Cardiometabolic Effects			
Limited and supportive evidence from epidemiologic and toxicological studies for effects on cardiovascular disease risk provides biological plausibility	Associations of NO_2 exposure with some markers of vascular damage were observed in epidemiologic studies.	Lenters et al. (2010) Rivera et al. (2013)	Mean: 18.3
	Dyslidemia—increased triglycerides and decreased HDL—in rats.	Takano et al. (2004)	Rats: 160

Table 6-11 (Continued): Summary of evidence, which is suggestive, but not sufficient, to infer a causal relationship between long-term nitrogen dioxide (NO2) exposure and cardiovascular and related metabolic effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations (ppb) Associated with Effects[c]
Some evidence for key events within the mode of action		Li et al. (2011), Section 4.3.2.9, Figure 4-3	Rats: 5,320 but not 2,660
	Limited and supportive evidence of increased oxidative stress in rats with relevant short-term and long-term NO_2 exposures (i.e., MDA, TBARS, GPx, GST) and in plasma from NO_2-exposed humans (i.e., LOX-1).	de Burbure et al. (2007)	Rats: 1,000
		Channell et al. (2012)	Healthy adults: 500
	Limited and supportive toxicological evidence of increased transcription of some inflammatory mediators in vitro after short-term exposure to NO_2 (i.e., IL-8, ICAM-1, VCAM-1) and in rats (i.e., ICAM-1, TNF-α).	Channell et al. (2012)	Human cells exposed to plasma from healthy adults: 500
		Li et al. (2011)	Rats: 2,660 and 5,320
	Limited and inconclusive evidence in controlled human exposure studies (i.e., IL-6, IL-8, ICAM-1).	Huang et al. (2012)	Adults: 350
		Riedl et al. (2012)	Adults: 500

Table 6-11 (Continued): Summary of evidence, which is suggestive, but not sufficient, to infer a causal relationship between long-term nitrogen dioxide (NO2) exposure and cardiovascular and related metabolic effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations (ppb) Associated with Effects[c]
Some evidence for key events within the mode of action (continued)	Inconsistent epidemiologic evidence for increases in CRP and IL-6 in adults.	Section 6.3.7	

BC = black carbon; CHD = coronary heart disease; CO = carbon monoxide; CRP = C-reactive protein; GPx = glutathione peroxidase; GST = glutathione s-transferase; EC = elemental carbon; HDL = high-density lipoprotein; ICAM = intercellular adhesion molecule 1; IDW = inverse distance weighting; IGM = impaired glucose metabolismn; IHD = ischemic heart disease; IL-6 = interleuken-6; IL-8 = interleukin-8; LOX-1 = lectin-like oxidized low density lipoprotein receptor 1; LUR = land-use regression; MDA = malondialdehyde; MI = myocardial infarction; NO_2 = nitrogen dioxide; NO_X = sum of NO and NO_2; NR = no quantitative results reported; $PM_{2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 2.5 μm; TBARS = thiobarbituric acid reactive substances; TNF-α = tumor necrosis factor alpha; VCAM-1 = vascular cell adhesion molecule 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.

[b]Describes the key evidence and references that contribute most heavily to causal determination, and where applicable, to uncertainties and inconsistencies. References to earlier sections indicate where the full body of evidence is described.

[c]Describes the NO_2 concentrations with which the evidence is substantiated (for experimental studies, below 5,000 ppb).

6.4 Reproductive and Developmental Effects

6.4.1 Introduction

The body of literature characterizing the health effects associated with exposure to NO_2 is large and continues to grow; much of the research focuses on birth outcomes, for which the body of evidence has grown considerably since the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008). Due to the growth in the quantity of literature, as well as in the breadth of the health endpoints evaluated, the reproductive and developmental effects will be divided into three separate categories: (1) Fertility, Reproduction, and Pregnancy (i.e., the ability to achieve and maintain a healthy pregnancy, with emphasis on the health of potential parents); (2) Birth Outcomes [i.e., measures of birth weight and fetal growth, preterm birth (PTB), birth defects, and infant mortality, with emphasis on the perinatal health of the child] and (3) Developmental Effects (i.e., effect on development through puberty/adolescence). Separate causal determinations are made for each of these categories at the end of this section. Among the epidemiologic studies of birth outcomes, various measures of birth weight and fetal growth, such as low birth weight (LBW), small for gestational age (SGA), intrauterine growth restriction (IUGR), and preterm birth (<37-week gestation) have received more attention in air pollution research, while

congenital malformations are less studied. There is some examination of effects on fertility and pregnancy conditions; however, studies on any particular endpoint remain limited. The toxicological studies of outcomes analogous to fetal growth and birth weight in humans measured litter size and birth weight in animals. Nervous system and respiratory outcomes after early life exposures to NO_2 are examined in the developmental toxicological and epidemiologic literature.

A major issue in studying environmental exposures and reproductive and developmental effects (including infant mortality) is selecting the relevant exposure period because the biological mechanisms leading to these outcomes and the critical periods of exposure are poorly understood. To account for this, many epidemiologic studies evaluate multiple exposure periods (including long-term exposure periods, such as the entirety of pregnancy, individual trimesters or months of pregnancy; or short-term (days to weeks) exposure periods, such as the days and weeks immediately preceding birth). Due to the shorter length of gestation in rodents (18−24 days, on average), animal toxicological studies investigating the effects of NO_2 on pregnancy generally utilize short-term exposure periods, which cover an entire lifestage. Thus, a study in humans that uses the entire pregnancy as the exposure period is considered to have a long-term exposure period (about 40 weeks, on average), while a toxicological study conducted with rats that also uses the entire pregnancy as the exposure period (about 18−24 days, on average) is defined as a short-term exposure. In order to characterize the weight of evidence for the effects of NO_2 on reproductive and developmental effects in a consistent, cohesive, and integrated manner, results from both short-term and long-term exposure periods are included in this section and are identified accordingly in the text and tables throughout this section.

Due to the paucity of data for biological mechanisms and uncertainty regarding relevant exposure periods, all of the studies of reproductive and developmental outcomes, including infant mortality, are evaluated in this section. Exposures proximate to death may be most relevant if exposure causes an acute effect. However, exposure occurring in early life might affect critical growth and development, with results observable later in the first year of life, or cumulative exposure during the first year of life may be the most important determinant. In dealing with the uncertainties surrounding these issues, studies have considered several exposure metrics based on different periods of exposure, including both short- and long-term exposure periods. These studies are characterized here as they contribute to the weight of evidence for an effect of NO_2 on reproductive and developmental effects.

Although the biological mechanisms are not fully understood, several hypotheses have been proposed for the effects of NO_2 on reproductive and developmental outcomes; these

include oxidative stress, systemic inflammation, vascular dysfunction, and impaired immune function. The study of these outcomes can be difficult given the need for detailed exposure data and potential residential movement of mothers during pregnancy. Air pollution epidemiologic studies reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) examined impacts on birth-related endpoints, including intrauterine, perinatal, post-neonatal, and infant deaths; premature births; intrauterine growth restriction; very low birth weight (weight <1,500 g) and low birth weight (weight <2,500 g); and birth defects. However, in the limited number of studies included in the 2008 ISA for Oxides of Nitrogen, no associations were found between NO_2 and birth outcomes, with the possible exception of birth defects. Overall, the evidence evaluated in the 2008 ISA for Oxides of Nitrogen was inconsistent and lacked coherence and plausibility, and was determined to be inadequate to infer the presence or absence of a causal relationship.

Several recent articles reviewed methodological issues relating to the study of outdoor air pollution and adverse birth outcomes (Chen et al., 2010; Woodruff et al., 2009; Ritz and Wilhelm, 2008; Slama et al., 2008). Some of the key challenges to interpretation of these study results include the difficulty in assessing exposure as most studies use existing monitoring networks to estimate individual exposure to ambient air pollution; the inability to control for potential confounders such as other risk factors that affect birth outcomes (e.g., smoking); evaluating the exposure window (e.g., trimester) of importance; and limited evidence on the physiological mechanism of these effects (Ritz and Wilhelm, 2008; Slama et al., 2008).

Overall, the number of studies examining the association between exposure to ambient NO_2 and reproductive and developmental outcomes has increased tremendously, yet evidence for an association with these outcomes remains relatively uncertain. Recently, an international collaboration was formed to better understand the relationships between air pollution and adverse birth outcomes and to examine some of these methodological issues through standardized parallel analyses in data sets from different countries (Woodruff et al., 2010). Initial results from this collaboration have examined PM and birth weight (Parker et al., 2011); work on NO_2 has not yet been performed. Although early animal studies (Shalamberidze and Tsereteli, 1971a, b) found that exposure to NO_2 during pregnancy in rats led to some abnormal birth outcomes, human studies to date have reported inconsistent results for the association of ambient NO_2 concentrations and a range of reproductive and developmental outcomes, though the evidence has been generally supportive for some particular outcomes (e.g., fetal growth).

6.4.2 Fertility, Reproduction, and Pregnancy

6.4.2.1 Effects on Sperm

A limited amount of research has been conducted to examine the association between air pollution and male reproductive outcomes, specifically semen quality. To date, the epidemiologic studies have considered various exposure durations before semen collection that encompass either the entire period of spermatogenesis (i.e., 90 days) or key periods of sperm development that correspond to epididymal storage, development of sperm motility, and spermatogenesis.

An occupational study of male motorway company employees reported that men with the highest NO_2 exposures in the workplace (near-road environment, ~160 ppb) had lower sperm motility, but no difference in sperm count, compared to men with lower exposures [~80 ppb; (Boggia et al., 2009)]. Two epidemiologic studies evaluated the relationship between ambient concentrations of NO_2 and sperm quality and observed no associations (Rubes et al., 2010; Sokol et al., 2006); while a cross-sectional study observed associations between NO_2 and some semen quality parameters (Zhou et al., 2014). No recent toxicological studies have examined the effect of NO_2 exposure on male reproductive outcomes, specifically semen quality. Kripke and Sherwin (1984) found no significant effects on spermatogenesis, or on germinal and interstitial cells of the testes of a small group of LEW/f mai rats (n = 6) after 21 days of exposure to a single concentration of NO_2, 1,000 ppb 7 h/day, 5 days/week (Table 6-13). Overall, there is little epidemiologic evidence for an association and no toxicological evidence of effects of NO_2 exposure on sperm or semen quality.

6.4.2.2 Effects on Reproduction

Several recent studies have examined the association between exposure to air pollutants during pregnancy and the ability to reproduce. Gametes (i.e., ova and sperm) may be even more at risk, especially outside of the human body, as occurs with assisted reproduction. Smokers require twice the number of in vitro fertilization (IVF) attempts to conceive as nonsmokers (Feichtinger et al., 1997), suggesting that a preconception exposure can be harmful to pregnancy. A recent study estimated daily concentrations of criteria pollutants at addresses of women undergoing their first IVF cycle and at their IVF labs from 2000 to 2007 in the northeastern U.S. (Legro et al., 2010). Increasing NO_2 concentration at the patient's address during ovulation induction (short-term exposure, ~12 days) was associated with a decreased chance of live birth (OR: 0.80 [95% CI: 0.71,

0.91] per 10-ppb increase). Similar risks were observed when the exposure period was the daily concentration averaged over the days from oocyte retrieval through embryo transfer, and the days from embryo transfer through the pregnancy test (14 days). The authors also observed a decreased odds of live birth when exposed from embryo transfer to live birth [long-term exposure, ~200 days; OR: 0.76 [95% CI: 0.56, 1.02] per 10-ppb increase). After adjusting for O_3 in a copollutant model, NO_2 continued to be associated with IVF failure. The results of this study suggest that both short- and long-term exposure to NO_2 during ovulation and gestation was detrimental and reduced the likelihood of a live birth. In a more general population, increased NO_2 exposure in the 30 days before initiation of unprotected intercourse also was associated with reduced fecundability [fecundability ratio per 10 ppb: 0.50 [95% CI: 0.32, 0.76]) (Slama et al., 2013)]. Similarly, in a cross-sectional study of fertility rates, Nieuwenhuijsen et al. (2014) observed decreased fertility in areas with higher NO_2 and NO_X concentrations.

In contrast, NO_2 exposure has not been shown to induce such effects in animals. Breeding studies by Shalamberidze and Tsereteli (1971b) and Shalamberidze and Tsereteli (1971a) with exposures of animals to 67 or 1,300 ppb NO_2 12 h/day for 3 months found that long-term NO_2 exposure had no effect on fertility; NO_2 exposure produced no change in the number of dams that became pregnant after mating with an unexposed male. At the higher dose, Shalamberidze and Tsereteli (1971b) and Shalamberidze and Tsereteli (1971a) did see impaired estrous cyclicity (cycle prolongation, increased duration of diestrus, decreased number of normal and total estrus cycles), and the exposed females had a decreased number of ovarian primordial follicles.

6.4.2.3 Effects on Pregnancy

Epidemiologic Evidence

Evidence suggests that exposure to air pollutants may affect maternal and fetal health during pregnancy. One such health effect, systemic inflammation, has been proposed as a potential biological mechanism through which air pollution could result in other adverse pregnancy outcomes (Slama et al., 2008; Kannan et al., 2006). Recent studies have investigated the relationship between CRP, a marker for systemic inflammation, measured in maternal blood during early pregnancy and in umbilical cord blood (as a measure of fetal health) and the association with NO_2 concentrations. van den Hooven et al. (2012a) observed generally null associations between exposure to NO_2 and elevated maternal CRP levels but did observe a positive, linear relationship between quartiles of NO_2 exposure and elevated fetal CRP levels. This association was evident when exposure was measured 1, 2, and 4 weeks prior to delivery but was strongest when exposure to

NO_2 was measured over the entire pregnancy. Similarly, Lee et al. (2011a) observed generally null associations between short-term exposure (i.e., 1 to 29 days) to NO_2 and elevated maternal CRP levels.

Pregnancy-associated hypertension is a leading cause of perinatal and maternal mortality and morbidity. A large body of research has linked changes in blood pressure to ambient air pollution; however, evidence is inconsistent for NO_2 (see Sections 5.3.5 and 6.3.5). A few recent studies have examined whether increases in NO_2 concentrations are associated with blood pressure changes in women who are pregnant. The results of these studies were not consistent. Hampel et al. (2011) observed that increases in NO_2 were associated with decreases in systolic blood pressure but found no clear associations between NO_2 concentrations and diastolic blood pressure. Lee et al. (2012a) observed associations between exposure to NO_2 and changes in blood pressure that were null for the entire population and when the population was restricted to nonsmokers. van den Hooven et al. (2011) observed small increases in systolic blood pressure associated with increases in NO_2 concentrations across all three trimesters of pregnancy but did not observe a similar association with diastolic blood pressure. Mobasher et al. (2013) observed a positive association between exposure to NO_2 during the first trimester and hypertensive disorders of pregnancy, though the association was imprecise and was reduced when exposure was averaged over the second and third trimesters. The same pattern was observed when analyses were restricted to nonobese women, but among obese women, the effect estimate was below 1.00 for each trimester. Xu et al. (2014) observed positive associations between NO_2 exposure during the entire pregnancy and first trimester and hypertensive disorders of pregnancy; associations remained positive after adjustment for O_3, CO, SO_2, or $PM_{2.5}$.

New-onset gestational hypertension can contribute to pre-eclampsia, a common pregnancy complication diagnosed after 20 weeks of pregnancy. Wu et al. (2009) observed a 45% increase (95% CI: 23%, 64%) in the risk of pre-eclampsia associated with a 20-ppb increase in NO_X measured over the entire pregnancy; when the exposure was examined categorically, the association between pre-eclampsia risk and NO_X concentration was consistent with a linear concentration-response relationship. Similarly, NO_2 concentrations during pregnancy were associated with an increased risk of pre-eclampsia among a cohort of Australian women (Pereira et al., 2013), with the strongest association observed when exposure was limited to the third trimester. Malmqvist et al. (2013) also observed a positive association between NO_X concentrations in the third trimester of pregnancy and pre-eclampsia consistent with a linear concentration-response relationship in a Swedish cohort. Dadvand et al. (2013) observed increases in odds of pre-eclampsia, particularly late-onset pre-eclampsia, with increased NO_2 exposure during the third and first trimesters, and with entire pregnancy exposures.

A number of other studies, of similar study quality and using similar study designs, did not observe positive associations for NO_2 exposure and risks of pregnancy-induced hypertension or pre-eclampsia across different exposure periods including exposure over entire pregnancy (Nahidi et al., 2014; van den Hooven et al., 2011) and the first trimester exposure (Olsson et al., 2013)]. Exposure to NO_X (Malmqvist et al., 2013; Wu et al., 2009) or NO_2 (Dadvand et al., 2013; Pereira et al., 2013) was estimated at each subject's residential address using LUR or dispersion models. Details on these exposure assessment techniques can be found in Sections 3.2.1.1 and 3.2.3, respectively. Information in Section 3.4.5.2 aids interpreting these methods with regard to potential exposure measurement error. A meta-analysis of pre-eclampsia studies reported a combined OR for NO_2 of 1.23 (95% CI: 1.04, 1.42), though there was a large amount of heterogeneity between studies particularly in outcome definition; removal of an influential study produced an OR of 1.11 (95% CI: 1.04, 1.17) with no observed heterogeneity (Pedersen et al., 2014).

Other pregnancy complications that have recently been evaluated and found to be associated with NO_2 include gestational diabetes (Malmqvist et al., 2013) and markers of placental growth and function (van den Hooven et al., 2012c). Overall, the evidence for the effects of NO_2 on pregnancy effects is inconsistent. Key studies examining the association between exposure to NO_2 and pregnancy-related effects can be found in Table 6-12. A supplemental Table S6-2 (U.S. EPA, 2013b) provides an overview of all of the epidemiologic studies of pregnancy-related health effects.

Table 6-12 Key reproductive and developmental epidemiologic studies for nitrogen dioxide (NO_2).

Study	Location Sample Size	Mean NO_2[a] or NO_X (ppb)	Exposure Assessment	Selected Effect Estimates[b] (95% CI)
Fertility, Reproduction, and Pregnancy				
Pereira et al. (2013)	Perth, Australia N = 23,452	NR	LUR model	Pre-eclampsia T1: 1.04 (0.94, 1.16) T2: 1.02 (0.91, 1.15) T3: 1.17 (1.04, 1.32) Entire pregnancy: 1.22 (1.02, 1.49)
Wu et al. (2009)	Southern California N = 81,186	NO_X: Entire pregnancy: 7.23 T1: 7.45 T2: 7.29 T3: 7.14	NO_X from dispersion model.	Pre-eclampsia Entire pregnancy: 1.44 (1.23, 1.68)
Malmqvist et al. (2013)	Sweden N = 81,110	NO_X: 7.5	NO_X from dispersion model with a spatial resolution of 500 × 500 m.	Pre-eclampsia Third trimester Q1: (reference) Q2: 1.28 (1.13, 1.46) Q3: 1.33 (1.17, 1.52) Q4: 1.51 (1.32, 1.73) Gestational diabetes Third trimester Q1: (reference) Q2: 1.19 (0.99, 1.44) Q3: 1.52 (1.28, 1.82) Q4: 1.69 (1.41, 2.03)
Dadvand et al. (2013)	Barcelona, Spain N = 8,398	T1: 30 T2: 31 T3: 31 EP: 30	LUR model	Pre-eclampsia T1: 1.07 (0.94, 1.22) T2: 1.03 (0.90, 1.19) T3: 1.11 (0.99, 1.23) EP: 1.09 (0.94, 1.27)

Table 6-12 (Continued): Key reproductive and developmental epidemiologic studies for nitrogen dioxide (NO_2).

Study	Location Sample Size	Mean NO_2[a] or NO_X (ppb)	Exposure Assessment	Selected Effect Estimates[b] (95% CI)
Legro et al. (2010)	Northeastern U.S. N = 7,403	19	Spatially interpolated concentrations from kriging based on monitoring data.	Odds of live birth following IVF Medication start to oocyte retrieval: 0.80 (0.71, 0.91) Oocyte retrieval to embryo transfer: 0.87 (0.79, 0.96) Embryo transfer to pregnancy test (14 days): 0.76 (0.66, 0.86) Embryo transfer to live birth: 0.76 (0.56, 1.02)
Slama et al. (2013)	Teplice, Czech Republic N = 1,916	19	Central site monitor (within 12 km) of residence.	Fecundity ratio Last 30 days before unprotected intercourse (Lag 1): 0.50 (0.32, 0.76) Last 30 days before Lag 1 (Lag 2): 1.10 (0.69, 1.80) Lag 1 + Lag 2: 0.52 (0.28, 0.94) Mo post-outcome: 1.17 (0.76, 1.85)
Birth Outcomes				
Aguilera et al. (2010)	Catalonia, Spain N = 562	16.9−17.2	LUR	Fetal length (% change) T1: −2.04 (−7.01, 2.95) T2: −1.69 (−7.05, 3.69) T3: 0.33 (−4.06, 4.72) Head circumference (% change) T1: 0.25 (−5.42, 5.91) T2: 1.70 (−3.69, 7.07) T3: 0.23 (−4.32, 4.77) Abdominal circumference (% change) T1: −2.82 (−8.24, 2.59) T2: −0.13 (−5.64, 5.38) T3: 0.74 (−3.926, 5.40) Biparietal diameter (% change) T1: 3.87 (−2.04, 9.75) T2: 4.90 (−0.34, 10.11) T3: 1.48 (−3.41, 6.35) Estimated fetal weight (% change) T1: −2.22 (−7.39, 2.98) T2: 0.46 (−5.82, 6.72) T3: 0.91 (−3.65, 5.45)

Table 6-12 (Continued): Key reproductive and developmental epidemiologic studies for nitrogen dioxide (NO_2).

Study	Location Sample Size	Mean NO_2[a] or NO_X (ppb)	Exposure Assessment	Selected Effect Estimates[b] (95% CI)
Ballester et al. (2010)	Valencia, Spain N = 785	19.1−20.2	LUR	Head circumference (cm) Entire pregnancy: −0.11 (−0.25, 0.03) Birth length (cm) Entire pregnancy: −0.09 (−0.27, 0.10) SGA—weight Entire pregnancy: 1.59 (0.89, 2.84) SGA—length Entire pregnancy: 1.48 (0.628, 3.49)
Estarlich et al. (2011)	Asturias, Gipuzkoa, Sabadell, Valencia, Spain N = 2,337	Overall: 15.5 Urban: 15.9 Rural: 8.7	LUR	Birth length (cm) Entire pregnancy: −1.69 (−0.34, −0.02) Head circumference (cm) Entire pregnancy: −0.01 (0.13, 0.11)
Hansen et al. (2007)	Brisbane, Australia N = 26,617	Median: 7.8 75th: 11.4 Max: 24.2	Central sites, city-wide avg.	Head circumference (cm) T1: 0.05 (−0.05, 0.17) T2: 0.08 (−0.02, 0.19) T3: 0.00 (−0.10, 0.10) Crown-heel length (cm) T1: 0.24 (0.05, 0.42) T2: 0.07 (−0.10, 0.24) T3: −0.15 (−0.25, −0.05)
Hansen et al. (2008)	Brisbane, Australia N = 15,623	9.8	Closest central site monitor (within 2−14 km of 1 of 17 monitors).	Head circumference (mm) M1: 0.54 (−1.88, 2.94) M2: −0.16 (−2.54, 2.20) M3: −0.60 (−3.18, 2.00) M4: −0.30 (−2.30, 1.68) Biparietal diameter (mm) M1: 0.14 (−0.62, 0.88) M2: −0.20 (−0.88, 0.50) M3: −0.12 (−0.82, 0.58) M4: −0.16 (−0.74, 0.42) Abdominal circumference (mm) M1: 0.48 (−1.98, 2.94) M2: 0.98 (−1.40, 3.34) M3: 0.20 (−2.12, 2.52) M4: 0.30 (−1.80, 2.40) Femur length (mm) M1: 0.06 (−0.50, 0.62) M2: −0.18 (−0.78, 0.44) M3: 0.02 (−0.52, 0.56) M4: −0.26 (−0.80, 0.26)

Table 6-12 (Continued): Key reproductive and developmental epidemiologic studies for nitrogen dioxide (NO_2).

Study	Location Sample Size	Mean NO_2[a] or NO_X (ppb)	Exposure Assessment	Selected Effect Estimates[b] (95% CI)
Iñiguez et al. (2012)	Valencia, Spain N = 818	Median: 20.2	LUR model	Fetal length (% change) T1: 0.97 (0.92, 1.02) T2: 0.96 (0.92, 1.00) T3: 0.97 (0.92, 1.02) Abdominal circumference (% change) T1: 0.96 (0.92, 0.99) T2: 0.98 (0.94, 1.02) T3: 0.98 (0.94, 1.03) Biparietal diameter (% change) T1: 0.96 (0.92, 1.00) T2: 0.97 (0.92, 1.01) T3: 0.98 (94, 1.02) Estimated fetal weight (% change) T1: 0.96 (0.92, 1.00) T2: 0.98 (0.94, 1.02) T3: 0.97 (0.93, 1.02)
van den Hooven et al. (2012b)	Rotterdam, Netherlands N = 7,772	Mean: 21.2 Median: 21.1 75th: 22.4 Max: 30.3	Combination of continuous monitoring data and GIS-based dispersion modeling techniques.	Head circumference (mm) T3: Q1: (reference) Q2: −0.40 (−1.00, 0.20) Q3: −0.81 (−1.42, −0.20) Q3: −1.28 (−1.96, −0.61) Length (mm) T3: Q1: Ref Q2: −0.02 (−0.17, 0.13) Q3: −0.09 (−0.24, 0.06) Q4: −0.33 (−0.50, −0.16) SGA Entire pregnancy: Q1: ref Q2: 0.93 (0.66, 1.31) Q3: 1.25 (0.90, 1.73) Q4: 1.35 (0.94, 1.94)
Ritz et al. (2014)	Los Angeles, CA LUR: n = 501; Monitors: n = 98	22.7−39.3	LUR model	Biparietal diameter (mm) GW 0−19: −0.41 (−1.07, 0.23) GW 19−29: 0.39 (−0.25, 1.02) GW 29−37: −0.50 (−1.23, 0.23)
			Central site monitors combined by IDW.	GW 0−19: −4.45 (−10.55, 1.55) GW 19−29: 4.92 (0.03, 9.83) GW 29−37: −8.33 (−13.83, −2.83)

Table 6-12 (Continued): Key reproductive and developmental epidemiologic studies for nitrogen dioxide (NO_2).

Study	Location Sample Size	Mean NO_2[a] or NO_X (ppb)	Exposure Assessment	Selected Effect Estimates[b] (95% CI)
Bell et al. (2007)	Connecticut and Massachusetts N = 358,504	17.4	County-level average of central site monitors.	Birth weight (g) Entire Pregnancy: −18.54 (−22.50, −14.58) Black mothers: −26.46 (−37.50, −15.63) White mothers: −17.29 (−21.67 −13.13) LBW: 1.06 (1.00, 1.11)
Darrow et al. (2011)	Atlanta, GA N = 406,627	23.6	Population-weighted spatial average.	Birth weight (g) Entire pregnancy: −18.40 (−28.00, −9.00) First 28 days: 0.8 (−3.60, 5.20) T3: −9.00 (−17.00, −1.20) Non-Hispanic white: −9.20 (−18.60, 0.20) Non-Hispanic black: −7.8 (−17.40, 1.60) Hispanic: −11.60 (−24.80, 1.40)
Postnatal Development				
Guxens et al. (2014)	Ruhr, Germany	Medians NR	LUR—home Prenatal exposure	Change in cognitive or motor function score Mental development: −3.61 (−8.53, 1.32) Motor function: −5.04 (−11, 0.49)
	Heraklion, Greece	6.1		Mental development: 1.90 (−2.33, 6.13) Motor function: −0.83 (−5.39, 3.74)
	Asturias, Spain	NR		Mental development: −1.39 (−3.12, 0.34) Motor function: −2.03 (−3.82, −0.24)
	Gipuzkoa, Spain	NR		Mental development: −1.11 (−6.65, 4.44) Motor function: 0.17 (−1.73, 5.34)
	Valencia, Spain	NR		General cognition: −1.35 (−3.74, 1.03) Motor function: −3.72 (−6.37, −1.07)
	Sabadell, Spain	23.4		General cognition: −0.15 (−2.42, 2.12) Motor function: 0.71 (−1.71, −3.14)
	Granada, Spain	NR		General cognition: 3.18 (−0.26, 6.62) Motor function: 1.80 (−1.73, 5.34)
	Rotterdam, Netherlands	NR		Motor function: −0.17 (−1.60, 1.26)
	Poitiers, France	NR		Motor function: −0.64 (−6.75, 5.47)
	Nancy, France	NR		Motor function: −2.84 (−5.64, −0.04)
	Rome, Italy	NR		Motor function: −1.97 (−4.44, 0.49)

Table 6-12 (Continued): Key reproductive and developmental epidemiologic studies for nitrogen dioxide (NO_2).

Study	Location Sample Size	Mean NO_2[a] or NO_X (ppb)	Exposure Assessment	Selected Effect Estimates[b] (95% CI)
Guxens et al. (2014) (continued)	Cohorts combined	NR		Motor function All subjects: −1.28 (−2.35, −0.21) Birth dates temporally matched with NO_2 monitoring: −0.79 (−1.84, 0.26)
Freire et al. (2010)	Spain N = 210	11.1	LUR—home Concurrent exposure	Change in score in group with NO_2 >13.2 ppb compared with group with NO_2 <8.2 ppb General cognitive index: −4.19 (−14.02, 5.64) Verbal: −3.09 (−13.31, 7.13) Quantitative: −6.71 (−17.91, 4.49) Memory: −5.52 (−16.18, 5.13) Executive function: −4.93 (−14.90, 5.05) Gross motor function: −8.61 (−18.96, 1.74) Fine motor skills: 0.91 (−10.22, 12.05)
van Kempen et al. (2012)	the Netherlands N = 485	School: 16.5 Home: 16.4	LUR—school Concurrent exposure	Change in score, adjusted for traffic noise Memory: −0.30 (−0.55, 0.04) Measures of attention SRTT, reaction time (ms): −2.23 (−22.1, 17.7) SAT block, # errors: −0.02 (−0.42, 0.38) SAT block, reaction time (ms): 13.9 (−16.7, 43.9) SAT switch, # errors: −1.19 (−3.62, 1.26) SAT switch, reaction time (ms): 21.5 (−45.2, 88.2) Locomotion: 0.08 (−0.08, 0.25)
			LUR—home Concurrent exposure	Change in score, adjusted for traffic noise Memory: 0.17 (−0.08, 0.42) Measures of attention SRTT, reaction time (ms): −2.11 (−21.0, 16.7) SAT block, # errors: −0.04 (−0.40, 0.32) SAT block, reaction time (ms): 15.9 (−11.3, 43.0) SAT switch, # errors: −1.23 (−3.32, 0.87) SAT switch, reaction time (ms): −20.2 (−74.9, 34.5) Locomotion: 0.06 (−0.08, 0.21)

Table 6-12 (Continued): Key reproductive and developmental epidemiologic studies for nitrogen dioxide (NO_2).

Study	Location Sample Size	Mean NO_2[a] or NO_X (ppb)	Exposure Assessment	Selected Effect Estimates[b] (95% CI)
Clark et al. (2012)	U.K. N = 719	22.7	LUR—school Concurrent exposure	Change in score, adjusted for traffic noise Reading comprehension: 0.08 (−0.17, 0.34) Information recall: 0.28 (−0.62, 1.17) Working memory: 0.06 (−5.55, 5.66) Physiological distress: 0.47 (−0.62, 1.56)
Guxens et al. (2012)	Spain N = 1,889	Overall: 15.4 Valencia: 19.6 Sabadell: 17.1 Asturias: 12.3 Gipuzkoa: 10.7	LUR Prenatal exposure	Change in mental development index[c] Location All regions: −0.95 (−3.90, 1.89) Gipuzkoa: −5.15 (−8.04, −2.27) Asturias: 0.17 (−2.71, 3.04) Sabadell: 1.98 (−1.69, 5.66) Valencia: −0.43 (−2.86, 2.01) Maternal fruit and vegetable intake ≤405 g/day: −4.13 (−7.06, −1.21) >405 g/day: 0.25 (−3.63, 4.12) Maternal Vitamin D circulation Low: −2.49 (−6.87, 1.89) Medium: −0.55 (−3.48, 2.39) High: −0.11 (−2.72, 2.49)
Volk et al. (2013)	California n = 524	NO_X: Q1: ≤9.7 Q2: 9.7−16.9 Q3: 16.9−31.8 Q4: ≥31.8	NO_X dispersion model (within 5 km of child's home).	Odds ratio for autism relative to Q1 First yr of life Q2: 0.91 (0.56, 1.47) Q3: 1.00 (0.62, 1.62) Q4: 3.10 (1.76, 5.57) Average prenatal Q2: 1.26 (0.77, 2.06) Q3: 1.09 (0.67, 1.79) Q4: 1.98 (1.20, 3.31)
			NO_2 from central sites within 50 km of homes, combined by IDW.	Odds ratio for autism First yr of life: 1.67 (1.25, 2.23) Average prenatal: 1.52 (1.16, 2.00)

Table 6-12 (Continued): Key reproductive and developmental epidemiologic studies for nitrogen dioxide (NO_2).

Study	Location Sample Size	Mean NO_2[a] or NO_X (ppb)	Exposure Assessment	Selected Effect Estimates[b] (95% CI)
Becerra et al. (2013)	California n = 83,385	30.8	LUR—home	Odds ratio for autism Average prenatal: 1.05 (0.98, 1.12) T1: 1.03 (0.98, 1.08) T2: 1.03 (0.98, 1.08) T3: 1.04 (0.98, 1.09)
			Nearest central site monitor to birth residence.	Average prenatal: 1.04 (0.98, 1.10) T1: 1.04 (0.99, 1.08) T2: 1.01 (0.97, 1.06) T3: 1.02 (0.97, 1.07)

CI = confidence interval; EP = entire pregnancy; GIS = geographic information system; GW = gestational week; IDW = inverse distance weighting; IVF = in vitro fertilization; LBW = low birth weight; LUR = land-use regression; M1 = Month 1; M2 = Month 2; M3 = Month 3; M4 = Month 4; NO_2 = nitrogen dioxide; NO_X = sum of NO and NO_2; NR = No quantitative results reported; Q1 = first quartile; Q2 = second quartile; Q3 = third quartile; Q4 = fourth quartile; SAT = Switching Attention Test ; SGA = small for gestational age; SRTT = Simple Reaction Time Test; T1 = first trimester; T2 = second trimester; T3 = third trimester.

[a]NO_2 unless otherwise specified.

[b]Relative risk per 10-ppb change in NO_2, or 20-ppb change in NO_X, unless otherwise noted.

[c]Per doubling in NO_2 concentration estimated using IDW.

Toxicological Evidence

Evidence from animal toxicological studies suggests that exposure to NO_2 may affect pregnancy. Maternal toxicity or deficits in maternal weight gain during gestation was reported in pregnant rats with inhalation exposure to 5,300 ppb NO_2 for 6 h/day 7 days/week throughout gestation [21 days; (Tabacova et al., 1984)]. Another study reported dam weight gain over pregnancy as a percentage of body weight at conception or GD0, which is an unusual metric, and found no differences in maternal weight gain with 1,500 or 3,000 ppb NO_2 exposure over the duration of pregnancy (Di Giovanni et al., 1994).

Fetal lethality in toxicological studies is measured by counting pup loss or resorption sites. This directly affects litter size, or number of live pups born. Shalamberidze and Tsereteli (1971b) and Shalamberidze and Tsereteli (1971a) reported decreased litter sizes (fewer pups born) to dams that received 1,300 ppb NO_2 12 h/day for 3 months during pregnancy. Litter size was not affected in dams exposed to 1,500 or 3,000 ppb NO_2 exposure over the duration of pregnancy (Di Giovanni et al., 1994).

6.4.3 Birth Outcomes

6.4.3.1 Fetal Growth

Fetal growth is influenced by maternal, placental, and fetal factors. The biological mechanisms by which air pollutants may influence the developing fetus remain largely unknown. LBW has often been used as an outcome measure because it is easily available and accurately recorded on birth certificates. However, LBW may result from either short gestation or inadequate growth in utero. Most of the studies investigating air pollution exposure and LBW limited their analyses to term infants to focus on inadequate growth. A number of studies were identified that specifically addressed growth restriction in utero by identifying infants who failed to meet specific growth standards. Usually, these infants had birth weight less than the 10th percentile for gestational age, using an external standard.

A limitation of environmental studies that use birth weight as a proxy measure of fetal growth is that patterns of fetal growth during pregnancy cannot be assessed. This is particularly important when investigating pollutant exposures during early pregnancy as birth weight is recorded many months after the exposure period. The insult of air pollution may have a transient effect on fetal growth, where growth is hindered at one point in time but catches up at a later point. For example, maternal smoking during pregnancy can alter the growth rate of individual body segments of the fetus at variable developmental stages, as the fetus experiences selective growth restriction and augmentation (Lampl and Jeanty, 2003).

The terms SGA, which is defined as a birth weight <10th percentile for gestational age (and often sex and/or race), and IUGR are often used interchangeably. However, this definition of SGA does have limitations. For example, using it for IUGR may overestimate the percentage of "growth-restricted" neonates as it is unlikely that 10% of neonates have growth restriction (Wollmann, 1998). On the other hand, when the 10th percentile is based on the distribution of live births at a population level, the percentage of SGA among PTB is most likely underestimated (Hutcheon and Platt, 2008). Nevertheless, SGA represents a statistical description of a small neonate, whereas the term IUGR is reserved for those with clinical evidence of abnormal growth. Thus, all IUGR neonates will be SGA, but not all SGA neonates with be IUGR (Wollmann, 1998). In the following section, the terms SGA and IUGR are referred to as each cited study used the terms.

The 2008 ISA for Oxides of Nitrogen reviewed three studies that evaluated the relationship between exposure to NO_2 and fetal growth (Mannes et al., 2005; Salam et al.,

2005; Liu et al., 2003) and concluded that they "did not consistently report associations between NO_2 exposure and intrauterine growth retardation" [(U.S. EPA, 2008); p. 3−73].

In recent years, a number of studies have examined various metrics of fetal growth restriction. Several of these more recent studies have used anthropometric measurements (e.g., head circumference, abdominal circumference) measured via ultrasound at different periods of pregnancy in order to evaluate patterns of fetal growth during pregnancy and to detect growth restriction that may occur early in pregnancy, but which may no longer be detectable at birth. In a mother and child cohort study conducted in Spain, ultrasound measurements were recorded at 12, 20, and 32 weeks of gestation, and these anthropometric measurements were recorded again at birth (Iñiguez et al., 2012; Aguilera et al., 2010). Aguilera et al. (2010) observed that exposure to NO_2 early in pregnancy was associated with impaired growth in head circumference from Weeks 12 to 20 of gestation and abdominal circumference and estimated fetal weight from Weeks 20 to 32. Similarly, Iñiguez et al. (2012) reported decreased fetal length and decreased biparietal diameter measured by ultrasound in association with exposure to NO_2 during Weeks 12−20 of gestation. Decreased birth length and head circumference measured at birth were also associated with exposure to NO_2 during this same period. Examining fetal growth characteristics assessed by ultrasound during each trimester of pregnancy, van den Hooven et al. (2012b) observed decreases in head circumference and fetal length in the second and third trimesters associated with exposure to NO_2. Hansen et al. (2008) used ultrasound measurements during Weeks 13−26 of pregnancy and did not observe associations between exposure to relatively low concentrations of NO_2 (mean = 9.8 ppb) and head circumference, biparietal diameter, abdominal circumference, or fetal length. Ritz et al. (2014) used multiple ultrasound measures to examine fetal growth parameters across gestation and observed that higher exposure to NO_2 during Gestational Weeks 29−37 was associated with decrements in biparietal diameter at 37 weeks; no consistent associations were found for head circumference, femur length, or abdominal circumference.

Several studies made use of anthropometric measurements made immediately after birth to evaluate fetal growth. Estarlich et al. (2011), Ballester et al. (2010), and Hansen et al. (2007) observed decreases in body length associated with exposure to NO_2. This association persisted when NO_2 exposure was estimated for each trimester of pregnancy in the study by Estarlich et al. (2011). Ballester et al. (2010) observed the strongest association with NO_2 exposure during the first trimester, while Hansen et al. (2007) reported that the association was strongest for NO_2 exposure measured at the end of the pregnancy.

When using SGA as an indicator of fetal growth restriction, several studies observed associations with exposure to NO_2, NO_X, or NO (Sathyanarayana et al., 2013; Le et al., 2012; Pereira et al., 2012; Malmqvist et al., 2011; Ballester et al., 2010; Rich et al., 2009; Brauer et al., 2008; Mannes et al., 2005). These associations were most often observed for exposure to NO_2 during the second trimester (Pereira et al., 2012; Ballester et al., 2010; Rich et al., 2009; Mannes et al., 2005). Gehring et al. (2011a), Hansen et al. (2007), Olsson et al. (2013), Kashima et al. (2011), and Hannam et al. (2014) did not observe an increased risk of SGA associated with exposure to NO_2. All of the studies that used IUGR as an indicator of fetal growth restriction observed an association with exposure to NO_2, and this association was strongest for exposures at the beginning of pregnancy [i.e., first month or first trimester; (Liu et al., 2007; Salam et al., 2005; Liu et al., 2003)]. Generally, studies of fetal growth restriction did not examine the potential for confounding by traffic-related copollutants.

When evaluating the association between fetal growth and exposure to NO_2, many studies relied on modeled concentrations of NO_2 at maternal residence coming from LUR models (Iñiguez et al., 2012; Pereira et al., 2012; Estarlich et al., 2011; Gehring et al., 2011a; Aguilera et al., 2010; Ballester et al., 2010; Brauer et al., 2008) and emissions or dispersion models (van den Hooven et al., 2012b; Malmqvist et al., 2011). Generally, the results of studies that relied on estimates of NO_2 from LUR models were not substantially different from those that estimated exposure to NO_2 using concentrations measured at central site monitors. However, in a study that assigned exposure to NO_2 using both a LUR model and IDW of measured NO_2 concentration from monitors, Brauer et al. (2008) found higher risks for SGA using the monitoring data (OR: 1.28 [95% CI: 1.18, 1.36]) compared to the risks observed with the NO_2 estimates from the LUR model (OR 0.94 [95% CI: 0.80, 1.10]). Given the differences among the study designs, it cannot be concluded that the inconsistencies are related to exposure assessment method or length of follow-up periods. In general, studies using central site monitors for exposure estimates carry uncertainty in long-term NO_2 exposure studies because the exposure error resulting from spatial misalignment between subjects' and monitor locations can overestimate or underestimate associations with health effects (Section 3.4.5.2).

Several studies were able to incorporate data on activity patterns in order to help reduce uncertainty related to exposure assessment. Some analyses attempted to decrease the potential exposure measurement error associated with exposure to ambient NO_2 by limiting inclusion to subjects that spent 15 or more hours per day at home or subjects that spent less than 2 hours a day in an outdoor environment other than at their primary residence. In such analyses, Aguilera et al. (2010) and Estarlich et al. (2011) found stronger associations between measures of decreased fetal growth and exposure to NO_2. In contrast, when Gehring et al. (2011a) limited their analyses to participants that did not

move during pregnancy or did not have paid employment outside of the home, there were no consistent associations between SGA and exposure to NO_2.

In summary, there is generally consistent evidence for an association between exposure to NO_2 and fetal growth restriction, including recent evidence from studies that have used fetal anthropometric measurements made via ultrasound and anthropometric measurements made immediately after birth. These are consistent with the studies of the clinical measurement of IUGR and the statistical definition of SGA. The evidence is less certain when it comes to assessing the time period of pregnancy when exposure to NO_2 is associated with the highest risks. Some studies find the highest risks associated with NO_2 when NO_2 is measured in early pregnancy, while in other studies, the time period associated with the greatest risk is toward the end of pregnancy. Others find the greatest risk when exposure is assigned for the entire pregnancy period. Key studies examining the association between exposure to NO_2 and fetal growth effects can be found in Table 6-12. A supplemental Table S6-3 (U.S. EPA, 2013c) provides an overview of all of the epidemiologic studies of fetal growth effects.

6.4.3.2 Preterm Birth

PTB is a syndrome (Romero et al., 2006) that is characterized by multiple etiologies. It is, therefore, unusual to be able to identify an exact cause for each PTB. In addition, PTB is not an adverse outcome in itself but an important determinant of health status (i.e., neonatal morbidity and mortality). Although some overlap exists for common risk factors, different etiologic entities related to distinct risk factor profiles and leading to different neonatal and post-neonatal complications are attributed to PTB and measures of fetal growth. Although both restricted fetal growth and PTB can result in LBW, prematurity does not have to result in LBW or growth restricted babies.

A major issue in studying environmental exposures and PTB is selecting the relevant exposure period because the biological mechanisms leading to PTB and the critical periods of vulnerability are poorly understood (Bobak, 2000). Short-term exposures proximate to birth may be most relevant if exposure causes an acute effect. However, exposure occurring in early gestation might affect placentation, with results observable later in pregnancy, or cumulative exposure during pregnancy may be the most important determinant. The studies reviewed have dealt with this issue in different ways. Many have considered several exposure metrics based on different periods of exposure. Often the time periods used are the first month (or first trimester) of pregnancy and the last month (or 6 weeks) prior to delivery. Using a time interval prior to delivery introduces an additional problem because cases and controls are not in the same stage of

development when they are compared. For example, a preterm infant delivered at 36 weeks is a 32-week fetus 4 weeks prior to birth, while an infant born at term (40 weeks) is a 36-week fetus 4 weeks prior to birth.

Recently, investigators have examined the association of PTB with both short-term (i.e., hours, days, or weeks) and long-term (i.e., months or years) exposure periods. Time-series studies have been used to examine the association between air pollution concentrations during the days immediately preceding birth. An advantage of these time-series studies is that this approach can remove the influence of covariates that vary across individuals over a short period of time. Retrospective cohort and case-control studies have been used to examine long-term exposure periods, often averaging air pollution concentrations over months or trimesters of pregnancy.

Studies of PTB fail to show consistency in the periods during pregnancy when pollutants are associated with an effect. For example, while some studies find the strongest effects associated with exposures early in pregnancy, others report effects when the exposure is limited to the second or third trimester. However, the effect of air pollutant exposure during pregnancy on PTB has a biological basis. There is an expanding list of possible mechanisms that may explain the association between NO_2 exposure and PTB.

Many studies of PTB compare exposure in quartiles, using the lowest quartile as the reference (or control) group. No studies use a truly unexposed control group. If exposure in the lowest quartile confers risk, then it may be difficult to demonstrate additional risk associated with a higher quartile. Thus, negative studies must be interpreted with caution.

Preterm birth occurs both naturally (idiopathic PTB), and as a result of medical intervention (iatrogenic PTB). Ritz et al. (2000) excluded all births by Cesarean section to limit their studies to idiopathic PTB. No other studies attempted to distinguish the type of PTB, although air pollution exposure maybe associated with only one type. This is a source of potential effect misclassification. One study examined preterm premature rupture of membranes, observing positive ORs with NO_2 exposure (Dadvand et al., 2014a).

A number of recent studies have evaluated the association between exposure to NO_2 and PTB, and the results have generally been inconsistent. The body of literature that has observed an association between NO_2 and PTB (Gehring et al., 2014; Trasande et al., 2013; Le et al., 2012; Olsson et al., 2012; Wu et al., 2011; Llop et al., 2010; Darrow et al., 2009; Wu et al., 2009; Jiang et al., 2007; Leem et al., 2006; Maroziene and Grazuleviciene, 2002; Bobak, 2000) is generally the same (in both the quantity and quality of studies) to those that find no consistent pattern in the association between NO_2 and PTB (Hannam et al., 2014; Olsson et al., 2013; Gehring et al., 2011a; Gehring et al.,

2011b; Kashima et al., 2011; Basu et al., 2010; Brauer et al., 2008; Jalaludin et al., 2007; Ritz et al., 2007; Hansen et al., 2006; Liu et al., 2003; Ritz et al., 2000). Among the studies that observe an association between exposure to NO_2 and PTB, the association seems to be strongest for exposure to NO_2 late in pregnancy, including the third trimester (Llop et al., 2010; Leem et al., 2006; Bobak, 2000), the last 8 weeks of pregnancy (Jiang et al., 2007), the last 6 weeks of pregnancy (Darrow et al., 2009), month of birth (Trasande et al., 2013), or the last week of pregnancy (Olsson et al., 2012).

Several studies examined very preterm birth (VPTB, <30 weeks gestation), and observed positive associations with NO_2 for VPTB when none were observed for PTB (Brauer et al., 2008), or observed stronger associations for VPTB compared to those for PTB (Wu et al., 2011; Wu et al., 2009).

When evaluating the association between PTB and exposure to NO_2 at maternal residence, several studies relied on estimates of NO_2 concentrations coming from LUR models (Gehring et al., 2014; Gehring et al., 2011a; Gehring et al., 2011b; Kashima et al., 2011; Wu et al., 2011; Llop et al., 2010; Brauer et al., 2008) and dispersion models (Wu et al., 2011; Wu et al., 2009). Generally, the results of studies that relied on modeled estimates of NO_2 were similarly inconsistent, and not substantially different from those that used measured NO_2 concentrations. In a study that assigned exposure to NO_2 using both a LUR model and IDW of measured NO_2 concentration from monitors, Brauer et al. (2008) found generally comparable risk estimates per 10 ppb for VPTB using the monitoring data (OR: 1.24, [95% CI: 0.80, 1.88]) and NO_2 estimates from the LUR model (OR: 1.16 [95% CI: 0.93, 1.61]). Given the differences among the study designs, it cannot be concluded that the inconsistencies are related to exposure assessment method or length of follow-up periods. In general, studies using central site monitors for exposure estimates carry uncertainty in long-term NO_2 exposure studies because the exposure error resulting from spatial misalignment between subjects' and monitor locations can overestimate or underestimate associations with health effects (Section 3.4.5.2).

In summary, the evidence is generally inconsistent, with some studies observing associations between NO_2, using both estimates from LUR models and concentrations measured at central site monitors to estimate exposure, and PTB. These studies are characterized in supplemental Table S6-4 (U.S. EPA, 2013d).

6.4.3.3 Birth Weight

With birth weight routinely collected in vital statistics and being a powerful predictor of infant mortality, it is the most studied outcome within air pollution-birth outcome

research. Air pollution researchers have analyzed birth weight as a continuous variable and/or as a dichotomized variable in the form of LBW [<2,500 g (5 lbs, 8 oz)].

Birth weight is primarily determined by gestational age and intrauterine growth but also depends on maternal, placental, and fetal factors as well as on environmental influences. In both developed and developing countries, LBW is the most important predictor for neonatal mortality and is an important determinant of post-neonatal mortality and morbidity. Studies report that infants who are smallest at birth have a higher incidence of diseases and disabilities, which continue into adulthood (Hack and Fanaroff, 1999).

A number of recent studies have evaluated the association between exposure to NO_2 and birth weight, and the results have generally been inconsistent. When examining birth weight as a continuous variable, several studies have observed decreases in birth weight associated with increases in NO_2 exposure (Gehring et al., 2014; Laurent et al., 2014; Savitz et al., 2014; Darrow et al., 2011; Estarlich et al., 2011; Ballester et al., 2010; Morello-Frosch et al., 2010; Bell et al., 2007). Generally, these studies observed the largest decreases in birth weight when exposure to NO_2 was averaged over the entire pregnancy. There were also a number of studies that examined birth weight as a continuous variable that found no consistent decreases in birth weight associated with increases in NO_2 exposure averaged over the entire pregnancy or specific trimesters of pregnancy (Hannam et al., 2014; Sellier et al., 2014; Pedersen et al., 2013; Geer et al., 2012; Rahmalia et al., 2012; Gehring et al., 2011a; Gehring et al., 2011b; Kashima et al., 2011; Lepeule et al., 2010; Aguilera et al., 2009; Hansen et al., 2007; Salam et al., 2005; Gouveia et al., 2004). With low birth weight examined as the risk of having a baby weighing less than 2,500 g, the study results remained inconsistent, with some study authors observing an association between LBW and exposure to NO_2 (Dadvand et al., 2014c; Ebisu and Bell, 2012; Ghosh et al., 2012a; Wilhelm et al., 2012; Morello-Frosch et al., 2010; Brauer et al., 2008; Bell et al., 2007; Lee et al., 2003), while others reported no consistent association (Pedersen et al., 2013; Kashima et al., 2011; Slama et al., 2007; Salam et al., 2005; Wilhelm and Ritz, 2005; Gouveia et al., 2004; Liu et al., 2003; Maroziene and Grazuleviciene, 2002; Bobak, 2000). One study observed decreases in effect estimates for both LBW and change in birth weight with increases in NO_2 exposure (Laurent et al., 2013). Generally, the studies that observed the largest risks for LBW averaged exposure to NO_2 over the entire pregnancy.

Several studies were able to incorporate data on activity patterns in order to help reduce uncertainty related to exposure assessment based on maternal residence. In analyses limited to subjects that spent 15 or more hours per day at home or subjects that spent less than 2 hours a day in an outdoor environment other than at their primary residence, Estarlich et al. (2011) found stronger associations between birth weight and exposure to

NO_2 at the maternal residence. These sensitivity analyses did not consistently change the associations observed by (Aguilera et al., 2009). When Gehring et al. (2011a) limited their analyses to participants that did not move during pregnancy, or did not have paid employment outside of the home, they continued to observe no consistent associations between birth weight and exposure to NO_2.

When evaluating the association between birth weight and exposure to NO_2, several studies relied on estimated residential concentrations of NO_2 coming from LUR models (Ghosh et al., 2012a; Wilhelm et al., 2012; Estarlich et al., 2011; Gehring et al., 2011a; Gehring et al., 2011b; Kashima et al., 2011; Ballester et al., 2010; Lepeule et al., 2010; Aguilera et al., 2009; Brauer et al., 2008; Slama et al., 2007) and dispersion models (Rahmalia et al., 2012; van den Hooven et al., 2012c; Madsen et al., 2010). Generally, the results of studies that relied on estimates of NO_2 from LUR models were similarly inconsistent, and not substantially different from those that used NO_2 concentrations measured at central site monitors. Several studies compared the use of statistical models and the use of routinely collected monitoring data to assign exposure to NO_2, and concluded that while the monitoring data may include larger errors in estimated exposure, these errors had little impact on the association between exposure to NO_2 and birth weight calculated using the two different methods for exposure assessment (Lepeule et al., 2010; Madsen et al., 2010). Details on deriving exposure estimates using LUR and dispersion models can be found in Sections 3.2.1.1 and 3.2.3, respectively. Given the differences among the study designs, it cannot be concluded that the inconsistencies are related to exposure assessment method or length of follow-up periods. In general, studies using central site monitors for exposure estimates carry uncertainty in long-term NO_2 exposure studies because the exposure error resulting from spatial misalignment between subjects' and monitor locations can overestimate or underestimate associations with health effects (Section 3.4.5.2). However, an association was observed with residential NO_2 exposure estimated for subjects' homes, and the improved spatial resolution of the exposure estimate lends more confidence in the association.

In animal toxicological studies by Shalamberidze and Tsereteli (1971b) and Shalamberidze and Tsereteli (1971a), albino rats with exposures to 67 or 1,300 ppb NO_2 12 h/day for 3 months prior to breeding produced pups with significantly decreased birth weights. These body-weight decrements continued to be significantly decreased at post-natal day (PND)4 and PND12.

In summary, the evidence is generally inconsistent, with some studies observing associations between NO_2 exposure and birth weight, while other studies observe no consistent pattern of association. Key studies examining the association between exposure to NO_2 and birth weight can be found in Table 6-12. A supplemental Table S6-5

(U.S. EPA, 2013e) provides an overview of all of the epidemiologic studies of birth weight.

6.4.3.4 Birth Defects

Despite the growing body of literature evaluating the association between ambient air pollution and various adverse birth outcomes, relatively few studies have investigated the effect of temporal variations in ambient air pollution on birth defects. Heart defects and oral clefts have been the focus of the majority of these recent studies, given their higher prevalence than other birth defects and associated mortality. Mechanistically, air pollutants could be involved in the etiology of birth defects via a number of key events.

A recent study investigated the association between NO or NO_2 and cardiac birth defects (Padula et al., 2013a) and other noncardiac birth defects (Padula et al., 2013b) in the San Joaquin Valley in California. The authors observed no associations between heart defects and NO or NO_2 but did observe an association between neural tube defects and both NO and NO_2. In a further analysis of noncardiac/nonneural tube defects, Padula et al. (2013c) observed no associations between NO or NO_2 and any of the defects studied. A nine-state cardiac birth defect case-control study observed associations between NO_2 and coarctation of the aorta, pulmonary valve stenosis, and left ventricular outflow tract obstructions (Stingone et al., 2014). A Barcelona, Spain-based case-control study of 18 congenital anomaly groups found coarctation of the aorta and digestive system defects associated with increases in NO_2 (Schembari et al., 2014). Two studies examining trisomy risk observed no correlations/associations with NO_2, and a correlation between NO and trisomy 21 (Chung et al., 2014; Jurewicz et al., 2014). In general, however, studies of birth defects have focused on cardiac defects, and the results from these studies are not entirely consistent. This inconsistency could be due to the absence of true associations between NO_2 and risks of cardiovascular malformations; it could also be due to differences in populations, pollution concentrations, outcome definitions, or analytical approaches. The lack of consistency of associations between NO_2 and cardiovascular malformations might be due to issues relating to statistical power or measurement error. A recent meta-analysis of air pollution and congenital anomalies observed elevated summary effects for NO_2 and coarctation of the aorta (OR: 1.17 (1.00, 1.36)), Tetralogy of Fallot (OR: 1.20 (1.02, 1.42)), and atrial septal defects (1.10 (0.91, 1.33)); ventral septal defects exhibited an elevated summary estimate but also high heterogeneity between studies (Vrijheid et al., 2011). Another meta-analysis found association only between coarctation of the aorta and NO_2 (Chen et al., 2014). These authors note that heterogeneity in the results of these studies may be due to inherent differences in study location, study design, and/or analytic methods, and comment that these studies have not

employed some recent advances in exposure assessment used in other areas of air pollution research that may help refine or reduce this heterogeneity. These studies are characterized in supplemental Table S6-6 (U.S. EPA, 2013f).

6.4.3.5 Early Life Mortality

An important question regarding the association between NO_2 and infant mortality is the critical window of exposure during development for which infants are at risk. Several age intervals have been explored: neonatal (<1 month); post-neonatal (1 month to 1 year); and an overall interval for infants that includes both the neonatal and post-neonatal periods (<1 year). During the neonatal and post-neonatal periods, the developing lung is highly sensitive to environmental toxicants. The lung is not well developed at birth, with 80% of alveoli being formed post-natally. The studies below reflect a variety of study designs, exposure periods, regions, and included relevant potential confounders. As discussed below, a handful of studies have examined the effect of ambient air pollution on neonatal and post-neonatal mortality, with the former the least studied. These studies varied somewhat with regard to the outcomes and exposure periods examined and study designs employed.

Overall, the evidence for an association between exposure to NO_2 and infant mortality is inconsistent. In an animal toxicological study, Tabacova et al. (1985) examined post-natal development of rodent pups from dams that were exposed to 50, 500, or 5,300 ppb NO_2 [6 h/day, 7 days/week, gestation day (GD)0−GD21]. Significantly decreased pup viability was seen at PND21 with 5,300 ppb NO_2. Another study in which male pups received prenatal exposure to NO_2 via a different daily exposure duration (dam continuous exposure to 1,500 or 3,000 ppb, GD0−GD20) showed no significant effects on pup post-natal mortality to PND21 (Di Giovanni et al., 1994). Recent epidemiologic studies have examined the association between long-term exposure to NO_2 measured at central site monitors and stillbirths, with one study (Faiz et al., 2012) observing an association and another (Hwang et al., 2011) observing associations near the null value. Faiz et al. (2013) observed positive ORs for stillbirth with NO_2 exposures 2 days before birth. A case-control study of spontaneous abortion before 14 weeks of gestation found a positive OR for NO_2 exposure (Moridi et al., 2014). Hou et al. (2014) also found positive ORs for fetal loss before 14 weeks of gestation with NO_2 exposure; however, these estimates have large confidence intervals and may not be reliable. Enkhmaa et al. (2014) found high correlations between air pollutants and fetal loss before 20 weeks in Mongolia, China, including NO_2, but these results were unadjusted for other factors or copollutants. One study investigated the association between short-term exposure to NO_2 and mortality during the neonatal period (Lin et al., 2004) and did not observe a positive association.

More studies have examined the association between exposure to NO_2 and mortality during the post-neonatal period. Son et al. (2008), Tsai et al. (2006), and Yang et al. (2006) examined the association between short-term exposure to NO_2 and post-neonatal mortality, while Ritz et al. (2006) investigated the association between long-term exposure to NO_2 and post-neonatal mortality; none observed a consistent, positive association. Finally, two studies examined the association between NO_2 and sudden infant death syndrome. Dales et al. (2004) and Ritz et al. (2006) observed positive associations with short-term and long-term exposure to NO_2, respectively. Supplemental Table S6-8 (U.S. EPA, 2013h) provides a brief overview of the epidemiologic studies of infant mortality

6.4.4 Postnatal Development

The role of prenatal air pollution exposure has assumed increasing importance over time for effects on post-natal development. Ambient air pollution exposures of pregnant women have been associated with negative birth outcomes. Additionally, the prenatal and early post-natal periods are critical periods for extensive growth and development, and air pollution exposures during this period have been linked to health effects in the first years of life. Thus, air pollution-related effects in both the developing fetus and infant have implications for effects on post-natal development. This evaluation of the relationship of post-natal developmental with NO_2 exposure consists primarily of neurodevelopmental outcomes and limited toxicological information on physical development. Studies examining the effects of NO_2 exposure on development of the respiratory system (Sections 6.2.6 and 6.2.7) inform the evaluation of the relationship between long-term NO_2 exposure and respiratory effects (Section 6.2.9).

6.4.4.1 Neurodevelopmental Effects

Epidemiologic studies of neurodevelopment in children were not available for the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008), but several have been published since then. As described in the sections that follow, associations with NO_2 are inconsistent for cognitive function, which was most extensively examined, and for attention-related behaviors, motor function, psychological distress, and autism, which were examined in a few studies each. Table 6-12 details the key studies, and Supplemental Table S6-7 (U.S. EPA, 2013g) provides an overview of all of the epidemiologic studies of neurodevelopmental effects. Strengths of the studies overall are assessment of neurodevelopment with widely used, structured neuropsychological tests, spatial alignment of ambient NO_2 concentrations to subjects' school or home locations, and

examination of potential confounding by multiple SES indicators. While some studies considered birth outcomes and traffic noise exposure as potential confounding factors, smoking and stress were inconsistently or not considered. Further, in most studies, NO_2 was the only air pollutant examined, and uncertainty remains regarding potential confounding by copollutants that are well-characterized risk factors for decrements in neurodevelopmental function such as lead, $PM_{2.5}$, or PM components such as polycyclic aromatic hydrocarbons.

Cognitive Function

NO_2 is not consistently associated with cognition in children. Among children age 4 years, indoor home NO_2 at age 3 months was associated with multiple measures of cognitive function, from a general cognition index to memory, verbal, and quantitative skills (Morales et al., 2009). These associations were limited to children with a glutathione s-transferase P (GSTP1) valine (Val)-105 allele [isoleucine (Ile)/Val or Val/Val vs. Ile/Ile genotype], which is associated with lower oxidative metabolism. In contrast with indoor NO_2, ambient NO_2 assessed concurrently with cognitive function or for the prenatal period was not clearly associated with cognitive function in schoolchildren or infants (Guxens et al., 2014; Clark et al., 2012; Guxens et al., 2012; van Kempen et al., 2012; Freire et al., 2010; Wang et al., 2009a). Within studies, results were inconsistent among the multiple indices of cognitive function examined. Results also were inconsistent across studies, including those for indices of memory, which was examined in most studies. As was done in the 2013 ISA for Lead (U.S. EPA, 2013a), evidence is evaluated separately for cognition in schoolchildren and infants.

A common strength of studies conducted in schoolchildren is the assessment of NO_2 exposures for home or school locations using well-validated LUR models (see Section 3.2.1.1 for details on using LUR models to assign exposure). van Kempen et al. (2012) was particularly noteworthy for assessing exposures outside both home and school and examining potential confounding by traffic noise. The LUR model well predicted ambient NO_2 concentrations in the study area (R^2 for cross-validation = 0.85). School NO_2, not home NO_2, was associated with memory with adjustment for noise (Table 6-12). Neither school nor home NO_2 was associated with ability to process information. A similar study found school-based aircraft noise but not NO_2 to be associated with cognitive function (Clark et al., 2012). NO_2 estimated for home locations also was inconsistently associated with cognitive function. Another study of concurrent NO_2 exposure observed that higher home outdoor NO_2 was associated with poorer cognitive function, but the wide 95% CIs call into question the reliability of findings (Freire et al., 2010). Associations for prenatal residential NO_2 exposure were similarly inconsistent among cohorts in three cities in Spain, with negative, null, and positive

associations observed with cognition [(Guxens et al., 2014); Table 6-12]. In these studies, NO_2 exposures were estimated from LUR models that varied in performance across locations; however, the inconsistency in findings does not appear to be related to model performance. In Freire et al. (2010), the LUR model performed better in the nonurban than urban locations (R^2 for building = 0.75 and 0.45, respectively; R^2 for validation = 0.64 for locations combined), and 84% of subjects lived in nonurban areas. Additionally, NO_2 was not associated with decrements in cognitive function in cities in Spain where LUR models predicted ambient NO_2 concentrations well [cross-validation R^2 = 0.75, 0.77; (Guxens et al., 2012; Estarlich et al., 2011)]. Wang et al. (2009a) also produced inconsistent findings but has weaker implications because of its ecological comparison of school locations that differed in ambient NO_2 concentrations not direct analysis of NO_2.

The Bayley Scales of Infant Development is a widely used and reliable test for infant development. The mental development index is a measure of sensory acuity, memory, and early language skills. However, the Bayley Scales of Infant Development scores are not necessarily correlated with development of children at older ages, and the tests at age 1 year or younger do not assess many outcomes that are analogous to those assessed at 2 and 3 years old. Across studies, mental development in infants ages 6 to 24 months was not associated with trimester-specific prenatal NO_2 exposure or NO_2 exposure from birth to age 6 months (Lin et al., 2014) and was inconsistently associated with NO_2 averaged over pregnancy (Guxens et al., 2014; Kim et al., 2014; Guxens et al., 2012). In an analysis of cohorts across multiple European counties, associations were found in locations where the LUR model better predicted ambient NO_2 [cross-validation R^2 = 0.69−0.84 vs. 0.45, 0.51; (Beelen et al., 2013; Estarlich et al., 2011)]. Associations for four cohorts in Spain differed between publications [(Guxens et al., 2014; Guxens et al., 2012); Table 6-12]. Other than sample sizes and possibly different pregnancy addresses used to estimate NO_2 exposure, an explanation for divergent results is not clear (Guxens et al., 2014; Estarlich et al., 2011). Mental development of infants also was inconsistently associated with NO_2 exposure assessed by averaging concentrations across city central site monitors (Lin et al., 2014) or combining concentrations by IDW (Kim et al., 2014). The uncertainty of these methods in capturing the spatial heterogeneity in ambient NO_2 concentrations is illustrated by the weak or moderate correlation (r = 0.42, 0.21) observed between ambient NO_2 estimated by IDW and measured outside homes in a subset of subjects in Kim et al. (2014). Another uncertainty in these studies is the lack of examination of potential confounding by benzene (Guxens et al., 2012) or PM_{10} (Kim et al., 2014), which showed a similar pattern of association as did NO_2 and were highly or moderately correlated with NO_2 (r = 0.70 and 0.40, respectively).

Attention-Related Behaviors

The few studies of attention-related behaviors produced contrasting results for associations with NO_2. Morales et al. (2009) observed that exposure to gas appliances and higher indoor NO_2 at age 3 months were associated with elevated odds ratios for symptoms of Attention Deficit Hyperactivity Disorder at age 4 years. The association was attributable mainly to inattention, as hyperactivity was not associated with NO_2. As was observed for cognitive function, associations were limited to children with a GSTP1 Val-105 allele. In contrast, outdoor school and home NO_2 concentrations (with or without adjustment for road traffic and aircraft noise) were not associated with poorer performance on multiple tests of sustained and switching attention (van Kempen et al., 2012). There was some evidence of an NO_2-road traffic noise interaction, as home outdoor NO_2 was associated with poorer attention switching among children in the highest noise category. Home NO_2 and road traffic noise were moderately correlated (r = 0.30). The ecological study did not find attention performance test results to differ consistently with respect to school locations (Wang et al., 2009a).

Motor Function

Evidence does not strongly indicate that NO_2 exposure affects motor function of children. Whereas higher indoor home NO_2 exposure at age 3 months was associated with poorer motor function in 4-year olds (Morales et al., 2009), findings were inconsistent for ambient NO_2 exposure. A combined analysis of multiple European cohorts found that NO_2 exposure ascertained for the birth address by LUR was associated with poorer motor function overall, but associations were limited to half of the individual cohorts (Guxens et al., 2014). $PM_{2.5}$, $PM_{2.5}$ absorbance (an indicator of EC), PM_{10}, and coarse PM were not associated with motor function in all of the same cohorts as NO_2. Thus, confounding by these copollutants does not seem to fully explain the NO_2 associations. There was no clear pattern of association by gross or fine motor function or by age at which motor function was assessed. NO_2 was associated with poorer motor function among children between ages 1−6 years in some locations but not others. The inconsistency in findings does not appear to depend on the adequacy of the LUR models to represent ambient NO_2 concentrations in the study area. LUR models have shown a similar range in performance [cross-validation R^2 = 0.49 to 0.87; (Beelen et al., 2013; Estarlich et al., 2011)] in locations where associations were observed and not observed. A limitation of the LUR models is that they were constructed based on ambient concentrations measured after the birth of some subjects. When the analysis was restricted to subjects whose birth dates coincided with the period of ambient monitoring, the effect estimate decreased; however, there was evidence of association between NO_2 and motor function for the combined cohorts (Table 6-12).

Results also are inconsistent for concurrent exposure. Among children ages 9−11 years, neither concurrent NO_2 nor noise exposure, alone or combined, at school or home, was associated with fine motor function (van Kempen et al., 2012). Among children age 4 years, higher concurrent outdoor home NO_2 exposure was associated with poorer gross motor function but not fine motor skills (Freire et al., 2010). Children attending schools with higher ambient NO_2 had poorer motor function compared to children attending schools with lower NO_2 (Wang et al., 2009a); however, attributing the findings to NO_2 versus another factor that differed between schools is not possible. Like home- or school-based exposure measures, NO_2 exposure assessed from central sites was not clearly associated with motor function in infants. Prenatal and lifetime exposure was inconsistently associated with motor function at 6 months of age, and no associations were observed in infants ages 12 to 24 months (Kim et al., 2014; Lin et al., 2014).

Limited evidence from toxicological studies also shows mixed effects of NO_2 exposure on motor function. Tabacova et al. (1985) found deficits in motor function and postural gait in rat pups exposed gestationally to 50, 500, or 5,300 ppb NO_2 (dam exposure, 6 h/day, 7 days/week, Gestation Day 0−20). In the open field test, female and male animals exhibited retarded locomotor development, with stronger effects earlier in life (testing done until 3 months of age). On PND9, reductions were noted in horizontal motility and head raising with prolonged periods of immobility, hypotonia, tremor, and equilibrium deficits. Gait deficits including hindlimb dragging, crawling en lieu of walking, pivoting, and impaired body raising ability were observed out to PND14 even in animals in the lowest dose group. Tabacova et al. (1985) also found deficits in righting reflex and the auditory startle reflex. In a separate study, prenatal exposure to 1,500 or 3,000 ppb NO_2 [(Di Giovanni et al., 1994) dam exposure over GD 0−20 of pregnancy] did not significantly affect motor function in 10- to 15-day-old male pups as measured by infrared sensors.

Psychological Distress

The two available studies produced equivocal evidence for the effects of NO_2 exposure on psychological distress. Among children ages 9−10 years, an index of emotional, social, and conduct problems was not associated with concurrent NO_2 or with aircraft or road traffic noise at school, either alone or after mutual adjustment (Clark et al., 2012). In rats, Di Giovanni et al. (1994) reported that 3,000 ppb continuous NO_2 exposure of dams during GD0−GD21 resulted in decreased pup vocalization, an indicator of emotionality, in males removed from the nest at PND5, PND10, or PND15.

Autism

Autism is a neurodevelopmental disorder characterized by impaired social interaction, verbal and nonverbal communication deficits, and repetitive or stereotypic behavior. Although the causes of autism are not fully understood, genetic conditions, family history, and older parental age have been implicated as risk factors. Case-control studies in California observed that higher NO_2 concentrations during the prenatal period and during the first year of life were associated with higher odds ratios for autism in children ages 24−71 months (Becerra et al., 2013; Volk et al., 2013). In both studies, cases were identified from regional referral centers contracted by the Department of Developmental Services. Controls were selected as birth certificate records not having a matching record of autism with the referral centers. Controls were matched to cases by age, sex, and wide geographic area. However, matching by area of residence was based only on birth addresses. These studies also observed stronger associations for autism among children with mothers with less than a high school education compared with higher education (Becerra et al., 2013) and children with the CC MET genotype compared with CC/GG genotype (Volk et al., 2014). The CC MET genotype is associated with decreased MET protein in the brain and has been associated with autism risk.

Between studies, inference about NO_2 is stronger in (Becerra et al., 2013). Residential NO_2 exposure was assessed using a well-validated LUR model [cross-validation R^2 = 0.87; (Su et al., 2009)]. In contrast, Volk et al. (2013) examined central site ambient NO_2 concentrations and NO_X estimated with a dispersion model. There are large uncertainties with these exposure measures. Central site NO_2 concentrations were assigned from a site 5 km from homes, if available, or by inverse distance weighting over a 50-km area. The authors did not report what proportion of subjects were assigned NO_2 exposures at the 5-km or 50-km scale, but neither scale may adequately capture the spatial heterogeneity in NO_2 concentrations (Section 3.4.5.2). Inference is poorer for NO_X as it was nearly perfectly correlated (r ~ 0.99) with EC and CO. In each study, $PM_{2.5}$ also was associated with autism, and (Becerra et al., 2013) found that NO_2 associations were robust to adjustment for traffic-related copollutants $PM_{2.5}$ or CO as well as the copollutants O_3 or PM_{10}. However, the reliability of the copollutant model results is uncertain as copollutant concentrations were assessed from central sites, and exposure measurement error likely varies between central site copollutant concentrations and residential NO_2.

Neuronal Degeneration and Nervous System Oxidative Stress

A recent study found that short-term NO_2 exposure induced neuronal degeneration and oxidative stress in the brains of adult male Wistar rats. Seven-day (6 h/day) exposure to

2,500−10,000 ppb NO_2 (Li et al., 2012) had no effect on body weight; however, concentration-dependent reductions in brain-to-body-weight ratios were observed, with statistically significant differences at 5,320 and 10,000 ppb NO_2. Histopathological analysis of cerebral cortex demonstrated a concentration-dependent increase in swollen or shrunken nuclei and a concentration-dependent, statistically significant increase in apoptotic cell number in all NO_2-exposed rats. Statistically significant changes in antioxidant enzyme activities [Cu/zinc (Zn) SOD, MnSOD, and glutathione peroxidase], protein carbonyls, and malondialdehyde were observed in response to 5,320 and 10,000 ppb NO_2. While rats exposed to 2,500 ppb NO_2 demonstrated a statistically significant increase in the protein level of p53, rats exposed to the higher concentrations exhibited statistically significant increases in mRNA and protein levels of c-fos, c-jun, p-53, and bax. These results are consistent with oxidative stress especially at higher concentrations of NO_2.

6.4.4.2 Physical Development

Limited information from toxicological studies does not clearly indicate that NO_2 exposure affects physical post-natal development. Distinct exposure periods and test endpoints produced disparate results in two studies on post-natal body-weight gain in pups whose dams were exposed to NO_2. Shalamberidze and Tsereteli (1971b) and Shalamberidze and Tsereteli (1971a) showed decrements in post-natal body weight at PND4 and 12 in albino rats with prenatal exposures to 67 or 1,300 ppb NO_2 during the 3 months prior to breeding for 12 hours a day. Continuous NO_2 exposure during gestation [continuous exposure of dam to 1,500 or 3,000 ppb NO_2, GD0−GD20; (Di Giovanni et al., 1994)] produced no significant differences in weight gain at PND1, 11, or 21 in male Wistar rat pups. Tabacova et al. (1985) saw concentration-dependent delays in eye opening and incisor eruption in rodents after maternal exposure to NO_2 during pregnancy (dam exposure: GD0−GD21, 5 h/day, 25, 50, 500, or 5,300 ppb NO_2) (Table 6-13).

6.4.4.3 Summary of Postnatal Development

The collective evidence does not consistently indicate a relationship between NO_2 exposure and effects on post-natal development. Very few outcomes were similar between epidemiologic and toxicological studies. Physical development, examined as post-natal weight gain, eye opening, and incisor eruption in only a few studies of rats, was not clearly affected by prenatal NO_2 exposures in the range of 53 to 5,320 ppb. As examined primarily in epidemiologic studies, prenatal, early life, or concurrent school-age NO_2 exposure was not consistently associated with cognitive function,

attention-related behaviors, motor function, or psychological distress in infants or schoolchildren. While epidemiologic associations were observed for indoor home NO_2 (Morales et al., 2009), evidence is equivocal for ambient NO_2, including exposure metrics spatially aligned with subjects' home and school locations using LUR models that well represented the spatial heterogeneity in the study areas (Guxens et al., 2014; van Kempen et al., 2012; Freire et al., 2010). In limited examination of children in California, autism was associated with residential prenatal NO_2 exposure (Becerra et al., 2013).

Table 6-13 Reproductive and developmental toxicological studies for nitrogen dioxide (NO_2).

Reference	Concentration NO_2 (ppb)	Species (Strain); Age; Sex; n	Exposure Conditions	Endpoints Examined
Tabacova et al. (1985)	25, 50, 500, or 5,300 ppb	Rats (Wistar); adult; F; n = 20	Developmental exposure with post-natal neurotoxicity testing. 0, 25, 50, 500, or 5,300 ppb, 5 h/day during Gestational Days 0 through 21; progeny followed up to PND60.	Pup viability, developmental endpoints (eye opening, incisor eruption); neuromotor (righting reflex, postural gait, geotaxis); hepatic lipid peroxidation; hepatic drug-metabolizing enzyme activity.
Shalamberidze and Tsereteli (1971a), Shalamberidze and Tsereteli (1971b)	1,300 ppb	Rats (albino); adult; F; n = 7	1,300 ppb for 12 h/day for 3 mo (further specifics unavailable).	Litter size, birth weight, post-natal weight gain (body weight).
Di Giovanni et al. (1994)	1,500 or 3,000 ppb	Rats (Wistar); pups; M; n = 7	Direct exposure of dams at 0, 1,500, or 3,000 ppb continuously throughout Gestational Days 0−20, male offspring tested for vocalizations on PND5, PND10, and PND15.	Neurobehavior (ultrasonic vocalization), maternal body weight during pregnancy, litter size, post-natal body weight, early life mortality, motor function.
Kripke and Sherwin (1984)	1,000 ppb	Rats (LEW/f mai); young adults; M; n = 6	0 or 1,000 ppb for 7 h/day, 5 days/week for 21 days.	Spermatogenesis, germinal cells histology, and testicular interstitial cell histology.

NR = no quantitative results reported; PND = post-natal day.

In addition to the inconsistent or limited evidence for NO_2-related neurodevelopmental effects, there is uncertainty regarding confounding by factors spatially correlated with NO_2 at the level of individuals or communities. Studies observed associations with NO_2 with adjustment for SES indicators and birth outcomes. However, examination of

confounding was absent for stress and was very limited for smoking, noise, and traffic-related copollutants. In van Kempen et al. (2012), school NO_2 was associated with memory after adjustment for traffic noise, and there was evidence of a home NO_2-noise interaction for attention switching. The few studies that examined pollutants other than NO_2 found associations of neurodevelopmental effects with traffic-related copollutants such as $PM_{2.5}$, benzene, and CO. An association between NO_2 and autism was observed with adjustment for CO or $PM_{2.5}$ as well as for O_3 or PM_{10} (Becerra et al., 2013). However, the reliability of results is uncertain because of potential differential exposure measurement error between residential NO_2 and central site copollutants. Other pollutants characterized to be associated with neurodevelopment such as lead or polycyclic aromatic hydrocarbons (U.S. EPA, 2013a, 2009) were not examined as potential confounding copollutants. Toxicological evidence is far more limited than epidemiologic evidence and is similarly uncertain. Prenatal NO_2 exposure induced psychological distress (Di Giovanni et al., 1994) in rat offspring but showed mixed effects on motor function (Di Giovanni et al., 1994; Tabacova et al., 1985). Further, although some studies showed effects on post-natal development, there was very limited information to characterize possible modes of action for ambient-relevant NO_2 exposures. In a recent study of adult rats, short-term exposure to 5,320 ppb NO_2 induced increases in the neuronal apoptotic and oxidative stress markers (Li et al., 2012), which have been linked to cognitive function.

6.4.5 Summary and Causal Determination

Overall, the evidence is suggestive, but not sufficient, to infer a causal relationship between exposure to NO_2 and birth outcomes and is inadequate to infer the presence or absence of a causal relationship between exposure to NO_2 and fertility, reproduction and pregnancy, and post-natal development. Separate conclusions are made for these three smaller groups of outcomes because they are likely to have different etiologies and critical exposure patterns over different lifestages. In past reviews, a limited number of epidemiologic and toxicological studies had assessed the relationship between exposure to NO_2 and reproductive and developmental effects. The 2008 ISA for Oxides of Nitrogen concluded that there was not consistent evidence for an association between NO_2 and birth outcomes and concluded that evidence was inadequate to infer the presence or absence of a causal relationship with reproductive and developmental effects overall. The change in the causal determination for birth outcomes reflects the larger number of studies that have evaluated these outcomes, as well as the improved exposure assessment (e.g., LUR models) and outcome assessment (e.g., fetal growth measured throughout pregnancy via ultrasound) employed by these studies. Exposure assessment

was evaluated drawing upon discussions in Sections 3.2 and 3.4.5. In general, LUR model predictions have been found to correlate well with outdoor NO_2 concentration measurements (Section 3.2.1.1). A select number of recent studies have employed exposure assessment methods such as LUR to represent the spatial variability of NO_2. All available evidence, including more than 100 recent epidemiologic studies, examining the relationship between exposure to NO_2 and reproductive and developmental effects were evaluated using the framework described in Table II of the Preamble. The key evidence as it relates to the causal determination is summarized in Table 6-14.

Fertility, Reproduction, and Pregnancy

A number of studies examined the association between exposure to NO_2 concentrations measured at central site monitors or estimates of NO_2 or NO_X concentration from LUR models and effects on fertility, reproduction, and pregnancy. These types of health endpoints and their relationship with air pollution have only recently begun to be evaluated, and, thus, the number of studies for any one endpoint is limited. There is generally no evidence for an association between NO_2 concentrations and sperm quality in either epidemiologic or toxicology studies. One study (Legro et al., 2010) observed a decreased odds of live birth associated with higher NO_2 concentrations during ovulation induction and the period after embryo transfer; while another (Slama et al., 2013) observed decreased fecundability with higher NO_2 exposure near conception. There is inconsistent evidence for an association between NO_2 concentrations estimated from LUR models and pre-eclampsia, pregnancy-induced hypertension, gestational diabetes, and reduced placental growth and function. Maternal weight gain showed mixed effects during pregnancy with one of two studies reporting decrements in weight gain with NO_2 exposure; the same trend was seen with NO_2-dependent smaller litter size. Impaired estrus cyclicity in NO_2-exposed animals was reported as was a decrease in number of primordial follicles in NO_2-exposed rodents. Collectively, the limited evidence is of insufficient consistency and is inadequate to infer a causal relationship between exposure to NO_2 and effects on fertility, reproduction, and pregnancy.

Birth Outcomes

While the collective evidence for many of the birth outcomes examined is not entirely consistent, there are several well-designed, well-conducted studies that indicate an association between NO_2 and adverse birth outcomes. For example, the Spanish cohort that utilized anthropometric fetal measurements throughout pregnancy (Iñiguez et al., 2012; Estarlich et al., 2011; Aguilera et al., 2010; Ballester et al., 2010) observed small, yet consistent associations with impaired fetal growth and NO_2 concentrations.

NO_2-dependent decrements in pup birth weight were reported in an animal toxicology study (Shalamberidze and Tsereteli, 1971a) and provide supporting evidence for the associations with fetal growth restriction observed in epidemiologic studies. Studies that examined PTB, birth weight, birth defects, and infant mortality generally found inconsistent results, with some studies observing positive associations, while others observed negative associations, regardless of whether NO_2 or NO_X were used to estimate exposure. Many of the studies examining PTB observed associations very close to the null value. Generally, studies of birth outcomes did not evaluate other traffic-related pollutants along with NO_2 or NO_X in copollutants models, and this is a source of uncertainty that can not be addressed. Several different methods for exposure assessment were used in these studies of birth outcomes, but there were no trends observed across studies based on the method of exposure assessment. Collectively, the limited and inconsistent evidence is suggestive, but not sufficient, to infer a causal relationship between exposure to NO_2 and effects on birth outcomes, with the strongest evidence coming from studies of fetal growth restriction.

Postnatal Development Effects

There is inconsistent evidence from both epidemiologic and animal toxicological studies for a relationship between prenatal and childhood NO_2 exposure and post-natal development effects. Findings across the several recent epidemiologic studies of neurodevelopment do not consistently support associations of NO_2 with cognitive function, attention-related behaviors, motor function, and psychological distress in children. Many of these studies estimate ambient NO_2 exposures for children's homes or schools using LUR models that well represent the variability in ambient concentrations in study areas. Toxicological evidence for effects on neurodevelopment is limited, and evidence for impaired physical development shows mixed results including null findings. NO_2 exposures were related to autism in children in recent epidemiologic studies, but such findings are limited to a few studies. In the small group of epidemiologic studies observing associations with neurodevelopmental effects, potential confounding by traffic-related copollutants was not adequately examined. Motor function testing showed multiple endpoints affected by NO_2 exposure in animal models including reflexes (auditory startle and righting reflex), postural gait, impaired walking, and head raising. But one study found no motor function impairment with NO_2 exposure. Communication of psychological distress was impaired in NO_2-exposed pups. Collectively, the evidence is of insufficient consistency or quantity and is inadequate to infer a causal relationship between exposure to NO_2 and effects on post-natal development.

Table 6-14 Summary of evidence supporting the causal determinations for relationships between long-term nitrogen dioxide (NO_2) exposure and reproductive and developmental effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Fertility, Reproduction, and Pregnancy—Inadequate to Infer a Causal Relationship			
Available epidemiologic studies of pre-eclampsia are of insufficient consistency	Inconsistent associations when exposure is assessed across entire pregnancy and individual trimesters, between NO_2 concentration and pre-eclampsia, after adjustment for common potential confounders. Uncertainty regarding potential confounding by traffic-related copollutants.	Wu et al. (2009), Pereira et al. (2013), Malmqvist et al. (2013), Dadvand et al. (2013)	Mean NO_2: 7.2 ppb Mean NO_X: 23 ppb Mean NO_X: 7.5 ppb Mean NO_2: 30 ppb
Available epidemiologic and toxicological studies for other pregnancy-related health effects are of insufficient consistency	Limited and inconsistent epidemiologic evidence for associations with pregnancy-induced hypertension, gestational diabetes, and placental growth and function.	Hampel et al. (2011), Lee et al. (2012a), Mobasher et al. (2013), Malmqvist et al. (2013), Xu et al. (2014), van den Hooven et al. (2012c)	Means: 8.7−28.6 ppb
	Limited and inconsistent evidence in rats for deficits in maternal weight gain during pregnancy.	Tabacova et al. (1985), Di Giovanni et al. (1994)	1,300, 1500, and 3,000 ppb
Limited evidence for key events in mode of action	Impaired estrus cyclicity and decreased number of primordial follicles in rodents exposed to NO_2.	Shalamberidze and Tsereteli (1971a), (Shalamberidze and Tsereteli, 1971b)	67 or 1300 ppb
Available epidemiologic studies for in vitro fertilization failure are of insufficient consistency	Decreased odds of live birth associated with higher NO_2 concentrations during ovulation induction and the period after embryo transfer.	Legro et al. (2010), Slama et al. (2013)	Mean: 19 ppb Median: 19 ppb
Available toxicological and epidemiologic studies on sperm quality are of insufficient consistency	Overall, a limited number of toxicological and epidemiologic studies provide no evidence for an association between exposure to ambient NO_2 concentrations and effects on sperm.	Rats: Kripke and Sherwin (1984) Humans: Rubes et al. (2010), Sokol et al. (2006)	1,000 ppb 7 h/day, 5 days/week Mean ambient exposure averaged over 90-days: 16.8 ppb Mean ambient daily concentration: 30.1 ppb

Table 6-14 (Continued): Summary of evidence supporting the causal determinations for relationships between long-term nitrogen dioxide (NO2) exposure and reproductive and developmental effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Birth Outcomes—Suggestive, But Not Sufficient, to Infer a Causal Relationship			
Evidence from multiple epidemiologic studies of fetal growth restriction is generally supportive but not entirely consistent	Strongest evidence from well-conducted Spanish cohort studies that observe associations with NO_2 concentrations and fetal growth restriction. Supported by consistent evidence for SGA and IUGR. Outcomes assessed with anthropometric fetal measurements.	Section 6.4.3.31	Mean exposure averaged over trimesters: 7.8−36.1 ppb
Limited and inconsistent epidemiologic evidence for other birth outcomes	Some studies observe an association between NO_2 exposure and PTB, birth weight, birth defects, and infant mortality while other studies observe no consistent pattern of association.	Section 6.4.3.2 Section 6.4.3.3 Section 6.4.3.4 Section 6.4.3.5	Mean exposure averaged over trimesters: 8.8−37.6 ppb Mean exposure averaged over trimesters: 6.2−62.7 ppb Mean exposure averaged over early pregnancy (e.g., Weeks 3−8): 8.2−28.0 ppb Mean daily concentrations: 20.3−50.3 ppb
Limited and inconsistent toxicological evidence with relevant NO_2 exposures	Mixed evidence of effects on litter size and mixed evidence of late embryonic lethality in rats.	Shalamberidze and Tsereteli (1971a), Shalamberidze and Tsereteli (1971b) Di Giovanni et al. (1994) Tabacova et al. (1985)	1,300−5,300 ppb
Limited evidence for key events in mode of action			
Inflammation	Increase in CRP concentration in human umbilical cord blood associated with NO_2 concentration.	van den Hooven et al. (2012a)	Mean exposure averaged over week before delivery: 21.4 ppb

Table 6-14 (Continued): Summary of evidence supporting the causal determinations for relationships between long-term nitrogen dioxide (NO2) exposure and reproductive and developmental effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Postnatal Development—Inadequate to Infer a Causal Relationship			
Limited and inconsistent epidemiologic and toxicological evidence for effects on neurodevelopment	Some epidemiologic studies showed cognitive function decrements in infants and schoolchildren in association with NO_2 exposure. Uncertainty regarding potential confounding by traffic-related copollutants.	van Kempen et al. (2012), Morales et al. (2009), Guxens et al. (2012)	Mean concurrent: 16.5, 16.9 ppb Mean prenatal: 15.7 ppb
	Some studies did not indicate associations with cognitive function. Evidence is inconsistent for NO_2 exposures estimated for childrens' homes or schools.	Clark et al. (2012), Freire et al. (2010), Guxens et al. (2014)	
	More limited and inconsistent epidemiologic evidence for attention-related behaviors, motor function, psychological distress.	Section 6.4.4.1	
	In utero NO_2 exposure increased emotionality in rat pups, but effects on motor function inconsistent.	Tabacova et al. (1985), Di Giovanni et al. (1994)	50, 500 or 5,300 ppb 1,500 and 3,000 ppb
	Prenatal NO_2 exposure associated with autism in first yr of life or at ages 3−6 yr in California.	Becerra et al. (2013)	Mean: 30.8 ppb
Limited evidence for key events within mode of action Neurodegeneration Oxidative stress	Short-term exposure increased apoptotic factors and oxidative stress in brain of adult rats.	Li et al. (2012)	5,320 ppb for 7 days

Table 6-14 (Continued): Summary of evidence supporting the causal determinations for relationships between long-term nitrogen dioxide (NO2) exposure and reproductive and developmental effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Limited and inconsistent toxicological evidence for physical development	NO_2 exposure delayed post-natal eye opening and incisor eruption but had mixed effects on post-natal growth.	Tabacova et al. (1985), Di Giovanni et al. (1994)	530 and 5,300 ppb 1,500 and 3,000 ppb

CRP = C-reactive protein; IUGR = intrauterine growth restriction; NO_2 = nitrogen dioxide; NO_X = sum of NO and NO_2; PTB = preterm birth; SGA = small for gestational age.

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Tables I and II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to causal determination and where applicable to uncertainties and inconsistencies. References to earlier sections indicate where the full body of evidence is described.

[c]Describes the NO_2 concentrations with which the evidence is substantiated (for experimental studies, below ~5,000 ppb).

6.5 Total Mortality

In past reviews, a limited number of epidemiologic studies had assessed the relationship between long-term exposure to NO_2 and mortality in adults, including cause-specific and total mortality. The 2008 ISA for Oxides of Nitrogen concluded that the amount of evidence was "inadequate to infer the presence or absence of a causal relationship" (U.S. EPA, 2008). In the current ISA, findings for cause-specific mortality (i.e., respiratory, cardiovascular) are used to assess the continuum of effects and inform the causal determinations for respiratory and cardiovascular effects. The causal determination for total mortality contained herein (Section 6.5) is based primarily on the evidence for nonaccidental mortality but also is informed by the extent to which evidence for the spectrum of cardiovascular and respiratory effects provides biological plausibility for NO_2-related total mortality. A supplemental Table S6-9 (U.S. EPA, 2013i) provides an overview of the epidemiologic studies of long-term exposure to NO_2 or NO_X and mortality, including details on exposure assessment and mean concentrations from the study locations.

6.5.1 Review of Mortality Evidence from 2008 Integrated Science Assessment for Oxides of Nitrogen

Two seminal studies of long-term exposure to air pollution and mortality among adults have been conducted in the United States; the American Cancer Society (ACS) and the

Harvard Six Cities (HSC) cohorts have undergone extensive independent reanalyses and have reported extended results including additional years of follow-up. The initial reports from the ACS (Pope et al., 1995) and the HSC (Dockery et al., 1993) studies did not include results for gaseous pollutants. However, as reported in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008), in reanalyses of these studies, Krewski et al. (2000) examined the association between gaseous pollutants, including NO_2, and mortality. Krewski et al. (2000) observed a positive association between long-term exposure to NO_2 and mortality in the HSC cohort, with effect estimates[1] similar in magnitude to those observed with $PM_{2.5}$. The effect estimates were positive for different causes of mortality but were the strongest for cardiopulmonary and total mortality. In a re-analyses of the ACS cohort data (Krewski et al., 2000), long-term exposure to NO_2 estimated from central site monitors was not associated with mortality. An extended study of the ACS cohort (Pope et al., 2002) doubled the follow-up time and tripled the number of deaths compared to the original study but still observed no association between long-term exposure to NO_2 and mortality.

A series of studies (Lipfert et al., 2006a; Lipfert et al., 2006b; Lipfert et al., 2003, 2000) characterized a national cohort of over 70,000 male U.S. military veterans who were diagnosed as having hypertension in the mid-1970s and were followed through 2001. In the earlier studies, the authors reported increased risk of mortality associated with exposure to NO_2; these excess risks were in the range of 5−9% (Lipfert et al., 2003, 2000). In the later studies, the authors focused on traffic density in this cohort. Lipfert et al. (2006b) and Lipfert et al. (2006a) reported that traffic density was a better predictor of mortality than ambient air pollution variables, though they still observed a positive association between mortality and NO_2 exposure. The results from the series of studies characterizing the Veterans cohort are indicative of a traffic-related air pollution effect on mortality, but the study population (lower SES, males with hypertension and a very high smoking rate) was not representative of the general U.S. population.

In another cohort conducted in the U.S. [the California Seventh-day Adventist cohort (AHSMOG)], Abbey et al. (1999) enrolled young adult, nonsmoking Seventh-day Adventists throughout California. Generally, NO_2 was not associated with total, cardiopulmonary, or respiratory mortality in either men or women. The authors observed large risk estimates for lung cancer mortality for most of the air pollutants examined, including NO_2, but the number of lung cancer deaths in this cohort was very small (12 for

[1] Quantitative effect estimates from studies reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) can be found alongside effect estimates from more recent studies in Figures 6-8, 6-9, and 6-10 and the corresponding Tables 6-15, 6-16, and 6-17, respectively).

females and 18 for males out of a total of 5,652 subjects); therefore, it is difficult to interpret these results.

Several studies conducted in European countries have examined the relationship between long-term exposure to traffic-related pollutants (including NO_2 and NO_X) and mortality among adults. Hoek et al. (2002) observed an association between NO_2 and mortality in the Netherlands Cohort Study on Diet and Cancer (NLCS), though the association with living near a major road was stronger in magnitude. On the other hand, Gehring et al. (2006) observed that NO_2 was generally more strongly associated with mortality than an indicator for living near a major road in a cohort of women from Germany. Results from the Air Pollution and Chronic Respiratory Diseases survey conducted in France, demonstrated increased risk between long-term exposure to NO_2 and total, cardiopulmonary, and lung cancer mortality (Filleul et al., 2005). Similarly, Nafstad et al. (2004) observed an association between NO_X and total mortality, as well as deaths due to respiratory causes, lung cancer, and ischemic heart disease in a cohort of Norwegian men. Nyberg et al. (2000) observed similar results for lung cancer mortality in a case-control study of men in Stockholm, Sweden. Naess et al. (2007) investigated the concentration-response relationships between NO_2 and cause-specific mortality among a cohort from Oslo, Norway, aged 51−90 years. Total mortality, as well as death due to cardiovascular causes, lung cancer, and COPD were associated with NO_2 for both men and women in two different age groups, 51−70 and 71−90 years. Naess et al. (2007) reported that the effects appeared to increase at NO_2 levels higher than 21 ppb in the younger age group (with little evidence of an association below 21 ppb), while a linear effect was observed between 10 and 31 ppb in the older age group.

The results from these studies led to the conclusion that the evidence was inadequate to infer the presence or absence of a causal relationship in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008). The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) noted that potential confounding by copollutants was an important uncertainty when interpreting the evidence for the association between long-term exposure to NO_2 and mortality. Collinearity among criteria pollutants is another uncertainty that needs to be considered; several studies reported moderate-to-high correlations between NO_2 and PM indices (i.e., >0.5). The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) also acknowledged that NO_2 could be a surrogate or marker for traffic-related pollution. These uncertainties do not preclude the possibility of an independent effect of NO_2, or of NO_2 playing a role in interactions among traffic-related pollutants.

6.5.2 Recent Evidence for Mortality from Long-term Exposure to Oxides of Nitrogen

Several recent studies provide extended analyses of existing cohort studies of adult populations. In a reanalysis that extended the follow-up period for the ACS cohort to 18 years (1982−2000), Krewski et al. (2009) reported generally null associations between long-term exposure to NO_2 estimated from central site monitors and total and cause-specific mortality, similar to what was reported in the initial reanalysis of this cohort (Krewski et al., 2000). In an update to the ACS study including cohort members residing in California, Jerrett et al. (2013) used LUR models to predict long-term (i.e., 15 years) exposures to NO_2 at the home addresses of each of the cohort members. The authors observed positive associations between predicted NO_2 exposures and total, CVD, IHD, stroke, and lung cancer mortality, but not for respiratory mortality. The strongest associations were observed for deaths due to lung cancer and stroke. The associations with CVD and IHD mortality were attenuated in copollutant models that included $PM_{2.5}$ (also estimated from an LUR model), while the association with lung cancer was generally unchanged in two-pollutant models. In an update to the Veterans cohort study, Lipfert et al. (2009) looked at markers for specific emission sources, including NO_X as a marker of traffic, and their relationship with mortality, utilizing a 26-year follow-up period now available for this cohort. The authors observed an association between long-term exposures to NO_X estimated from a plume-in-grid model and mortality, and noted that this association was stronger among men living in areas with high traffic density compared to men living in areas with lower traffic density. The authors also demonstrate that traffic-related air pollutants (including NO_X) are better predictors of mortality than a measure of traffic density in this cohort. Updated results have also been reported for the NLCS cohort [the same effect estimates are reported by both Beelen et al. (2008b) and Brunekreef et al. (2009)]. Consistent with previous results from this cohort, the authors observe an association with total mortality. In the updated results, the authors observe the strongest effect between long-term exposure to NO_2 estimated from central site monitors and respiratory mortality; this association is stronger than any observed with the traffic variables and total or cause-specific mortality.

In updates to a cohort of women in Germany (Gehring et al., 2006), Schikowski et al. (2007) observed a positive association between ambient NO_2 concentrations measured at central site monitors and cardiovascular mortality among older women, though this association was not modified by lung function status (i.e., normal vs. impaired lung function). Heinrich et al. (2013) includes five additional years of follow-up and twice as many fatalities compared to the original analysis. In the updated analyses, the authors observed positive associations between NO_2 concentrations measured at central site monitors and total and cardiopulmonary mortality. The effect estimates were highest for

women living within 50 m of a road with median daily traffic volume of 5,000 cars or greater. The effect estimates for the associations between total and cardiopulmonary mortality and NO_2 were generally lower for the follow-up period compared to the original analysis.

Several recent U.S. cohort studies examined the association between long-term exposures to NO_2 and mortality in occupational cohorts. Hart et al. (2010) examined the association between residential exposure to NO_2 estimated from a spatial smoothing model and mortality among men in the U.S. trucking industry in the Trucking Industry Particle Study (TrIPS). The authors observed an increase in cardiovascular disease mortality and a decrease in COPD mortality associated with NO_2 exposure. The association between NO_2 exposure and total mortality was robust to the inclusion of PM_{10} or SO_2 in copollutant models. This association was stronger when the cohort was restricted to truck drivers that maintained local routes, and long haul drivers were excluded. COPD mortality was positively associated with NO_2 exposure in the sensitivity analysis excluding long haul drivers. The associations for other causes of death (i.e., lung cancer, IHD, respiratory disease) were generally positive. Another recent U.S. cohort study, The California Teachers Study (Lipsett et al., 2011) examined the association between long-term exposure to NO_X and NO_2 measured at central site monitors and mortality among current and former female public school teachers. The authors observed the strongest associations between IHD mortality and exposure to NO_X and NO_2; the associations for other causes of death (i.e., CVD, cerebrovascular, respiratory, lung cancer and total) were less consistent and generally close to the null value. Hart et al. (2013) examined the association between long-term exposure to NO_2 and total mortality among a cohort of female nurses in the Nurses' Health Study. The authors used spatial modeling to estimate exposure to NO_2 and observed a small increase in the risk of total mortality. In a sensitivity analysis examining women that moved during study follow-up, the authors observed even higher risks among women that moved to areas with higher concentrations of NO_2.

A number of recent studies have examined the association between long-term exposure to NO_2 and mortality in Canadian cities. Chen et al. (2013) conducted a cohort study of air pollution and cardiovascular mortality in three cities in Ontario. They used LUR models to assign exposure to NO_2 and observed that long-term exposure to NO_2 was associated with an increased risk of cardiovascular mortality. The association was stronger when mortality from IHD was evaluated separately. In a single-city study conducted in Toronto, Ontario, Jerrett et al. (2009) examined the association between long-term exposure to NO_2 (estimated from a LUR model) and total mortality among subjects from a respiratory clinic. The authors observed positive associations with total and circulatory mortality; the associations with respiratory and lung cancer mortality were also positive,

though less precise. In a model that included both NO_2 and proximity to traffic, the effect estimate for NO_2 remained robust, and the effect attributable to traffic was attenuated. In a single-city study conducted in Vancouver, British Columbia, Gan et al. (2013) and Gan et al. (2011) conducted a population-based cohort study to evaluate the association between traffic-related pollutants and risk of mortality due to CHD and COPD, respectively. LUR models were used to estimate exposure over a 5-year period, (1994−1998) and the cohort was followed for 4 years (1999−2002). The authors observed the strongest associations (i.e., highest magnitude) for exposures to NO_2 and CHD mortality; however, these associations were greatly attenuated when $PM_{2.5}$ and BC were included in the model. The correlations between NO_2 and $PM_{2.5}$ and BC were low to moderate (i.e., <0.5). The authors observed positive associations between both NO and NO_2 concentrations and COPD mortality, which were slightly attenuated when $PM_{2.5}$ and BC were included in the model.

A recent multicenter European study pooled data from 22 existing cohort studies and used a strictly standardized protocol to investigate the associations between long-term concentrations of NO_2 and NO_X and total (Beelen et al., 2014a), respiratory, (Dimakopoulou et al., 2014) and cardiovascular (Beelen et al., 2014b) mortality. The authors used LUR models to assign exposure and observed generally null associations with total, respiratory, and cardiovascular mortality. The total mortality associations were evaluated in copollutants models and remained unchanged after adjustment for $PM_{2.5}$ and $PM_{10-2.5}$.

Several studies have examined the association between long-term exposure to NO_2 and mortality in England. Carey et al. (2013) conducted a cohort study using an emissions-based model to assign exposure. Model validation was good, and model estimates for NO_2 were highly correlated with PM_{10} and $PM_{2.5}$ (r = 0.9). The authors observed positive associations with total mortality; these associations were stronger for respiratory and lung cancer deaths, and somewhat attenuated when restricted to cardiovascular deaths. Tonne and Wilkinson (2013) evaluated the association between long-term exposure to NO_2 and NO_X estimated from a Gaussian dispersion model among survivors of hospital admissions for acute coronary system in England and Wales and observed evidence of a null association after adjustment for $PM_{2.5}$. In a single-city study, Maheswaran et al. (2010) compiled a cohort of stroke survivors and modeled NO_2 concentrations estimated from an emissions model across London. The authors observed a nearly 30% increase in total mortality associated with exposure to NO_2.

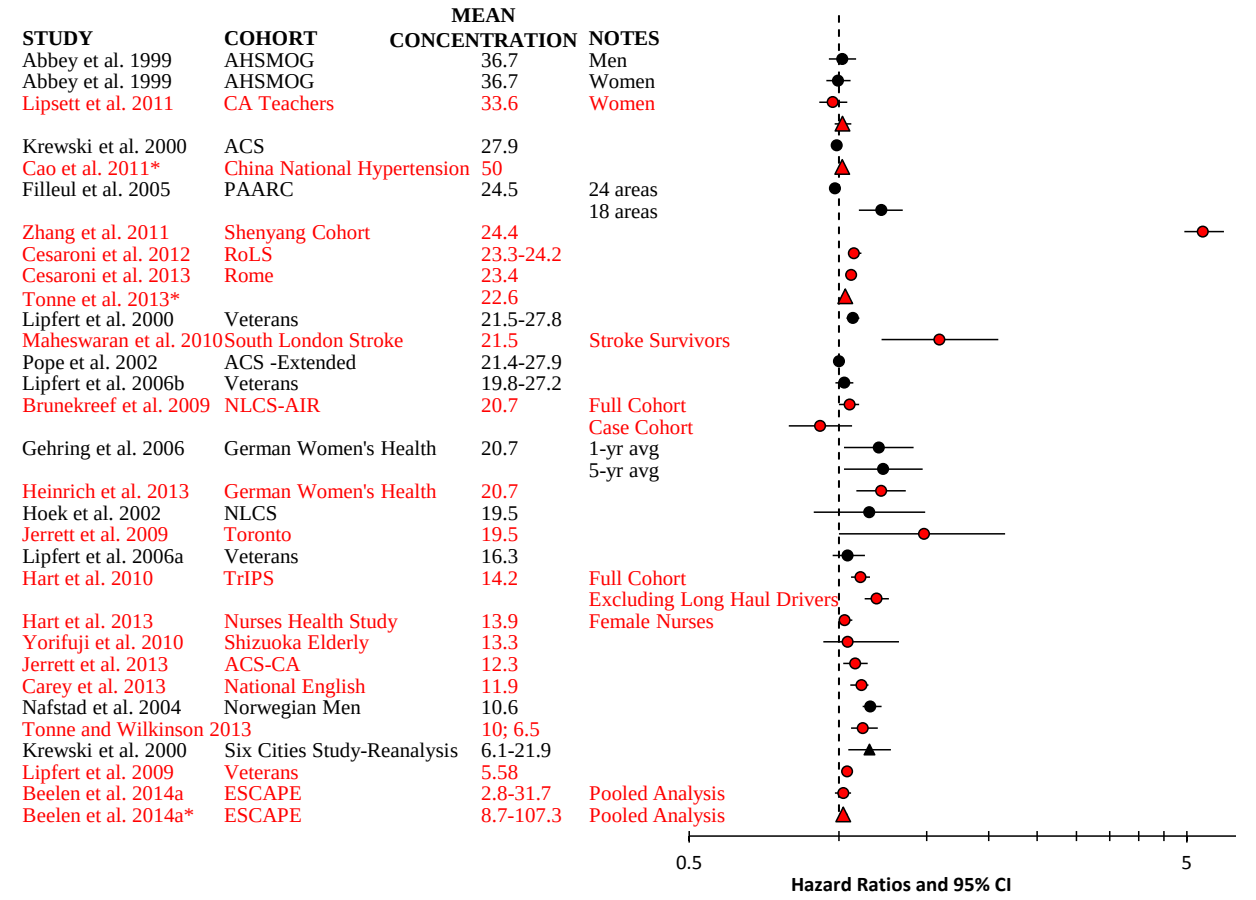
Rome, Italy was the setting for a number of single-city cohort studies. Cesaroni et al. (2013) observed positive associations between long-term exposure to NO_2 estimated from an LUR model and total, cardiovascular, IHD, respiratory, and lung cancer mortality

among the adult population in the Rome Longitudinal Study (RoLS). These associations were robust to the inclusion of $PM_{2.5}$ in the model. Later, these authors used several different LUR models to predict NO_2 in Rome (Cesaroni et al., 2012) and observed that the modest, positive association between total mortality and NO_2 concentrations was consistent across all models evaluated. Rosenlund et al. (2008) conducted a cohort study in Rome to investigate the effects of long-term exposure to NO_2 and cardiovascular deaths, including mortality among previous MI survivors. The authors observed a positive association between long-term exposures to NO_2 estimated from an LUR model and fatal coronary events, though they did not observe an association with mortality among survivors of a first coronary event.

A Danish study evaluated the association between long-term exposure to NO_2 (estimated from a dispersion model) and diabetes-related mortality Raaschou-Nielsen et al. (2013b). The authors reported a 30% increase in risk of diabetes-related mortality associated with NO_2 concentrations. In Brisbane, Australia, Wang et al. (2009b) examined the association between long-term exposure to NO_2 estimated from central site monitors and cardio-respiratory mortality. The relative risk for NO_2 and cardio-respiratory mortality was near the null value.

A number of studies were conducted in Asian countries to evaluate the association between long-term-exposure to NO_2 and mortality. In a national study covering 16 provinces in eastern China, Cao et al. (2011) observed positive associations between ambient NO_X concentrations from central site monitors and total, cardiovascular, respiratory, and lung cancer mortality. The association between total mortality and NO_X was relatively unchanged in a copollutant model with total suspended particles (TSP) but was reduced by half in a copollutants model with SO_2. The associations between NO_X and cardiovascular, respiratory, and lung cancer mortality were all attenuated in copollutants models including either TSP or SO_2. In a single-city study in Shenyang, China, the authors observed a strong, positive association between long-term exposure to NO_2 estimated from central site monitors and respiratory mortality (Dong et al., 2012) and total, cardiovascular, and cerebrovascular mortality (Zhang et al., 2011). In Shizuoka, Japan, Yorifuji et al. (2010) observed positive associations between NO_2 estimated from an LUR model and total, cardiopulmonary, IHD, and respiratory disease mortality, with the strongest effects observed for IHD mortality. When the analysis was restricted to nonsmokers, a positive association was observed with lung cancer mortality. Similar observations were reported for lung cancer by Katanoda et al. (2011) among a cohort in Tokyo, Japan, and Liu et al. (2008) for a study of women living in Taiwan. In a related study, Liu et al. (2009) also observed a positive association between long-term exposure to NO_2 and bladder cancer mortality.

These quantitative results of these studies are characterized in Figures 6-8, 6-9, and 6-10; and Tables 6-15, 6-16, and 6-17.



ACS = American Cancer Society; AHSMOG = California Seventh-day Adventists Cohort; ESCAPE = European Study of Cohorts for Air Pollution Effects; NLCS = Netherlands Cohort Study on Diet and Cancer; NLCS-AIR = Netherlands Cohort Study on Air Pollution and Mortality; PAARC = Air Pollution and Chronic Respiratory Diseases; RoLS = Rome Longitudinal Study; TrIPS = Trucking Industry Particle Study.

Note: Red = recent studies; black = studies reviewed in the 2008 ISA for Oxides of Nitrogen. Hazard ratios are standardized to a 10-ppb increase in NO_2 and a 20-ppb increase in NO_X concentration.

[a]Effect estimates from studies measuring NO_X in $\mu g/m^3$ have not been standardized. Studies are presented in descending order, with the largest mean concentration (ppb) at the top and the smallest at the bottom of the figure. Circles = NO_2; triangles = NO_X.

Figure 6-8 Results of studies of long-term exposure to nitrogen dioxide (NO_2) or the sum of nitric oxide and NO_2 (NO_X) and total mortality.

Table 6-15 Corresponding risk estimates for Figure 6-8.

Study	Location	Notes	Hazard Ratio[a] (95% CI)
Abbey et al. (1999)	U.S.	Men	1.02 (0.95, 1.08)
Abbey et al. (1999)	U.S.	Women	0.99 (0.94, 1.05)
Lipsett et al. (2011)	California	Women, NO_2	0.97 (0.94, 1.05)
Lipsett et al. (2011)	California	Women, NO_X	1.02 (0.98, 1.06)
Krewski et al. (2000)	U.S.		0.99 (0.99, 1.00)
Cao et al. (2011)[b]	China	NO_X	1.02 (1.00, 1.03)
Filleul et al. (2005)	France	24 areas	0.98 (0.96, 1.00)
Filleul et al. (2005)	France	18 areas	1.22 (1.10, 1.34)
Zhang et al. (2011)	China		5.39 (4.94, 5.94)
Cesaroni et al. (2012)	Italy		1.07 (1.05, 1.11)
Cesaroni et al. (2013)	Italy		1.06 (1.04, 1.06)
Tonne and Wilkinson (2013)[b]	England and Wales	NO_X	1.03 (1.01, 1.05)
Lipfert et al. (2000)	U.S.		1.07 (1.04, 1.10)
Maheswaran et al. (2010)	England	Stroke survivors	1.59 (1.22, 2.09)
Pope et al. (2002)	U.S.		1.00 (0.98, 1.02)
Lipfert et al. (2006b)	U.S.		1.03 (0.98, 1.02)
Brunekreef et al. (2009)	the Netherlands	Full cohort	1.05 (1.00, 1.10)
Brunekreef et al. (2009)	the Netherlands	Case cohort	0.92 (0.79, 1.06)
Gehring et al. (2006)	Germany	1-yr avg	1.20 (1.02, 1.41)
Gehring et al. (2006)	Germany	5-yr avg	1.23 (1.02, 1.47)
Heinrich et al. (2013)	Germany		1.21 (1.08, 1.36)
Hoek et al. (2002)	the Netherlands		1.15 (0.89, 1.49)
Jerrett et al. (2009)	Canada		1.48 (1.00, 2.16)
Lipfert et al. (2006a)	U.S.		1.04 (0.97, 1.13)
Hart et al. (2010)	U.S.	Full cohort	1.10 (1.06, 1.15)
Hart et al. (2010)	U.S.	Excluding long haul drivers	1.19 (1.13, 1.26)

Table 6-15 (Continued): Corresponding risk estimates for Figure 6-8.

Study	Location	Notes	Hazard Ratio[a] (95% CI)
Hart et al. (2013)	U.S.	Female nurses	1.03 (1.00, 1.06)
Yorifuji et al. (2010)	Japan		1.04 (0.93, 1.32)
Jerrett et al. (2013)	U.S.		1.08 (1.02, 1.14)
Carey et al. (2013)	England		1.11 (1.05, 1.15)
Nafstad et al. (2004)	Norway		1.16 (1.12, 1.22)
Tonne and Wilkinson (2013)	England and Wales		1.12 (1.06, 1.20)
Krewski et al. (2000)	U.S.	NO_X	1.15 (1.04, 1.27)
Lipfert et al. (2009)	U.S.		1.04 (1.03, 1.05)
Beelen et al. (2014a)	Europe	NO_2-pooled analysis	1.02 (0.98, 1.06)
Beelen et al. (2014a)[c]	Europe	NO_X-pooled analysis	1.02 (1.00, 1.04)

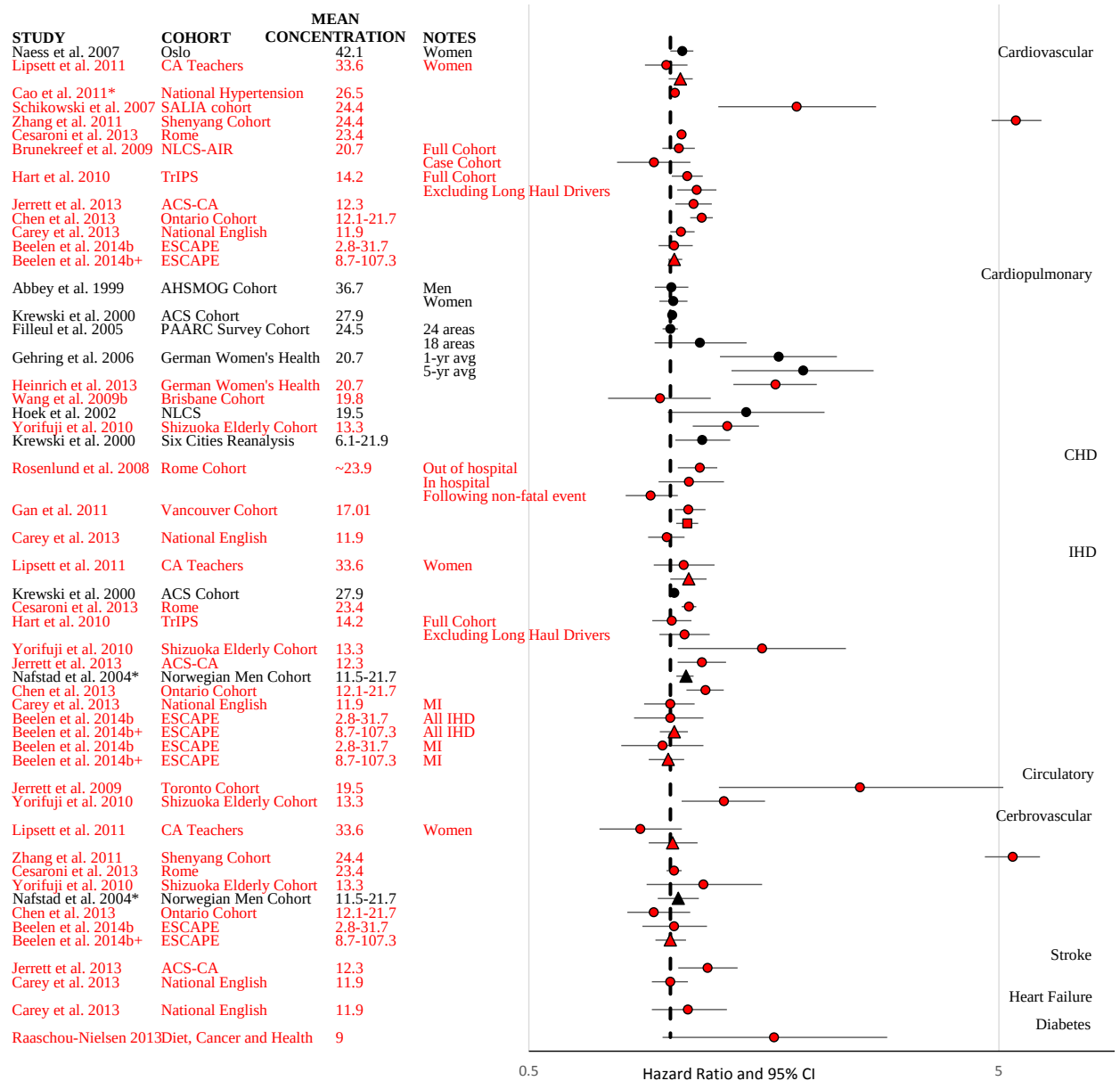
CI = confidence interval; NO_2 = nitrogen dioxide; NO_X = sum of NO and NO_2

Note: Studies correspond to those presented in Figure 6-8.

[a]Effect estimates are standardized to a 10-ppb increase in NO_2 and a 20-ppb increase in NO_X concentration.

[b]NO_X measured in $\mu g/m^3$. Effect estimate is per 10 $\mu g/m^3$ increase.

[c]NO_X measured in $\mu g/m^3$. Effect estimate is per 20 $\mu g/m^3$ increase.



ACS = American Cancer Society; AHSMOG = California Seventh-day Adventists Cohort; ESCAPE = European Study of Cohorts for Air Pollution Effects; NLCS = Netherlands Cohort Study on Diet and Cancer; NLCS-AIR = Netherlands Cohort Study on Air Pollution and Mortality; PAARC = Air Pollution and Chronic Respiratory Diseases; RoLS = Rome Longitudinal Study; TrIPS = Trucking Industry Particle Study.

Note: Red = recent studies/studies not reviewed in 2008 ISA; black = studies reviewed in the 2008 ISA for Oxides of Nitrogen. Hazard ratios are standardized to a 10-ppb increase in NO_2 and NO, and a 20-ppb increase in NO_X concentration.

* NO_X measured in μg/m^3. Effect estimate is per 10 μg/m^3 increase. + NO_X measured in μg/m^3. Effect estimate is per 20 μg/m^3 increase.Studies are presented in descending order, with the largest mean concentration (ppb) at the top and the smallest at the bottom of the figure. Circles = NO_2; triangles = NO_X; Squares = NO.

Figure 6-9 Results of studies of long-term exposure to nitrogen dioxide (NO_2), nitric oxide (NO), or the sum of NO and NO_2 (NO_X) and cardiovascular mortality.

Table 6-16 Corresponding risk estimates for Figure 6-9.

Study	Location	Notes	Hazard Ratio[a] (95% CI)
Cardiovascular Disease			
Naess et al. (2007)	Norway	Women	1.06 (1.00, 1.12)
Lipsett et al. (2011)	California	Women, NO_2	0.98 (0.88, 1.09)
Lipsett et al. (2011)	California	Women, NO_X	1.05 (0.99, 1.12)
Cao et al. (2011)[b]	China	NO_X	1.02 (1.01, 1.04)
Schikowski et al. (2007)	Germany		1.86 (1.26, 2.74)
Zhang et al. (2011)	China		5.43 (4.82, 6.16)
Cesaroni et al. (2013)	Italy		1.06 (1.04, 1.08)
Brunekreef et al. (2009)	the Netherlands	Full cohort	1.04 (0.96, 1.13)
Brunekreef et al. (2009)	the Netherlands	Case cohort	0.92 (0.77, 1.10)
Hart et al. (2010)	U.S.	Full cohort	1.09 (1.01, 1.17)
Hart et al. (2010)	U.S.	Excluding long haul drivers	1.14 (1.03, 1.25)
Jerrett et al. (2013)	U.S.		1.12 (1.02, 1.22)
Chen et al. (2013)	Canada		1.17 (1.10, 1.23)
Carey et al. (2013)	England		1.05 (1.00, 1.13)
Beelen et al. (2014b)	Europe	Pooled analysis, NO_2	1.02 (0.94, 1.12)
Beelen et al. (2014b)[c]	Europe	Pooled analysis, NO_X	1.02 (0.99, 1.06)
Cardiopulmonary Disease			
Abbey et al. (1999)	U.S.	Men	1.01 (0.93, 1.09)
Abbey et al. (1999)	U.S.	Women	1.02 (0.95, 1.09)
Krewski et al. (2000)	U.S.		1.01 (1.00, 1.02)
Filleul et al. (2005)	France	24 areas	1.00 (0.96, 1.04)
Filleul et al. (2005)	France	18 areas	1.16 (0.93, 1.45)
Gehring et al. (2006)	Germany	1-yr avg	1.70 (1.28, 2.26)
Gehring et al. (2006)	Germany	5-yr avg	1.92 (1.35, 2.71)
Heinrich et al. (2013)	Germany		1.67 (1.36, 2.05)

Table 6-16 (Continued): Corresponding risk estimates for Figure 6-9.

Study	Location	Notes	Hazard Ratio[a] (95% CI)
Wang et al. (2009b)	Australia		0.95 (0.74, 1.22)
Hoek et al. (2002)	the Netherlands		1.45 (0.99, 2.13)
Yorifuji et al. (2010)	Japan		1.32 (1.12, 1.54)
Krewski et al. (2000)	U.S.		1.17 (1.03, 1.34)
CHD			
Rosenlund et al. (2008)	Italy	Out of hospital	1.16 (1.04, 1.26)
Rosenlund et al. (2008)	Italy	In hospital	1.10 (0.94, 1.30)
Rosenlund et al. (2008)	Italy	Following nonfatal coronary event	0.91 (0.80, 1.04)
Gan et al. (2011)	Canada	NO_2	1.09 (1.02, 1.19)
Gan et al. (2011)	Canada	NO	1.09 (1.03, 1.15)
Carey et al. (2013)	England		0.98 (0.90, 1.07)
IHD			
Lipsett et al. (2011)	California	Women	1.07 (0.92, 1.24)
Lipsett et al. (2011)	California	Women, NO_X	1.09 (1.00, 1.19)
Krewski et al. (2000)	U.S.		1.02 (1.00, 1.03)
Cesaroni et al. (2013)	Italy		1.10 (1.06, 1.14)
Hart et al. (2010)	U.S.	Full cohort	1.01 (0.92, 1.11)
Hart et al. (2010)	U.S.	Excluding long haul drivers	1.07 (0.95, 1.21)
Yorifuji et al. (2010)	Japan		1.57 (1.04, 2.36)
Jerrett et al. (2013)	U.S.		1.17 (1.04, 1.31)
Nafstad et al. (2004)[b]	Norway	NO_X	1.08 (1.03, 1.12)
Chen et al. (2013)	Canada		1.19 (1.08, 1.30)
Carey et al. (2013)	England	MI only	1.00 (0.88, 1.13)
Beelen et al. (2014b)	Europe	Pooled analysis, all IHD; NO_2	1.00 (0.84, 1.18)
Beelen et al. (2014b)[c]	Europe	Pooled analysis all IHD; NO_X	1.02 (0.95, 1.09)
Beelen et al. (2014b)	Europe	Pooled analysis, MI only; NO_2	0.96 (0.79, 1.18)
Beelen et al. (2014b)[c]	Europe	Pooled analysis, MI only; NO_X	0.99 (0.90, 1.07)

Table 6-16 (Continued): Corresponding risk estimates for Figure 6-9.

Study	Location	Notes	Hazard Ratio[a] (95% CI)
Circulatory Disease			
Jerrett et al. (2009)	Canada		2.53 (1.27, 5.11)
Yorifuji et al. (2010)	Japan		1.30 (1.06, 1.59)
Cerebrovascular Disease			
Lipsett et al. (2011)	California	Women, NO_2	0.86 (0.71, 1.06)
Lipsett et al. (2011)	California	Women, NO_X	1.01 (0.90, 1.14)
Zhang et al. (2011)	China		5.35 (4.67, 6.11)
Cesaroni et al. (2013)	Italy		1.02 (0.98, 1.06)
Yorifuji et al. (2010)	Japan		1.18 (0.89, 1.57)
Nafstad et al. (2004)[b]	Norway	NO_X	1.04 (0.94, 1.15)
Chen et al. (2013)	Canada		0.92 (0.81, 1.10)
Beelen et al. (2014b)	Europe	Pooled analysis, NO_2	1.02 (0.87, 1.20)
Beelen et al. (2014b)[c]	Europe	Pooled analysis, NO_X	1.00 (0.93, 1.08)
Stroke			
Jerrett et al. (2013)	U.S.		1.20 (1.04, 1.39)
Carey et al. (2013)	England		1.00 (0.91, 1.09)
Heart Failure			
Carey et al. (2013)	England		1.09 (0.91, 1.32)
Diabetes			
Raaschou-Nielsen et al. (2013b)	Denmark		1.66 (0.96, 2.89)

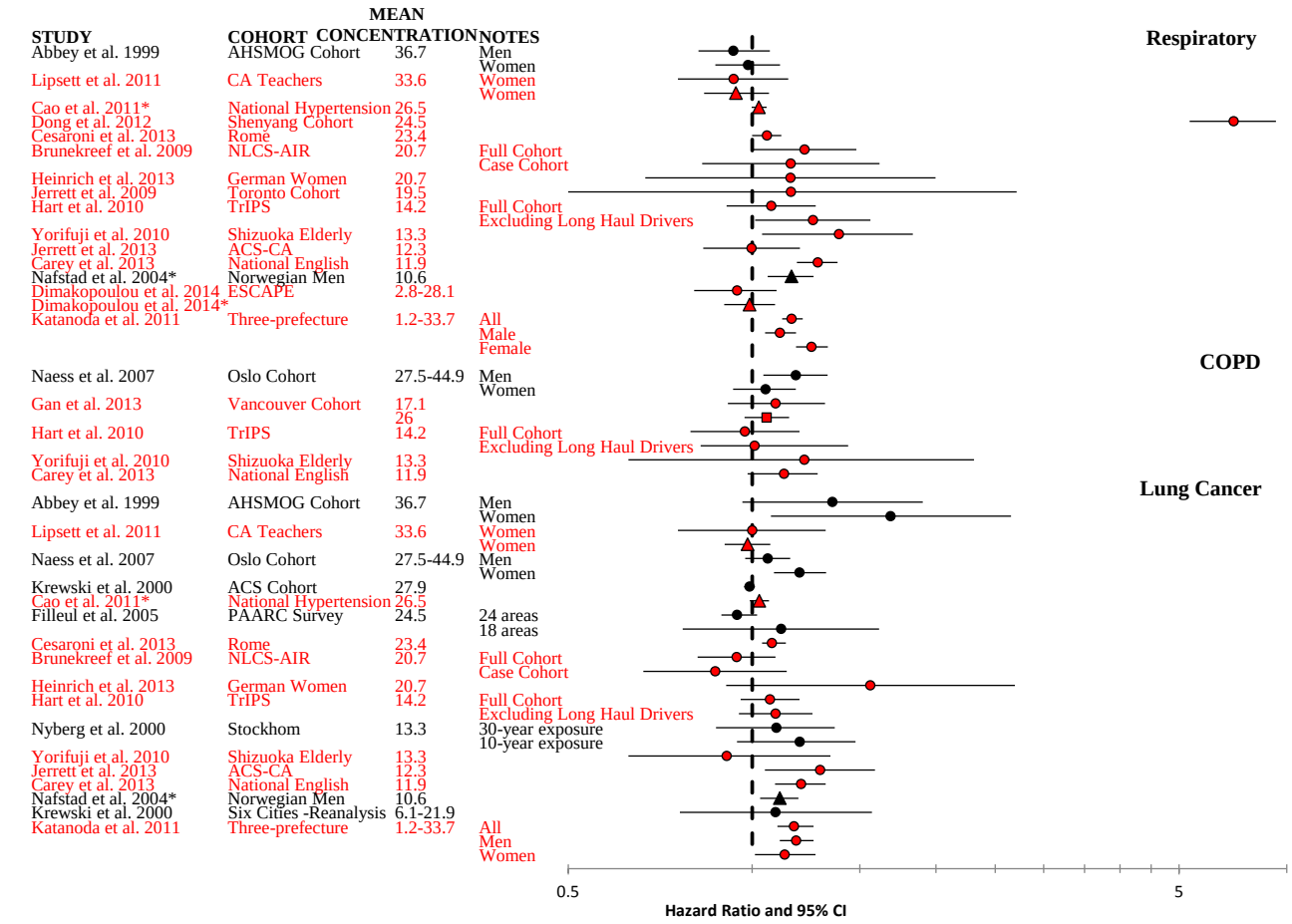
CHD = coronary heart disease; CI = confidence interval; IHD = ischemic heart disease; MI = myocardial infarction; NO = nitric oxide; NO_2 = nitrogen dioxide; NO_X = sum of NO and NO_2.

Note: Studies correspond to those presented in Figure 6-9.

[a]Effect estimates are standardized to a 10-ppb increase in NO_2 and NO, or a 20-ppb increase in NO_X concentration.

[b]NO_X measured in $\mu g/m^3$. Effect estimate is per 10 $\mu g/m^3$ increase.

[c]NO_X measured in $\mu g/m^3$. Effect estimate is per 20 $\mu g/m^3$ increase.



ACS = American Cancer Society; AHSMOG = California Seventh-day Adventists Cohort; ESCAPE = European Study of Cohorts for Air Pollution Effects; NLCS-AIR = Netherlands Cohort Study of Air Pollution and Mortality; PAARC = Air Pollution and Chronic Respiratory Diseases; TrIPS = Traffic Industry Particle Study.

Note: Red = recent studies; black = studies reviewed in the 2008 ISA for Oxides of Nitrogen. Hazard ratios are standardized to a 10-ppb increase in NO_2 and NO, and a 20-ppb increase in NO_X concentration. *Effect estimates from studies measuring NO_X in $\mu g/m^3$ have not been standardized. Studies are presented in descending order, with the largest mean concentration (ppb) at the top and the smallest at the bottom of the figure. Circles = NO_2; triangles = NO_X; squares = NO.

Figure 6-10 Results of studies of long-term exposure to nitrogen dioxide (NO_2), nitric oxide (NO), or the sum of NO and NO_2 (NO_X), and respiratory mortality.

Table 6-17 Corresponding risk estimates for Figure 6-10.

Study	Location	Notes	Hazard Ratio[a] (95% CI)
Respiratory			
Abbey et al. (1999)	U.S.	Men	0.93 (0.82, 1.07)
Abbey et al. (1999)	U.S.	Women	0.98 (0.87, 1.11)
Lipsett et al. (2011)	California	Women, NO_2	0.93 (0.76, 1.15)
Lipsett et al. (2011)	California	Women, NO_X	0.94 (0.83, 1.07)
Cao et al. (2011)[b]	China	NO_X	1.03 (1.00, 1.06)
Dong et al. (2012)	China		6.1 (5.2, 7.2)
Cesaroni et al. (2013)	Italy		1.06 (1.00, 1.12)
Brunekreef et al. (2009)	the Netherlands	Full cohort	1.22 (1.00, 1.48)
Brunekreef et al. (2009)	the Netherlands	Case cohort	1.16 (0.83, 1.62)
Heinrich et al. (2013)	Germany		1.15 (0.67, 2.00)
Jerrett et al. (2009)	Canada		1.16 (0.37, 2.71)
Hart et al. (2010)	U.S.	Full cohort	1.07 (0.91, 1.27)
Hart et al. (2010)	U.S.	Excluding long haul drivers	1.26 (1.01, 1.56)
Yorifuji et al. (2010)	Japan		1.39 (1.04, 1.83)
Jerrett et al. (2013)	U.S.		1.00 (0.83, 1.20)
Carey et al. (2013)	England		1.28 (1.18, 1.38)
Nafstad et al. (2004)	Norway	NO_X	1.16 (1.06, 1.26)
Dimakopoulou et al. (2014)	Europe	Pooled analysis, NO_2	0.94 (0.80, 1.10)
Dimakopoulou et al. (2014)[c]	Europe	Pooled analysis, NO_X	0.99 (0.90, 1.09)
Katanoda et al. (2011)	Japan	All	1.16 (1.12, 1.21)
Katanoda et al. (2011)	Japan	Men	1.11 (1.05, 1.18)
Katanoda et al. (2011)	Japan	Women	1.25 (1.18, 1.33)
COPD			
Naess et al. (2007)	Norway	Men	1.18 (1.04, 1.33)
Naess et al. (2007)	Norway	Women	1.05 (0.93, 1.18)

Table 6-17 (Continued): Corresponding risk estimates for Figure 6-10.

Study	Location	Notes	Hazard Ratio[a] (95% CI)
Gan et al. (2013)	Canada	NO_2	1.09 (0.91, 1.32)
Gan et al. (2013)	Canada	NO	1.06 (0.97, 1.15)
Hart et al. (2010)	U.S.	Full cohort	0.97 (0.79, 1.19)
Hart et al. (2010)	U.S.	Excluding long haul drivers	1.01 (0.82, 1.44)
Yorifuji et al. (2010)	Japan		1.22 (0.63, 2.31)
Carey et al. (2013)	England		1.13 (0.98, 1.28)
Lung Cancer			
Abbey et al. (1999)	U.S.	Men	1.35 (0.96, 1.90)
Abbey et al. (1999)	U.S.	Women	1.69 (1.07, 2.65)
Lipsett et al. (2011)	California	Women, NO_2	1.00 (0.76, 1.32)
Lipsett et al. (2011)	California	Women, NO_X	0.98 (0.90, 1.07)
Naess et al. (2007)	Norway	Men	1.06 (0.97, 1.15)
Naess et al. (2007)	Norway	Women	1.20 (1.09, 1.32)
Krewski et al. (2000)	U.S.		0.99 (0.97, 1.01)
Cao et al. (2011)b	China	NO_X	1.03 (0.99, 1.07)
Filleul et al. (2005)	France	24 areas	0.94 (0.89, 1.02)
Filleul et al. (2005)	France	18 areas	1.12 (0.77, 1.61)
Cesaroni et al. (2013)	Italy		1.08 (1.04, 1.14)
Brunekreef et al. (2009)	the Netherlands	Full cohort	0.94 (0.81, 1.09)
Brunekreef et al. (2009)	the Netherlands	Case cohort	0.87 (0.66, 1.14)
Heinrich et al. (2013)	Germany		1.56 (0.91, 2.69)
Hart et al. (2010)	U.S.	Full cohort	1.07 (0.96, 1.19)
Hart et al. (2010)	U.S.	Excluding long haul drivers	1.09 (0.95, 1.25)
Nyberg et al. (2000)	Sweden	30-yr exposure	1.10 (0.87, 1.36)
Nyberg et al. (2000)	Sweden	10-yr exposure	1.20 (0.94, 1.48)
Yorifuji et al. (2010)	Japan		0.91 (0.63, 1.34)
Jerrett et al. (2013)	U.S.		1.29 (1.05, 1.59)

Table 6-17 (Continued): Corresponding risk estimates for Figure 6-10.

Study	Location	Notes	Hazard Ratio[a] (95% CI)
Carey et al. (2013)	England		1.20 (1.09, 1.32)
Nafstad et al. (2004)[b]	Norway	NO_X	1.11 (1.03, 1.19)
Krewski et al. (2000)	U.S.		1.09 (0.76, 1.57)
Katanoda et al. (2011)	Japan	All	1.17 (1.10, 1.26)
Katanoda et al. (2011)	Japan	Men	1.18 (1.11, 1.26)
Katanoda et al. (2011)	Japan	Women	1.13 (1.01, 1.27)

CI = confidence interval; COPD = chronic obstructive pulmonary disease; NO = nitric oxide; NO_2 = nitrogen dioxide; NO_X = sum of NO and NO_2.

Note: Studies correspond to those presented in Figure 6-10.

[a]Effect estimates are standardized to a 10-ppb increase in NO_2 and NO or a 20-ppb increase in NO_X concentration.

[b]NO_X measured in $\mu g/m^3$. Effect estimate is per 10 $\mu g/m^3$ increase.

[c]NO_X measured in $\mu g/m^3$. Effect estimate is per 20 $\mu g/m^3$ increase.

6.5.3 Summary and Causal Determination

Collectively, the evidence is suggestive, but not sufficient, to infer a causal relationship between long-term exposure to NO_2 and mortality among adults. The strongest evidence comes from cohort studies conducted in the U.S., Canada, and Europe, which show consistent, positive associations with total mortality, as well as deaths due to respiratory and cardiovascular disease (Chen et al., 2013; Gan et al., 2013; Hart et al., 2013; Heinrich et al., 2013; Jerrett et al., 2013; Gan et al., 2011; Lipsett et al., 2011; Hart et al., 2010; Brunekreef et al., 2009; Jerrett et al., 2009; Beelen et al., 2008b; Schikowski et al., 2007; Krewski et al., 2000). The results from these studies are coherent with studies that have observed associations between long-term exposure to NO_2 and respiratory hospital admissions (Andersen et al., 2012a; Andersen et al., 2011) and cardiovascular effects (Lipsett et al., 2011; Hart et al., 2010). Additionally, the evidence for short- and long-term respiratory and cardiovascular morbidity provides some biological plausibility for mortality.

Many of the studies evaluating the associations between long-term exposure to NO_2 and mortality have used concentrations measured at central site monitors to assign exposure. A select number of recent studies have employed exposure assessment methods such as LUR to represent the spatial variability of NO_2. There was no distinguishable pattern or trend in the results of this body of evidence that could be attributed to the use of central site monitors or LUR in order to assign exposure. Exposure assessment was evaluated

drawing upon discussions in Sections 3.2 and 3.4.5. In general, LUR model predictions have been found to correlate well with outdoor NO_2 concentration measurements (Section 3.2.1.1), which may explain why the results for this evidence base were consistent across these exposure assessment types.

In past reviews, a limited number of epidemiologic studies had assessed the relationship between long-term exposure to NO_2 and mortality in adults. The 2008 ISA for Oxides of Nitrogen concluded that the scarce amount of evidence was "inadequate to infer the presence or absence of a causal relationship" (U.S. EPA, 2008). Recent studies provide evidence for an association between long-term exposure to NO_2 or NO_X and mortality from extended analyses of existing cohorts as well as original results from new cohorts in the U.S., Europe, and Asia. Recent studies have examined the potential for copollutant confounding by evaluating copollutant models that include $PM_{2.5}$, PM_{10}, $PM_{10-2.5}$, and SO_2 (Beelen et al., 2014a; Jerrett et al., 2013; Hart et al., 2010). These recent studies address a previously identified data gap. The NO_2 results from these models were generally attenuated with the inclusion of copollutants, though a key traffic-related copollutant (i.e., EC) was not evaluated. It remains difficult to disentangle the independent effect of NO_2 from the potential effect of the traffic-related pollution mixture or other components of that mixture.

While the results were generally consistent across studies, there were several well-designed, well-conducted studies that did not observe an association between long-term exposure to NO_2 and mortality (Beelen et al., 2014a; Beelen et al., 2014b; Dimakopoulou et al., 2014; Krewski et al., 2009; Pope et al., 2002; Abbey et al., 1999). All available evidence for mortality due to long-term exposure to NO_2 or NO_X was evaluated using the framework described in Table II of the Preamble. The key evidence as it relates to the causal determination is summarized in Table 6-18. The overall evidence is suggestive, but not sufficient, to infer a causal relationship between long-term exposure to NO_2 and total mortality among adults.

Table 6-18 Summary of evidence, which is suggestive, but not sufficient, to infer a causal relationship between long-term nitrogen dioxide (NO_2) exposure and total mortality.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_X or NO_2 Concentrations Associated with Effects[c]
High-quality epidemiologic studies are generally supportive but not entirely consistent	Positive association between long-term exposure to NO_2 and mortality in the HSC cohort and a subset of the ACS cohort, with effect estimates similar in magnitude to those observed with $PM_{2.5}$, even after adjustment for common potential confounders.	Krewski et al. (2000)	Mean concentrations across cities (1980): 6.1−21.9 ppb
		Jerrett et al. (2013)	Mean (1988−2002): 12.3 ppb
	Updated results from the NLCS report a positive association with total mortality, effects for respiratory mortality stronger than any observed with traffic variables and total or other cause-specific mortality.	Beelen et al. (2008b)	Mean (1987−1996): 20.7 ppb
		Brunekreef et al. (2009)	Max: 35.5 ppb
	Updated results from the German women's cohort report positive associations with total and cardiopulmonary mortality.	Heinrich et al. (2013)	Mean: 20.7 ppb
		Schikowski et al. (2007)	Median: 24.4 ppb
	Recent cohort studies in the U.S. observe increases in total mortality and mortality due to cardiovascular disease in separate cohorts of men and women.	Hart et al. (2010)	Mean (1985−2000): 14.2 ppb
		Lipsett et al. (2011)	Mean (1996−2005): 33.6 ppb; Max: 67.2 ppb
		Hart et al. (2013)	Median (2000): 13.9 ppb
	Positive associations with total, cardiovascular, respiratory, and lung cancer mortality in Canadian cities.	Chen et al. (2013)	Mean (across cities): 12.1−21.7 ppb
		Jerrett et al. (2009)	Median: 22.9 ppb
		Gan et al. (2011)	Mean: 17.0 ppb
		Gan et al. (2013)	Mean: 17.1 ppb

Table 6-18 (Continued): Summary of evidence, which is suggestive, but not sufficient, to infer a causal relationship between long-term nitrogen dioxide (NO2) exposure and total mortality.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_X or NO_2 Concentrations Associated with Effects[c]
Uncertainty remains regarding independent effects of NO_2	Associations with mortality generally attenuated with adjustment for PM_{10}, $PM_{2.5}$, $PM_{10-2.5}$, or SO_2, but analysis is limited and does not include other traffic-related copollutants (i.e., EC). When reported, correlations with copollutants were highly variable (low to high).	Beelen et al. (2014a), Jerrett et al. (2013), Hart et al. (2010)	Mean 2.8−31.7 ppb Mean (1988−2002): 12.3 ppb Mean (1985−2000): 14.2 ppb
Some studies show no association	No association in several reanalyses of the ACS cohort.	Krewski et al. (2000), Pope et al. (2002), Krewski et al. (2009)	Mean (1982−1998): 21.4−27.9 ppb Mean (1982−1998) 27.9 ppb; Max 51.1 ppb
	No association observed in a multicenter European study of pooled data from 22 existing cohort studies for total, respiratory, or cardiovascular mortality.	Beelen et al. (2014a), Dimakopoulou et al. (2014), Beelen et al. (2014b)	Range of means across cohorts: 2.8−31.7 ppb
	No association with total, cardiopulmonary, or respiratory mortality in the AHSMOG.	Abbey et al. (1999)	Mean (1973−1992): 36.8 ppb
Limited coherence with evidence for respiratory and cardiovascular morbidity	Limited evidence for respiratory hospitalizations in adults coherent with evidence for respiratory mortality.	Andersen et al. (2011), Andersen et al. (2012a)	35-yr mean: 9.0 ppb 25-yr mean: 9.5 ppb
	Some inconsistencies reported for cardiovascular morbidity. Evidence for MI and heart failure coherent with evidence for cardiovascular mortality.	Lipsett et al. (2011)	Mean: 33.6 ppb; Max: 67.2 ppb
		Atkinson et al. (2013)	Mean: 12.0 ppb; Max: 32.3 ppb

ACS = American Cancer Society; AHSMOG = Adventist Health Study of Smog; EC = elemental carbon; HSC = Harvard Six Cities; MI = myocardial infarction; NLCS = Netherlands Cohort Study on Diet and Cancer; 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; $PM_{10-2.5}$ = particulate matter with a nominal aerodynamic diameter less than or equal to 10 µm and greater than a nominal diameter of 2.5 µm; SO_2 = sulfur dioxide.

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Tables I and II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to causal determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where the full body of evidence is described.

[c]Describes the NO_X or NO_2 concentrations with which the evidence is substantiated (for experimental studies, below 5,000 ppb).

6.6 Cancer

The 1993 AQCD for Oxides of Nitrogen and the 2008 ISA for Oxides of Nitrogen reported that there was no clear evidence that NO_2 or other oxides of nitrogen act as a complete carcinogen. The U.S. Department of Health and Human Services, the International Agency for Research on Cancer, and the U.S. Environmental Protection Agency (EPA) have not classified nitrogen oxides for potential carcinogenicity. The American Conference of Industrial Hygienists has classified NO_2 as A4 (Not classifiable for humans or animals). The 2008 Oxides of Nitrogen ISA (U.S. EPA, 2008) included a few epidemiologic studies of oxides of nitrogen and cancer, both examining lung cancer incidence and reporting positive associations. Since the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008), additional studies have been published exploring this relationship. In addition, epidemiologic studies have been performed examining the relationship between NO_2 and leukemia, bladder cancer, breast cancer, and prostate cancer. These are all described in more detail in supplementary Table S6-10 (U.S. EPA, 2013j), which includes information on the exposure assessment and duration, as well as effect estimates. Many studies assigned exposure using local air monitors, but others derived exposure estimates using LUR and dispersion models. Details on these methods of exposure assessment can be found in Sections 3.2.1.1 and 3.2.3, respectively. Information in Section 3.4.5.2 aids interpreting these methods with regard to potential exposure measurement error.

6.6.1 Lung Cancer

6.6.1.1 Epidemiologic Studies

Lung Cancer Incidence

Two previous studies included in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) reported positive associations between NO_2 or NO_X and lung cancer incidence (Nafstad et al., 2003; Nyberg et al., 2000). Nyberg et al. (2000) reported an association between NO_2 and lung cancer at the highest 10-yr avg concentrations of NO_2 with a 20-year lag. This association was robust to inclusion of SO_2, which was not observed to be associated with lung cancer (Pearson correlation coefficient between SO_2 and NO_2 ranged from 0.5 to 0.7). Nafstad et al. (2003) performed a study with 24 years of follow-up and reported a positive association between NO_X concentrations and lung cancer incidence during the

early years of the study, but the authors report more recent years had weaker associations (results were not provided). The Pearson r between NO_X and SO_2 was 0.63, and no association was observed between SO_2 concentration and cancer.

A recent study examined the association between NO_2 concentration and lung cancer incidence within the NLCS using over 11 years of follow-up (Brunekreef et al., 2009; Beelen et al., 2008a). The researchers observed no association in analyses using case-cohort and full cohort approaches. The associations between lung cancer and SO_2 (correlation coefficient with NO_2 >0.6) and $PM_{2.5}$ (correlation coefficient with NO_2 >0.8) were also examined and found to be null.

A meta-analytical study in Europe combined individual estimates from cohort studies across nine countries in Europe (Raaschou-Nielsen et al., 2013a). Although positive associations between concentrations of NO_X and NO_2 and lung cancer were detected in models adjusting for age, sex, and calendar time, these associations became null when other confounders, such as smoking-related covariates, fruit intake, and area-level SES, were included. No associations were observed for $PM_{2.5}$, $PM_{2.5}$ absorbance, and $PM_{10-2.5}$, but PM_{10} was positively associated with lung cancer.

A Danish study combined three cohorts and reported an association between increased NO_X concentrations and lung cancer incidence (Raaschou-Nielsen et al., 2010a). This increased incidence with NO_X exposure persisted in some models of specific cancer types, such as squamous cell carcinomas. When examining the associations by sex, length of education, and smoking status, the precision was decreased (i.e., wider 95% CI), and no differences were observed between the groups. One of these cohorts was used in another study where the follow-up period was extended 5 years to include more cases (Raaschou-Nielsen et al., 2011b). This study detected an increased incidence rate of lung cancer in the highest quartile of NO_X concentrations. Further analyses evaluated interactions with sex, smoking status, length of school attendance, and daily fruit intake. An increased association between NO_X concentration and lung cancer incidence was observed among individuals with at least 8 years of schooling, but no association was apparent among those with less schooling.

A case-control study of molecular changes and genetic susceptibility in relation to air pollution reported on nonsmokers and lung cancer incidence (Papathomas et al., 2011). This study used multiple statistical analysis techniques to evaluate the associations between air pollutants and lung cancer incidence. Although profile regression analyses reported higher NO_2 exposures for the higher risk grouping, logistic regression analyses did not find an association between NO_2 and lung cancer incidence. The same was true of PM_{10}. In another statistical model by the authors, NO_2 was not chosen as a predictor, whereas PM_{10} concentration was chosen.

A study in Canada reported a positive association between NO_2 concentrations from national spatiotemporal models and lung cancer incidence (Hystad et al., 2013). When examining types of lung cancer, the association was present for adenocarcinomas but not squamous cell carcinoma. Confidence intervals for estimates of small cell and large cell carcinomas were wide and included the null. Associations were not present between O_3 and lung cancer. $PM_{2.5}$ demonstrated some associations with lung cancer incidence, especially in the third and fourth, but not fifth, quintiles of exposures. When NO_2 and O_3 were considered in copollutant models, the ORs increased for both pollutants. Copollutant models were not examined for NO_2 and $PM_{2.5}$ because of the high correlation between the pollutants. Odds of lung cancer increased in association with NO_2 concentrations when the analysis was limited to the closest monitor within 50 km, but the authors state that NO_2 estimates, "are also capturing a component of $PM_{2.5}$, due to the correlation between the two pollutants." The authors also believe it could be the result of more accurate exposure assessment or restriction of the study area. In stratified analyses, associations appear to be greater among men, with null results among women. No differences were clear in stratified analyses of education or smoking status. Another Canadian study also reported a positive association between NO_2 concentrations and lung cancer when using a population-based control group (Villeneuve et al., 2014). The association was present when using NO_2 concentration at the time of the interview, from 10 years prior, and from a time-weighted average. However, in analyses adjusted for personal (e.g., age, smoking, BMI) and ecological (e.g., neighborhood unemployment rate) covariates using hospital-based and population-based controls, no association was detected. Associations were observed between lung cancer and benzene and total hydrocarbons when using population-based controls but only for total hydrocarbons and time-weighted average of benzene when using all controls.

An ecologic study examined NO_2 and NO concentrations in relation to lung cancer rates (Tseng et al., 2012). No associations were observed for NO_2 concentrations and all lung cancer cases combined, adenocarcinomas, or squamous cell carcinomas. A positive association was observed in the highest quartile of NO for adenocarcinomas but not for squamous cell carcinomas. Associations were also reported for SO_2 concentrations but not O_3 concentrations, CO concentrations, or PM_{10} concentrations.

In summary, multiple studies have examined the associations between concentrations of oxides of nitrogen and lung cancer incidence. Positive associations were reported in multiple studies, but other studies reported no associations. The inconsistency observed between studies does not appear to be related to the length of the exposure or follow-up period. Inconsistency is observed with NO_2 exposure assessed from central site monitors/spatiotemporal pollutant models (Brunekreef et-al., 2009; Beelen et-al., 2008a, Papathomas et-al., 2011, Hystad et-al., 2013, Tseng et-al., 2012), NO_2 estimated for

subject's residences using LUR (Raaschou-Nielsen et-al., 2013, Villeneuve et-al., 2014), and NO_2 estimated for subject's residences using dispersion models (Raaschou-Nielsen et-al., 2010a, Raaschou-Nielsen et-al., 2011b, Nafstad et-al., 2003; Nyberg et-al., 2000). Given the differences among the study designs, it cannot be concluded that the inconsistencies are related to exposure assessment method or length of follow-up periods. In general, studies using central site monitors for exposure estimates carry uncertainty in long-term NO_2 exposure studies because the exposure error resulting from spatial misalignment between subjects' and monitor locations can overestimate or underestimate associations with health effects (Section 3.4.5.2). However, an association was observed with residential NO_2 exposure estimated for subjects' homes, and the improved spatial resolution of the exposure estimate lends more confidence in the association.

Lung Cancer Mortality

Two HEI Research Reports have investigated the association between NO_2 concentration and lung cancer mortality using large cohorts with follow-ups of at least 10 years. Brunekreef et al. (2009) (see also, Beelen et al., 2008b) reported no association between NO_2 and lung cancer mortality using the NLCS, and results were not changed with the inclusion of a traffic-intensity variable. No association was observed between lung cancer mortality and other pollutants (SO_2, correlation coefficient with NO_2 >0.6, or $PM_{2.5}$, correlation coefficient with NO_2 >0.8). Krewski et al. (2009) utilized an extended follow-up of the American Cancer Society Study and reported no associations between NO_2 and lung cancer mortality. An association with lung cancer mortality for $PM_{2.5}$ was noted in this report but not for CO, O_3, or SO_2. However, a study utilizing the American Cancer Society's Cancer Prevention Study II cohort reported a positive association between NO_2 concentrations and lung cancer mortality (Jerrett et al., 2013). Pearson correlation coefficients were about 0.55 for the association between NO_2 and $PM_{2.5}$ as well as for the association between NO_2 and O_3. The positive association between NO_2 and lung cancer mortality was robust to adjustment with $PM_{2.5}$ or O_3, although when both $PM_{2.5}$ and O_3 were included in the model with NO_2, the 95% CIs widened.

Inconsistent findings between NO_2 and lung cancer mortality have been reported in studies conducted across Europe. A positive association was observed between NO_2 and lung cancer mortality in a large study conducted in Rome, Italy (Cesaroni et al., 2013). The association demonstrated a linear relationship. No effect measure modification was apparent by age, sex, educational level, area-based socioeconomic position, or moving history. NO_2 was highly correlated with $PM_{2.5}$, which was also associated with lung cancer mortality. In England, a study using a large nationally representative database reported a positive association between NO_2 concentrations and lung cancer mortality (Carey et al., 2013). Among other pollutants, no associations were observed in fully

adjusted models for PM_{10}, $PM_{2.5}$, and O_3. SO_2 concentrations were positively associated with lung cancer mortality in some adjusted analyses. A study in France reported a positive association between NO_2 and lung cancer mortality only after exclusion of areas with air monitoring sites reporting a high ratio of NO to NO_2 [which implied a strong influence of heavy traffic near the monitor that may not represent the air pollution concentrations in the entire area (Filleul et al., 2005)]. Correlations between NO_2 and other air pollutants ranged from 0.22 to 0.86. No other air pollutants examined in the study (SO_2, total suspended particles, black smoke, and NO) were associated with lung cancer mortality. A study in Norway examined 4 years of air pollution and mortality data (Naess et al., 2007). Positive associations between NO_2 and lung cancer mortality were observed among women aged 51−70 years and 71−90 years but not among men in these age groups (although a positive association was reported in the crude HR for 71−90 year-old men). Correlations between the pollutants examined (NO_2, PM_{10}, and $PM_{2.5}$) were not reported individually but ranged from 0.88 to 0.95. Associations between lung cancer and the other pollutants were similar to those observed for NO_2. In a nonparametric smooth analysis that combined the sexes, the increase in log odds for lung cancer appears to begin around 21.3 ppb for 51−70 year-olds while the increase appears to be at lower concentrations among those aged 71−90 years. A large study of women from Germany followed up women who were originally enrolled in cross-sectional studies in the 1980s and 1990s (Heinrich et al., 2013). Using NO_2 concentration from their address at the baseline examination, the authors reported no association between NO_2 concentration and lung cancer mortality. The Spearman's correlation coefficient for PM_{10}, which was observed to be associated with lung cancer, and NO_2 was 0.5. A large cohort of men employed by the U.S. trucking industry in 1985 were matched to records in the National Death Index through 2000 (Hart et al., 2011). Using NO_2 concentrations at their residential address, the association with lung cancer mortality was examined. No association was detected, and this persisted when long-haul drivers who are away from the home at least one night per week were excluded from the analyses. Similar results were observed for PM_{10} and SO_2.

Multiple studies of NO_2 and lung cancer mortality have been conducted in Asia. A study in Japan followed individuals aged 65−84 years at enrollment for about 6 years (Yorifuji et al., 2010). No overall association was reported between NO_2 concentration and lung cancer mortality. In stratified analyses, the association between NO_2 concentration and lung cancer mortality was higher among nonsmokers compared to former/current smokers, but the findings were imprecise and the 95% confidence intervals overlapped. No difference in the association was observed among other stratification variables (age, sex, BMI, hypertension, diabetes, and financial ability). A continuation of this study was conducted using additional years of follow-up and NO_2 concentrations assigned at the year of the outcome (Yorifuji et al., 2013). This follow-up reported a positive association

between NO_2 concentration and lung cancer mortality. Results were similar for nonsmokers compared to former and current smokers. When examining exposure over various years (ranging from 1 year before death to an average from baseline to death), the association remained. Another study in Japan followed individuals for 10 years and observed a positive association between NO_2 concentration and lung cancer mortality (Katanoda et al., 2011). An association was also observed for suspended PM (Pearson correlation coefficient with NO_2 = 0.26) but not for SO_2. When the association between NO_2 concentration and lung cancer mortality was examined by region, the association appears to persist only in the areas of study with the highest NO_2 concentration (data on association by region only presented in figures; numerical estimates not provided). A national study of urban areas in China had a follow-up of less than 10 years and reported no association between NO_X and lung cancer mortality (Cao et al., 2011). This lack of association was robust to inclusion of TSP or SO_2, of which SO_2 concentrations were found to be associated with lung cancer mortality. A study performed in Taiwan used a case-control approach, comparing women who died of lung cancer or other nonrespiratory related causes (Liu et al., 2008). The highest tertile of NO_2 concentration was positively associated with lung cancer mortality. Associations between pollution concentrations and lung cancer mortality were also observed for CO, but not SO_2, PM_{10}, or O_3. A combined exposure category was created, examining those women with estimated exposure concentrations of CO and NO_2 in the highest tertiles compared to those in the lowest tertiles. The results were similar to those of the single-pollutant estimates.

Overall, there are inconsistent findings among studies of NO_2 and lung cancer mortality (see Table S6-10 for quantitative results). Most of these studies controlled for confounders, such as smoking. Inconsistency is observed with NO_2 exposure assessed from central site monitors/spatiotemporal pollutant models (Brunekreef et al., 2009; Beelen et al., 2008a,, Krewski et al. (2009), Filleul et al., 2005, Heinrich et al., 2013, Katanoda et al., 2011, Cao et al., 2011, Liu et al., 2008), NO_2 estimated for subject's residences using LUR (Cesaroni et al., 2013, Jerrett et al., 2013, Hart et al., 2011, Yorifuji et al., 2010, Yorifuji et al., 2013), and NO_2 estimated for subject's residences using dispersion models (Carey et al., 2013, Naess et al., 2007). Given the differences among the study designs, it cannot be concluded that the inconsistencies are related to exposure assessment method or length of follow-up periods. In general, studies using central site monitors for exposure estimates carry uncertainty in long-term NO_2 exposure studies because the exposure error resulting from spatial misalignment between subjects' and monitor locations can overestimate or underestimate associations with health effects (Section 3.4.5.2). However, an association was observed with residential NO_2 exposure estimated for subjects' homes, and the improved spatial resolution of the exposure estimate lends more confidence in the association.

6.6.1.2 Animal Toxicological Studies

Lung Tumors with Co-exposure with Known Carcinogens

The 1993 AQCD for Oxides of Nitrogen and the 2008 ISA for Oxides of Nitrogen detailed NO_2 co-exposure with known carcinogens. NO_2 has been reported to act as a tumor promoter at the site of contact, i.e., in the respiratory tract after inhalation exposure. Toxicological studies of NO_2 and carcinogenicity and genotoxicity are described in Table 6-18. This is consistent with mechanistic evidence of observed hyperplasia of respiratory epithelium with NO_2 exposure (see Section 6.2.6). Rats injected with the carcinogen N-bis (2-hydroxy-propyl) nitrosamine (BHPN) and continuously exposed to 40, 400, or 4,000 ppb NO_2 for 17 months developed a nonstatistically significant fivefold increase in incidence of adenomas or adenocarcinomas of the lungs versus control animals [4,000 ppb NO_2; (Ichinose et al., 1991)]. Another study by the same lab (Ichinose and Sagai, 1992) showed statistically significant increases in BHPN-induced lung tumors with combined NO_2 + O_3 exposure, a multipollutant effect absent with exposure to either single pollutant (BHPN injection followed the next day by either clean air 0% NO_2, 500 ppb NO_2, 50 ppb NO_2 + 400 ppb O_3, or 400 ppb O_3 + 1 mg/m^3 H_2SO_4 for 13 months, and then recovery with clean air for another 11 months); continuous NO_2 exposure, 11 h/day H_2SO_4, or O_3 exposure).

Another study with coexposure of F344 male rats to diesel exhaust particle extract-coated carbon black particles (DEPcCBP) and NO_2 and/or SO_2 found significantly increased incidences of lung tumors (alveolar adenomas) for the animals coexposed to DEPcCBP and NO_2 and/or SO_2 but not in those with DEPcCBP exposure alone (Ohyama et al., 1999). The National Toxicology Program's Report on Carcinogens has stated DEP is reasonably anticipated to be a human carcinogen (NTP, 2011). Exposed rats received intratrachael (IT) installation of DEPcCBP once per week for 4 weeks, and 6,000 ppb NO_2, 4,000 ppb SO_2, or 6,000 ppb NO_2 + 4,000 ppb SO_2 was administered 16 h/day for 8 months, and followed by 8 months of clean air exposure.

Lung Tumors in Animals with Spontaneously High Tumor Rates

The previous ISA and AQCDs described studies in animals with spontaneously high tumor rates including strain A/J mice, AKR/cum mice, and CAF1/Jax mice. Strain A/J mice exposed to 10,000 ppb NO_2 for 6 h/day, 5 days/week for 6 months (Adkins et al., 1986) had a small, but statistically significant increase in pulmonary adenomas (increased tumor multiplicity) with NO_2 exposure (1,000 and 5,000 ppb NO_2 had no effect). In another study, increased survival rates of NO_2-exposed animals were reported in a model of spontaneous T cell lymphoma, i.e., AKR/cum mice that were exposed intermittently

(7 h/day, 5 days/week) to 250 ppb NO_2 for up to 26 weeks (Richters and Damji, 1990). Another study using CAF1/Jax mice (Wagner et al., 1965) showed that continuous exposure to 5,000 ppb NO_2 produced significant increases in the number of year-old animals with pulmonary tumors when compared with control; this finding was no longer significant at 14 or 16 months exposure.

Facilitation of Lung Cancer Metastases

The previous ISA and AQCDs summarized a group of experiments by one lab that focused on the role of NO_2 in metastases facilitation. Richters and Kuraitis (1981), Richters and Kuraitis (1983), and Richters et al. (1985) exposed mice to multiple concentrations and durations of NO_2, and after exposure, the mice were injected intravenously (I.V.) with the B16 melanoma cell line. Lung tumors were then counted, with results of some of the experiments showing significantly increased numbers of tumors.

Genotoxicity in Airway Cells

Ex vivo exposure of human nasal epithelial mucosa cells cultured at the air-liquid interface to 10 ppb NO_2 (Koehler et al., 2013; Koehler et al., 2010) or 100 ppb NO_2 (Koehler et al., 2011) produced increased deoxyribonucleic acid (DNA) fragmentation measured with the single cell gel electrophoresis (COMET) assay as early as 30 minutes after exposure and micronuclei formation after 3-hour exposure to 100 ppb NO_2 (Koehler et al., 2011). Percentage of DNA content in the tail as detected with the COMET assay decreased with increasing exposure duration [0.5, 1, 2, and 3-hour exposure; (Koehler et al., 2013)]. Of the in vivo assays reported in the previous ISA [see U.S. EPA (2008); Annex Tables 4-11, 4-12, and 4-13, on pages 4-36 and 4-37 of the 2008 Annex], results were mixed with positive findings of genotoxicity seen in two studies that employed rat lung cells (mutations and chromosome abnormalities, 50,000−560,000 ppb NO_2 >12 days; 27,000 ppb NO_2, 3 hours) and negative findings of genotoxicity seen in tests employing Drosophila recessive lethals (500,000−7,000,000 ppb NO_2, 1 hour), Drosophila wing spot test (50,000−280,000 ppb NO_2, 2 days), mouse bone marrow micronuclei (20,000 ppb, 23 hours), and mouse spermatocyte and lymphocyte chromosomal aberrations (100−10,000 ppb NO_2, 6 hours). In vitro exposures to NO_2 yielded positive findings in a majority of the tests in rodent (2,000−3,000 ppb NO_2, 10 minutes) and human cell lines, bacteria (5,000−90,000 ppb NO_2, 30 minutes), and plants (5,000 ppb NO_2, 24 hours).

Table 6-19 Animal toxicological studies of carcinogenicity and genotoxicity with exposure to nitrogen dioxide (NO_2).

Reference	Concentration NO_2	Species (Strain); Age; Sex; n	Exposure Conditions	Endpoints Examined
Koehler et al. (2013)	10 ppb	Human cells	Ex vivo cell culture at the air liquid interface, primary human nasal epithelia cells from n = 10 donors, NO_2 exposure for 0, 0.5, 1, 2 and 3 h.	Comet assay, micronucleus formation, proliferation assay, apoptosis, necrosis, cytotoxicity.
Koehler et al. (2010)	100, 1,000, or 10,000 ppb	Human cells	Ex vivo cell culture at the air liquid interface, primary human nasal epithelia cells from n = 10 donors, NO_2 exposure for 0 or 0.5 h.	Comet assay, micronucleus formation, proliferation assay, cytotoxicity.
Koehler et al. (2011)	100 ppb	Human cells	Ex vivo cell culture at the air liquid interface, primary human nasal epithelia cells from n = 10 donors, NO_2 exposure for 0, 0.5, 1, 2, and 3 h.	Comet assay, micronucleus formation, proliferation assay, apoptosis, necrosis, cytotoxicity.
Ohyama et al. (1999)	1,000, 5,000, or 6,000 ppb	Rats (F344); Adult M, n=26	Exposure to DEPcCBP and NO_2. IT installation of DEPcCBP 1 x/week for 4 weeks. 6,000 ppb NO_2 was administered 16 h/day for 8 mo, and followed by 8 mo of clean air exposure.	Lung tumor incidence (alveolar adenomas).
Adkins et al. (1986)	10,000 ppb	Mice (A/J); adult F, n=30.	Exposure of mice with spontaneous high tumor rates to NO_2 for 6 h/day, 5 days/week for 6 mo.	Lung tumor multiplicity (pulmonary adenomas).
Richters and Damji (1990)	250 ppb	Mice (AKR/cum), adult female, n=50	Exposure of mice intermittently (7 h/day, 5 days/week) to NO_2 for up to 26 weeks.	Rodent survival rate.
Wagner et al. (1965)	1000, or 5,000 ppb	Mice (CAF1/Jax), adult male, n=20.	Continuous exposure to 1,000 or 5,000 ppb NO_2.	Lung tumor multiplicity at 12, 14, and 16 mo.

Table 6-19 (Continued): Animal toxicological studies of carcinogenicity and genotoxicity with exposure to nitrogen dioxide (NO2).

Reference	Concentration NO_2	Species (Strain); Age; Sex; n	Exposure Conditions	Endpoints Examined
Richters and Kuraitis (1981)	400 or 800 ppb	Mice (Swiss Webster); adult M; n = 24 Mice (C57BL/6J); Adult M; n = 90	NO_2 exposure 8 h/day, 5 days/week for 10 weeks (Swiss mice) or 12 weeks (C57BL/6J mice); then all animals were infused i.v. with B16 melanoma cells that are known to metastasize to the lung. 3 weeks post-infusion, animals were sacrificed and lungs scored for tumor incidence.	Facilitation of lung tumor metastasis (incidence of lung tumors).
Richters and Kuraitis (1983)	300, 400, or 500 ppb	Mice (C57BL/6J); adult M; n = 25, 51, 23	NO_2 exposure 7 h/day, 5 days/week for 10 weeks. Then all animals were infused i.v. with B16 melanoma cells that are known to metastasize to the lung. 3 weeks post-infusion, animals were sacrificed and lungs scored for tumor incidence.	Facilitation of lung tumor metastasis (incidence of lung tumors).
Richters et al. (1985)	400 ppb	Mice (C57BL/6J); adult M, n=48.	12 weeks of continuous exposure to NO_2. Then all animals were infused i.v. with B16 melanoma cells. 3 weeks post-infusion, animals were sacrificed and lungs scored for tumor incidence.	Facilitation of lung tumor metastasis (incidence of lung tumors).
Ichinose et al. (1991)	40, 400, or 4,000 ppb	Rats (Wistar); adult M; n=30.	Coexposure with carcinogen BHPN and NO_2. NO_2 exposure for 17 mo.	Incidence of BHPN-induced lung tumors (adenoma or adenocarcinomas).
Ichinose and Sagai (1992)	500 ppb NO_2; 50 ppb NO_2 + 400 ppb O_3	Rats (Wistar); adult M; n=36.	Carcinogen exposure plus air pollutant mixture exposure (O_3 + NO_2). 500 ppb NO_2, 50 ppb NO_2 + 400 ppb O_3, for 13 mo, and then recovery with clean air for another 11 mo; continuous NO_2 exposure, 11 h/day O_3 exposure.	Incidence of BHPN-induced lung tumors (adenoma or adenocarcinomas).

DEPcCBP = diesel exhaust particle extract-coated carbon black particles; COMET = single cell gel electrophoresis; I.V. = intravenously; IT = intratrachael instillation; BHPN = N-bis(2-hydroxypropvl) nitrosamine; NO_2 = nitrogen dioxide; O_3 = ozone.

6.6.2 Leukemia Incidence and Mortality

A study of acute leukemia incidence identified cases from the French National Registry of Childhood Blood Malignancies. Controls were randomly selected from the population with a distribution of age and sex that matched that of the cases (Amigou et al., 2011). NO_2 concentrations over 6.5 ppb were positively associated with the odds of leukemia incidence. This was also observed for specific types of leukemia. There was no difference in results based on urban or rural residence. The authors stated that results were strengthened when including only children who had been in the residences utilized in the study for at least 2 years (data not included in the paper). In a case-control study in Italy, no association was observed between NO_2 concentrations and leukemia incidence (Badaloni et al., 2013). This was true in analyses limited to children aged 0−4 years and children who never moved. NO_2 concentrations were highly correlated with $PM_{2.5}$ (0.78) and inversely correlated with O_3 (−0.74). No association was reported between leukemia incidence and these other pollutants. A study in Denmark reported no association between NO_X and leukemia incidence (Raaschou-Nielsen et al., 2011a). One key difference between this and the other studies described in this section is the age of the study participants, with this study including adults, whereas the prior studies included children.

A study performed in the U. S. examined the associations between NO, NO_2, and NO_X concentrations during pregnancy with multiple cancers among children ages 0−5 years old (Ghosh et al., 2013). The exposure period examined the overall pregnancy, as well as each trimester. Positive associations were observed between NO, NO_2, and NO_X and acute lymphoblastic leukemia for the entire pregnancy and the first and second trimesters but not the third trimester. Similar associations were not reported for acute myeloid leukemia; results were null for all oxides of nitrogen during all trimesters.

A study in Taiwan matched children with a cause of death related to leukemia to children with a cause of death unrelated to neoplasms or respiratory problems based on sex, year of birth, and year of death (Weng et al., 2008). NO_2 concentrations were positively associated with the odds of leukemia mortality.

These studies used multiple methods of exposure assessment, such as LUR (Badaloni et-al., 2013; Ghosh et-al., 2013), dispersion models (Raaschou-Nielsen et al., 2011a), and air monitors (Weng et al., 2008). There were not enough studies within each type of exposure metric to examine consistency across studies. Given the differences among the study designs, it cannot be concluded that the inconsistencies are related to exposure assessment method or length of follow-up periods. In general, studies using central site monitors for exposure estimates carry uncertainty in long-term NO_2 exposure studies because the exposure error resulting from spatial misalignment between subjects' and

monitor locations can overestimate or underestimate associations with health effects (Section 3.4.5.2). However, an association was observed with residential NO_2 exposure estimated for subjects' homes, and the improved spatial resolution of the exposure estimate lends more confidence in the association.

6.6.3 Bladder Cancer Incidence and Mortality

A study in Denmark examined the association between NO_X concentration and bladder cancer incidence (Raaschou-Nielsen et al., 2011a). This longitudinal study calculated incidence rate ratios for various types of cancer, and no association was demonstrated between NO_X concentration and bladder cancer incidence.

A study performed in Taiwan examined mortality records, comparing individuals (matched on sex, year of birth, and year of death) with and without mortality due to bladder cancer (Liu et al., 2009). Increased odds of bladder cancer mortality was associated with increased NO_2 concentrations. This trend was also observed for SO_2. The highest tertile of PM_{10} concentration was also associated with bladder cancer mortality, but no association was observed for CO or O_3 concentrations. Liu et al. (2009) further examined a three-level variable, with the lowest level being individuals in the lowest tertile of SO_2 and NO_2 concentrations (≤4.32 ppb and ≤20.99 ppb, respectively), the highest level being individuals in the highest tertile of SO_2 and NO_2 concentrations (>6.49 ppb and >27.33 ppb, respectively), and all others being categorized in the middle. The resulting ORs, adjusted for urbanization of residential area and marital status, were 1.37 (95% CI: 1.03, 1.82) for the middle level and 1.98 (95% CI: 1.36, 2.88) for the highest level. Although the point estimates for NO_2 and SO_2 combined are higher than those observed for NO_2 or SO_2 alone [see Supplemental Table S6-10; (U.S. EPA, 2013j)], the 95% confidence intervals overlap. Therefore, the conclusion that NO_2 and SO_2 combined contribute to higher odds of mortality than either alone cannot be drawn.

6.6.4 Breast Cancer Incidence

A Canadian study of post-menopausal breast cancer incidence using a hospital-based case-control study design estimated NO_2 concentrations at residential addresses using two methods: (1) extrapolating data from fixed-site monitoring stations or (2) extrapolating data from predicted concentrations determined with LUR using a dense network of air samplers (Crouse et al., 2010). Although point estimates were elevated for some of the associations between NO_2 concentrations and post-menopausal breast cancer incidence, most of the associations were not statistically significant (see Table S6-10). In sensitivity

analyses limited to subjects who were residents of the same address for at least 10 years prior to the study, the point estimates were slightly higher, but precision was reduced. The results of this study may be biased due to the selection of controls, which were hospital-based and limit the generalizability of the study. This study suggests a possible association between post-menopausal breast cancer incidence and NO_2 concentration. A study in Denmark reported no association between NO_X and breast cancer incidence (Raaschou-Nielsen et al., 2011a). This study, unlike the one performed in Canada, included all breast cancer cases instead of limiting to post-menopausal cases.

An ecologic study was performed by Wei et al. (2012) using data from the Surveillance, Epidemiology, and End Results program to determine the breast cancer incidence rate of various states and metropolitan areas and data from the EPA's Geographic Area AirData to determine NO_X emissions. Results of Pearson's correlations demonstrated a relationship between NO_X emissions and breast cancer incidence. The state with the highest NO_X emissions also had the highest breast cancer incidence rate, and the state with the lowest emissions had the lowest breast cancer incidence rate. However, this study is limited by its ecologic nature and the lack of individual-level data. There is no control for potential confounders or examination of factors other than air pollutants (of which CO, SO_2, and VOCs, but not PM_{10}, also had positive correlations) that could be associated with breast cancer incidence rates.

6.6.5 Prostate Cancer Incidence

Men enrolled in the Prostate Cancer and Environment Study were included in an investigation of NO_2 concentration and prostate cancer incidence (Parent et al., 2013). Cases were men diagnosed with prostate cancer and recruited through pathology departments. Population-based controls were identified through electoral lists and frequency matched by 5-year age groups. A positive association was observed between recent NO_2 concentration and odds of prostate cancer. The association was also observed using back-extrapolated estimates of NO_2 10 years prior. Multiple sensitivity analyses were performed, including back-extrapolation of NO_2 estimates for 20 years, addition of smoking and alcohol consumption as confounders, exclusion of proxy subjects, exclusion of subjects without a prostate cancer screening in the past 5 years, exclusion of subjects at their residence for less than 10 years, and comparisons of subjects with geo-coding to their exact address or to a centroid of their postal code. The results, while not always statistically significant (in some parts due to decreases in sample size and precision), were similar to the overall results reported. However, a study in Denmark examined the association between NO_X concentration and prostate cancer incidence using the Diet,

Cancer and Health cohort study (Raaschou-Nielsen et al., 2011a). No association was reported between prostate cancer and NO_X concentrations in the study.

6.6.6 Other Cancers Incidence and Mortality

A study in Denmark utilized participants in the Diet, Cancer and Health cohort and examined the relationship between NO_X concentrations and multiple cancers, including many not examined in other studies (Raaschou-Nielsen et al., 2011a). A positive association was demonstrated between NO_X concentrations and cervical and brain cancer incidences. No association was observed between NO_X concentrations and buccal cavity/pharynx cancer, esophageal cancer, stomach cancer, colon cancer, rectal cancer, liver cancer, pancreatic cancer, laryngeal cancer, uterine cancer, ovarian cancer, kidney cancer, melanoma, non-Hodgkin lymphoma, or myeloma.

A study performed in the United States examined maternal exposure to air pollution (i.e., NO, NO_2, and NO_X concentrations during pregnancy) and multiple cancers in children at age 0−5 years (Ghosh et al., 2013). Elevated point estimates were observed for NO and NO_X, but not NO_2, in the third trimester and incidence of bilateral retinoblastoma. Null associations were found for all other cancers examined with NO_X (non-Hodgkin lymphoma, central nervous system tumors, ependymoma, astrocytoma, intracranial and intraspinal embryonal tumors, primitive neuroectodermal tumor, other gliomas, neuroblastoma, retinoblastoma, unilateral retinoblastoma, Wilms tumor, hepatoblastoma, rhabdomyosarcomas, germ cell tumors, extracranial and extragonadal germ cell tumors, and teratoma).

Finally, a study in Canada reported the association between daily NO_2 concentrations and cancer mortality [any cancer type; (Goldberg et al., 2013)]. A positive association was observed between cancer mortality and NO_2 as well as for some other pollutants (warm season O_3 and SO_2). No association was reported for CO or $PM_{2.5}$. This study is limited by the use of daily concentrations and the grouping of all types of cancer together.

6.6.7 Production of N-Nitroso Compounds and other Nitro Derivatives

Daily chemical transformations involving ultraviolet, NO_2 and hydrocarbons, products of automobile exhaust, and oxygen/ozone can generate peroxyacetyl nitrate (PAN) in the gas fraction as part of photochemical smog. Mutagenicity assays demonstrated that PAN is weakly mutagenic in the lungs of the highly susceptible big Blue (R) mice and in Salmonella and that PAN produces a unique signature mutation (DeMarini et al., 2000).

N-nitroso compounds can be generated endogenously in the human body from NO_2 via processes that generate nitrite (NO_2^-) or nitrate. Further, NO_2 is known to react with amines to produce nitrosamines, known animal carcinogens. The possibility that NO_2 could produce cancer via nitrosamine formation has been investigated and was reported in the 2008 ISA for Oxides of Nitrogen U.S. EPA (2008).

6.6.8 Genotoxicity

6.6.8.1 Toxicological Studies

A number of animal toxicology studies have examined the genotoxicity of NO_2. Ex vivo exposure of human nasal epithelial mucosa cells cultured at the air-liquid interface to 10 ppb NO_2 (Koehler et al., 2013; Koehler et al., 2010) or 100 ppb NO_2 (Koehler et al., 2011) produced increased DNA fragmentation measured with the COMET assay as early as 30 minutes after exposure and micronuclei formation after 3-hours of exposure to 100 ppb NO_2 (Koehler et al., 2011). Percentage of DNA content in the tail as detected with the COMET assay decreased with increasing exposure duration [0.5, 1, 2, and 3-hour exposure; (Koehler et al., 2013)]. Of the in vivo assays reported in the previous ISA [see U.S. EPA (2008), Annex Tables 4-11, 4-12, and 4-13, on pages 4-36 and 4-37 of the 2008 Annex], results were mixed with positive findings of genotoxicity seen in two studies that employed rat lung cells (mutations and chromosome abnormalities, 50,000−560,000 ppb NO_2 >12 days; 27,000 ppb NO_2, 3 hours) and negative findings of genotoxicity seen in tests employing Drosophila recessive lethals (500,000−7,000,000 ppb NO_2, 1 hour), Drosophila wing spot test (50,000−280,000 ppb NO_2, 2 days), mouse bone marrow micronuclei (20,000 ppb, 23 hours), and mouse spermatocyte and lymphocyte chromosomal aberrations (100−10,000 ppb NO_2, 6 hours). In vitro exposures to NO_2 yielded positive findings in a majority of the tests in rodent (2,000−3,000 ppb NO_2, 10 minutes) and human cell lines, bacteria (5,000−90,000 ppb NO_2, 30 minutes), and plants (5,000 ppb NO_2, 24 hours) (Table 6-19).

NO_2-induced genotoxicity in various organs of male rats was demonstrated after inhalation exposure to 2,660, 5,320, or 10,640 ppb NO_2 for 6 h/day for 7 days (Han et al., 2013). In the COMET assay, NO_2 inhalation exposure generated significant increases in DNA damage in all dose groups in the liver, lung, and kidney; brain and spleen had significantly increased DNA damage at the two highest doses. Bone marrow PCE micronuclei testing, a marker of chromosomal damage, revealed significant increases in Mn formation with NO_2 inhalation across all dose groups. DNA-protein cross-links, another form of DNA damage, was significantly increased in all dose groups in the brain

and liver; in the two highest dose groups in the heart and spleen; and in the highest dose group in the lung and kidney (Han et al., 2013).

6.6.8.2 Epidemiologic Studies

A study in Italy of children living near chipboard industries examined the relationship between NO_2 concentrations and genotoxicity (Marcon et al., 2014). NO_2 concentrations were not associated with results of Comet Assays (i.e., tail length, moment, or intensity) but were associated with some results of micronucleus assays. A 10-ppb increase in NO_2 concentration was associated with a 1.16% (95% CI: 0.6%, 1.7%) change in binucleated cells. A 10-ppb increase in NO_2 concentration was also associated with an increased risk of nuclear buds [risk ratio (RR): 3.72 [95% CI: 1.67, 7.73). The other pollutant examined, formaldehyde, was also associated with nuclear buds but not with binucleated cells. Additionally, formaldehyde was associated with Comet tail intensity and moment. Although this study is limited by potential for bias by confounding, this study supports the findings that NO_2 concentrations may be associated with cancer, as some measures of genotoxicity were associated with NO_2 concentrations and genotoxicity may lead to cancer.

6.6.9 Summary and Causal Determination

The overall evidence for long-term NO_2 exposure and cancer is suggestive, but not sufficient, to infer a causal relationship. This conclusion is based on evidence from some prospective epidemiologic studies reporting associations between NO_2 exposure and cancer incidence and mortality, with the strongest evidence coming from studies of lung cancer incidence and mortality. Animal toxicology studies employing NO_2 exposure with other known carcinogens provide further supporting evidence, showing that inhaled NO_2 can increase tumor load in laboratory rodents. Nonetheless, toxicological data provide no clear evidence of NO_2 acting as a complete carcinogen, and not all epidemiologic studies report positive associations.

In past reviews, a limited number of epidemiologic studies had assessed the relationship between long-term NO_2 or NO_X exposure and cancer incidence and mortality. The 2008 ISA for Oxides of Nitrogen concluded that the evidence was “inadequate to infer the presence or absence of a causal relationship” (U.S. EPA, 2008). Recent studies include evidence on lung cancer as well as new types of cancer, evaluating both incidence and mortality. All available evidence for cancer due to long-term NO_2 or NO_X exposure was evaluated using the framework described in Table II of the Preamble. The key evidence

as it relates to the causal determination is summarized in Table 6-20 and demonstrates the addition of recent studies, some of which support an association but are not consistent throughout the body of literature, as well as limited toxicological evidence providing coherence.

Epidemiologic studies of NO_2 or NO_X and lung cancer incidence have had mixed results, with some studies reporting no associations while other studies report positive associations. Most of these studies included large sample sizes, similar NO_X or NO_2 concentrations, and control for many potential confounders, including smoking exposures, although many studies lacked investigation into potential copollutant confounding. Most studies of NO_2 or NO_X and lung cancer mortality reported no association, but some studies reported positive associations. Evidence for cancer in other organ systems is accumulating, but is more difficult to interpret due to questions regarding biological plausibility. Recent studies of leukemia have reported associations with NO_2 concentration. Similarly, a study of bladder cancer mortality reported an association with NO_2. Breast cancer incidence was positively correlated with NO_X concentration in an ecologic analysis, but a study of post-menopausal women observed no increase in odds with higher NO_2 concentrations. A positive association was observed between NO_2 concentration and prostate cancer incidence. Overall, the epidemiologic studies use multiple methods, such as nearest air monitor and dispersion models, to estimate NO_X concentrations. No patterns or trends are apparent in the results based on the type of exposure assessment method. Toxicological data provide no clear evidence of NO_2 acting as a complete carcinogen, and agencies that classify carcinogens including the Department of Health and Human Services, the International Agency for Research on Cancer, and the EPA have not classified oxides of nitrogen for potential carcinogenicity. The American Conference of Industrial Hygienists has classified NO_2 as A4 (not classifiable for humans or animals). However, in some animal toxicological models, NO_2 may act as a tumor promoter at the site of contact, possibly due to its ability to produce cellular damage, induce respiratory epithelial hyperplasia (Section 6.2.6), or promote regenerative cell proliferation. Genotoxic and mutagenic studies with NO_2 have mixed results. Some studies with coexposure to other known carcinogens demonstrated that inhaled NO_2 can increase tumor burden in rodents. Collectively, while some studies observed no associations, the evidence from several toxicological and epidemiologic studies is suggestive, but not sufficient, to infer a causal relationship between long-term exposure to NO_2 and cancer incidence and mortality.

Table 6-20 Summary of evidence, which is suggestive, but not sufficient, to infer a causal relationship between long-term nitrogen dioxide (NO_2) exposure and cancer.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 or NO_X Concentrations Associated with Effects[c]
Evidence from epidemiologic studies generally supportive but not consistent	Positive associations were observed between overall lung cancer incidence and mortality in multiple studies conducted in the U.S., Canada, Europe and Asia.	Nafstad et al. (2003), Nyberg et al. (2000), Raaschou-Nielsen et al. (2010a), Raaschou-Nielsen et al. (2011b), Cesaroni et al. (2013), Filleul et al. (2005), Carey et al. (2013), Jerrett et al. (2013), Hystad et al. (2013)	Means varied with some studies including areas estimating concentrations of NO_2 or NO_X as low as 1.2 ppb to studies with areas estimated at 33.7 ppb.
	No associations were observed between overall lung cancer incidence and mortality in multiple other studies conducted in the United States, Europe, and Asia.	Brunekreef et al. (2009), Beelen et al. (2008a), Papathomas et al. (2011), Beelen et al. (2008b), Cao et al. (2011), Hart et al. (2011), Heinrich et al. (2013), Krewski et al. (2009), Raaschou-Nielsen et al. (2013a)	Means varied with estimated concentrations of NO_2 NO_X ranging from 2.8 to 34.1 ppb.
	Positive associations were also observed in some studies of NO_2 concentrations and leukemia, bladder cancer, and prostate cancer.	Amigou et al. (2011), Weng et al. (2008), Liu et al. (2009), Parent et al. (2013), Ghosh et al. (2013), Raaschou-Nielsen et al. (2011a)	Associations observed at levels as low as 6.5−8.6 ppb for leukemia.

Table 6-20 (Continued): Summary of evidence, which is suggestive, but not sufficient, to infer a causal relationship between long-term nitrogen dioxide (NO_2) exposure and cancer.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 or NO_X Concentrations Associated with Effects[c]
Limited toxicological evidence provides coherence	Studies of facilitation of metastasis and coexposures with known carcinogens show NO_2 related effects. Studies of NO_2 as a direct carcinogen are lacking.	Adkins et al. (1986), Richters and Damji (1990), Wagner et al. (1965), Richters and Kuraitis (1981), Richters and Kuraitis (1983), Richters et al. (1985), Ichinose et al. (1991), Ichinose and Sagai (1992) Sections 6.6.1 and 6.6.8	10,000 ppb 250 ppb 5,000 ppb 400, 800 ppb 300, 400, 500 ppb 400 ppb 4,000 ppb 500 ppb
Limited supporting evidence of carcinogenesis, mutagenesis, or genotoxicity provides biological plausibility	Finding of mutagenicity and micronucleus formation in ex vivo culture of primary human nasal epithelial cells exposed to NO_2.	Koehler et al. (2013), Koehler et al. (2011), Koehler et al. (2010) Section 6.6.7	100, 1,000, 10,000 ppb
	Mixed findings of mutagenicity and carcinogenicity in various models of NO_2 exposure in older studies, mainly in nonhuman species.	U.S. EPA (2008), Annex Table AX4-11, Table AX 4-12, and Table AX 4-13	

NO_2 = nitrogen dioxide; NO_X = sum of NO and NO_2.

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Tables I and II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to causal determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where the full body of evidence is described.

[c]Describes the NO_2 concentrations with which the evidence is substantiated.

References for Chapter 6

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CHAPTER 7 POPULATIONS AND LIFESTAGES POTENTIALLY AT RISK FOR HEALTH EFFECTS RELATED TO NITROGEN DIOXIDE EXPOSURE

7.1 Introduction

Interindividual variation in human responses to air pollution exposure can result in some groups being at increased risk for detrimental effects in response to ambient exposure to an air pollutant. The NAAQS are intended to protect public health with an adequate margin of safety. 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., NO_2) (see Preamble to the ISA). The scientific literature has used a variety of terms to identify factors and subsequently populations that may be at increased risk of an air pollutant-related health effect including susceptible, vulnerable, sensitive, and at-risk, with recent literature introducing the term response-modifying factor (Vinikoor-Imler et al., 2014) (see Preamble to the ISA). Due to the inconsistency in definitions for these terms across the scientific literature and the lack of a consensus on terminology in the scientific community, as detailed in the Preamble to this ISA, this chapter focuses on identifying those populations potentially "at-risk" of an NO_2-related health effect. This leads to a focus on the identification, evaluation, and characterization of factors to address the main question of what populations and lifestages are at increased risk of an NO_2-related health effect. It is recognized that some factors may lead to a reduction in risk, and these are recognized during the evaluation process, but for the purposes of identifying those populations or lifestages at greatest risk to inform decisions on the NAAQS, the focus of this chapter is on characterizing those factors that may increase risk.

Individuals, and ultimately populations, could be at increased risk of an air pollutant-related health effect via multiple avenues. As discussed in the Preamble, risk may be modified by intrinsic or extrinsic factors, differences in internal dose, or differences in exposure to air pollutant concentrations. It is important to note that the emphasis of this chapter is on identifying, evaluating, and characterizing the evidence for factors that potentially increase the risk of health effects related to exposure to NO_2, regardless of whether the change in risk is due to intrinsic factors, extrinsic factors, increased internal dose, increased exposure, or a combination. Some studies examined potential at-risk populations or lifestages based on NO_X exposures, but these studies are not discussed in this chapter because they do not directly inform whether a population is at increased risk of an NO_2-related health effect (Section 1.1). It is important to note that

although individual factors that may increase the risk of an NO_2-related health effect are discussed in this chapter, it is likely in many cases that portions of the population are at increased risk of an NO_2-related health effect due to a combination of multiple factors (e.g., residential location and SES), but information on the interaction among factors remains limited. Thus, the following sections indentify, evaluate, and characterize the overall weight-of-evidence for individual factors to determine if they potentially lead to increased risk for NO_2-related health effects (see Preamble to the ISA).

7.2 Approach to Evaluating and Characterizing the Evidence for At-Risk Factors

The systematic approach used to evaluate factors that may increase the risk of a population or specific lifestage to an air pollutant-related health effect is described in more detail in the Preamble. The evidence evaluated includes relevant studies discussed in Chapter 5 and Chapter 6 of this ISA building on the evidence presented in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) and the 1993 Air Quality Criteria for Oxides of Nitrogen (U.S. EPA, 1993). Additionally, within this chapter each factor is evaluated using the current weight-of-evidence framework to charcaterize whether the factor may lead to increased risk of an air pollutant-related health effect in a population or specific lifestage as detailed in previous ISAs (U.S. EPA, 2013a, b). In general, the current approach builds on the causal framework used throughout the ISA. The characterization of each factor consists of evaluating the evidence across scientific disciplines for individual health effects and assessing the overall weight-of-evidence to detail the level of confidence that a specific factor may result in a population or lifestage being at increased risk of an NO_2-related health effect.

As discussed in the Preamble, this evaluation focuses on epidemiologic studies that conduct stratified analyses to compare populations or lifestages exposed to similar air pollutant concentrations within the same study design. During the evaluation of these studies, important considerations include whether the stratified analyses were planned a priori or were post-hoc analyses, whether the study conducted multiple comparisons, and whether there were small sample sizes in individual strata. These study design issues can increase the probability of finding associations by chance or reduce power to detect associations in subgroup analyses. Experimental studies in human subjects or animal models that focus on factors, such as genetic background or health status, are also important lines of evidence to evaluate because they inform coherence and biological plausibility of effects observed in epidemiologic studies, as well as the independent effect of NO_2. Additionally, studies examining whether factors may result in differential

exposure to NO_2 and subsequently increased risk of an NO_2-related health effects are also included.

The objective of this chapter is to identify, evaluate, and characterize the evidence regarding factors that may increase the risk an NO_2-related health effect in a population or lifestage, building on the conclusions drawn in the ISA with respect to NO_2 exposure and health effects. The factors that are evaluated in this chapter include pre-existing disease (Section 7.3), genetic background (Section 7.4), sociodemographics (Section 7.5), and behavioral and other factors (Section 7.6). These categories are described in more detail in Table 7-1, and a summary of the characterization of the evidence for each factor considered that may increase the risk of NO_2-related health effects is presented in Section 7.7.

Table 7-1 Characterization of evidence for factors potentially increasing the risk for nitrogen dioxide-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 evidene includes coherence across disciplines. Evidence includes multiple high-quality studies.

7.3 Pre-Existing Disease/Conditions

Individuals with pre-existing disease may be considered at greater risk for some air pollution-related health effects because they may be in a compromised biological state depending on the disease and severity. The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) concluded that those with pre-existing pulmonary conditions were likely to be at

greater risk for NO_2-related health effects, especially individuals with asthma. Among recent studies evaluating effect modification by pre-existing disease, the largest group examined asthma (Section 7.3.1). Several studies are available on other diseases, including chronic pulmonary respiratory disease (COPD, Section 7.3.2), cardiovascular disease (CVD, Section 7.3.3), diabetes (Section 7.3.4) and obesity (Section 7.3.5). Table 7-2 presents the prevalence of these diseases according to the Center for Disease Control's National Center for Health Statistics (Schiller et al., 2012), 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 cardiovascular diseases, indicates the potential public health impact of characterizing the risk of NO_2-related health effects for affected populations.

Table 7-2 Prevalence of respiratory diseases, cardiovascular diseases, diabetes, and obesity among adults by age and region in the U.S. in 2010.

Chronic Disease/Condition	Adults (18+)	Age (%)[a]				Region(%)[b]			
	N (in thousands)	18−44	45−64	65−74	75+	North-east	Midwest	South	West
All (N, in thousands)	229,505	110,615	80,198	21,291	17,401	40,577	53,316	81,721	53,891
Selected respiratory diseases									
Asthma[c]	18,734	8.1	8.4	8.7	7.4	8.7	8.2	7.7	8.4
COPD	–	–	–	–	–	–	–	–	–
Chronic bronchitis	9,883	3.0	5.3	6.0	6.3	3.8	4.7	4.7	3.1
Emphysema	4,314	0.3	2.1	5.4	6.3	1.7	2.3	1.9	1.2
Selected cardiovascular diseases/conditions									
All heart disease	27,066	4.4	13.2	24.3	37.1	10.7	12.2	12.3	10.1
Coronary heart disease	15,262	1.4	7.3	16.5	25.8	6.1	6.6	7.2	5.4
Hypertension	59,259	9.3	34.4	54.2	57.3	24.0	24.7	27.1	21.7
Stroke	6,226	0.6	3.0	6.1	10.7	2.0	2.9	2.9	2.5
Diabetes	20,974	2.8	12.3	22.0	21.7	7.1	8.9	10.1	8.3
Obesity	62,026	25.4	32.8	31.5	18.2	25.1	29.6	29.4	24.4

[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: Schiller et al. (2012); National Center for Health Statistics: Data from Tables 1 and 2; Tables 3 and 4; and Tables 7 and 8 of the Centers for Disease Control and Prevention report.

7.3.1 Asthma

Approximately 8.2% of adults and 9.5% of children (age <18 yr) in the U.S. currently have asthma (Schiller et al., 2012; Bloom et al., 2011), and it is the leading chronic illness affecting children. This ISA concludes that a causal relationship exists short-term NO_2 exposure and respiratory effects, based primarily on evidence for effects on asthma exacerbation (Section 5.2.9). The evidence demonstrating NO_2-induced asthma exacerbation formed the basis for the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) concluding that individuals with pre-existing pulmonary conditions, particularly those with asthma, are likely at greater risk of NO_2-related health effects. This section evaluates controlled human exposure and epidemiologic studies that compare NO_2-related health effects between groups with or without asthma (Tables 7-3 and 7-4). As a whole, results of these comparisons are variable; however, controlled human exposure studies provide compelling evidence that people with asthma are at greater risk for NO_2-related respiratory effects than people without asthma.

Controlled human exposure studies demonstrating NO_2-induced increases in airway responsiveness in adults with asthma provide key evidence for an independent, causal relationship between NO_2 exposure and respiratory effects (Section 5.2.9). This evidence also demonstrates greater sensitivity of adults with asthma to short-term NO_2 exposure compared to adults without asthma. A meta-analysis conducted by Folinsbee (1992) demonstrates that NO_2 exposures in the range of 100−300 ppb increased airway responsiveness in adults with asthma [(Folinsbee, 1992); Table 7-3]. In this study Folinsbee (1992) combined groups that varied with respect to respiratory symptoms and medication use at the time of assessment but had high prevalence (50−100%) of atopic asthma. Additionally, in many studies these participants were characterized as having mild asthma. Although fewer studies of healthy adults examined airway responsiveness for NO_2 exposures below 300 ppb, results were statistically significant only for NO_2 exposures >1,000 ppb. In comparison to airway responsiveness, there is inconsistent evidence for the effects of short-term NO_2 exposure on lung function in adults with asthma in the absence of a challenge agent, and the limited evidence is inconclusive in demonstrating differences in response between healthy adults and those with asthma [(Vagaggini et al., 1996; Jörres et al., 1995; Linn et al., 1985b); Table 7-3].

Epidemiologic evidence does not clearly show differences in NO_2-related health effects between children with and without asthma (Table 7-4). Studies characterized as having strong exposure assessment, such as monitoring NO_2 at or near children's school, also did not show differences in NO_2-related respiratory effects between children with and without asthma [(Lin et al., 2011; Flamant-Hulin et al., 2010; Holguin et al., 2007);

Table 7-4]. However, asthma is a heterogeneous disease as demonstrated in Section 5.2.2, and a limitation of epidemiologic studies that may obscure potential differences among people with and without asthma is the practice often used of grouping all people with asthma together even though there are varying phenotypes and triggers of asthma as well as varying degrees of response among people with asthma. Additionally, compared to controlled human exposure studies, when examining potential differences among people with and without asthma, epidemiologic studies examined a more diverse set of respiratory outcomes and asthma phenotypes.

Several lines of evidence indicate that people with asthma are at increased risk for NO_2-related health effects. The causal relationship determined for short-term NO_2 exposure and respiratory effects is based on the evidence for asthma exacerbation (Section 5.2.9). Controlled human exposure studies demonstrate that NO_2 has an independent effect on increasing airway responsiveness in adults with asthma and show increased sensitivity of adults with asthma compared to healthy adults. People with asthma also tend to have oronasal breathing, although the implications on differential uptake of NO_2 in the respiratory tract are not known (Section 4.2.2). Epidemiologic studies do not clearly demonstrate differences between populations with and without asthma. The study populations represent an array of asthma phenotypes, and limited epidemiologic evidence indicates larger NO_2-related respiratory effects in children with asthma not using asthma medication. Thus, the epidemiologic results are not necessarily incoherent with experimental findings from populations of mostly mild, atopic asthma. Because of clear evidence for an effect of NO_2 exposure on asthma exacerbation and for increased sensitivity of adults with asthma to NO_2-induced increases in airway responsiveness in controlled human exposure studies, there is adequate evidence to conclude that people with asthma are at increased risk for NO_2-related health effects.

Table 7-3 Controlled human exposure studies evaluating pre-existing asthma.

Factor Evaluated	Reference Category	Direction of Effect Modification or Effect[a]	Outcome	Study Population	Study Details	Study
Asthma n = 33 ≤300 ppb NO_2	Healthy n = 36 <1,000 ppb NO_2	↑	Airway respons-iveness	N = 355	Range: 100 ppb NO_2 for 1 h to 7,500 ppb NO_2 for 2 h of exposure; Exposures at rest	Folinsbee (1992)
Asthma n = 12	Healthy n = 8	–	Airway inflamma-tion	N = 20 Ages 1−33 yr	1,000 ppb for 3 h; Exercise 10 min on/10 min off at individual's maximum workload	Jörres et al. (1995)
		↑	Lung function decrement			
Asthma n = 4	Healthy n = 7	–	Lung function decrement	N = 11 Mean age: 31.5 yr	300 ppb for 1 h; Exercise at $\dot{V}_E$ = 25 L/min	Vagaggini et al. (1996)
Asthma n = 23	Healthy n = 25	–	Airway resistance	N = 48 Ages 18−36 yr	4,000 ppb for 75 min; Two 15-min periods of exercise at $\dot{V}_E$ = 25 L/min and 50 L/min	Linn et al. (1985b)
Atopic asthma	None	–	Lung function decrement Allergen response	N = 11 Mean age: 31.2 yr	(1) 200 ppb NO_2 for 6 h (2) 200 ppb NO_2 + 100 ppb O_3 for 6 h (3) 400 ppb NO_2 for 3 h (4) 400 ppb NO_2 + 200 ppb O_3 for 3 h (1−4) Exercise 10 min on/40 min off at $\dot{V}_E$ = 32 L/min	Jenkins et al. (1999)
Asthma	None	–	Lung function decrement	N = 41 Mean age: 31 yr	200 ppb for 2 h; Exercise 15 min on/15 min off at $\dot{V}_E$ = ~2 times resting	Kleinman et al. (1983)
Asthma	None	↑	Lung function decrement	N = 15 Mean age: 33 yr	300 ppb for 30 min Exercise 10 min on/20 min off at $\dot{V}_E$ >3 times resting	Bauer et al. (1986)
Asthma	None	–	Airway resistance	N = 11 Ages 7−55 yr	250 ppb for 30 min; Exercise 10 min on/20 min off/ at $\dot{V}_E$ 3 times resting	Jörres and Magnussen (1991)

[a]Up facing arrow indicates that the effect of NO_2 is greater (e.g., larger lung function decrement, larger increase in airway inflammation) in the group with the factor evaluated than in the reference group. Down facing arrow indicates that the effect of NO_2 is smaller in the group with the factor evaluated than in the reference group. A dash indicates no difference in NO_2-related health effect between groups. In some studies, only a population with pre-existing disease was examined; therefore, the arrow or dash represents the direction of the effect in that population after exposure to NO_2 relative to exposure to filtered air.

Table 7-4 Epidemiologic studies evaluating pre-existing asthma.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Short-term exposure						
Asthma n = 57	No asthma n = 192	↑	Respiratory symptoms	N = 249 Ages 14−20 yr	New York, NY, 2003−2005	Patel et al. (2010)
Asthma n = 50, 89% with atopy	No asthma n = 158, 72% with atopy	↓	Pulmonary inflammation, lung function decrement	N = 208 Ages 7.9−11 yr	Mexico City, 2003−2005	Barraza-Villarreal et al. (2008)
Asthma n = 8	No asthma n = 30	–	Pulmonary inflammation	N = 38 Ages 9−12 yr	Beijing, China, 2008	Lin et al. (2011)
Asthma n = 100	No asthma n = 100	–	Pulmonary inflammation	N = 200 Ages 6−12 yr	Ciudad Juarez, Mexico, 2001−2002	Holguin et al. (2007)
Asthma n = 34	No asthma n = 70	–	Pulmonary inflammation	N = 104 Mean age: 10.3 yr	Clermont-Ferrand, France	Flamant-Hulin et al. (2010)
Asthma n = 169	No asthma n = 2,071	↓	Pulmonary inflammation	N = 2,240 Ages 5−7 yr	Southern California, 2004−2005	Berhane et al. (2011)
Long-term exposure						
Asthma n = 50	No asthma n = 1,934	↑	Incident stroke	N = 1,984 Ages 50−65 yr at baseline	Copenhagen, Aarhus counties, Denmark, 1993−2006 Long-term NO_2	Andersen et al. (2012a)
		↑	Fatal stroke			
Asthma n = 1,273	No asthma n = 50,545	–	Diabetes	N = 51,818 Ages 50−65 yr at baseline	Copenhagen, Aarhus counties, Denmark, 1993−2006 Long-term NO_2	Andersen et al. (2012b)

[a]Up facing arrow indicates that the effect of NO_2 is greater (e.g., larger lung function decrement, larger increase in pulmonary inflammation) in the group with the factor evaluated than in the reference group. Down facing arrow indicates that the effect of NO_2 is smaller in the group with the factor evaluated than in the reference group. A dash indicates no difference in NO_2-related health effect between groups.

7.3.2 Chronic Obstructive Pulmonary Disease

Chronic lower respiratory disease, including COPD, was ranked as the third leading cause of death in the United States in 2011 (Hoyert and Xu, 2012). COPD comprises chronic bronchitis and emphysema which affect approximately 9.9 million and 4.3

million adults in the U.S., respectively [Table 7-2; (Schiller et al., 2012)]. Given that people with COPD have compromised respiratory function and systemic inflammation, they may be at increased risk for an array of NO_2-related health effects. Evidence for differential NO_2-related respiratory or cardiovascular effects between adults with COPD and those without COPD is weak (Table 7-5 and 7-6). Based on the ability to inform a direct effect of NO_2, controlled human exposure studies of lung function provide a stronger basis for drawing conclusions about whether pre-existing COPD leads to increased risk of NO_2-related health effects.

Compared with asthma exacerbation, there is greater uncertainty regarding a relationship between short-term NO_2 exposure and COPD exacerbation (Section 5.2.2.4). This is illustrated by the lack of consistent evidence from controlled human exposure studies for changes in lung function or pulmonary inflammation in adults with COPD following NO_2 exposure (Gong et al., 2005; Linn et al., 1985a). Among the limited number of studies that compared adults with COPD and healthy adults, only some indicated larger NO_2-induced decrements in lung function in adults with COPD (Table 7-5). Among adults with COPD, NO_2 exposures of 300 ppb for 1 or 4 hours induced decreases in lung function of 4.8, 8.2 (Morrow et al., 1992) or 10% (Vagaggini et al., 1996) relative to air control exposures. In contrast, in healthy adults, NO_2 did not have any effect on lung function or resulted in increased lung function. However, adults with COPD were older than healthy adults and had a higher prevalence of smoking, which could have influenced results (Table 7-5). For example, in one study, smokers had larger NO_2-induced decrements in lung function independently of COPD (Morrow et al., 1992).

COPD was not observed to modify associations between long-term NO_2 exposure and diabetes (Eze et al., 2014; Andersen et al., 2012b), but some epidemiologic studies show larger association between short-term exposures and cardiovascular-related emergency department (ED) visits and decreases in heart rate variability (HRV) among adults with COPD, as well as long-term exposures and stroke (Table 7-6). Inference is limited from studies of cardiovascular effects because of lack of comparison to healthy groups in the short-term exposure studies (Suh and Zanobetti, 2010; Peel et al., 2007) and uncertainty regarding an independent relationship for both short-term and long-term NO_2 exposure with cardiovascular effects (Sections 5.3.12 and 6.3.9).

In conclusion, some but not all epidemiologic evidence points to larger NO_2-related cardiovascular effects in adults with COPD, and there is uncertainty as to whether the findings reflect an independent effect of NO_2. Controlled human exposure studies do not clearly demonstrate that NO_2 exposure induces respiratory effects in adults with COPD, and the limited findings for larger lung function decrements in adults with COPD relative to healthy adults may be influenced by differences between groups in age or smoking.

The limited and inconsistent evidence for NO_2-related changes in lung function in adults with COPD is inadequate to determine whether people with COPD are at increased risk for NO_2-related health effects.

Table 7-5 Controlled human exposure studies evaluating pre-existing COPD.

Factor Evaluated	Reference Category	Direction of Effect Modification or Effect[a]	Outcome	Study Population	Study Details	Study
COPD n = 20	Healthy n = 20	↑	Lung function decrements	N = 40 Mean age 59.9 yr	300 ppb for 4 h; Three 7 min periods of exercise at $\dot{V}_E$ = ~4 times resting	Morrow et al. (1992)
		–	Symptoms, Respiratory conductance			
COPD n = 7	Healthy n = 7	↑	Lung function decrements	N = 14 Mean age COPD: 58 yr Healthy: 34 yr	300 ppb for 1 h; Exercise at $\dot{V}_E$ = 25 L/min	Vagaggini et al. (1996)
		–	Sputum cell counts, symptoms			
COPD n = 18	Healthy n = 6	–	Lung function decrements, heart rate, blood pressure, symptoms	N = 24 Mean age COPD: 72 yr Healthy: 68 yr	(1) 400 ppb NO_2 for 2 h (2) 200 µg/m^3 CAPs for 2 h (3) 400 ppb NO_2 + 200 µg/m^3 CAPs for 2 h (1−3) Exercise 15 min on/15 min off at $\dot{V}_E$ = ~2 times resting	Gong et al. (2005)
COPD	None	–	Lung function decreases, heart rate, symptoms	N = 22 Mean age: 60.8 yr	500, 1,000, and 2,000 ppb for 1 h; Exercise 15 min on/15 min off $\dot{V}_E$ = 16 L/min	Linn et al. (1985a)

[a]Up facing arrow indicates that the effect of NO_2 is greater (e.g., larger FEV_1 decrement, larger increase in airway inflammation) in the group with the factor evaluated than in the reference group. Down facing arrow indicates that the effect of NO_2 is smaller in the group with the factor evaluated than in the reference group. A dash indicates no difference in NO_2-related health effect between groups. In some studies, only a population with pre-existing disease was examined; therefore, the arrow or dash represents the direction of the effect in that population after exposure to NO_2 relative to exposure to filtered air.

Table 7-6 Epidemiologic studies evaluating pre-existing COPD.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Short-term exposure						
COPD n = 18	Recent myocardial infarction n = 12	↑	HRV pNN50 decrements	N = 30 Ages not reported	Atlanta, GA, 1999−2000	Suh and Zanobetti (2010)
		–	HRV r-MSSD decrements			
COPD n = 8% ED visits	No COPD n = 92% ED visits	↑	Cardiovascular-related ED visits	31 participating hospitals, 103,551 ED visits for cardiovascular disease	Atlanta, GA, 1993−2000	Peel et al. (2007)
Long-term exposure						
COPD n = 121	No COPD n = 1,863	–	Incident stroke	N = 1,984 Ages 50−65 yr at baseline	Copenhagen, Aarhus counties, Denmark, 1993−2006	Andersen et al. (2012a)
COPD n = 6	No COPD n = 136	↑	Fatal stroke	N = 142 Ages 50−65 yr at baseline		
COPD n = 2,058	No COPD n = 49,760	–	Diabetes	N = 51,818 Ages 50−65 yr at baseline	Copenhagen, Aarhus counties, Denmark, 1993−2006	Andersen et al. (2012b)
COPD n = 1,268	No COPD n = 5,124	–	Diabetes	N = 6,392 Ages 29−73 yr	Switzerland 2002	Eze et al. (2014)

[a]Up facing arrow indicates that the effect of NO_2 is greater (e.g., larger decrement in HRV, larger increase in ED visits) in the group with the factor evaluated than in the reference group. Down facing arrow indicates that the effect of NO_2 is smaller in the group with the factor evaluated than in the reference group. A dash indicates no difference in NO_2-related health effect between groups.

7.3.3 Cardiovascular Disease

Cardiovascular disease is the primary cause of death in the U.S., and approximately 12% of adults report a diagnosis of heart disease. In addition, hypertension has been diagnosed in roughly 25% of the adult U.S. population [Table 7-2; (Schiller et al., 2012)]. For both short-term (Section 5.3.12) and long-term (Section 6.3.9) NO_2 exposure, the collective body of evidence is suggestive, but not sufficient to infer a causal relationship with cardiovascular effect. These conclusions were primarily based on the uncertainty in distinguishing an independent effect of NO_2 on cardiovasucular effects. In addition to this uncertainty, the evidence base does not consistently show that pre-existing CVD

increases the risk for NO_2-related health effects (Table 7-7). Evidence is equally inconsistent for short-term and long-term NO_2 exposure.

For short-term exposure, NO_2-related mortality was higher among individuals with CVD (Chiusolo et al., 2011); however, the majority of evidence, which is for cardiovascular hospital admissions and ED visits, is inconsistent (Table 7-7). The strongest evidence for a relationship between short-term NO_2 exposure and cardiovascular effects is for myocardial infarction (MI, Section 5.4.8), and most studies show no difference in the association in groups with hypertension (Tsai et al., 2012; Peel et al., 2007; D'Ippoliti et al., 2003), arrhythmia (Tsai et al., 2012; Mann et al., 2002), or congestive heart failure (Tsai et al., 2012; D'Ippoliti et al., 2003). Many studies of long-term NO_2 exposure compared groups with and without hypertension and found no difference in the association with diabetes (Eze et al., 2014; Andersen et al., 2012b) and no consistent difference in the association with stroke (Andersen et al., 2012a). For studies that examined hypertension or blood pressure examined as an outcome, associations with long-term NO_2 exposure were larger in groups with pre-existing CVD (Foraster et al., 2014; Sørensen et al., 2012).

In studies examining subclinical cardiovascular effects such as changes in HRV, interleukin (IL)-6, or arrhythmic events recorded on electrocardiograms, most did not observe that associations with short- or long-term NO_2 exposure differed between groups with or without pre-existing CVD, whether defined as any CVD, ischemic heart disease (IHD), or hypertension (Panasevich et al., 2009; Felber Dietrich et al., 2008; Ljungman et al., 2008). Risk factors for CVD, including higher systemic inflammation and hypercholesterolemia, do not consistently modify NO_2-related cardiovascular effects (Andersen et al., 2012a; Huang et al., 2012). Experimental studies (Table 7-8) also do not clearly support greater NO_2-induced subclinical cardiovascular effects as examined in adults with CVD (Scaife et al., 2012) and a mouse model of CVD (Campen et al., 2010).

For associations with short-term and long-term NO_2 exposure, people with and without pre-existing CVD have been compared with respect to an array of cardiovascular diseases, events, and subclinical effects. Studies are also diverse in the conditions by which they define pre-existing CVD. No consistent difference in NO_2-related cardiovascular effects is demonstrated between groups with and without pre-existing CVD. Additionally, there was limited biological plausibility from the experimental evidence demonstrating NO_2-related health effects in response to pre-existing cardiovascular conditions. In conclusion, the large evidence base lacks sufficient consistency in demonstrating that pre-existing CVD modifies NO_2-related cardiovascular effects, and an independent effect of NO_2 is uncertain overall. Therefore, the current

evidence is inadequate to determine whether people with CVD are at increased risk for NO_2-related health effects.

Table 7-7 Epidemiologic studies evaluating pre-existing cardiovascular disease.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Short-term exposure						
Hypertension n = 30% visits	No hypertension n = 70% visits	↑	Arrhythmia ED visits	31 participating hospitals N = 103,551 ED visits for cardiovascular disease	Atlanta, GA, 1993−2000	Peel et al. (2007)
		–	ED visits for IHD or congestive heart failure (CHF)			
Hypertension n = 40% visits	No hypertension n = 60% visits	–	Myocardial infarction hospital admission	N = 27,563 hospital admissions	Taipei, Taiwan 1999−2009	Tsai et al. (2012)
CHF n = 15% visits	No CHF n = 85% visits	–				
Cardiac arrhythmia n = 11% visits	No cardiac arrhythmia n = 89% visits	–				
Hypertension n = 1,648	No hypertension n = 4,883	–	First acute myocardial infarction hospital admission	N = 6,531 hospital records	Rome, Italy, 1995−1997	D'Ippoliti et al. (2003)
Heart conduction disorder n = 414	No heart conduction disorder n = 6,117	↑				
Cardiac dysrhythmia n = 1,296	No cardiac dysrhythmia n = 5,235	–				
Heart failure n = 703	No heart failure n = 5,828	–				
Secondary diagnosis of arrhythmia n = 34.5% admissions	No secondary diagnosis of arrhythmia n = 65.5% admissions	–	Hospital admission for IHD	N = 54,863 hospital admissions	Southern California, 1988−1995	Mann et al. (2002)
Secondary diagnosis of CHF n = 14.1% admissions	No secondary diagnosis of CHF n = 85.9% admissions	↑				

Table 7-7 (Continued): Epidemiologic studies evaluating pre-existing cardiovascular disease.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Pre-existing heart disease n = 525 with stroke	No pre-existing heart disease n = 2,214 with stroke	↑	Hospital admission for ischemic stroke	N = 5,927 hospital admissions	Edmonton, Canada, 2003−2009	Villeneuve et al. (2012)
		–	Hospital admission for hemorrhagic stroke			
IHD n = 56	No IHD n = 32	–	Ventricular tachy-arrhythmia	N = 88 with implantable cardioverter defibrillators Age 28−85 yr	Gothenburg, Stockholm, Sweden, 2001−2006	Ljungman et al. (2008)
Pre-existing CVD n = 1.2−14%	No pre-existing CVD n = 86−98.8%	↑	Total mortality	N 276,205 natural deaths	10 Italian cities 12% of population 2001−2005	Chiusolo et al. (2011)
Long-term exposure						
Hypertension n = 575	No hypertension n = 1,409	–	Incident stroke	N = 1,984 Ages 50−65 yr at baseline	Copenhagen, Aarhus counties, Denmark, 1993−2006	Andersen et al. (2012a)
Hypertension n = 38	No hypertension n = 104	–	Fatal stroke	N = 142 Ages 50−65 yr at baseline		
Hypercholesterolemia n = 230	No hypercholesterolemia n = 1,754	–	Incident stroke (confirmed by hospital admission)	N = 1,984 Ages 50−65 yr at baseline	Copenhagen, Aarhus counties, Denmark, 1993−2006	Andersen et al. (2012a)
Hypercholesterolemia n = 19	No hypercholesterolemia n = 123	–	Fatal stroke (confirmed by hospital admission)	N = 142 Ages 50−65 yr at baseline		
Pre-existing CVD n = 269	No pre-existing CVD n = 3,431	↑	Systolic/ diastolic blood pressure	N = 3,700 Ages 35−83 yr	Girona, Spain	Foraster et al. (2014)
Hypertension n = 867	No hypertension n = 669	–	Blood IL-6 levels	N = 1,536 Ages 45−70 yr	Stockholm county, Sweden, 1992−1994	Panasevich et al. (2009)
Hypertension n = 19.4%	No hypertension n = 80.6%	–	Diabetes	N = 6,392 Ages 29−73 yr	Switzerland, 2002	Eze et al. (2014)

[a]Up facing arrow indicates that the effect of NO_2 is greater (e.g., larger FEV_1 decrement, larger increase in airway inflammation) in the group with the factor evaluated than in the reference group. Down facing arrow indicates that the effect of NO_2 is smaller in the group with the factor evaluated than in the reference group. A dash indicates no difference in NO_2-related health effect between groups.

Table 7-8 Controlled human exposure and toxicological studies informing pre-existing cardiovascular disease.

Factor Evaluated	Reference Category	Direction of Effect[a]	Outcome	Study Population/ Animal Model	Study Details	Study
Stable coronary heart disease or impaired left ventricular systolic function	None	–	Heart rate, HRV decrement	N = 18 humans Mean age 68 yr	400 ppb NO_2 for 1 h	Scaife et al. (2012)
Athero-sclerosis	None	↑	Oxidative stress in heart tissue	N = 5−10 mice/group, $ApoE^{-/-}$	200 or 2,000 ppb NO_2, 6 h/day, 7 days High fat diet	Campen et al. (2010)

[a]These studies only examined subjects with cardiovascular disease and have no reference group. A dash indicates that NO_2 was not observed to induce an effect in the group with cardiovascular disease evaluated relative to clean air exposure. An up-facing arrow indicates that NO_2 induced an effect on the outcome (e.g., cause a decrement in HRV) in the group with cardiovascular disease.

7.3.4 Diabetes

Diabetes mellitus is a group of diseases characterized by high blood glucose levels that result from defects in the body's ability to produce and/or use insulin. An estimated 20 million Americans had diagnosed diabetes mellitus in 2010, representing 9.1% of the adult population (Schiller et al., 2012). High blood glucose levels can damage blood vessels, increasing the risk of people with diabetes for heart disease or stroke. Diabetes and cardiovascular disease are also linked by common risk factors such as hypertension and obesity. These relationships provide support for diabetes influencing the risk of cardiovascular disease; however, diabetes has not consistently been observed to modify epidemiologic associations in studies of short-term or long-term NO_2 exposure and cardiovascular effects (Table 7-9). No difference by diabetes was observed in studies of short-term NO_2 exposure with hospital admissions or ED visits for IHD or MI (Tsai et al., 2012; Filho et al., 2008) or of long-term NO_2 exposure with stroke (Andersen et al., 2012a). Diabetes also did not clearly modify associations of short-term or long-term NO_2 exposure with the subclinical effects heart rate variability, ventricular tachyarrhythmia, blood pressure, and blood IL-6 levels (Foraster et al., 2014; Huang et al., 2012; Panasevich et al., 2009; Ljungman et al., 2008). Associations of short-term and long-term NO_2 exposure with total mortality also did not consistently differ between people with and without diabetes (Faustini et al., 2013; Chiusolo et al., 2011; Maheswaran et al., 2010).

Across studies there was not evidence of a consistent difference in associations of NO_2 exposure with cardiovascular effects between people with and without diabetes. Additionally, there was no consistent pattern observed for short-term or long-term exposure or for a particular cardiovascular endpoint (Table 7-9). In conclusion, the large evidence base lacks sufficient consistency in demonstrating that diabetes modifies NO_2-related cardiovascular effects along with evidence demonstration an independent effect of NO_2 on cardiovascular outcomes (Sections 5.3.12 and 6.3.9). As a result, the evidence is inadequate to determine whether people with diabetes are at increased risk for NO_2-related health effects.

Table 7-9 Epidemiologic studies evaluating pre-existing diabetes.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Short-term exposure						
Diabetes 29.6% admission	No diabetes 70.4% admission	–	Hospital admission for MI	N = 27,563 hospital admissions	Taipei, Taiwan 1999−2009	Tsai et al. (2012)
Diabetes n = 700 ED visits	No diabetes n = 44,300 ED visits	↑	ED visits for hypertension and cardiac ischemic disease	N = 45,000 ED visits	Sao Paulo Hospital, January 2001–July 2003	Filho et al. (2008)
Diabetes n = 9	No diabetes n = 31	↓	HRV decrements	N = 40 with CVD Mean age 66 yr	Beijing, China, 2008	Huang et al. (2012)
Diabetes n = 12	No diabetes n = 76	–	Ventricular tachy-arrhythmia	N = 88 with implantable cardioverter defibrillators Ages 28–85 yr	Gothenburg, Stockholm, Sweden, 2001−2006	Ljungman et al. (2008)
Diabetes n = 30,260	No diabetes n = 245,945	↑	Total mortality	N = 276,205 natural deaths	10 Italian cities 2001–2005	Chiusolo et al. (2011)
Diabetes n = 11.3% admission	No diabetes n = 88.7% admission	–	Respiratory hospital admissions	N = 100,690 hospital admissions	6 Italian cities, 2001–2005	Faustini et al. (2013)
Long-term Exposure						
Diabetes n = 97	No diabetes n = 1,887	–	Incident stroke	N = 1,984 Ages 50–65 yr at baseline	Copenhagen, Aarhus counties, Denmark, 1993−2006	Andersen et al. (2012a)
Diabetes n = 9	No diabetes n = 133	–	Fatal stoke	N = 142 Age 50–65 yr at baseline		

Table 7-9 (Continued): Epidemiologic studies evaluating pre-existing diabetes.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Diabetes n = 580	No diabetes n = 3,120	–	Systolic blood pressure	N = 3,700 Ages 35–83 yr	Girona, Spain	Foraster et al. (2014)
		↓	Diastolic blood pressure			
Diabetes n = 121	No diabetes n = 1,415	–	Relative IL-6 levels	N = 1,536 Ages 45–70 yr	Stockholm county, Sweden 1992–1994	Panasevich et al. (2009)
Diabetes n = 315	No diabetes n = 1,541	↓	Total mortality	N = 3,320 Mean age 70 yr	London, England Follow-up: 1995−2005 NO_2 assessed for 2002	Maheswaran et al. (2010)
Diabetes n = 1,045	No diabetes n = 12,399	–	Lung cancer mortality	N = 13,444 Mean age 74 yr	Shizuok, Japan 1999–2006	Yorifuji et al. (2010)

[a]Up facing arrow indicates that the effect of NO_2 is greater (e.g., larger risk of ED visit, larger decrement in HRV) in the group with the factor evaluated than in the reference group. Down facing arrow indicates that the effect of NO_2 is smaller in the group with the factor evaluated than in the reference group. A dash indicates no difference in NO_2-related health effect between groups.

7.3.5 Obesity

Obesity can be defined as a body mass index (BMI) of 30 kg/m^2 or greater. It is a public health issue of increasing importance as obesity rates in adults have continually increased over several decades in the U.S., reaching an estimated 27% in 2010 (Schiller et al., 2012). Obesity or high BMI could increase the risk of NO_2-related health effects through multiple mechanisms including persistent, low-grade inflammation. Obesity often occurs with poor diet and chronic diseases. As a result, the combination of these factors could be part of the pathway by which obesity increases the risk of NO2-related health effects or they could act in combination with obesity to increase risk.

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) did not evaluate obesity as a potential factor that could increase the risk of NO_2-related health effects. More recently studies have included obesity or BMI as a potential effect measure modifier, but overall the evidence is inconsistent as to whether obesity leads to increased risk of NO_2-related health effects. Most studies examined whether obesity increases the risk of NO_2-related cardiovascular and related metabolic effects. Obesity has been shown to be a risk factor for both cardiovascular disease and diabetes, providing biological plausibility for the potential increased risk of both diseasse in response to NO_2 exposure. A study in rats provides evidence that long-term NO_2 exposure has larger effects on dyslipidemia, a known risk factor for cardiovascular disease, in obese rats compared to nonobese rats

[(Takano et al., 2004); Table 7-10]. However, differences between obese and nonobese strains were limited to 160 ppb NO_2 and not observed at higher NO_2 exposures.

The epidemiologic evidence does not provide coherence with the results from toxicological studies. There is some indication of larger NO_2-associated cardiovascular or diabetes-related mortality in obese groups (Raaschou-Nielsen et al., 2012). However, most studies did not provide evidence that associations between long-term NO_2 exposure and cardiovascular disease or diabetes morbidity differed between obese and nonobese groups [(Eze et al., 2014; Atkinson et al., 2013; Hart et al., 2013; Mobasher et al., 2013; Andersen et al., 2012b; Andersen et al., 2012a); Table 7-23]. Most studies used a similar definition of obese, i.e., BMI > 30 kg/m^2. The limited number of studies that examined NO_2 assocaitions with mortality from respiratory causes or lung cancer also did not provide any evidence of differences by obesity (Dimakopoulou et al., 2014; Yorifuji et al., 2010). Studies that examined short-term and long-term NO_2 exposure with subclinical cardiovascular effects found that most associations did not differ between obese and nonobese groups of people (Dadvand et al., 2014; Huang et al., 2012; Baja et al., 2010; Ljungman et al., 2008).

Toxicological evidence from a single study in rats demonstrated that obesity increases dyslipidemia in relation to long-term NO_2 exposure. However, the majority of epidemiologic studies reported that associations of long-term NO_2 exposure with cardiovascular diseases, events, and mortality as well as diabetes mostly did not differ between obese and nonobese adults. In addition to the limited evidence indicating that obese people may be at increased risk of NO_2-related health effects uncertainties remain in the overall body of evidence regarding the independent effects of NO_2 on cardiovascular and related metabolic effects (Section 6.3.9) and mortality (Section 6.5.3). Therefore, the evidence is inadequate to determine whether obese individuals are at increased risk for NO_2-related health effects.

Table 7-10 Toxicological study evaluating pre-existing obesity.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Animal Model	Study Details	Study
Obesity n = 9−13	No obesity n = 10−14	↑	Triglycerides, HDL, total cholesterol, blood sugar	Rats (OLETF and LETO) N = 10−14 males/group		Takano et al. (2004)

[a]Up facing arrow indicates that the effect of NO_2 is greater (e.g., larger increase in triglycerides) in the group with the factor evaluated than in the reference group.

Table 7-11 Epidemiologic studies evaluating pre-existing obesity.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Short-term exposure						
High BMI (≥27) n = 29	Low BMI (<27) n = 69	–	Ventricular arrhythmia	N = 98 with implantable cardioverter defibrillators Age 28−85 yr	Gothenburg, Stockholm, Sweden, 2001−2006	Ljungman et al. (2008)
High BMI (≥25) n = 16	Low BMI (<25) n = 24	–	HRV decrements	N = 40 nonsmoking adults with CVD Mean age 66 yr	Beijing, China, 2007−2008	Huang et al. (2012)
High BMI (≥30) n = 27.6%	Low BMI (<30) n = 72.4%	↑	Change in ventricular repolarization	N = 580 males Mean age 75 yr	Boston, MA area, Follow-up: 2000−2008	Baja et al. (2010)
Long-term exposure						
High BMI (≥30)[b]	Low BMI (<30)[b]	–	Incidence MI	N = 84,562 Ages 30−55 yr at enrollment	U.S., 1990−2008	Hart et al. (2013)
High BMI (>30) n = 109,104	Low BMI (25−30) n = 243,556	–	Heart failure	N = 836,557 Ages 40−89 yr in 2003	England, 2003−2007	Atkinson et al. (2013)
High BMI (≥30) n = 84	Low BMI (<30) n = 158	–	C-reactive protein	N = 242 adults with clinically stable COPD Mean age 68 yr	Barcelona, Spain, 2004−2006	Dadvand et al. (2014)
		–	Tumor necrosis factor (TNF)-α			
		–	IL-6			
		↓	IL-8			
		–	Fibrinogen			
		–	Hepatocyte Growth Factor			
High BMI (≥30) n = 366	Low BMI (<30) n = 1,618	–	Incident stroke	N = 1,984 Ages 50−65 yr at baseline	Copenhagen, Aarhus counties, Denmark, 1993−2006	Andersen et al. (2012a)
High BMI (≥30) n = 19	Low BMI (<30) n = 123	–	Fatal stroke	N = 142 Ages 50−65 yr at baseline		
High (≥25−<30) and very high BMI (≥30) n = 28,937	Low BMI (<25) n = 22,881	–	Diabetes	N = 51,818 Ages 50−65 yr at baseline	Copenhagen, Aarhus counties, Denmark, 1993−2006	Andersen et al. (2012b)

Table 7-11 (Continued): Epidemiologic studies evaluating pre-existing obesity.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
High waist-to-hip ratio (>0.90 male, >0.85 female) n = 26,183	Low waist-to-hip ratio (≤0.90 male, ≤0.85 female) n = 25,635	↑				
High BMI[b] (>30)	Low BMI[b] (≤30)	–	Diabetes	N = 6,392 Ages 29−73 yr	Switzerland, 2002	Eze et al. (2014)
High BMI (>30) n = 68	Low BMI (<30) n = 213	–	Hypertensive disorders of pregnancy	N = 298 predominantly Hispanic women	Los Angeles, CA, 1996−2008	Mobasher et al. (2013)
High BMI[b] (>30)	Low BMI[b] (<30)	↓	Respiratory mortality	N = 307,553 Mean age at baseline 41.9 to 73.0 yr across 16 cohorts	Europe Follow-up: 1985−2007 NO_2 exposure assessed for 2008−2011	Dimakopoulou et al. (2014)
High BMI (>25) n = 1,950	Low BMI (<18.5) n = 1,010	–	CVD mortality	N = 9,941, 256 deaths Ages 35−103 yr	Shenyang, China Follow-up: 1998−2009 NO_2 exposure assessed for 1998−2009	Zhang et al. (2011)
High BMI (>30) n = 96,076 person-years	Low BMI (<25) n = 298,503 person-years	↑	Diabetes-related mortality	N = 52,061 Ages 50−64 yr	Denmark Follow-up: 1971−2009 NO_2 exposure assessed for 1971−2009	Raaschou-Nielsen et al. (2012)
High BMI (>21.8)[b]	Low BMI (<21.8)[b]	–	Lung cancer mortality	N = 14,001 Ages >65 yr	Shizuoka, Japan, 1999−2006	Yorifuji et al. (2010)

[a]Up facing arrow indicates that the effect of NO_2 is greater (e.g., larger change in ventricular repolarization) in the group with the factor evaluated than in the reference group. Down facing arrow indicates that the effect of NO_2 is smaller in the group with the evaluated factor than in the reference group. A dash indicates no difference in NO_2-related health effect between groups.
[b]Sample size not reported.

7.4 Genetic Factors

Genetic variation in the human population is known to contribute to numerous diseases and differential physiologic responses. The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) concluded that "it remains plausible that there are genetic factors that can influence health response to NO_2, through the few available studies did not provide specific support." Since then a growing body of studies have examined whether specific genetic

polymorphisms increase the risk of NO_2-related health effects. A strength of these studies is that they evaluate genetic factors using a targeted approach to focus on specific genes that potentially are involved in signaling pathways involved in biological responses to NO_2. For example, NO_2 exposure can lead to the formation of oxidation products (Section 4.3.2.1) and also modulate immune function (Section 4.3.2.6), and studies examined variants for genes encoding antioxidant enzymes [e.g., glutathione S-transferases (GST)M1 and GSTP1]) and mediators of immune response [e.g., tumor necrosis factor-alpha (TNF-α)]. A potential limitation for drawing conclusions about genetic variants is the large number of variants examined within studies which increases the probability of finding associations by chance alone. Thus, consistency in findings across genetic variants is considered. Further, the functional or biological consequence of some of the gene variants is unknown, and some variants may be surrogates for another linked gene or a group of related genes. Thus, where available, the variant effect is described (Table 7-12) and considered in conclusions. With in this section gene variants have been examined primarily for NO_2-associated respiratory effects, with a few studies examining cardiovascular effects and cognitive function. Because evidence supporting an independent effect of NO_2 exposure is strongest for respiratory effects (causal for short-term NO_2 exposure, Section 5.2.9) and there is uncertainty for relationships with other health effects, conclusions for genetic variants emphasize respiratory effects evidence.

Oxidative stress has been described as a key process underlying the respiratory effects attributed to NO_2 exposure (Section 4.3.2.1); however, studies that examined functional variants of GSTM1 or GSTP1 estimated similar (Castro-Giner et al., 2009) or lower (Romieu et al., 2006) effects of short-term or long-term NO_2 exposure on asthma symptoms or asthma prevalence in groups with variants encoding enzymes with null or reduced oxidative metabolizing activity (Table 7-12). These variants are common in the population, and NO_2-related health effects were compared between groups with fairly similar numbers of people (Table 7-12). Genetic variants with the potential for elevated oxidative stress have been observed to increase NO_2-related subclinical cardiovascular and metabolic effects [(Kim and Hong, 2012; Baja et al., 2010); Table 7-12]. A strength of the studies of cardiovascular effects is that rather than performing multiple comparisons of individual variants, genetic variants were analyzed as a cumulative index of oxidative stress potential, either the number of variants with increased oxidative stress potential (Baja et al., 2010). Further, independent relationships between short-term NO_2 exposure and cardiovascular and metabolic effects are uncertain. Thus, it is not clear the extent to which the findings for modification by gene variants can be attributed to NO_2 specifically.

Similar to variants with known functional differences, no clear evidence exists that genetic variants in oxidative metabolism enzymes with unknown functional differences increase the risk of NO_2-related respiratory effects. Associations of long-term NO_2 exposure with decrements in lung development in children were larger for some variants in glutathione metabolism pathway genes, such as glutathione synthetase [GSS; (Breton et al., 2011)]. However, results were not consistent across the multiple gene variants of glutathione examined [glutathione reductase (GSR), glutamate-cysteine ligase, modifier subunit (GCLM), glutamate-cysteine ligase, catalytic subunit (GCLC)] or NADPH-quinone oxidoreductase (NQO1) (rs10517). Variants for NQO1 (Castro-Giner et al., 2009) were observed to increase associations with asthma prevalence in adults. However, NQO1 was one among many variants examined.

Mediators of immune response including TNF-α and toll-like receptor (TLR)4 are known to have a role in oxidant-induced inflammation and asthma pathogenesis, but variants in these genes were not observed to modify associations of long-term NO_2 exposure with asthma prevalence [(Castro-Giner et al., 2009); Table 7-12]. Variants in immune response genes also did not modify associations of long-term NO_2 exposure with myocardial infarction (Panasevich et al., 2013).

The beta-2-adrenergic receptor (ADRB2) is an encoded G protein-coupled receptor that plays an important role in regulation of airway smooth muscle tone and is the pharmacological target of beta-agonist asthma medications (Hizawa, 2011). NO_2 exposure has been shown to increase airway responsiveness in adults with asthma (Section 5.2.2.1), providing a plausible role for variants in ADRB2 in modifying the risk of NO_2-associated respiratory effects. The association between ambient NO_2 estimated for residential locations and asthma prevalence in adults was not modified by ADRB2 variants with unknown functional differences (Castro-Giner et al., 2009). However, higher methylation of the ADRB2 promoter, which is associated with reduced expression of the receptor, was observed to increase the risk of asthma severity in children associated with indoor residential NO_2 exposure (Fu et al., 2012). There is mixed evidence for beta-agonist medication use in modifying NO_2-associated respiratory effects (Section 5.2.2.2), and it is not known whether the response to beta-agonists is influenced by genetic variants in ADRB2.

There is evidence for independent relationships of short-term and long-term NO_2 exposure, respectively, with exacerbation (Section 5.2.9) and development (Section 6.2.9) of asthma, and antioxidant modulation, immune-mediated inflammation, and airway responsiveness are described as key events in the underlying modes of action (Section 4.3.5). Evidence in rodents that dietary antioxidants modify NO_2-induced pulmonary oxidative stress (Section 7.6.1) would suggest a role for variants in oxidative

metabolism genes in modifying NO_2-related respiratory effects. However, gene variants with greater potential for oxidative stress were not observed to modify associations of short-term NO_2 with asthma-related effects. Variants in antioxidant and immune-related genes did modify some associations of long-term NO_2 exposure with respiratory effects, but results are inconsistent for any particular gene variant or respiratory effect. Further, several results are based on multiple comparisons and post-hoc analyses. There is more limited but consistent evidence for gene variants for antioxidant enzymes and inflammatory cytokines modifying subclinical cardiovascular and related metabolic effects but not myocardial infarction. Overall, the findings for effect measure modification by genetic variants are inconsistent for respiratory effects and the interpretation of results from studies of cardiovascular and related metabolic effects is complicated by the uncertainty as to whether the effects can be attributed specifically to NO_2 exposure. Therefore, the evidence is inadequate to determine whether genetic variants, particularly for antioxidant enzymes and immune responses, increase the risk for NO_2-related health effects.

Table 7-12 Epidemiologic studies evaluating genetic factors.

Factor Evaluated/Gene Function	Reference Category	Direction of Effect Modification[a]	Outcome	Study Location and Population	Study
GSTM1 null, n = 58 Null oxidant metabolizing capacity	GSTM1 positive, n = 93	↓	Respiratory symptoms and medication use	Mexico City, Mexico N = 151 children with asthma Ages not reported	Romieu et al. (2006) Short-term NO_2
GSTM1 null n = 49% Null oxidant metabolizing capacity	GSTM1 positive, n = 51%	–	Asthma prevalence	Umea and Uppsala, Sweden; Ipswich and Norwich, U.K.; Albacete, Barcelona, Huelva, Galdakao, Oviedo, Spain; Erfurt, Germany; Paris, Grenoble, France; Antwerp, Belgium N = 2,920 Mean age 43 yr	Castro-Giner et al. (2009) Long-term NO_2
GSTM1 null, n = 299 Null oxidant metabolizing capacity	GSTM1 positive, n = 225	↑	Fasting glucose	Seoul, Korea N = 560 Ages 60−87 yr	Kim and Hong (2012) Short-term NO_2
		↑	Insulin levels		
GSTT1 null, n = 270 Null oxidant metabolizing capacity	GSTT1 positive, n = 254	↑	Fasting glucose		
		↑	Insulin levels		

Table 7-12 (Continued) Epidemiologic studies evaluating genetic variants.

Factor Evaluated/Gene Function	Reference Category	Direction of Effect Modification[a]	Outcome	Study Location and Population	Study
GSTP1 Val105Val or Ile105Val (rs1695), n = 179 Reduced oxidant metabolizing capacity (Val/Val)	GSTP1 Ile105Ile, n = 359	↑	Fasting glucose		
		–	Insulin levels		
GSTP1 Val105Val, n = 54 (rsID 947894) Reduced oxidant metabolizing capacity	Ile105Ile, n = 97	↓	Respiratory symptoms and medication use	Mexico City, Mexico N = 151 children with asthma Ages not reported	Romieu et al. (2006) Short-term NO_2
GSTP1 Val105Val (rsID 1695) n = 32% Reduced oxidant metabolizing capacity	GSTP1 Ile105Ile or Ile105Val n = 68%	–	Asthma prevalence	Multiple European countries (see above) N = 2,920 Mean age 43 yr	Castro-Giner et al. (2009) Long-term NO_2
≥4 variants with increased oxidative stress potential[b] (GSTT1, GSTP1, GSTM1, HMOX, NQO1, HFE)	<4 variants with increased oxidative stress potential[b]	↑	Heart rate-corrected QT interval (ventricular repolarization)	Boston, MA area N = 580 males Mean age 75 yr	Baja et al. (2010)
GSTP1 Val105Val or Ile105Val n = 198 Reduced oxidant metabolizing capacity	GSTP1 Ile/Ile n = 152	↑	Cognitive function decrements	Menorca, Spain N = 350 children followed from birth to age 4 yr	Morales et al. (2009) Long-term indoor NO_2
GSS haplotype 0100000, n = 1,010 (rs1801310) Unknown function	Other haplotypes, n = 1,096	↑	Lung development decrements	Alpine, Atascadero, Lake Elsinore, Lake Arrowhead, Lancaster, Lompoc, Long Beach, Mira Loma, Riverside, San Dimas, Santa Maria, Upland, CA N = 2,106 children followed ages 10−18 yr	Breton et al. (2011) Long-term NO_2
GSR, various SNPs n = 3−21% Unknown function	Other haplotypes n = 3−21%	–			
GCLM, various SNPs, n = 6−35% Unknown function	Other haplotypes, n = 6−35%	–			
GCLC, various SNPs, n = 4−54% Unknown function	Other haplotypes, n = 4−54%	–			
NQO1 CC (rs2917666) n = 32% Unknown function	NQO1 GC or GG, n = 68%	↑	Asthma prevalence	Multiple European countries (see above) N = 2,920	Castro-Giner et al. (2009) Long-term NO_2

Table 7-12 (Continued) Epidemiologic studies evaluating genetic variants.

Factor Evaluated/Gene Function	Reference Category	Direction of Effect Modification[a]	Outcome	Study Location and Population	Study
TLR4 GG (rs11536889) n = 14% Unknown function	TLR4 GC or CC n = 86%	↑		Mean age 43 yr	
TNF-α 308 GA/AA (rs1800629) n = 16% Increased expression	TNF-α 308 GG n = 84%	–	Asthma prevalence	Multiple European countries (see above) N = 2,920 Mean age 43 yr	Castro-Giner et al. (2009) Long-term NO_2
TNF-α 308 GA/AA Increased expression n = 17%	TNF-α 308 GG n = 83%	–	Myocardial infarction	Stockholm County, Sweden N = 2,698 Ages 45−70 yr	Panasevich et al. (2013) Long-term NO_2
IL-6 174 CC n = 48% Increased blood IL-6 levels	IL-6 174 GG n = 52%	–			
IL-6 598 AA n = 47% Increased blood IL-6 levels	IL-6 598 GG n = 53%	–			
ADRB2[b] Intermediate or high methylation levels Reduced expression	ADRB2[b] Low methylation levels	↑	Asthma severity	CT and Springfield, Worcester, MA N = 182 Ages 5−12 yr, followed for 1 yr	Fu et al. (2012) Long-term indoor NO_2
ADRB2 rs1042713 G/G rs1042714 C/C rs1042718 C/C rs1042719 G/G n = 18−40% Unknown function	G/A or A/A C/G or G/G C/A or A/A G/C or C/C n = 60−82%	–	Asthma prevalence	Multiple European countries (see above) N = 2,920 Mean age 43 yr	Castro-Giner et al. (2009) Long-term NO_2
MET Tyrosine receptor kinase CC (rs1858830) n = 102	MET Tyrosine receptor kinase CG/GG n = 305	↑	Autism	Multiple unspecified locations, California N = 252 with autism, 156 without Ages 2−5 yr	Volk et al. (2014)

[a]Up facing arrow indicates that the effect of NO_2 is greater (e.g., larger increase in symptoms) in the group with the factor evaluated than in the reference group. Down facing arrow indicates that the effect of NO_2 is smaller (e.g., smaller increase in symptoms) in the group with the factor evaluated than in the reference group. A dash indicates no difference in NO_2-related health effect between groups.
[b]sample size not reported

7.5 Sociodemographic Factors

7.5.1 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). The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) indicated there was supporting evidence for increased risk of health effects related to NO_2 exposure among different lifestages, i.e., children and older adults. Differential health effects of NO_2 across lifestages theoretically could be due to several factors:

6) The human respiratory system is not fully developed until 18−20 years of age, and therefore, it is plausible to consider children to have intrinsic risk for respiratory effects due to potential perturbations in normal lung development.
7) 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 7-2).
8) Exposure/internal dose of NO_2 may vary across lifestages due to varying ventilation and time-activity patterns.

Studies in this ISA add to the evidence presented in the 2008 ISA indicating increased risk of NO_2-related health effects for children and older adults. Further, this evaluation of lifestage as a factor that may lead to increased risk for NO_2-related health effects draws upon information about time activity patterns and ventilation patterns among different lifestages to assess the potential for differences in NO_2 exposure or internal dose among lifestages.

7.5.1.1 Children

According to the 2010 census, 24% of the U.S. population is less than 18 years of age, with 6.5% less than age 6 (Howden and Meyer, 2011). The large proportion of children within the U.S. supports the public health significance of characterizing the risk of NO_2-related health effects among children.

In evaluating risk for NO_2-related health effects in children, it is important to consider exposure or dose differences; however, these are not well characterized. Children and adults differ with respect to time-activity patterns, which are determinants of inter-individual variability in NO_2 exposure [(Mölter et al., 2012; Kousa et al., 2001) and

Section 3.4.4]. In a comparison of children (mostly less than age 8 years), adults mostly under age 55 years, and adults older than age 55 years, a larger proportion of children reported spending over 30 minutes performing vigorous outdoor physical activity (Wu et al., 2011). However, there was little variation among groups in time spent in various microenvironments (Wu et al., 2011). Although a recent meta-analysis suggested a weaker association between ambient NO_2 concentrations and personal NO_2 exposure of children (Meng et al., 2012), some studies found ambient NO_2-related respiratory effects in children for whom there were moderate personal-ambient correlations [*r* values of 0.43 and 0.63; (Delfino et al., 2006; Linn et al., 1996)]. Such personal-ambient NO_2 relationships are consistent with greater time spent outdoor among children and could be an explanation for larger risk of NO_2-related health effects for children. A recent analysis found children more likely than adults to take part in vigorous activity or aerobic exercise [indoors and outdoors; (Wu et al., 2011)]. Higher activity along with higher ventilation rates relative to lung volume could potentially result in greater NO_2 penetration to the lower respiratory tract of children; however, this has not been examined for NO_2 (Section 4.2.2.3).

Epidemiologic evidence across diverse locations including the U.S., Europe, Asia, and Australia consistently demonstrates that short-term increases in ambient NO_2 concentration are associated with larger increases in asthma-related hospital admissions, ED visits, or outpatient visits among children than adults [(Son et al., 2013; Sinclair et al., 2010; Ko et al., 2007; Hinwood et al., 2006; Peel et al., 2005; Atkinson et al., 1999; Anderson et al., 1998); Table 7-13]. Most results are based on comparisons between children ages 0−14 years and people ages 15−64 years, and these found that NO_2-associated increases in asthma hospital admissions were 1.8 to 3.4 fold greater in children (Son et al., 2013; Ko et al., 2007; Atkinson et al., 1999; Anderson et al., 1998). Not all results demonstrated increased risk for children, with some studies of asthma hospital admissions, outpatient visits, and medication sales showing no difference in association with NO_2 between children and adults or no association in either group (Burra et al., 2009; Laurent et al., 2009; Migliaretti et al., 2005; Petroeschevsky et al., 2001). A few results point to larger NO_2-related increases in asthma hospital admissions or ED visits among younger children (e.g., age 0−4 years, 2−4 years) than older children ages 5−14 years (Samoli et al., 2011; Villeneuve et al., 2007); however, inference from these findings is limited because of the questionable reliability of asthma diagnosis in children below the age of 5 years (Section 5.2.2.4). Limited comparisons of lifestage in toxicological studies do not indicate larger NO_2-related effects on lung injury, inflammation, or lung host defense among juvenile than mature rodents (Azoulay-Dupuis et al., 1983) or between rodents with prenatal/weaning exposure and exposure only during weaning [(Kumae and Arakawa, 2006); Table 7-14]. The endpoints examined in

experimental animals do not have direct coherence with asthma-related effects and are not considered to be in conflict with epidemiologic evidence.

Risk may vary among children according to the time window of exposure because there are differences in lung development over the course of childhood. In this ISA, critical time windows of exposure for NO_2-related health effects in children were assessed from longitudinal studies that permitted within-subject comparisons as children were followed over time. Across studies, respiratory effects were associated with long-term NO_2 exposures assessed for various time windows, including birth, the first year of life, year of asthma diagnosis, and lifetime exposure (Section 6.2.2.1). In limited comparisons of time periods, no single critical time window of exposure was identified for the association of short-term or long-term NO_2 exposure and asthma exacerbation or diagnosis in children. In cohorts of children diagnosed with asthma at a median age of 2 or 5 years, NO_2 in the first year of life was associated with similar or lower risk of asthma compared with NO_2 assessed for later in childhood [average of ages 1−3 years or average in year of diagnosis; (Nishimura et al., 2013; Clougherty et al., 2007)]. The young age of diagnosis in most of these children limits inference about critical time windows of NO_2 exposure. In the Children's Health Study (CHS) cohorts, both exposures and respiratory outcomes were examined at various ages during follow-up from ages 5 or 10 years to 18 years. The heterogeneity among studies in exposure assessment methods, statistical methods, and examination of incidence or prevalence of outcomes is not amenable to quantitative comparisons. However, NO_2 exposure was associated with asthma and respiratory symptoms in childhood (ages 9−13 or 10 years) and into adolescence [ages 13−16 years or 10−18 years; (Jerrett et al., 2008; McConnell et al., 2006; Gauderman et al., 2005; McConnell et al., 2003; McConnell et al., 1999)], also pointing to risk of NO_2-associated respiratory effects throughout childhood.

In conclusion, epidemiologic evidence generally demonstrates that NO_2-related asthma exacerbation is greater in children compared to adults. In a few cases, no difference was observed by age, i.e., for NO_2-associated asthma outpatient visits and medication use. However, there is sufficient consistency for asthma hospital admissions and ED visits and for similar age comparisons (ages 0−14 years vs. 15−64 years). Limited toxicological results suggest greater NO_2-induced pulmonary injury and impaired host defense in mature compared to juvenile animals, but these endpoints are not directly related to asthma and are not considered to contradict epidemiologic evidence. As examined for asthma incidence or prevalence in children, no single critical time window of exposure (e.g., infancy, later childhood) has been identified. Children have different time-activity and ventilation patterns than adults, but it is not clear whether these contribute to higher NO_2 exposure or internal dose or increased risk for NO_2-related asthma exacerbation in children. Overall, the consistent epidemiologic evidence for larger NO_2-related asthma

exacerbation is adequate to conclude that children are at increased risk for NO_2-related health effects.

Table 7-13 Epidemiologic studies evaluating childhood lifestage.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Age of health effect: short-term NO_2 exposure						
Childhood Ages 0−14 yr n = 23,596	Adulthood Ages 15−65 yr n = 21,204	↑	Asthma hospital admissions	15 hospitals N = 69,176 admissions	Hong Kong, 2000−2005	Ko et al. (2007)
Childhood Ages 0−14 yr	Adulthood Ages 15−64 yr	↑	Asthma hospital admissions	Database accounting for 48% of Korean population	8 South Korean cities, 2003−2008	Son et al. (2013)
Childhood Ages 0−4 yr	Childhood Ages 5−14 yr	↑	Asthma hospital admissions	3 children's hospitals	Athens, Greece, 2001−2004	Samoli et al. (2011)
Childhood Ages 2−4 yr n = 7,247	Childhood Ages 5−14 yr n = 13,145	↑	Asthma ED visits	5 hospitals N = 57,192 visits	Edmonton, Canada, 1992−2002	Villeneuve et al. (2007)
Childhood Ages not reported n = 28,487	Adulthood Ages not reported n = 19,085	↑	Asthma outpatient visits	Records from managed care organization for 270,000 people	Atlanta, GA, 1998−2002	Sinclair et al. (2010)
Childhood Ages 1−17 yr	Adulthood Ages 18−64 yr	–	Asthma outpatient visits		Toronto, Canada, 1992−2001	Burra et al. (2009)
Childhood Ages 0−19 yr n = 7,774	Adulthood Ages 20−39 yr n = 7,347	–	Asthma medication sales		Strasbourg, France, 2004	Laurent et al. (2009)
Time window of exposure: long-term exposure						
Exposure in year of diagnosis	Exposure in first year of life	↑	Asthma incidence Median age of diagnosis: 5 yr	N = 417 children	Boston, MA, Follow up: prenatally (1987−1993) to 1997	Clougherty et al. (2007)
Exposure in first year of life	Exposure in first 3 yr of life	–	Asthma prevalence Median age of diagnosis: 2 yr	N = 4,320 children enrolled between ages of 8 and 21 yr	5 U.S. cities, 1996−2001 Central site NO_2	Nishimura et al. (2013)

[a]Up facing arrow indicates that the effect of NO_2 is greater (e.g., larger increase in hospital admission) in the group with the factor evaluated than in the reference group. Down facing arrow indicates that the effect of NO_2 is smaller in the group with the factor evaluated than in the reference group. A dash indicates no difference in NO_2-related health effect between groups.

Table 7-14 Toxicological studies informing childhood lifestage.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Animal Model	Study Details	Study
Prenatal/ weanling exposure	Weanling exposure	↑	Alveolar macrophage activity	Rats (Brown Norway) N = 5−7/group Females	Prenatal/weaning exposure: Breeding pairs mated in 200, 500, or 2,000 ppb NO_2. Litters continuously exposed until 8 and 12 weeks Weanling exposure: 5 week old rats exposed to 200, 500, or 2,000 ppb NO_2 continuously until 8 and 12 weeks	Kumae and Arakawa (2006)
Juvenile age	Adult age	–	Mortality	Rats (Wistar) N = 5−8/group	2,000 ppb for 3 days at 5, 10, 21, 45, 55, and 60 days of age	Azoulay-Dupuis et al. (1983)
Juvenile age	Adult age	↓		Guinea pigs (Hartley) N = 5−8/group		
Juvenile age	Adult age	–	Lung injury, inflammation	Rats (Wistar) N = 5−8/group	2,000 ppb for 3 days at 5, 10, 21, 45, 55, and 60 days of age	
Juvenile age	Adult age	↓		Guinea pigs (Hartley) N = 5−8/group		

[a]Up facing arrow indicates that the effect of NO_2 is greater (e.g., greater increase in alveolar macrophage activity) in the group with the factor evaluated than in the reference group. Down facing arrow indicates that the effect of NO_2 is smaller in the group with the factor evaluated than in the reference group. A dash indicates no difference in NO_2-related health effect between groups.

7.5.1.2 Older Adults

According to the 2008 National Population Projections issued by the U.S. Census Bureau, approximately 12.9% of the U.S. population is age 65 years or older, and by 2030, this fraction is estimated to grow to 20% (Vincent and Velkoff, 2010). Thus, this lifestage represents a substantial proportion of the U.S. population that is potentially at increased risk for health effects related to NO_2 exposure.

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) indicated that compared with younger adults, older adults (ages 65 years and older) may be at increased risk for NO_2-related respiratory effects and morality but not cardiovascular effects. Recent epidemiologic findings add to this body of evidence (Table 7-15). As described in the preceding section, time-activity patterns were found to differ across age groups; however, there were no differences in time spent in particular microenvironments or time in vigorous or outdoor activity (Wu et al., 2011) that might inform differences in NO_2

exposure among older adults than younger adults. Comparisons of older and younger adults with respect to NO_2-related asthma exacerbation are limited and generally show larger (one- to three-fold) effects in adults ages 65 years or older than among individuals ages 15−64 years or 15−65 years (Ko et al., 2007; Villeneuve et al., 2007; Migliaretti et al., 2005; Anderson et al., 1998). A few studies showed no difference between older and younger adults [(Son et al., 2013; Hinwood et al., 2006); Table 7-15]. Results for all respiratory hospital admissions combined generally also showed larger associations with NO_2 among older adults ages 65 years or older (Arbex et al., 2009; Wong et al., 2009; Hinwood et al., 2006; Atkinson et al., 1999). Evidence for increased risk in older adults for NO_2-related respiratory outcomes is more limited in controlled human exposure studies, where only statistically nonsignificant decrements in lung function were found with NO_2 exposure in healthy, older adults [(Gong et al., 2005; Morrow et al., 1992); Table 7-16].

For nonrespiratory effects, associations of short-term NO_2 with total mortality in most studies were larger in adults ages 65 or older than in younger adults Table 7-15), with evidence pointing to elevated risk among the oldest adults ages greater than 75 or 85 years (Chen et al., 2012; Cakmak et al., 2011; Chiusolo et al., 2011). Studies of long-term NO_2 exposure do not provide strong evidence of elevated risk of health effects among older adults, with inconsistent effect modification observed for total or cause-specific mortality (Dimakopoulou et al., 2014; Carey et al., 2013; Cesaroni et al., 2013; Zhang et al., 2011; Maheswaran et al., 2010; Yorifuji et al., 2010) and generally no difference by age group observed for associations with cardiovascular effects or diabetes (Eze et al., 2014; Atkinson et al., 2013; Rivera et al., 2013; Wichmann et al., 2013; Andersen et al., 2012a; Rosenlund et al., 2009a; Ljungman et al., 2008; Min et al., 2008). The age to define older adults varied among mortality and cardiovascular effect studies from 50 to 75 years. Further, it is uncertain the extent to which the inconsistent findings can be attributable to NO_2 because of uncertainty in whether NO_2 has independent relationships with cardiovascular effects (Sections 5.3.12 and 6.3.9) and mortality (Sections 5.4.8 and 6.5.3).

There is substantial and consistent evidence for larger NO_2-related respiratory effects in older adults compared to younger adults. Such evidence is based on hospital admissions and ED for asthma in a few cases, all respiratory conditions combined in several cases, and comparisons between adults ages 65 years or older and individuals 15−64 years of age. Controlled human exposure studies do not indicate NO_2-induced respiratory effects in older, healthy adults, but evidence is far more limited compared with epidemiologic evidence. Older adults had larger NO_2-related increases in total mortality but not cardiovascular effects. However, inferences about the risk for older adults from the total mortality and cardiovascular effects evidence is limited because of uncertainties

regarding the independent effect of NO_2 on those outcomes. Overall, the consistent epidemiologic evidence for larger NO_2-related asthma and all respiratory hospital admissions and ED visits is adequate to conclude that older adults are at increased risk for NO_2-related health effects.

Table 7-15 Epidemiologic studies evaluating older adult lifestage.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Short-term exposure						
Older adulthood Ages >65 yr n = 24,916	Younger adulthood Ages 15−65 yr n = 21,204	↑	Asthma hospital admissions	15 hospitals N = 69,176 admissions	Hong Kong, 2000−2005	Ko et al. (2007)
Older adulthood Ages >65 yr n = 4,705	Younger adulthood Ages 15−64 yr n = 32,815	↑	Asthma ED visits	5 hospitals N = 57,912 visits	Edmonton, Canada, 1992−2002	Villeneuve et al. (2007)
Older adulthood Ages ≥65 yr	Younger adulthood Ages 15−64 yr	–	Asthma and allergic disease hospital admissions	Hospital admission database accounting for 48% of Korean population	8 South Korean cities, 2003−2008	Son et al. (2013)
Older adulthood Ages ≥65 yr	All ages	–	COPD hospital admissions	14 hospitals	Hong Kong, 1996−2002	Wong et al. (2009)
		↑	COPD hospital admissions with influenza			
		–	Acute respiratory disease hospital admissions			
		↑	Cardiovascular hospital admissions			
Older adulthood Ages >64 yr, n = 789	Younger adulthood Ages 40−64 yr, n = 980	↑	COPD ED visits	40 hospitals	Sao Paulo, Brazil, 2001−2003	Arbex et al. (2009)
Older adulthood Ages ≥65 yr[b]	Younger adulthood, childhood Ages 5−64 yr[b]	↑	Total mortality	Data from Municipal Center for Disease Control and Prevention	17 Chinese cities	Chen et al. (2012)

Table 7-15 (Continued) Epidemiologic studies evaluating older adult lifestage.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Older adulthood Ages ≥65 yr, n = 187,608	Younger adulthood, childhood Ages <65 yr, n = 91,253	↑	Total mortality	Mean daily mortality across locations 7.29 to 15.8	Santiago Province, Chile (7 urban centers), 1997−2007	Cakmak et al. (2011)
Older adulthood Ages ≥85 yr n = 90,070	Younger adulthood Ages 35−64 yr n = 181,031	↑	Total mortality	N = 276,205 natural deaths	10 Italian cities, 2001−2005	Chiusolo et al. (2011)
Older adulthood Ages 65−74 yr n = 52,689	Younger adulthood Ages 35−64 yr n = 35,803	↓				
Long-term exposure						
Older adulthood Ages ≥75 yr[b]	Younger adulthood Ages <60 yr[b]	↓	Total mortality, Cardiovascular mortality	N = 1,265,058 Ages ≥30 yr	Rome, Italy, 2001−2010	Cesaroni et al. (2013)
		–	Lung cancer mortality			
Older adulthood Ages ≥75 yr[a]	Younger adulthood Ages 65−75 yr[a]	–	Lung cancer or cardiopulmonary mortality	N = 14,001	Shizuoka, Japan, 1999−2006	Yorifuji et al. (2010)
Older adulthood Ages ≥60 yr n = 365,368	Younger adulthood Ages 40−60 yr n = 470,239	↑	Total Mortality	N = 835,607 deaths Ages 40−89 yr	England Follow-up: 2003−2007 NO_2 exposure assessed for 2002	Carey et al. (2013)
Older adulthood Ages >60 yr n = 4,061	Younger adulthood Ages ≤60 yr n = 5,880	–	Cardiovascular mortality	N = 9,941, 256 deaths Ages 35−103 yr	Shenyang, China Follow-up: 1998−2009 NO_2 exposure assessed for 1998−2009	Zhang et al. (2011)
Older adulthood Ages ≥60 yr[b]	Younger adulthood Ages <60 yr[b]	↑	Respiratory Mortality	16 cohorts N = 307,553 Mean age at baseline 41.9 to 73.0 yr across cohorts	Europe Follow-up: 1985−2007 NO_2 exposure assessed for 2008−2011	Dimakopoulou et al. (2014)
Older adulthood Ages >70 yr n = 1,329	Younger adulthood Ages ≤70 yr n = 527	↓	Total mortality	N = 3,320 Mean age 70 yr	London, England Follow-up: 1995−2005 NO_2 exposure assessed for 2002	Maheswaran et al. (2010)
Older adulthood Ages >50 yr n = 635	Younger adulthood Ages 20−50 yr n = 242	↑	HRV decrements in low frequency domain	N = 1,349 healthy subjects Mean age 44 yr	Taein Island, South Korea, 2003−2004	Min et al. (2008)

Table 7-15 (Continued) Epidemiologic studies evaluating older adult lifestage.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Older adulthood Ages >75 yr n = 1,995	Younger adulthood Ages <60 yr, n = 1,252	–	Out-of-hospital cardiac arrest	N = 4,657 events	Copenhagen, Denmark, 2000−2010	Wichmann et al. (2013)
	Younger adulthood Ages 60−75 yr, n = 1,410	↑				
Older adulthood Ages ≥65 yr n = 50	Younger adulthood Ages <65 yr n = 60	–	Ventricular arrhythmia	N = 211 with implantable cardioverter defibrillators Age 28−85 yr	Gothenburg, Stockholm, Sweden, 2001−2006	Ljungman et al. (2008)
Older adulthood Ages ≥65 yr n = 137,184	Younger adulthood Ages <65 yr n = 417,156	–	Myocardial infarction	N = 43,275 cases, 511,065 controls Ages 15−79 yr	Stockholm County, Sweden, 1985−1996	Rosenlund et al. (2009a)
Older adulthood Ages 65−89 yr[b]	Younger adulthood Ages 40−64 yr[b]	–	Heart failure	N = 836,557 Ages 40−89 yr at baseline	England, 2003−2007	Atkinson et al. (2013)
Older adulthood Ages ≥56 yr n = 1,297	Younger adulthood Ages <56 yr n = 687	–	Incidence stroke	N = 1,984 Ages 50−65 yr at baseline	Copenhagen, Aarhus counties, Denmark, 1993−2006	Andersen et al. (2012a)
Older adulthood Ages ≥56 yr, n = 106	Younger adulthood Ages <56 yr, n = 36	–	Fatal stroke	N = 142 Ages 50−65 yr at baseline		
Older adulthood Ages ≥60 yr Female[b]	Younger adulthood Ages <60 yr Female[b]	–	Intimedia thickness cca	N = 2,780 Median age: 58 yr	Girona Province, Spain, 2007−2010	Rivera et al. (2013)
		↓	Intimedia thickness 6seg			
Male[b]	Male[b]	↑	Intimedia thickness cca			
		–	Intimedia thickness 6seg			
Older adulthood Ages ≥65 yr n = 2,234	Younger adulthood Ages 55−65 yr n = 3,913	–	Prevalent hypertension	N = 24,845 Mean age 45.59 yr	Shenyan, Anshan and Jinzhou, China, 2006−2008	Dong et al. (2013b)
Older adulthood Ages >50 yr[b]	Younger adulthood Ages ≤50 yr[b]	–	Diabetes	N = 6,392 Ages 29−73 yr	Switzerland, 2002	Eze et al. (2014)

[a]Up facing arrow indicates that the effect of NO_2 is greater (e.g., larger risk of hospital admission, larger decrement in HRV) in the group with the factor evaluated than in the reference group. Down facing arrow indicates that the effect of NO_2 is smaller in the group with the factor evaluated than in the reference group. A dash indicates no difference in NO_2-related health effect between groups.
[b]Sample size not reported.

Table 7-16 Controlled human exposure studies informing older adult lifestage.

Factor Evaluated	Reference Category	Direction of Effect[a]	Outcome	Study Population	Study Details	Study
Older adulthood	None	–	Lung function	N = 20 (10 males, 10 females) Mean age 61 yr	300 ppb NO_2 for 4 h with exercise	Morrow et al. (1992)
Older adulthood	None	–	Lung function, airway inflammation	N = 6 (2 males, 4 females) Mean age 68 yr	400 ppb NO_2 for 2 h with exercise	Gong et al. (2005)

[a]These studies only examined older adults and have no reference group. A dash indicates that NO_2 was not observed to induce an effect in the older adults relative to clean air exposure.

7.5.2 Socioeconomic Status

SES is a composite measure that usually consists of economic status, measured by income; social status measured by education, and work status measured by occupation. Persons with lower SES have been generally found to have a higher prevalence of pre-existing diseases; potential inequities in access to resources such as healthcare; and possibly increased nutritional deficiences, which may increase this population's risk to NO_2-related health effects. According to U.S. Census data, 15.9% (approximately 48.5 million) of Americans were of poverty status in 2011 as defined by household income, which is one metric used to define SES (Bishaw, 2012). Across the indicators of SES examined (e.g., education level, employment status, insurance status, social deprivation, and access to health care) there is some evidence indicating higher NO_2 exposure and larger risk of NO_2-related health effects among low SES groups in the population, but these relationships are not uniformly observed (Table 7-15). A challenge in synthesizing findings across studies is the array of SES indicators examined. Further, many studies were conducted outside of the U.S., and definitions of SES can vary across countries based on population demographics, bureaucracy, and the local economy which can contribute to varying degrees of deprivation or inequities.

Several studies relate higher NO_2 exposure with indicators of low SES, but the relationship varies across communities, levels of SES, and indicators of SES. Results from studies conducted in the U.S., Canada, and Europe point to a relationship between higher ambient NO_2 exposure among populations of low SES as determined by household income, job class (e.g., unskilled, professional, skilled manual labor), or education. Higher ambient NO_2 concentrations have been measured in communities in Montreal, Canada and Los Angeles, CA with high proportions of nonwhite residents and low SES residents (Su et al., 2012; Molitor et al., 2011; Crouse et al., 2009; Su et al.,

2009). While many of these studies examined community-level correlations based on census block or tract SES and NO_2 modeled at the neighborhood scale (Clark et al., 2014; Deguen and Zmirou-Navier, 2010; Namdeo and Stringer, 2008; Kruize et al., 2007; Chaix et al., 2006; Mitchell, 2005), studies with data from individuals also found relationships between higher residential or personal NO_2 and lower SES (Llop et al., 2011; Deguen and Zmirou-Navier, 2010). A U.S.-wide analysis of census blocks suggests inequities in NO_2 exposure in the low SES communities by age, with higher exposures indicated for children and older adults (Clark et al., 2014).

While most results indicate higher NO_2 exposure in low SES groups, some indicate that the relationship between NO_2 exposure and SES varies in strength and direction. In some cases, a nonlinear relationship is observed with either no difference in NO_2 concentrations among communities in the higher end of the income distribution [e.g., top 50%; (Kruize et al., 2007)] or higher NO_2 concentrations in some affluent communities in the downtown core of a city (Crouse et al., 2009). Other studies find that the relationship varies across communities (Stroh et al., 2005) and among particular SES indicators [e.g., education but not occupation, country of birth but not education; (Stroh et al., 2005; Rotko et al., 2001)]. The relationship between NO_2 exposure and SES also may weaken over time as was forecasted for Leeds, U.K. Over the period 1993−2005, fleet renewal and congestion pricing were predicted to reduce the discrepancy in NO_2 exposures between groups with high and low deprivation index [combining unemployment, noncar ownership, nonhome ownership, and household overcrowding; (Mitchell, 2005)]. O'Neill et al. (2003) noted that several factors might alter the relationship between NO_2 exposure and SES, including changing development, migration, and transportation patterns all of which could result in individuals of high socioeconomic status having high NO_2 exposures.

There is also the possibility that a multitude of factors may interact to influence the risk of NO_2-related health effects in populations of low SES. The hypothesis of "double jeopardy" describes interactions between higher air pollution exposure and social inequities in health, whereby risk of health effects for low SES and/or nonwhite populations may be increased because of increased exposure and psychosocial stress or access to health services (O'Neill et al., 2003). An index combining risk factors such as air pollution concentrations, including NO_2, with nonwhite population and low SES population has been constructed for communities in a few California cities (Su et al., 2012; Su et al., 2009). However, relationships between such indices and health effects have not been examined, and for the studies evaluated in this ISA, the risk for certain SES or nonwhite populations resulting from multiple stressors have not been characterized.

While there is strong evidence for relationships between short-term and long-term NO_2 exposure and asthma exacerbation (Section 5.2.9) and development (Section 6.2.9), evidence does not clearly indicate differences among groups of varying SES (Table 7-15). Children with higher psychosocial stress due to exposure to community violence were observed to have increased risk for asthma incidence related to long-term NO_2 exposure (Clougherty et al., 2007). Associations between short-term NO_2 exposure and asthma-related effects mostly do not differ by SES (Burra et al., 2009; Laurent et al., 2009; Kim et al., 2007; Lin et al., 2004), although a stronger association was found among children in Phoenix, AZ with no insurance (Grineski et al., 2010). The latter study observed interactions between race/ethnicity and SES. NO_2-related asthma hospital admissions did not differ between Hispanic and white children, except in the group without health insurance (Grineski et al., 2010). Such results indicate the potential for multiple co-occurring factors in certain populations to influence risk of NO_2-related health effects.

Several multicity studies in various countries found larger associations between short-term NO_2 exposures and total mortality in low SES compared to high SES groups as indicated by education, income, or employment [(Chen et al., 2012; Cakmak et al., 2011; Chiusolo et al., 2011); Table 7-17]. Despite the consistency, there is uncertainty in the extent to which the findings can be attributed specifically to NO_2 because uncertainty regarding confounding by traffic-related copollutants is noted for a relationship between short-term NO_2 exposure and total mortality (Section 5.4.8).

Evidence that SES modifies associations of long-term NO_2 exposure with cardiovascular and related metabolic effects, mortality, reproductive effects, developmental effects, or cancer is unclear (Table 7-17). However, independent effects of NO_2 exposure on these health effects is uncertain (Sections 6.3.9, 6.4.5, 6.5.3, 6.6.9). Some studies found larger associations among lower SES groups (Becerra et al., 2013; Carey et al., 2013; Cesaroni et al., 2013; Morello-Frosch et al., 2010), but studies equally found no difference among SES groups (Eze et al., 2014; Andersen et al., 2012b; Guxens et al., 2012; Pereira et al., 2012; Zhang et al., 2011; Lenters et al., 2010; Yorifuji et al., 2010; Rosenlund et al., 2009a) or inconsistent effect modification among the outcomes examined (Foraster et al., 2014; Andersen et al., 2012a; Sørensen et al., 2012). A few studies observed weaker NO_2-related effects among lower SES groups (Atkinson et al., 2013; Rivera et al., 2013). A diverse set of SES indicators was examined, and results were inconsistent even among studies examining education or income.

Evidence indicates higher NO_2 exposure among low SES communities, although elevated concentrations are also reported for some high SES communities. For short-term or long-term NO_2 exposure, associations with respiratory effects, cardiovascular effects,

mortality, reproductive effects, developmental effects, and cancer do not consistently vary by SES. However, evidence consistently demonstrates larger associations between short-term NO_2 exposure and mortality among low SES groups. Interpreting this body of evidence is challenging given the diversity of SES indicators used across studies, breadth of countries where studies have been conducted, and varying certainty in the independent effect of NO_2 on the array of health effects examined. Overall, there is consistent evidence for larger NO_2-related increases in mortality in low SES groups, but uncertainty remains in attributing the findings specifically to NO_2. As a result, the evidence is suggestive that low SES populations are at increased risk for NO_2-related health effects.

Table 7-17 Epidemiologic studies evaluating socioeconomic status.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Short-term exposure						
No insurance n = 205	Insurance n = 2,508 private n = 2,015 Medicaid	↑	Asthma hospital admissions	Children Ages <14 yr	Phoenix, AZ, 2001−2003	Grineski et al. (2010)
Low income census tracts[b]	High income census tracts[b]	–	Asthma hospital admissions	N = 3,822 admissions Children 6−12 yr	Vancouver, Canada (13 subdivisions), 1987−1998	Lin et al. (2004)
Low income-based insurance premiums n = 24% quintile 1	High income-based insurance premiums n = 17% quintile 5	–	Asthma emergency outpatient visits	Mean 254 visits/day Prior asthma diagnosis required	Seoul, Korea, 2002	Kim et al. (2007)
Lowest quintile for census tract income n = 610,121	Highest quintile for census tract income n = 527,385	–	Asthma physician visits	Data from Ontario Health Insurance Plan	Toronto, Canada, 1992−2001	Burra et al. (2009)
Low SES composite of income, education, job, housing factors n = 43,674 lowest stratum	High SES n = 49,111 highest stratum	–	Asthma medication sales	N = 261,063 Ages 0−39 yr	Strasbourg, France, 2004	Laurent et al. (2009)

Table 7-17 (Continued): Epidemiologic studies evaluating socioeconomic status.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Low income census tracts <20th percentile n = 33,565	Middle income census tracts 20th−80th percentile n = 93,040	↑	Total mortality	N = 276,205 natural deaths Ages >35 yr SES available for 44% of study population	10 Italian cities, 2001−2005	Chiusolo et al. (2011)
Low or middle income census tracts n = 126,605	High income census tracts >80th percentile n = 38,681	–				
Low education (<primary school)[b]	High education (university diploma)[b]	↑	Total mortality	Mean daily mortality across locations 7.29 to 15.8	Santiago Province, Chile 7 urban centers 1997−2007	Cakmak et al. (2011)
Low income census area[b]	High income census area[b]	↑				
Un-employed[b]	White collar worker[b]	↑				
Low education (illiterate/ primary school)[b]	High education (middle school and above)[b]	↑	Total mortality	Data from Municipal Center for Disease Control and Prevention	17 Chinese cities	Chen et al. (2012)
Long-term exposure						
High exposure to violence[b]	Low exposure to violence[b]	↑	Asthma incidence	N = 417 children followed from prenatal period	Boston, MA, 1987−1993 Followed to 1997	Clougherty et al. (2007)
Blue collar work, Low-level white collar work n = 57.9%	High-level white collar work n = 42.1%	–	Myocardial infarction	N = 43,275 cases, 511,065 controls	Stockholm county, Sweden, 1985−1996	Rosenlund et al. (2009a)
Low income (<mean of controls) n = 44.4%	High income (>mean of controls) n = 55.6%	–				
Low education (<high school) n = 58.5%	High education (≥high school) n = 41.5%	–				
Low or medium education n = 65%	High education n = 35%	–	Atherosclerosis (carotid intima-media thickness)	N = 745 Ages 26−30 yr	Utrecht, Netherlands, 1999−2000	Lenters et al. (2010)

Table 7-17 (Continued): Epidemiologic studies evaluating socioeconomic status.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Primary or secondary school n = 2,234	Higher education/ technician n = 526	↓	Atherosclerosis (carotid intima meda thickness)	N = 2,780 Median age 58 yr	Girona Province, Spain, 2007−2010	Rivera et al. (2013)
Lowest deprivation index n = 20%	Highest deprivation index n = 20%	↓	Heart failure	N = 836,557, Ages 40−89 yr in 2003	England, 2003−2007	Atkinson et al. (2013)
Low or medium education (<10 yr) n = 1,628	High education (≥10 yr) n = 356	–	Incident stroke	N = 1984 Ages age 50−65 yr at baseline	Copenhagen, Aarhus counties, Denmark, 1993−2006	Andersen et al. (2012a)
Low or medium education (<10 yr) n = 110	High education (≥10 yr) n = 32	↑	Fatal stroke	N = 142 Ages 50−65 yr at baseline		
Low or medium education (<10 yr) n = 40,956	High education (≥10 yr) n = 10,862	–	Diabetes	N = 51,818 Ages 50−65 yr at baseline	Copenhagen, Aarhus counties, Denmark, 1993−2006	Andersen et al. (2012b)
Low or medium education (primary or secondary) n = 4,586	High education (college or university) n = 1,806	–	Diabetes	N = 6,392 Age 29−73 yr	Switzerland, 2002	Eze et al. (2014)
Low or medium education (<11 yr) n = 79%	High education (≥11 yr) n = 21%	↑	Hypertension (change in systolic BP)	N = 44,436 Ages 50−65 yr at baseline	Copenhagen, Aarhus counties, Denmark, 1993−2006	Sørensen et al. (2012)
Low/ medium SES municipality n = 78%	High SES municipality n = 22%	–				
Illiterate/ primary education n = 1,540	Secondary/ university education n = 2,160	–	Systolic blood pressure	N = 3,700, age 35−83 yr	Girona, Spain	Foraster et al. (2014)
		↑	Diastolic blood pressure			
Low income (<200 month) n = 1,817	High income (≥800 month) n = 2,618	–	Cardiovascular mortality	N = 9,941, 256 deaths Ages 35−103 yr	Shenyang, China Follow-up: 1998−2009 NO_2 assessed for 1998−2009	Zhang et al. (2011)
Low education n = 5,970	High education n = 3,971	–				

Table 7-17 (Continued): Epidemiologic studies evaluating socioeconomic status.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Low education (<8 yr) n = 33%	High education (<10 yr) n = 21%	–	Diabetes−related Mortality	N = 52,061 Ages 50−64 yr	Denmark Follow-up: 1971−2009 NO_2 exposure assessed for 1971−2009	Raaschou-Nielsen et al. (2012)
Low socio-economic position census block[b]	High or medium socio-economic position census block[b]	↑	Mortality – total, cardiovascular, IHD, lung cancer	N = 1,265,058 Ages ≥30 yr	Rome, Italy, 2001−2010	Cesaroni et al. (2013)
Lowest quintile for area-level income n = 12.5%	Lowest quintile for area-level income n = 24.7%	↑	Total Mortality	N = 835,607 Ages 40−89 yr	England Follow-up: 2003−2007 NO_2 exposure assessed for 2002	Carey et al. (2013)
Financially incapable (self-reported) n = 4,054	Financially capable (self-reported) n = 7,340	–	Lung cancer mortality	N = 14,001 Ages >65 yr	Shizuoka, Japan, 1999−2006	Yorifuji et al. (2010)
Lowest SES tertile n = 7,556	Highest SES tertile n = 7,941	–	Small for gestational age or intrauterine growth restriction	N = 23,452 women/infants	Perth, Western Australia, 2000−2006	Pereira et al. (2012)
High neighbor-hood level poverty[b]	Low neighbor-hood level poverty[b]	↑	Low birth weight	N = 3,545,177 births	California, 1996−2006	Morello-Frosch et al. (2010)
Low parental social class (semi-skilled/un-skilled occupation ≤primary education) n = 33%	High parental social class (managers/ technician occupation university degree education n = 41%	–	Mental development in infants at age 14 months	N = 1,889 children followed from prenatal period	4 Spanish cities, 2003−2008	Guxens et al. (2012)
Low maternal education (<high school) n = 1,725	High maternal education (>high school) n = 3,926	↑	Autistic disorder in children	N = 7,603 children with autism, 10 controls per case	Los Angeles, CA, 1998−2009	Becerra et al. (2013)

[a]Up facing arrow indicates that the effect of NO_2 is greater (e.g., larger risk of hospital admission) in the group with the factor evaluated than in the reference group. Down facing arrow indicates that the effect of NO_2 is smaller in the group with the factor evaluated than in the reference group. A dash indicates no difference in NO_2-related health effect between groups.
[b]Sample size not reported.

7.5.3 Race/Ethnicity

Based on the 2010 U.S. Census, 63.7% of the U.S. population identified themselves as non-Hispanic whites; 12.6% reported their race as non-Hispanic black; and 16.3% reported being Hispanic (Humes et al., 2011). Race and ethnicity are complex factors that are often closely correlated with other factors including particular genetics, diet, and socioeconomic status. Therefore, race and ethnicity may influence any potential differences in NO_2-related health effects through both intrinsic and extrinsic mechanisms.

Information characterizing racial/ethnic differences in NO_2 exposure is sparse but suggests higher exposure among nonwhite people independent of SES. For U.S. urban areas, the population-weighted mean annual average NO_2 for nonwhites was estimated to be 4.6 ppb (38%) higher than for whites (Clark et al., 2014). This difference was observed across the distribution of census block household income. However, NO_2 was estimated from a national scale LUR model and may reflect census block differences other than race or in combination with race.

In contrast with exposure, NO_2-related health effects have not been shown clearly to differ between groups of nonwhite and white populations (Table 7-18). This is the case for NO_2-related asthma ED visits among children, but interestingly there was a difference between Hispanic and white children when examining insurance status (Section 7.5.2) (Grineski et al., 2010). Additionally, Grineski et al. (2010) reported evidence of larger NO_2-related asthma ED visit associations among black children compared to Hispanic children. Racial and ethnic differences in NO_2-related health effects also are not consistently found for birth outcomes, although the implications of these findings are weak because an independent relationship between NO_2 exposure and birth outcomes is not certain. Some studies estimated larger effects on birth weight or gestational age among babies of black or Hispanic mothers (Rich et al., 2009; Bell et al., 2007); whereas others estimated larger effects for babies of white mothers (Morello-Frosch et al., 2010), or no difference among races (Darrow et al., 2011; Madsen et al., 2010).

There is some indication that NO_2 exposure may be higher among nonwhite compared to white populations, but information on NO_2 exposure at the individual-level is lacking. NO_2-related health effects do not consistently differ among racial and ethnic groups, particularly, for asthma exacerbation, which is concluded to have an independent relationship with short-term NO_2 exposure (Section 5.2.9). Additionally, it is unclear whether higher NO_2 exposure and higher prevalence of potential at-risk factors in combination impact the health of nonwhite populations (Section 7.5.2). Overall, the evidence for potential differences in the risk of NO_2-related health effects by race and

ethnicity is inconsistent and largely based on birth outcomes, for which an independent relationship with NO_2 exposure is uncertain. Therefore, the evidence is inadequate to determine whether race or ethnicity increases the risk for NO_2-related health effects.

Table 7-18 Epidemiologic studies evaluating race/ethnicity.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Short-term exposure						
Black race n = 635	White race n = 2,227	–	Asthma hospital admissions	N = 4,316 Children ages <14 yr	Phoenix, AZ, 2001−2003	Grineski et al. (2010)
Black race n = 635	Hispanic race n = 1,454	↑				
Hispanic race n = 1,454	White race n = 2,227	–				
Long-term exposure						
Black maternal race n = 10.7%	White maternal race n = 83.4%	↑	Birth weight decrements	N = 358,504 births	Massachusetts, Connecticut, 1999−2002	Bell et al. (2007)
Hispanic maternal race n = 14.3%	White maternal race n = 45.2%	–	Birth weight decrements	N = 406,627 full−term, singleton births	Atlanta, GA, 1994−2004	Darrow et al. (2011)
Non-Hispanic black maternal race n = 40.5%		–				
Non-Western ethnicity n = 24.3%	Western ethnicity n = 75.7%	–	Birth weight decrements	N = 25,229 Full-term, singleton births	Oslo, Norway	Madsen et al. (2010)
Hispanic maternal race n = 51.5%	Non-Hispanic white maternal race n = 32.2%	↓	Birth weight decrements	N = 3,545,177 singleton births, 37−44 week gestation	California, 1996−2006	Morello-Frosch et al. (2010)
Non-Hispanic black maternal race n = 5.8%		↓				

Table 7-18 (Continued): Epidemiologic studies evaluating race/ethnicity.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Hispanic maternal race n = 31%	White or African-American maternal race n = 69%	↑	Very small for gestational age (VSGA)	N = 178,198 singleton births, 37−42 week gestation, birth weight >500 g	New Jersey, 1999−2003	Rich et al. (2009)

[a]Up facing arrow indicates that the effect of NO_2 is greater (e.g., larger risk of hospital admission, larger decrement in birth weight) in the group with the factor evaluated than in the reference group. Down facing arrow indicates that the effect of NO_2 is smaller in the group with the factor evaluated than in the reference group. A dash indicates no difference in NO_2-related health effect between groups.

7.5.4 Sex

A vast number of health conditions and diseases have been shown to differ by sex with some indication that there may be differences by sex in the relationship between air pollution and health effects. The 2010 U.S. Census indicates an approximately equal distribution of males and females in the U.S.: 49.2% male and 50.8% female (Howden and Meyer, 2011). However, the distribution varies by age with a greater prevalence of females above 65 years of age compared to males. Thus, the public health implications of potential sex-based differences in air pollution-related health effects may vary among age groups within the population.

With respect to NO_2 exposure, limited evidence from a large (N = 1,634) multi-European country study indicates no difference between males and females as demonstrated by similar 2-week avg residential outdoor NO_2 concentrations (Sunyer et al., 2006). With respect to NO_2-related health effects, the strongest basis for inferring differences between males and females comes from studies of asthma exacerbation, for which an independent effect of short-term NO_2 exposure is determined (Section 5.2.9). Associations between short-term increases in ambient NO_2 concentration and asthma-related effects, as examined in children, did not clearly differ between males and females: with observations of larger effects in females (Lin et al., 2004), males (Mann et al., 2010), or no difference between sexes (Sarnat et al., 2012; Liu et al., 2009). Inconsistent evidence for potential differences by sex was also observed in studies examining associations between short-term NO_2 exposures and respiratory infections in children (Zemek et al., 2010; Lin et al., 2005). In contrast with findings for short-term NO_2 exposure, studies of long-term NO_2 exposure were consistent in showing that associations with respiratory effects such as asthma prevalence (Kim et al., 2004) and lung function decrements (Rosenlund et al., 2009b; Oftedal et al., 2008; Rojas-Martinez et al., 2007; Peters et al.,

1999) were larger in female than male children. The majority of evidence is for lung function decrements, and effect modification by sex is not explained by lower baseline lung function in females. While many studies estimated NO_2 exposures at or near (2 km) subjects' homes or schools (Rosenlund et al., 2009b; Oftedal et al., 2008; Rojas-Martinez et al., 2007), there is uncertainty regarding potential confounding by other traffic-related pollutants (Section 6.2.9). Thus, it is not clear to what extent the larger NO_2-related decreases in lung function among females reflect an independent effect of NO_2.

Beyond respiratory effects, the majority of studies observed no difference between males and females in associations of long-term NO_2 with an array of cardiovascular effects (e.g., myocardial infarction, heart failure, hypertension, stroke), diabetes, total mortality, cause-specific mortality, or lung cancer incidence as described in Table 7-19 (Beelen et al., 2014; Eze et al., 2014; Atkinson et al., 2013; Cesaroni et al., 2013; Dong et al., 2013a; Johnson et al., 2013; Andersen et al., 2012b; Raaschou-Nielsen et al., 2012; Raaschou-Nielsen et al., 2011; Zhang et al., 2011; Raaschou-Nielsen et al., 2010; Yorifuji et al., 2010; Rosenlund et al., 2009a; Abbey et al., 1999). In most cases, no difference between males and females was observed for NO_2 associations with subclinical effects such as changes in blood pressure, atherosclerosis, HRV, systemic inflammation, or insulin resistance (Bilenko et al., 2015; Foraster et al., 2014; Atkinson et al., 2013; Dong et al., 2013b; Rivera et al., 2013; Thiering et al., 2013; Lenters et al., 2010; Panasevich et al., 2009; Felber Dietrich et al., 2008). In the relatively small group of studies that found differences between males and females, most observed greater risk among females for associations of short-term NO_2 exposure with cardiac arrest (Wichmann et al., 2013) or total mortality (Cakmak et al., 2011; Kan et al., 2008) and for associations of long-term NO_2 exposure with total mortality or mortality from cardiovascular or respiratory causes or lung cancer (Carey et al., 2013; Katanoda et al., 2011; Naess et al., 2007; Abbey et al., 1999). Although most epidemiologic evidence indicates no difference between males and females for cardiovascular morbidity and mortality and total mortality related to long-term NO_2 exposure, similar to the evidence for long-term NO_2 exposure and lung function, there is uncertainty as to whether NO_2 has an effect independent of other traffic-related pollutants (Sections 6.3.9 and 6.5.3). Thus, the extent to which the lack of effect modification by sex can be attributable to NO_2 versus correlated copollutants is not clear.

The collective body of evidence does not clearly indicate that NO_2 exposure or NO_2-related health effects differ between males and females. Evidence demonstrates that short-term NO_2 exposure has an independent effect on asthma exacerbations, but the few studies do not indicate differences in associations between males and females. Additionally, there is limited but inconsistent evidence for short-term NO_2 exposure and cardiovascular disease and mortality, but uncertainty remains regarding an independent

effect of NO_2 on these outcomes. There is some support for larger associations in females for long-term NO_2 exposure and pulmonary function, cardiovascular disease, and mortality, though results are consistent only for pulmonary function. Therefore, the current evidence is suggestive that females may be at increased risk for NO_2-related health effects.

Table 7-19 Epidemiologic studies evaluating sex.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Short-term exposure						
Female n = 1,454	Male n = 2,368	↑	Asthma hospital admissions	N = 3,822 admissions Ages 6−12 yr	Vancouver, Canada, 1987−1998	Lin et al. (2004)
Female n = 2,137	Male n = 2,077	↑	Respiratory hospital admissions	4 hospitals	Windsor, Canada, 1995−2000	Luginaah et al. (2005)
Female n = 20	Male n = 38	–	Pulmonary inflammation	N = 58 children with asthma Ages 6−12 yr	Ciudad Juarez, Mexico and El Paso, TX	Sarnat et al. (2012)
Female n = 68	Male n = 114	–	Lung function decrements, Pulmonary inflammation	N = 182 children with asthma Ages 9−14 yr	Windsor, Canada, 2005	Liu et al. (2009)
Female n = 43.5%	Male n = 56.5%	↓	Wheeze	N = 315 children with asthma Ages 6−11 yr	Fresno, CA, 2000−2005	Mann et al. (2010)
Female n = 2,784	Male n = 3,998	–	Respiratory infection hospital admissions	N = 6,782 admissions in children Ages 0−14 yr	Toronto, Canada, 1998−2001	Lin et al. (2005)
Female n = 8,055	Male n = 6,472	↑	Otitis media ED visits	N = 14,527 ED visits in children Ages 1−3 yr	Edmonton, Canada, 1992−2002	Zemek et al. (2010)
Female n = 24	Male n = 16	↑	HRV	N = 40 nonsmoking adults with CVD Mean age: 65.6 yr	Beijing, China, 2007−2008	Huang et al. (2012)
Female n = 1,846	Male n = 2,811	↑	Out-of-hospital cardiac arrest	N = 4,657 events	Copenhagen, Denmark, 2000−2010	Wichmann et al. (2013)
Female n = 51.9%	Male n = 48.1%	↓	Total mortality	N = 276,205 natural deaths, >35 yr	10 Italian cities, 2001−2005	Chiusolo et al. (2011)

Table 7-19 (Continued): Epidemiologic studies evaluating sex.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Female[b]	Male[b]	↑	Total mortality	Mean daily mortality across locations 7.29 to 15.8	Santiago Province, Chile (7 urban centers), 1997−2007	Cakmak et al. (2011)
Female Mean daily deaths = 56.5	Male Mean daily deaths = 62.5%	↑	Total mortality	N = 173,911 deaths	Shanghai, China, 2001−2004	Kan et al. (2008)
Long-term exposure						
Female n = 49%	Male n = 51%	↑	Lung function decrements	N = 2,307 children Ages 9−10 yr	Oslo, Norway, 2001−2002	Oftedal et al. (2008)
Female n = 52.6%	Male n = 47.4%	↑	Asthma diagnosis	N = 1,109 children Grades 3−5	San Francisco, CA, 2001	Kim et al. (2004)
		–	Bronchitis			
Female n = 942−1,161	Male n = 890−1,160	↑	Lung development decrements	N = 3,170 healthy children Age 8 yr	Mexico City, Mexico, 1996−1999	Rojas-Martinez et al. (2007)
Female n = 648	Male n = 711	↑	Lung function decrements	N = 1,760 children Ages 9−14 yr	Rome, Italy, 2000−2001	Rosenlund et al. (2009b)
Female n = 832	Male n = 924	–	Respiratory symptoms	N = 1,756 full-term infants Assessed at ages 1 and 2 yr	3 German cities, 1995−1999	Gehring et al. (2002)
Female[b]	Male[b]	↑	Lung function decrements	N = 3,293 children Grades 4, 7, 10	Southern California, 1986−1990	Peters et al. (1999)
Female n = 49.4%	Male n = 50.6%	–	Myocardial infarction	N = 43,275 cases, 511,065 controls	Stockholm county, Sweden, 1985−1996	Rosenlund et al. (2009a)
Female n = 508	Male n = 1,028	–	Blood IL-6 levels	N = 1,536 Ages 45−70 yr	Stockholm county, Sweden, 1992−1994	Panasevich et al. (2009)
Female n = 395	Male n = 350	–	Mean carotid artery intima-media thickness	N = 745 Ages 26−30 yr	Utrecht, Netherlands, 1999−2000	Lenters et al. (2010)
Female n = 53.6%	Male n = 46.4%	–	Atherosclerosis (carotid intima media thickness)	N = 2,780 Median age 58 yr	Girona Province, Spain, 2007−2010	Rivera et al. (2013)
Female n = 1,980	Male n = 1,720	–	Systolic/diastolic blood pressure	N = 3,700 Age 35−83 yr	Girona, Spain	Foraster et al. (2014)
Female n = 431,388	Male n = 405,169	–	Heart failure	N = 836,557, Age 40−89 yr in 2003	England, 2003−2007	Atkinson et al. (2013)

Table 7-19 (Continued): Epidemiologic studies evaluating sex.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Female n = 573	Male n = 574	–	Systolic/diastolic blood pressure	N = 1,147 children Age 12 yr	The Netherlands, Long-term and short-term NO_2 exposure	Bilenko et al. (2013)
Female n = 23,092	Male n = 21,344	↓	Hypertension (change in systolic BP)	N = 44,436 Age 50−65 yr at baseline	Copenhagen, Aarhus, Denmark, 1993−2006	Sørensen et al. (2012)
Female n = 12,184	Male n = 12,661	– –	Incident CVD Incident stroke	N = 24,845 Mean age 41.7 yr	Shenyan, Anshan and Jinzhou, China, 2009	Dong et al. (2013a)
Female n = 12,184	Male n = 12,661	– –	Incidence hypertension Absolute increase in arterial blood pressure	N = 24,845 Mean age 45.59 yr	Shenyan, Anshan and Jinzhou, China, 2009−2010	Dong et al. (2013b)
Female n = 829	Male n = 1,155	–	Incident stroke	N = 1,984 Ages 50−65 yr at baseline	Copenhagen, Aarhus counties, Denmark, 1993−2006 (Long-term)	Andersen et al. (2012a)
Female n = 63	Male n = 79	–	Fatal stroke	N = 142 Ages 50−65 yr at baseline		
Female n = 18,085	Male n = 29,030	–	Stroke	N = 4,696 cases and 37,723 controls Age ≥20 yr	Edmonton, Alberta, Canada, 2007−2009	Johnson et al. (2013)
Female n = 725	Male n = 683	–	HRV decrements (SDNN)	N = 1,408 Ages ≥50 yr	Switzerland Followed 1991 to 2001−2003	Felber Dietrich et al. (2008)
Female with CVD n = 115	Male with CVD n = 121	–				
Female without CVD n = 610	Male without CVD n = 562	↑				
Female n = 27,273	Male n = 24,545		Diabetes	N = 51,818 Ages 50−65 yr at baseline	Copenhagen, Aarhus counties, Denmark, 1993−2006	Andersen et al. (2012b)
Female n = 51.3%	Male n = 48.7%	–	Diabetes	N = 6,392 Ages 29−73 yr	Switzerland, 2002	Eze et al. (2014)
Female n = 222	Male n = 175	–	Insulin resistance	N = 397 children Age 10 yr	Munich and Wesel, Germany	Thiering et al. (2013)

Table 7-19 (Continued): Epidemiologic studies evaluating sex.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Female	Male	–	Respiratory mortality	16 cohorts N = 307,553 Mean age at baseline 41.9 to 73.0 yr across cohorts	Europe Follow-up: 1985−2007 NO_2 exposure assessed for 2008−2011	Dimakopoulou et al. (2014)
Female n = 51.47%	Male n = 48.53%	–	CVD mortality	N = 9,941, 256 deaths Ages 35−103 yr	Shenyang, China Follow-up: 1998−2009 NO_2 exposure assessed for 1998−2009	Zhang et al. (2011)
Female n = 63%	Male n = 37%	–	CVD mortality		Europe Follow-up: 1985−2007 NO_2 exposure assessed for 2008−2011	Beelen et al. (2014)
Female n = 52.5%	Male n = 47.5%	–	Diabetes-related mortality	N = 52,061 Ages 50−64 yr	Denmark Follow-up: 1971−2009 NO_2 exposure assessed for 1971−2009	Raaschou-Nielsen et al. (2012)
Female n = 430,891	Male n = 404,716	↑	Total mortality	N = 835,607 deaths Ages 40−89 yr	England Follow-up: 2003−2007 NO_2 exposure assessed for 2002	Carey et al. (2013)
Female[b]	Male[b]	–	Mortality (total, CV, IHD, lung cancer)	N = 1,265,058 Ages ≥30 yr	Rome, Italy, 2001−2010	Cesaroni et al. (2013)
Female n = 111 deaths	Male n = 407 deaths	–	Lung cancer mortality	N = 63,520 Ages >40 yr	3 Japanese prefectures, 1983−1985	Katanoda et al. (2011)
		↑	Respiratory mortality			
Female n = 203–7,840 deaths	Male n = 233–4,531 deaths	↑	Lung cancer mortality	N = 138,977, 518 deaths Ages 51−90 yr	Oslo, Norway, 1992−1998	Naess et al. (2007)
		–	CVD mortality			
		↓	COPD mortality			

Table 7-19 (Continued): Epidemiologic studies evaluating sex.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Female n = 4,060	Male n = 2,278	↑	Lung cancer mortality	N = 6,338 nonsmoking, non-Hispanic adults Ages 27−95 yr	California, Follow-up: 1977−1992 NO_2 exposure assessed for 1973−1992	Abbey et al. (1999)
		–	Total mortality			
		–	Cardiopulmonary mortality			
		–	Respiratory mortality			
Female n = 49%	Male n = 51%	–	Lung cancer mortality	N = 14,001 Ages >65 yr	Shizuoka, Japan, 1999−2006	Yorifuji et al. (2010)
Female n = 1,206	Male n = 2,275	–	Lung cancer incidence	N = 3,481 Ages 20−93 yr at enrollment	Copenhagen, Aarhus counties, Denmark, 1970−1997	Raaschou-Nielsen et al. (2010)
Female n = 27,788	Male n = 25,182	–	Lung cancer incidence	N = 52,970 Ages 50−64 yr	Copenhagen, Aarhus counties, Denmark, 1993−1997	Raaschou-Nielsen et al. (2011)

[a]Up facing arrow indicates that the effect of NO_2 is greater (e.g., larger risk of hospital admission, larger decrement in HRV) in the group with the factor evaluated than in the reference group. Down facing arrow indicates that the effect of NO_2 is smaller in the group with the factor evaluated than in the reference group. A dash indicates no difference in NO_2-related health effect between groups.
[b]Sample size not reported.

7.5.5 Residence in Urban Areas

A majority (81%) of the U.S. populations lives in urban areas, and U.S. Census data indicate that the urban population grew 12% from 2000 to 2010. Higher ambient NO_2 concentrations in urban than suburban areas and the large numbers of people potentially having higher exposures highlights the public health significance of potential differences in NO_2-related health effects in urban residents. Higher ambient NO_2 concentrations have been described for downtown versus suburban areas [14.9 ppb vs. 11.7 ppb; (Rotko et al., 2001)]. Higher ambient NO_2 concentrations also were related to building characteristics such as high-rise building versus single family home and older versus newer construction (before or after 1970). Proximity to roads has been shown to be a determinant of personal NO_2 exposure (Section 7.5.6), and the higher road density in urban areas and proximity to major roads also may result in higher exposure of urban residents. The topography of urban communities also may contribute to higher NO_2 exposure among residents as the presence of street canyons enhances mixing at elevations closer to the street canyon-urban boundary layer interface, resulting in higher NO_2 concentrations at lower

elevations (Section 2.5.3). This may have implications for higher NO_2 exposures for pedestrians, outdoor workers, and those living on lower floors of buildings. The evidence indicates that residing in urban areas may lead to increased exposure to NO_2.

Although the potential for higher exposure to NO_2 of urban residents is well characterized, epidemiologic comparisons of NO_2-related health effects between urban and nonurban residents are limited and use variable definitions of urban and nonurban residence (Table 7-20). A larger association between short-term increases in NO_2 and lung function decrements among urban than suburban children was observed for children in the general population (Steerenberg et al., 2001) but not for children with asthma (Ranzi et al., 2004). Associations of long-term NO_2 and cardiovascular effects did not differ by urban residence (Atkinson et al., 2013; Sørensen et al., 2012), but there is uncertainty regarding the extent to which there is an independent effect of NO_2 exposure on cardiovascular effects (Section 6.3.9).

As detailed above, urban residents potentially have higher ambient NO_2 exposure. However, the limited epidemiologic evidence based on variable definitions of urban and nonurban residence does not provide a strong basis for inferring whether urban residence leads to increased risk for NO_2-related health effects. Overall, the limited number of epidemiologic studies that examined urban residence is inconsistent and based primarily on health effects for which independent relationships with NO_2 exposure are uncertain. As a result, the evidence is inadequate to determine whether residence in urban areas increases the risk for NO_2-related health effects.

Table 7-20 Epidemiologic studies evaluating urban residence.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Short-term exposure						
Urban residence n = 38	Suburban residence n = 44	↑	Lung function decrements	N = 82 Ages 8−13 yr	Utrecht, Bilthoven, the Netherlands	Steerenberg et al. (2001)
Urban-industrial residence n = 67	Rural residence n = 51	↓	Lung function decrements	N = 118 Ages 6−11 yr Children with asthma or respiratory symptoms	Urban & rural areas Emilia-Romagna, Italy, 1999	Ranzi et al. (2004)
Long-term exposure						
Residence in London n = 91,992	Residence in North/ South UK (excluding London) n = 744,565	–	Heart failure	N = 836,557 Ages 40−89 yr in 2003	England, 2003−2007	Atkinson et al. (2013)
Residence near city center n = 24,514	Residence outside of city center n = 19,922	–	Systolic blood pressure	N = 44,436 Ages 50−65 yr at baseline	Copenhagen, Aarhus counties, Denmark, 1993−2006	Sørensen et al. (2012)

[a]Up facing arrow indicates that the effect of NO_2 is greater (e.g., larger decrement in lung function) in the group with the factor evaluated than in the reference group. Down facing arrow indicates that the effect of NO_2 is smaller (e.g., smaller decrement in lung function) in the group with the factor evaluated than in the reference group. A dash indicates no difference in NO_2-related health effect between groups.

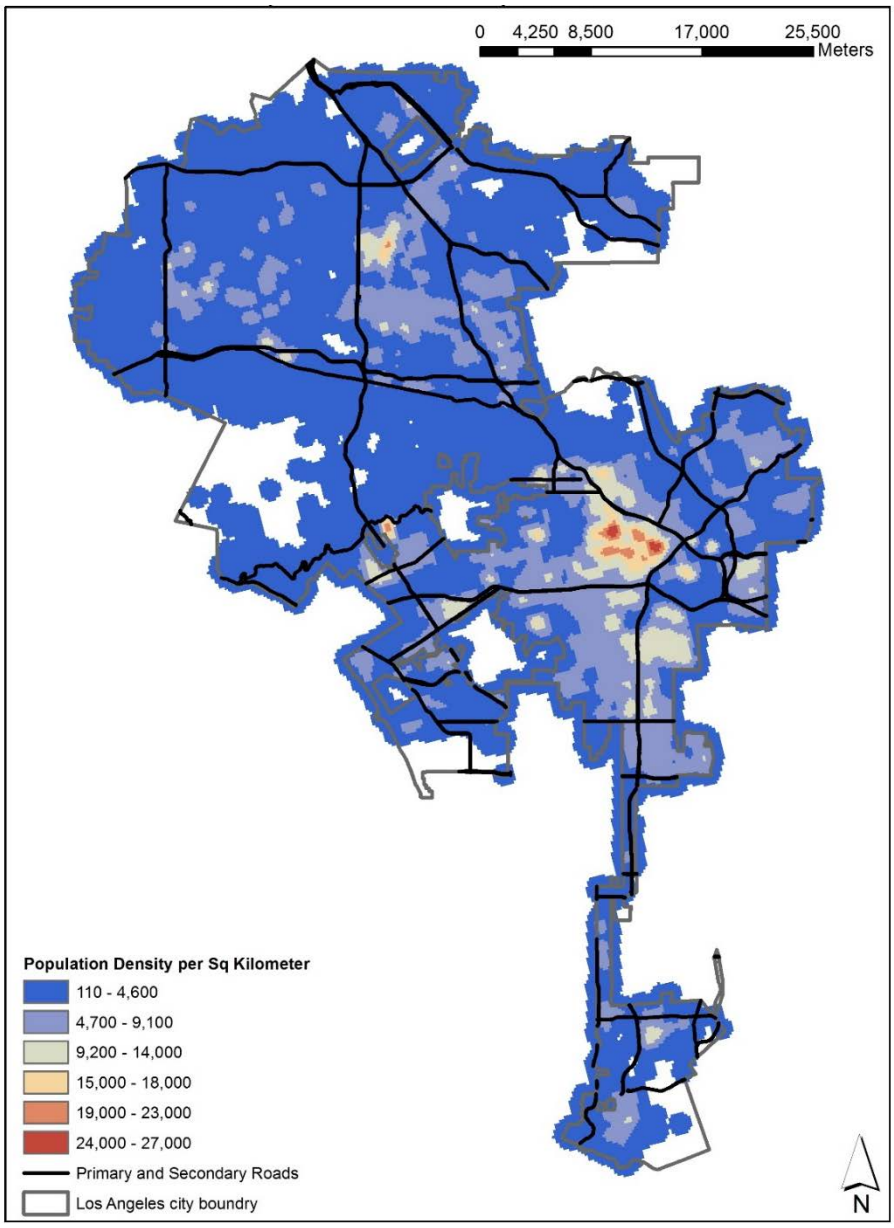
7.5.6 Proximity to Roadways

NO_2 concentrations can be 30 to 100% higher within 10−20 m of roads compared to locations 80−500 m away (Sections 2.5.3 and 3.3.1.1). Thus, individuals spending a substantial amount of time on or near high-traffic roadways, including those living or working near highways and commuters, are likely to be exposed to elevated NO_2 concentrations.

Large proportions of the U.S. population potentially have elevated NO_2 exposures as a result of proximity to roadways. Seventeen percent of U.S. homes are located within 91 m of a highway with four or more lanes, a railroad, or an airport (U.S. Census Bureau, 2009). Specific to road traffic, Rowangould (2013) found that over 19% of the U.S. population lives within 100 m of roads with an annual average daily traffic (AADT) of 25,000 vehicles, and 1.3% lives near roads with AADT greater than 200,000. The

proportion is much larger in certain parts of the country, mostly coinciding with urban areas. For example in Los Angeles, CA, primary and secondary roads run through neighborhoods with population density as high as 15,000−18,000 people per km^2 [(U.S. Census Bureau, 2014, 2013); Figure 7-1]. Among California residents, 40% lives within 100 m of roads with AADT of 25,000 (Rowangould, 2013).

Though far from generalizable across populations, there are examples indicating that residence near a busy roadway may be associated with higher NO_2 exposure. In the Southern California CHS cohort, closer proximity to a freeway showed a range of correlations with residential NO_2 measurements, with values of −0.73 to −0.90 in some communities (Gauderman et al., 2005). In a cohort of pregnant women who spent on average 60% of time home indoors, traffic intensity within 250 and 500 m of homes was moderately correlated with personal NO_2 exposures [r = 0.3, 0.4, respectively; (Schembari et al., 2013)]. However, the strongest correlation was not observed for traffic intensity in closest proximity to homes as the correlation between personal NO_2 and traffic intensity within a 100-m buffer was 0.2. Such results may be explained by the atmospheric chemistry of NO_2. Depending on atmospheric stability, NO_2 concentrations can dilute with distance from the road or extend beyond 1 km of the roadway (Section 2.5.3) and may be higher after some of the NO reacts photochemically to become NO_2.



Source: National Center for Environmental Assessment analysis of U.S. Census data (U.S. Census Bureau, 2014, 2013).

Figure 7-1 Map of population density in Los Angeles, CA in relation to primary and secondary roads.

Exposure to NO_2 in transport is found to be an important determinant of total personal exposure (Son et al., 2004; Lee et al., 2000), although time in transport makes up a relatively small proportion of people's activities. Such findings have implications for commuters as well as for occupational drivers. Among the populace working outside the home, 15.6% spend 45 minutes or more commuting to work each day (U.S. Census Bureau, 2007). Average one-way commuting times for the U.S. labor force working

outside the home are 19.3 minutes for bicyclists, 11.5 minutes for walkers, and 25.9 minutes for all other modes of transportation. The potential of higher NO_2 exposure of commuters and professional drivers is supported by many observations of in-vehicle NO_2 concentrations approaching roadside concentrations (Figure 3-2) and some evidence of higher personal NO_2 exposure during transport than in outdoor or indoor environments (Delgado-Saborit, 2012). The relationship between NO_2 exposure during commute or while driving for work and health effects is not well characterized. In the CHS cohort, increasing commuting time to school was associated with wheeze but not asthma onset; commute-time NO_2 exposures were not measured (McConnell et al., 2010).

Children are characterized to be at increased risk for NO_2-related health effects (Section 7.5.1.1), and time spent near major roads could potentially be a source of higher NO_2 exposure contributing to health effects. Attendance at schools or daycare near major roads may be an important determinant of NO_2 exposure, and ambient NO_2 concentrations at schools have been associated with respiratory effects in children with asthma (Sections 5.2.2.2 and 5.2.2.5). Seven percent of U.S. schools serving 3,152,000 school children are located within 100 m of a major roadway, and 15% of U.S. schools serving 6,357,000 school children are located within 250 m of a major roadway [not specifically defined in terms of AADT, number of lanes, or other criteria; (Kingsley et al., 2014)]. In California, 2.3% of public schools serving 150,323 children were estimated to be located within 150 m of high-traffic roads [≥50,000 vehicles per day; (Green et al., 2004)]. Also in California, 1,534 daycare facilities serving 57,173 (7% of those in daycare) children were within 200 m of roadways with AADT of ≥50,000, and 4,479 facilities serving 171,818 (21%) children were within 200 m of roadways with AADT of 25,000−49,999 (Houston et al., 2006). Though neither of these analyses assessed NO_2 exposures, they identify the large numbers of children potentially exposed to higher NO_2 concentrations in locations where they spend several hours per day.

There is some indication that traffic exposures differ among sociodemographic groups. In California, schools or daycare in close proximity to high-traffic roadways had a higher percentage of nonwhite students (Green et al., 2004) or tended to be located in areas with higher percentages of nonwhite residents (Houston et al., 2006). Analyses of U.S. Census blocks or tracts indicate associations of higher traffic or road density or proximity to roadways with higher proportion of nonwhite residents (Rowangould, 2013; Tian et al., 2013). In some (Rowangould, 2013; Green et al., 2004) but not all (Tian et al., 2013) cases, closer proximity to roadways or higher traffic density was associated with lower SES at the school or census block level. In analyses not considering proximity or density of traffic, higher NO_2 exposures are suggested among nonwhite (Section 7.5.3) or low SES (Section 7.5.2). However, it is not understood whether observations of higher NO_2 exposures in certain sociodemographic groups is related to disparities in traffic exposure.

Large proportions of the U.S. population live or attend school near roads or travel on roads, and some evidence indicates higher NO_2 exposure with proximity to roads. Traffic proximity may be more prevalent among nonwhite and low SES groups, but the influence of traffic proximity on differential NO_2 exposure in these groups is unclear. While traffic proximity (HEI, 2010) and NO_2 exposure near traffic (Section 5.2.9.3) are linked to asthma exacerbation or prevalence, evidence does not clearly indicate larger NO_2-related health effects in populations living near traffic (Table 7-21). Closer proximity to freeway was associated with larger NO_2-related decrements in lung development among children (Gauderman et al., 2007), but NO_2 concentrations as measured at central sites were weakly correlated with traffic counts near homes, and an independent effect of NO_2 exposure on lung development is uncertain. Additionally, results are inconclusive for cardiovascular effects and leukemia (Foraster et al., 2014; Hart et al., 2013; Amigou et al., 2011). The insufficient quantity and consistency of evidence and uncertainty regarding the independent effects of NO_2 exposure is inadequate to determine whether populations in close proximity to roadways are at increased risk for NO_2-related health effects.

Table 7-21 Epidemiologic studies evaluating proximity to roadways (all long-term exposure).

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Residence <500 m from freeway n = 440	Residence 1,000−1,500 m from freeway[b]	↑	Decrements in lung development (FEV_1 change over time)	N = 3,677 children followed ages 10−18 yr	Alpine, Lake Elsinore, Lake Arrowhead, Atascadero, Lancaster, San Dimas, Long Beach, Mira Loma, Lompoc, Riverside, Santa Maria, Upland, CA Follow-up: 1993/1996 to 2001/2004	Gauderman et al. (2007)
Near road n = 539 MI cases	Far from road (>50 m from secondary road [> 2 lanes] or >150 m from primary road [highway]) n = 2,841 MI cases	↑	Incident MI	N = 84,562 Age 30−55 yr at enrollment	U.S., 1990−2008	Hart et al. (2013)
Moved near road n = 48 MI cases		↑				
Moved away from road n = 603 MI cases		–				
Traffic intensity of nearest road >median	Traffic intensity of nearest road ≤median	–	Systolic blood pressure	N = 3,700 Age 35−83 yr	Girona, Spain	Foraster et al. (2014)
		–	Diastolic blood pressure			
Traffic load at 500 m >median	Traffic load at 500 m ≤median	↑	Systolic blood pressure			
		–	Diastolic blood pressure			
Residence near main roads (<500 m) n = 48	Residence away from all road types (≥500 m) n = 954	–	Leukemia	N = 763 cases, 1,681 controls Ages <15 yr	France, 2003−2004	Amigou et al. (2011)

[a]Up facing arrow indicates that the effect of NO_2 is greater (e.g., larger decrement in lung function, larger risk of MI) in the group with the factor evaluated than in the reference group. Down facing arrow indicates that the effect of NO_2 is smaller in the group with the factor evaluated than in the reference group. A dash indicates no difference in NO_2-related health effect between groups.
[b]Sample size not reported.

7.6 Behavioral and Other Factors

7.6.1 Diet

Diet is an important influence on health and thus, plausibly could influence air pollutant-related health effects. The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008) did not discuss whether diet influences the risk of NO_2-related health effects; however, evidence from previous experimental studies indicates reduced or greater respiratory effects in humans and rodents with supplementation of or deficiencies in antioxidant vitamins, respectively (Table 7-22). A controlled human exposure study demonstrated that healthy adults with diets supplemented with Vitamin C had less airway responsiveness following 2,000 ppb NO_2 for 1 hour compared to adults with a normal diet (Mohsenin, 1987). Airway responsiveness is a hallmark of asthma exacerbation (Figure 4-1). The evidence that higher antioxidant vitamin intake reduces NO_2-induced airway responsiveness is supported by experimental evidence in humans and rodents that higher dietary vitamin E and/or C reduces NO_2-induced pulmonary inflammation and modulates the oxidant/antioxidant balance [(Mohsenin, 1991; Hatch et al., 1986; Elsayed and Mustafa, 1982; Sevanian et al., 1982; Selgrade et al., 1981; Ayaz and Csallany, 1978); Table 7-22], which are early events in the mode of action for NO_2 effects on asthma exacerbation (Section 4.3.5, Figure 4-1). Despite the consistency and coherence of evidence, findings are limited, particularly for changes that are indicative of health effects. The changes in NO_2-induced lipid peroxidation, antioxidant levels, and antioxidant enzyme activity observed in relation to vitamin deficiencies or supplementation may or may not lead to health effects.

Epidemiologic studies have not examined whether diet modifies NO_2-related respiratory effects. Limited information indicates that associations of long-term NO_2 exposure with mental development in infants are larger in groups with low fruit intake (maternal prenatal or concurrent, respectively) than groups with high fruit intake [(Guxens et al., 2012); Table 7-23]. Fruits are a source of antioxidants; thus, the results for modification by fruit intake are consistent with those for dietary antioxidant vitamins. However, because evidence for NO_2-related neurodevelopmental effects is overall inconclusive (Section 6.4.5), the available epidemiologic evidence cannot adequately inform whether diet deficiencies increase the risk for NO_2-related health effects.

Experimental studies in humans and animals provide evidence that dietary intake of Vitamin C or E modifies airway responsiveness, pulmonary inflammation, and oxidant

balance following NO_2 exposure, with high vitamin intake reducing these effects and low intake increasing effects. Epidemiologic evidence is available only for health effects for which relationships with NO_2 are uncertain. Oxidative stress, pulmonary inflammation, and airway responsiveness are key events in the mode of action for asthma exacerbation (Figure 4-1); thus, a biologically plausible mechanism exists for dietary antioxidants to reduce the risk of NO_2-related health effects. Although biological plausibility exists for dietary deficiencies influencing the risk for NO_2-related health effects, most findings are for changes in oxidant/antioxidant balance rather than changes clearly indicative of health effects such as airway responsiveness. Therefore, there is suggestive evidence that insufficient dietary antioxidant intake increases the risk for NO_2-related health effects.

Table 7-22 Controlled human exposure and toxicological studies evaluating diet

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population/ Animal Model	Study Details	Study
Vitamin C supplemented diet 3 days n = 11	Normal diet n = 11	↓	Airway responsive-ness	Humans n = 8 male, 3 female Ages 18−37 yr	2,000 ppb for 1 hour, randomized, double-blind	Mohsenin (1987)
Vitamin C and E supplemented diet 4 weeks n = 10	Normal diet n = 9	↓	Lipid peroxidation in lavage fluid	Humans n = 10 male, 9 female Ages 21−33 yr	4,000 ppb for 3 hour	Mohsenin (1991)
Vitamin C supplemented diet n = 4−5	Vitamin C normal diet n = 9	↑	Pulmonary inflammation	Guinea pigs (Hartley) n = 2−6 males/group	400, 1,000, 3,000, or 5,000 ppb for 3 days	Selgrade et al. (1981)
Vitamin C deficient diet n = 5−8	Vitamin C supplemented diet n = 15	↑	Pulmonary inflammation, Antioxidant reduction	Guinea pigs (Hartley) n = 3−15 males/group	4,800 ppb for 3 hours	Hatch et al. (1986)
Vitamin E deficient diet, birth−adolescence n = 6−7	Vitamin E supplemented diet n = 6−8	↑	Lipid peroxidation, Pulmonary inflammation	Rats (Sprague-Dawley) n = 6−8/group	3,000 ppb for 1 week	Sevanian et al. (1982)
Vitamin E deficient diet, birth−adolescence n = 6	Vitamin E supplemented diet n = 6	↑	Lipid peroxidation, Induction of antioxidant enzymes	Rats (Sprague−Dawley) n = 6/group	3,000 ppb for 1 week	Elsayed and Mustafa (1982)
Vitamin E deficient diet n = 6−10	Vitamin E supplemented diet n = 6−10	↑	Glutathione peroxidase activity reduction	Mice (C57BL/6J) n = 120 females	500 or 1,000 ppb for 17 months	Ayaz and Csallany (1978)

[a]Up facing arrow indicates that the effect of NO_2 is greater (e.g., larger increase in airway responsiveness, larger increase in lipid peroxidation) in the group with the factor evaluated than in the reference group.

Table 7-23 Epidemiologic studies evaluating diet (all long-term exposure).

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Low healthy eating index (≤109)[b]	High healthy eating index (>109)[b]	–	MI Incidence	N = 84,562 Age 30−55 yr at enrollment	U.S., 1990−2008	Hart et al. (2013)
Low maternal fruit intake in 1st trimester (≤405 g/day) n = 33.5%	Medium/ high maternal fruit intake in 1st trimester (405 g/day) n = 66.5%	↑	Decrement in mental development score in infants at age 14 mo	N = 1,889 children followed from prenatal period	4 Spanish cities 2003−2008	Guxens et al. (2012)

[a]Up facing arrow indicates that the effect of NO_2 is greater (e.g., larger decrement in mental development score) in the group with the factor evaluated than in the reference group. A dash indicates no difference in NO_2-related health effect between groups.
[b]Sample size not reported.

7.6.2 Smoking

Smoking is a common behavior, the 2010 National Health Interview Survey estimated that within the U.S. adult population approximately 19.2% of individuals report being current smokers and 21.5% report being a former smoker (Schiller et al., 2012). Smoking is a well-documented risk factor for many diseases, but it is unclear whether smoking exacerbates health effects associated with air pollutant exposures, including NO_2.

Although many controlled human exposure studies report smoking status, comparisons between smokers and nonsmokers are infrequent due to small sample size. However, in limited examination, a 4-hour exposure to 300 ppb NO_2 induced a larger decrement in mean forced expiratory volume in 1 second (FEV_1) among 7 smoking, healthy adults than among 13 nonsmoking subjects (Morrow et al., 1992). There is a lack of epidemiologic studies to draw direct coherence with this experimental evidence for respiratory effects. As examined primarily for cardiovascular or diabetes morbidity and mortality, most associations with long-term NO_2 do not differ between smokers and nonsmokers (Dadvand et al., 2014; Atkinson et al., 2013; Hart et al., 2013; Rivera et al., 2013; Andersen et al., 2012a; Zhang et al., 2011; Lenters et al., 2010; Panasevich et al., 2009) or are larger among nonsmokers [(Carey et al., 2013; Andersen et al., 2012b; Raaschou-Nielsen et al., 2012; Maheswaran et al., 2010); Table 7-24]. A similar lack of difference between smokers and nonsmokers was observed for NO_2 associations with lung cancer incidence (Raaschou-Nielsen et al., 2011; Raaschou-Nielsen et al., 2010),

although the association with lung cancer mortality was larger in smokers when limited to males (Katanoda et al., 2011).

A controlled human exposure study demonstrated larger NO_2-induced decrements in lung function among smokers compared to nonsmokers, but epidemiologic evidence is not available for respiratory effects. Most epidemiologic studies examine NO_2-related cardiovascular effects, diabetes, or mortality and do not report differences between smokers and nonsmokers. Many studies similarly defined smoking as current or former smoking, providing a basis for comparisons across studies. Although there is lack of evidence for differences in risk for NO_2-related health effects by smoking status, there is also uncertainty as to whether NO_2 has an independent relationship with cardiovascular effects, diabetes (Section 6.3.9), and mortality (Section 6.5.3), the health effects for which smoking status was examined. Therefore, the evidence is inadequate to determine whether smoking increases the risk of NO_2-related health effects.

Table 7-24 Epidemiologic studies evaluating smoking status.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Short-term exposure						
Current or former smoking n = 74%	Never smoking n = 28.4%	↓	Change in ventricular repolarization	N = 580 males Mean age 75 yr	Boston, MA, Follow-up: 2000−2008	Baja et al. (2010)
Long-term exposure						
Current or former smoking[b]	Never smoking[b]	–	Incident MI	N = 84,562 Ages 30−55 yr at enrollment	U.S., 1990−2008	Hart et al. (2013)
Current or former smoking n = 313,487	Never smoking n = 396,647	–	Heart failure	N = 836,557 Ages 40−89 yr in 2003	England, 2003−2007	Atkinson et al. (2013)
Current or former smoking n = 28,612	Never smoking n = 15,824	↑	Hypertension (change in systolic BP)	N = 44,436 Ages 50−65 yr at baseline	Copenhagen, Aarhus counties, Denmark, 1993−2006	Sørensen et al. (2012)
Current or former smoking n = 1,503	Never smoking n = 481	–	Incident stroke	N = 1,984 Ages 50−65 yr at baseline	Copenhagen, Aarhus counties, Denmark, 1993−2006	Andersen et al. (2012a)
Current or former smoking n = 118	Never smoking n = 24	–	Fatal stroke	N = 142 Ages 50−65 yr at baseline		

Table 7-24 (Continued) Epidemiologic studies evaluating smoking status.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Current or former smoking n = 33,380	Never smoking n = 18,438	↓	Diabetes	N = 51,818 Ages 50−65 yr at baseline	Copenhagen, Aarhus counties, Denmark, 1993−2006	Andersen et al. (2012b)
Current or former smoking n = 45%	Never smoking n = 55%	–	Atherosclerosis (carotid artery intima-media thickness	N = 745 Ages 26−30 yr	Utrecht, the Netherlands, 1999−2000	Lenters et al. (2010)
Current or former smoking n = 45.5%	Never smoking n = 54.5%	–	Atherosclerosis (carotid intima media thickness)	N = 2,780 Median age 58 yr	Girona Province, Spain, 2007−2010	Rivera et al. (2013)
Current smoking n = 90	Former smoking n = 152	↓	C-reactive protein	N = 242 adults with clinically stable COPD Mean age 68 yr	Barcelona, Spain, 2004−2006	Dadvand et al. (2014)
		–	TNF-α			
		–	IL-6			
		–	IL-8			
		↓	Fibrinogen			
		–	Hepatocyte growth factor			
Current or former smoking n = 917	Never smoking n = 619	–	Blood IL-6 levels	N = 1,536 Ages 45−70 yr	Stockholm county, Sweden, 1992−1994	Panasevich et al. (2009)
Current or former smoking n = 25.5−65% across cohorts	Never smoking n = 35−74.5% across cohorts	↑	Respiratory mortality	N = 307,553 Mean age at baseline 41.9 to 73.0 yr across 16 cohorts	Europe Follow-up: 1985−2007 NO_2 exposure assessed for 2008−2011	Dimakopoulou et al. (2014)
Current or former smoking n = 2,850	Never smoking n = 4,359	–	CVD Mortality	N = 9,941, 256 deaths Ages 35−103 yr	Shenyang, China Follow-up: 1998−2009 NO_2 exposure assessed for 1998−2009	Zhang et al. (2011)
Current or former smoking n = 64%	Never smoking n = 36%	↓	Diabetes-related mortality	N = 52,061 Ages 50−64 yr	Denmark Follow-up: 1971−2009. NO_2 exposure assessed for 1971−2009	Raaschou-Nielsen et al. (2012)
Current smoking n = 608	Never or former smoking n = 1,248	↓	Total mortality	N = 3,320 Mean age 70 yr	London, England Follow-up: 1995−2005 NO_2 assessed for 2002	Maheswaran et al. (2010)

Table 7-24 (Continued) Epidemiologic studies evaluating smoking status.

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Current or former smoking n = 322,766	Never smoking n = 386,591	↓	Total mortality	N = 835,607 deaths Ages 40−89 yr	England Follow-up: 2003−2007 NO_2 exposure assessed for 2002	Carey et al. (2013)
Current smoking, male n = 292 deaths	Former smoking, male n = 90 deaths	↑	Lung cancer mortality	N = 63,520 Ages >40 yr	3 Japanese prefectures, 1983−1985	Katanoda et al. (2011)
Current or former smoking n = 3,713	No smoking n = 9,135	↓	Lung cancer or cardiopulmonary mortality	N = 14,001 Ages >65 yr	Shizuoka, Japan, 1999−2006	Yorifuji et al. (2010)
Current or former smoking n = 3,372	No smoking n = 109	–	Lung cancer incidence	N = 3,481 Ages 20−93 yr at enrollment	Copenhagen, Aarhus counties, Denmark, 1970−1997	Raaschou-Nielsen et al. (2010)
Current or former smoking n = 19,253	No smoking n = 33,717	–	Lung cancer incidence	N = 52,970 Ages 50−64 yr	Copenhagen, Aarhus counties, Denmark, 1993−1997	Raaschou-Nielsen et al. (2011)

[a]Up facing arrow indicates that the effect of NO_2 is greater (e.g., larger risk hypertension) in the group with the factor evaluated than in the reference group. Down facing arrow indicates that the effect of NO_2 is smaller in the group with the factor evaluated than in the reference group. A dash indicates no difference in NO_2-related health effect between groups.
[b]Sample size not reported.

7.6.3 Physical Activity

There is some evidence indicating that during physical activity, increased respiratory rate and oronasal breathing can increase the deposition of NO_2 in the lower respiratory tract (Section 4.2.2), which could have implications for increasing the risk of NO_2-related health effects. However, the effect of concurrent physical activity on NO_2-related health effects has not been characterized. Rather, physical activity has been examined as a modifier of health effects related to long-term NO_2 exposure, as an indicator of active versus sedentary lifestyle or fitness. Further, outdoor activity has not been assessed. The influence of general activity or fitness on NO_2 exposure and internal dose are not known.

Epidemiologic studies examined physical activity or exercise as a modifier of cardiovascular effects and mortality, for which independent relationships with NO_2 are uncertain (Sections 6.3.9 and 6.5.3). These studies have found inconsistent results with respect to whether physical activity increases the risk for NO_2-related health effects

(Table 7-25). Associations between long-term NO_2 exposure and mortality from diabetes was higher in the group not engaging in exercise (Raaschou-Nielsen et al., 2012), but risk of diabetes was similar or lower among those with low levels of physical activity (Eze et al., 2014; Andersen et al., 2012b). Similarly, NO_2-related cardiovascular mortality was greater in the group with no exercise (Zhang et al., 2011), but associations with other cardiovascular effects were similar between groups with low or high physical activity (Hart et al., 2013; Panasevich et al., 2009). Contributing to the uncertainty in the evidence base is the heterogeneity across studies in how physical activity was defined, for example, the frequency or intensity of activity. Overall, there is inconsistent evidence indicating that physical activity increases the risk for NO_2-related health effects. Therefore, the evidence is inadequate to determine whether low physical activity increases the risk for NO_2-related health effects.

Table 7-25 Epidemiologic studies evaluating physical activity (all long-term exposure).

Factor Evaluated	Reference Category	Direction of Effect Modification[a]	Outcome	Study Population	Study Details	Study
Low physical activity (<18 METS/ week)[b]	High physical activity (≥18 METS/ week)[b]	–	Incident MI	N = 84,562 Age 30−55 yr at enrollment	U.S., 1990−2008	Hart et al. (2013)
Physical inactivity n = 23,536	Physical activity or playing sports in leisure time n = 28,282	↓	Diabetes	N = 51,818 Ages 50−65 yr at baseline	Copenhagen, Aarhus counties, Denmark, 1993−2006	Andersen et al. (2012b)
Low physical activity (<0.5 h/ week) n = 38%	Physical activity (≥2 h/week) n = 28%	–	Diabetes	N = 6,392 Ages 29−73 yr	Switzerland, 2002	Eze et al. (2014)
Physical inactivity (inactive leisure time) n = 543	Physical activity n = 993	–	Blood IL-6 levels	N = 1,536 Age 45−70 yr	Stockholm county, Sweden, 1992−1994	Panasevich et al. (2009)
No Exercise n = 45.7%	Exercise n = 54.3%	↑	Diabetes−related mortality	N = 52,061 Ages 50−64 yr	Denmark Follow-up and NO_2 exposure assessed for 1971−2009	Raaschou-Nielsen et al. (2012)
No Exercise n = 5,795	Exercise n = 4,146	↑	CV mortality	N = 9,941, 256 deaths Ages 35−103 yr	Shenyang, China Follow-up and NO_2 exposure assessed for 1998−2009	Zhang et al. (2011)

[a]Up facing arrow indicates that the effect of NO_2 is greater (e.g., larger risk of mortality) in the group with the factor evaluated than in the reference group. Down facing arrow indicates that the effect of NO_2 is smaller in the group with the factor evaluated than in the reference group. A dash indicates no difference in NO_2-related health effect between groups.
[b]Sample size not reported.

7.7 Conclusions

This chapter evaluates factors that may characterize populations and lifestages at increased risk for health effects related to NO_2 exposure (Table 7-26). The evidence for each factor was classified based on judgments of the consistency, coherence, and biological plausibility of evidence integrated across epidemiologic, controlled human exposure, and toxicological studies using the weight-of-evidence approach detailed in Table 7-1. The evaluation also drew upon information presented in preceding chapters on

exposure, dosimetry, modes of action, and independent relationships of NO_2 exposure with health effects.

Consistent with observations made in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008), there is adequate evidence to conclude that people with asthma, children, and older adults are at increased risk for NO_2-related health effects. Not only does evidence consistently indicate increased risk for these groups, but the evidence is based on findings for short-term NO_2 exposure and respiratory effects (particularly asthma exacerbation), for which a causal relationship exists (Section 5.2.9). In addition to the strong evidence for a relationship between short-term NO_2 exposure and asthma exacerbation, the conclusion that people with asthma are at increased risk of NO_2-related health effects is supported by results from a meta-analysis of controlled human exposure studies demonstrating that NO_2 exposure increases airway responsiveness, a key feature of asthma exacerbation, at lower concentrations in people with asthma compared to healthy individuals. Epidemiologic evidence does not consistently demonstrate differences in NO_2-related respiratory effects in people with asthma. It is important to note that there is evidence of heterogeneity in asthma severity and triggers within study populations; thus, the epidemiologic evidence is not considered to be in conflict with experimental evidence. Children and older adults consistently have larger magnitude associations between NO_2 exposure and asthma hospital admissions and ED visits, compared to adults or the general population. There is not clear evidence from controlled human exposure studies that NO_2 induces respiratory effects in healthy, older adults, but examination is much more limited compared with epidemiologic evidence. Time-activity patterns and ventilation rates differ among age groups, but it is not understood whether these factors contribute to the increased risk of NO_2-related health effects for children and older adults.

Table 7-26 Summary of evidence for potential increased NO_2 exposure and increased risk of NO_2-related health effects.

Evidence Classification	Factor Evaluated	Rationale for Classification
Adequate evidence	Asthma (Section 7.3.1) Lifestage: (Section 7.5.1.1): Children (Section 7.5.1.2): Older adults	• Each factor: consistent evidence for increased risk for NO_2-related asthma exacerbation. • Asthma: evidence from controlled human exposure studies. • Lifestage: different time-activity patterns and ventilation patterns but unclear implications for differences in NO_2 exposure or internal dose.
Suggestive evidence	SES (Section 7.5.2): Low SES Sex (Section 7.5.4): Females Diet (Section 7.6.1): Reduced antioxidant intake	• Each factor: limited and generally supporting evidence for differences in NO_2-related health effects. • SES and females: findings based primarily on short-term NO_2 exposure and mortality for SES and long-term NO_2 exposure and lung function for females. Uncertainty in independent relationships with NO_2 for some health effects provides limited basis for inferences about differential risk. • Reduced dietary antioxidant vitamin intake: consistent evidence from experimental studies for modification of NO_2-related respiratory effects, but changes in oxidant balance may not necessarily indicate health effects.
Inadequate evidence	COPD (Section 7.3.2) Cardiovascular disease (Section 7.3.3) Diabetes (Section 7.3.4) Genetic background (Section 7.4) Obesity (Section 7.3.5) Smoking (Section 7.6.2) Physical activity (Section 7.6.3) Race/ethnicity (Section 7.5.3) Residence in urban areas (Section 7.5.5) Proximity to roadways (Section 7.5.6)	• Epidemiologic findings inconsistently show differences in NO_2-related health effects, show no difference, or are limited in quantity. • Findings based primarily on cardiovascular effects, diabetes, birth outcomes, and mortality. Uncertainty in independent relationships with NO_2 provides limited basis for inferences about differential risk. • Indication of higher NO_2 exposure among nonwhite populations, urban residents, and people spending time or living near roadways, but insufficient information to assess increased risk of NO_2-related health effects.
Evidence of no effect	None	

There is suggestive evidence that people with low antioxidant diets, people of low SES, and females are at increased risk for NO_2-related health effects because of some uncertainties in the evidence bases. While experimental studies indicate that dietary intake of Vitamin C or E modifies NO_2-related effects on airway responsiveness, much of

the evidence is for effects on oxidant balance, which is not necessarily indicative of health effects. Evidence indicates that low SES populations have higher NO_2 exposure and larger NO_2-related risk of mortality. For females, limited epidemiologic evidence points to greater risk for NO_2-related decrements in lung function. However, for low SES populations and females, the evidence is based on studies of health effects for which independent relationships with NO_2 exposure are uncertain (Sections 5.4.8 and 6.2.9).

There is inadequate evidence to determine whether pre-existing cardiovascular disease, diabetes, COPD, genetic variants, obesity, smoking, or physically active lifestyle increases the risk for NO_2-related health effects. Studies show either inconsistent or no modification of NO_2-related health effects by these factors, and information is based primarily on cardiovascular effects (Sections 5.3.12 and 6.3.9) and mortality (Sections 5.4.8 and 6.5.3) for which independent relationships with NO_2 are uncertain. Evidence also is inadequate to determine whether race/ethnicity, urban residence, or proximity to roadways increase the risk for NO_2-related health effects. While nonwhite populations, urban residents, and people with close proximity to roadways (i.e., living, attending school, working, or commuting on or near roadways) may have increased exposure to NO_2, there is limited or inconsistent evidence for larger NO_2-related health effects in these populations. Further, inferences about the potential differential risk for these populations are limited by evidence that is based on cardiovascular effects (Section 6.3.9) and birth outcomes (Section 6.4.5), for which independent effects of NO_2 exposure are uncertain. Additionally, it is important to note that many factors may be acting in combination, which may lead to a different public health impact than is reflected when evaluating any one factor in isolation. However, at this time information remains limited as to the impact of multiple factors and how they affect the risk for NO_2-related health effects.

In conclusion, evidence is adequate to conclude that people with asthma, children, and older adults are at increased risk for NO_2-related health effects. The large proportions of the U.S. potentially that encompass each of these groups and lifestages underscores the potential public health significance of NO_2-related health effects.

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