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Integrated Science Assessment for Oxides of Nitrogen– Health Criteria

(First External Review Draft)

November 2013

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, single term defined to express the influence of time-weighting and infiltration on NO_2 exposure
α-ATD	alpha 1-antitrypsin deficiency
AADT	annual average daily traffic
Abs	absorbance coefficient
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ACS	American Cancer Society
ADHD	attention deficit hyperactivity disorder
ADRB2	beta-2-adrenergic receptor
AERMOD	American Meteorological Society/Environmental Protection Agency Regulatory Model
AHR	airway hyperresponsiveness
a_i	air exchange rate
AIRES	Atlanta Aerosol Research Inhalation Epidemiology Study
AK	Alaska
AKR/J	mice strain with short life-span; often used as model for aging
AL	Alabama
ALRI	acute lower respiratory infection
a.m.	ante meridiem (before noon)
AM	alveolar macrophages
AM3	global scale, three-dimensional chemical tracer model
AMF	air mass factor
AMs	alveolar macrophages
APEX	Air Pollution Exposure
APHEA	Air Pollution and Health: a European Approach
APHEA-2	second, more recent APHEA study with more cities
APHENA	Air Pollution and Health: A European and North American Approach study
$ApoE^{-/-}$	apolipoprotein E knockout
AR	Arkansas
AQCD	Air Quality Criteria Document
AQI	Air Quality Index
AQM	air quality model
ATS	American Thoracic Society
avg	average
AZ	Arizona
β	beta
BAL	bronchoalveolar lavage
BALF	bronchoalveolar lavage fluid
BC	black carbon
BHPN	N-bis (2-hydroxy-propyl) nitrosamine
BL	bronchial lavage
BMI	body mass index
BP	blood pressure
BR^-	bromide
BS	black smoke
BSA	body surface area
BTEX	benzene, toluene, ethybenzene, xylene: traffic related VOCs
BW	body weight
C	Celsius, microenvironmental concentration
Ca	calcium
CA	California
C_a	ambient concentration
$C_{a,csm}$	ambient concentration at a central site monitor
CAA	Clean Air Act
CALINE4	California Department of Transportation's most recent line dispersion model

Acronym/Abbreviation	Meaning
CALPUFF	Non-steady-state meterological and air quality modeling system developed by the Atmospheric Studies Group at TRC
CAMP	Childhood Asthma Management Program
CAPES	China Air Pollution and Health Effects Study
CAPs	concentrated ambient particles
CAPS	cavity attenuated phase shift
CASAC	Clean Air Scientific Advisory Committee
CASNET	Clean Air Status and Trends Network
CBSA	Core Based Statistical Area
CC16	Clara Cell secretory protein
CDC	Centers for Disease Control
CDPFs	catalyzed diesel particle filters
CEMS	Continuous Emission Monitoring System
CFD	computational fluid dynamics
CHAD	Consolidated Human Activity Database
CHD	coronary heart disease
CHF	congestive heart failure
CHIMERE	regional chemistry transport model
C_i	average NO_2 concentration in the *i*th microenvironment
CI(s)	confidence interval(s)
Cl^-	chloride
CL/MC	catalytic converter
CL/PC	photolytic converter
$ClNO_2$	nitryl chloride
CMAQ	Community Multiscale Air Quality
CMSA	Consolidated Metropolitan Statistical Area
CO	carbon monoxide; Colorado
COPD	chronic obstructive pulmonary disease
C-R	concentration-response (relationship)
CRDS	diode laser based cavity ring down spectroscopy
CRP	C-reactive protein
CT	Connecticut
CTM	chemical transport models
CVD	cardiovascular disease
$Cys^{\bullet}$	cysteine radical
DBP	diastolic blood pressure
DC	District of Columbia
D-dimer	blood indicator of thrombosis
DE	Deleware
DEARS	Detroit Exposure and Aerosol Research Study
DEP	diesel exhaust particles
df	degrees of freedom
DHA	dehydroascorbate
DL	distributed lag
DLM	polynomial distributed lag model
DOAS	differential optical absorption spectroscopy
DOCs	diesel oxidation catalysts
e.g.	exempli gratia; for example
E_a	ambient NO_2 exposure
$\overline{E_a}$	average exposure to ambient NO_2
EBC	expired (exhaled) breath condensate
EC	elemental carbon
ECG	electrocardiography
ECP	eosinophil cationic protein
ED	emergency department
EGR	exhaust gas recirculation
E_i	indoor NO_2 exposures in the *i*th microenvironment
ELF	epithelial lining fluid, extracellular lining fluid
E_{na}	non-ambient NO_2 exposure
eNO	exhaled nitric oxide, endogenous nitric oxide

Acronym/Abbreviation	Meaning
eNOS	endothelial nitric oxide synthase
E_o	outdoor microenvironmental NO_2 exposures
EPA	U.S. Environmental Protection Agency
E-selectin	indicator of inflammation
ESR	erythrocyte sedimentation rate
E_T	total personal exposure
ET-1	vasoconstrictor endothelin-1
EXPOLIS	exposure in polis or cities
FA	filtered air
factor VII	enzyme in the coagulation cascade
$FEF_{25\%}$	forced expiratory flow at 25% of forced vital capacity
$FEF_{25-75\%}$	forced expiratory flow at 25-75% of exhaled volume
$FEF_{50\%}$	forced expiratory flow at 50% of forced vital capacity
FEM	Federal Equivalent Method
FeNO	fractional exhaled nitric oxide
FEV_1	forced expiratory volume in 1 second
FL	Florida
FRM	Federal Reference Method
FVC	forced vital capacity
γ	gamma; uptake coefficients
g	gram
g/bhp-h	grams per brake horsepower-hour
GA	Georgia
GAM	generalized additive models
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
GEE	generalized estimating equations
GIS	geographic information systems
GLM	generalized linear model
GLMM	generalized linear mixed model
GPx	glutathione peroxidase
$GS^{\bullet}$	glutathione radical
GSH	glutathione
GSR	glutathione reductase
GSS	glutathione synthetase
GST	glutathione S-transferase
GSTM1	glutathione s-transferase Mu 1
GSTP1	glutathione s-transferase P
h	hour, hours
hCAEC	human coronary artery endothelial cell
HDL	high-density lipoprotein
HDM	house dust mite
H&E staining	hematoxylin and eosin stain for histology analysis
HECT	hand eye coordination test
HEI	Health Effects Institute
HERO	Health and Environmental Research Online
HEV	hold-out evaluation
HF	high frequency component of HRV
HI	Hawai'i
HNO_2	nitrous acid
HNO_3	nitric acid
HNO_4	peroxynitric acid
HO-1	heme oxidase-1, heme oxygenase-1
HO_2	hydroperoxyl radical, perhydroxyl radical
HO_2NO_2	peroxynitric acid (PNA)
HONO	nitrous acid
HOONO	pernitrous acid, peroxynitrous acid
HR	hazard ratio(s)
HRV	heart rate variability
HSC	Harvard Six Cities
H_2SO_4	sulfuric acid

Acronym/Abbreviation	Meaning
IA	Iowa
i.e.	id est; that is
ICARTT	International Consortium for atmospheric research on Transport and Transformation
ICAS	Inner-City Asthma Study
ICD	International Classification of Diseases
ICR	mice strain
ICS	inhaled corticosteroids
ID	Idaho
IDW	inverse distance weighting
Ig	immunoglobulin
IgA	immunoglobulin A
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
IHD	ischemic heart disease
IL	interleukin; Illinois
IL-1	interleukin-1
IL-6	interleukin-6
IL-8	interleukin-8
Ile	isoleucine
IMSI	Integrated Mobile Source Indicator
IN	Indiana
INDAIR	probabilistic model for indoor pollution exposures
INF	infiltration of outdoor NO_2
iNOS	inducible nitric oxide synthase
INs	isoprene nitrates
IQR	interquartile range
ISA	Integrated Science Assessment
ISC3	Industrial Source Complex dispersion model
IUGR	intrauterine growth restriction
IVF	in-vitro fertilization
k	reaction rate
kg	kilogram

Acronym/Abbreviation	Meaning
k_i	decay rate
km	kilometer(s)
KS	Kansas
KY	Kentucky
LA	Louisiana; Los Angeles
LBW	low birth weight
LDH	lactate dehydrogenase
LF	low-frequency component of HRV
LF/HF	ratio of LF and HF components of HRV
LIF	laser induced fluorescence
LOOCV	leave one out cross-validation
LOPAP	long path absorption photometer
LOX-1	lectin-like oxLDL receptor
Lp-PLA_2	lipoprotein-associated phospholipase A2
LRI	lower respiratory infection
LRS	lower respiratory symptoms
LRTI	lower respiratory tract infection
LT	leukotrienes
LTO	landing and take-off cycles
LUR	land use regression
µ	mu; micro
µg/m^3	micrograms per cubic meter
m	meter
MA	Massachusetts
max	maximum
MCP-1	monocyte chemoattractant protein-1
MD	Maryland
MDA	malondialdehyde
ME	Maine
MESA	Multi-Ethnic Study of Atherosclerosis
MI	myocardial infarction, "heart attack"; myocardial ischemia
MI	Michigan
min	minimum

Acronym/Abbreviation	Meaning
MLI	mean linear intercept
MM5	meteorological mesoscale model
MMEF	maximum (or maximal) midexpiratory flow
MMP	matrix metalloproteinase
MMP-2	matrix metalloproteinase-2
MMP-9	matrix metalloproteinase-9
MN	Minnesota
mo	month, months
MO	Missouri
MoO_X	molybdenum oxide
MPO	myeloperoxidase
mRNA	messenger ribonucleic acid
ms; msec	millisecond
MS	Mississippi
MSA	Metropolitan Statistical Area
MSCA	McCarthy Scales of Children's Abilities
MT	Montana
n	sample size; total number of microenvironments
N	nitrogen; population number
NAAQS	National Ambient Air Quality Standards
NAB	North American Background
NaCl	sodium chloride
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NAL	nasal lavage
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
NES	Neurobehavioral Evaluation System
NH	New Hampshire
NH_3	ammonia
$(NH_4)_2SO_4$	ammonium sulfate
NHAPS	National Human Activity Pattern Survey
NJ	New Jersey
nm	nanometer
NM	New Mexico
NMMAPS	The National Morbidity Mortality Air Pollution Study
NMOR	N-nitrosomorpholine
NO	nitric oxide; nitrogen monoxide
NO_2	nitrogen dioxide
NO_2^-	nitrite
NO_3^-	nitrate
N_2O_5	dinitrogen pentoxide
NOS	nitric oxide synthase
NO_X	$NO + NO_2$
NO_Y	total oxides of nitrogen, $NO_X + NO_Z$
NO_Z	reactive oxides of nitrogen (e.g., HNO_3, HONO, PAN, particulate nitrates)
NQO1	NADPH-quinone oxidoreductase (genotype)
NR	not reported
n.s.	not significant
NSR	NO_X storage reduction
NV	Nevada
NY	New York
NYC	New York City
$O_2^{\bullet -}$	superoxide radical anion
O_3	ozone
OAQPS	Office of Air Quality Planning & Standards
OAR	Office of Air and Radiation
OC	organic carbon
OH	Ohio
8-OHdG	8-hydroxy-29-deoxyguanosine

Acronym/Abbreviation	Meaning
OK	Oklahoma
OMI	Ozone Monitoring Instrument
$ONOO^-$	peroxynitrite
$ONOOCO_2^-$	nitrosoperoxylcarbonate anion
OR	odds ratio(s); Oregon
ORD	Office of Research and Development
OTAG	Ozone Transport Assessment Group
p	probability
PA	Pennsylvania
PAH(s)	polycyclic aromatic hydrocarbon(s)
PAN	peroxyacetyl 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
PC_x	provocative concentration required to reduce/increase an effect by X%
PD	provocative dose
PD_x	provocative dose required to reduce/increase an effect by X%
PEACE	Pollution Effects on Asthmatic Children in Europe
PEF	peak expiratory flow
PFK	phosphofructokinase
P_i	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.

Acronym/Abbreviation	Meaning
$PM_{2.5}$	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
PMF	positive matrix factorization
PMN(s)	polymorphonuclear cell(s), polymorphonuclear leukocyte
PNC	particle number concentration
PNN50	proportion of successive NN intervals with difference >50 msec (NN50) out of the total number of NN intervals
pNO_3	particulate nitrate
ppb	parts per billion
ppm	parts per million
PPN	peroxypropionyl nitrate
ppt	parts per trillion
PSDs	passive sampling devices
P-selectin	platelet selectin, a marker of platelet activation
PTB	preterm birth
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
ρ	rho, Spearman correlation coefficient
r	Pearson correlation coefficient; Spearman correlation coefficient
RANTES	regulated on activation, normal T cell expressed and secreted (aka chemokine ligand 5, CCL5)
RBC	red blood cells
$RC(=O)OONO_2$	peroxynitrates, peroxyacetyl nitrates
REA	Risk and Exposure Assessment
RI	Rhode Island
RMS	ratios of the mean asthma scores
rMSSD	root mean square of successive differences; a measure of HRV
RNS	reactive nitrogen species
$RONO_2$	organic nitrates
$ROO^{\bullet}$	organic peroxyl radical
ROS	reactive oxygen species
RR	risk ratio(s), relative risk
RSNO	S-nitrosothiols
s	second(s)
S/N	signal to noise ratio
SA-LUR	Source-Area land use regression
SAT	Switching Attention Test
SBL	stable boundary layer
SBP	systolic blood pressure
SC	South Carolina
sCD62P	platelet activation biomarker
SCR	selective catalytic reduction
SD	standard deviation; South Dakota
SDNN	standard deviation of beat-to-beat (NN) intervals, an index of total HRV
SEARCH	Southeast Aerosol Research Characterization
sec	second(s)
SEER	Surveillance, Epidemiology, and End Results

Acronym/Abbreviation	Meaning
SES	socioeconomic status
SGA	small for gestational age
SHARP	Study of Houston Atmospheric Radical Precursors
SHEDS	Stochastic Human Exposure and Dose Simulation
SHS	secondhand smoke
sICAM-1	soluble intercellular adhesion molecule-1
SLAMS	State and Local Air Monitoring Stations
SMWAOs	small molecular weight antioxidants
SNCR	selective non-catalytic reduction
SNP	single nucleotide polymorphism
SNR	selective NO_X recirculation
SO_2	sulfur dioxide
SOA	secondary organic aerosols
SOD	superoxide dismutase
SPM	suspended PM, suspended particulate matter
SpO_2	blood oxygen saturation
sRaw	specific airway resistance
SRTT	Simple Reaction Time Test
s-TNFα-RII	soluble tumor necrosis factor α receptor II
ST-segment	measured from the J point to the end of the T wave in an ECG
sVCAM-1	soluble vascular adhesion molecule-1
t	fraction of time spent in a microenvironment, time
TBARS	thiobabituric acid reactive substances (species)
TC	total carbon
TEA	triethanolamine
Th2	T-derived lymphocyte helper 2
t_i	fraction of total time spent in the ith microenvironment
TIMP-1	tissue inhibitor of matrix metalloproteinase-1
TIMP-2	tissue inhibitor of matrix metalloproteinase-2
TLR2	Toll-like receptor 2
TLR4	toll-like receptor 4
TN	Tennessee
TNF	tumor necrosis factor
TP	total power of heart rate signal in an ECG
TRP	traffic-related pollution
TX	Texas
UFP	ultrafine particles
UH_2^-	urate
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
URI	upper respiratory infection
U.S.; USA	United States of America
USC	U.S. Code
U.S. EPA	U.S. Environmental Protection Agency
UT	Utah
VA	Virginia
Val	valine
$\dot{V}_E$	minute volume
VOCs	volatile organic compounds
VPTB	very preterm birth
VT	Vermont
vWF	von Willbrand factor
WHO	World Health Organization
WI	Wisconsin
wk	week, weeks
WRE-chem	Weather Research and Forecast model with chemistry
WV	West Virginia

Acronym/Abbreviation	Meaning
WY	Wyoming
y_i	time spent indoors
y_o	fraction of the day spent outdoors
yr	year(s)

PREAMBLE

1. Process of ISA Development

This preamble outlines the general process for developing an Integrated Science Assessment (ISA) including the framework for evaluating weight of evidence and drawing scientific conclusions and causal judgments. The ISA provides a concise review, synthesis, and evaluation of the most policy-relevant science to serve as a scientific foundation for the review of the National Ambient Air Quality Standards (NAAQS)[1] for the criteria air pollutants (i.e., carbon monoxide [CO], lead [Pb], nitrogen oxides, ozone [O_3], particulate matter [PM] and sulfur oxides) as defined by the Clean Air Act (CAA, 1990a, b). Figure I depicts the general NAAQS review process, and information for individual NAAQS reviews is available online[2].

The ISA is preceded by the release of an Integrated Review Plan (IRP) that discusses the planned scope and organization of the key NAAQS assessment documents (e.g., ISA), including policy-relevant questions, approaches for preparing documents, and the schedule for release and review of the documents. The policy-relevant questions included in the IRP serve to clarify and focus the NAAQS review on the critical scientific and policy issues, including uncertainties discussed during the previous review and newly emerging literature. The IRP is informed by an 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 science that should be considered in the ISA.

[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.

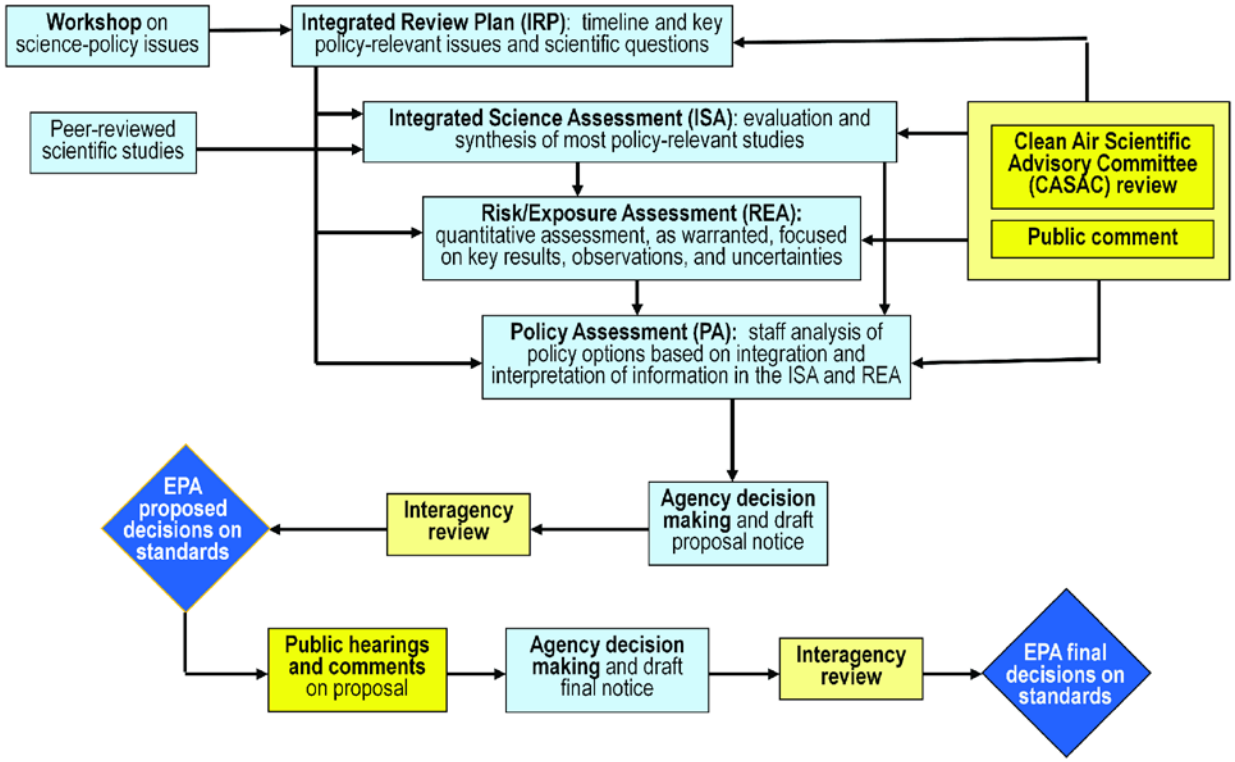

Figure I Schematic of the key steps in the process of the review of National Ambient Air Quality Standards.

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.

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.

In developing an ISA, the U.S. Environmental Protection Agency (EPA) reviews and summarizes the evidence from studies on atmospheric sciences, human exposure, animal

toxicology, controlled human exposure, epidemiology, and 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 health or welfare effects, involves collaboration between scientists from various disciplines. As an example, an evaluation of health effects evidence would include the integration of the results from epidemiologic, controlled human exposure, and toxicological studies, consideration of exposure assessment, and application of the causal framework (described below) to draw conclusions.

Integration of results on health or ecological effects that are logically or mechanistically connected (e.g., effects on the respiratory system) informs judgments of causality. 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 and completing the final ISA.

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

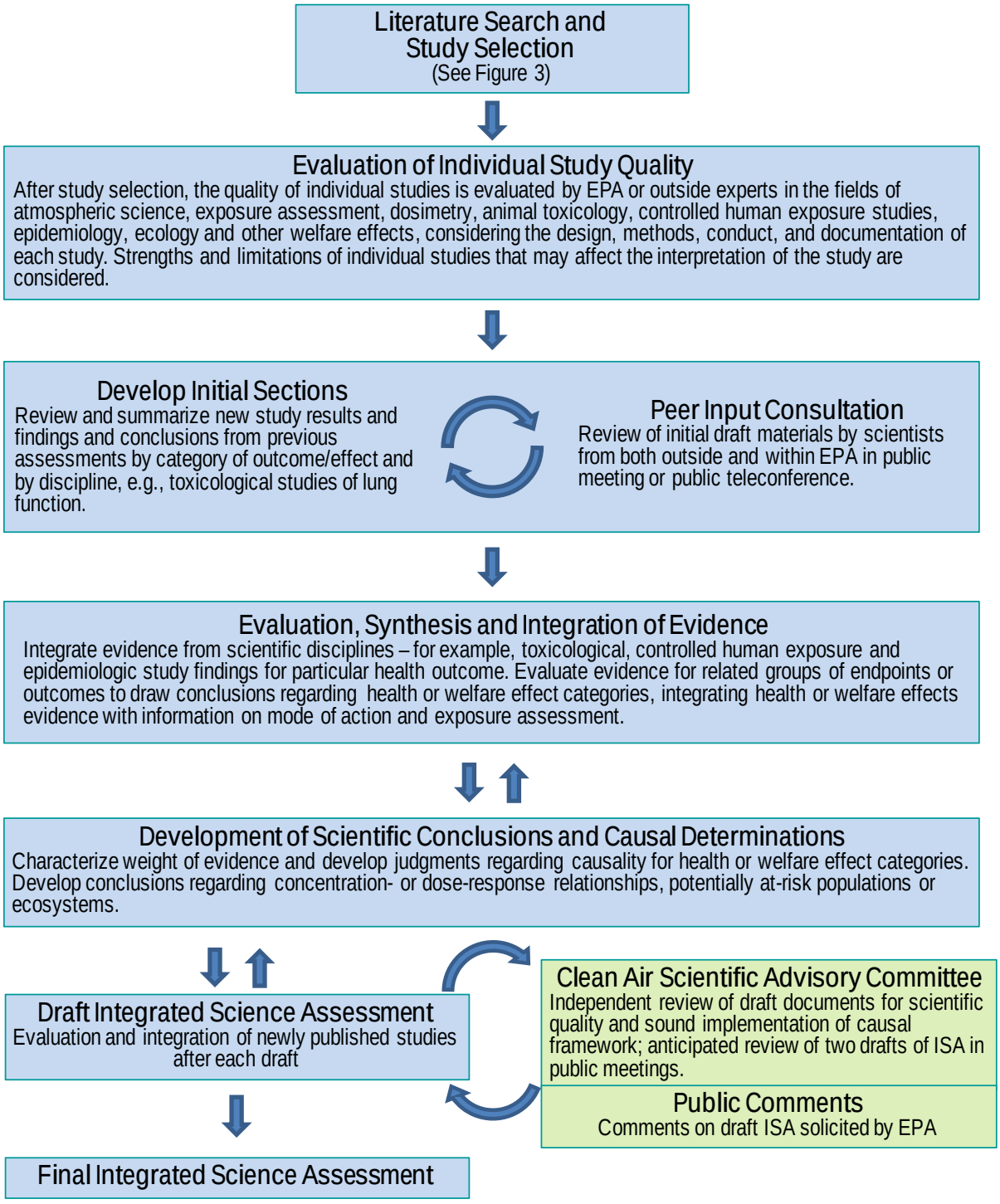

Figure II Characterization of the general process of ISA development.

2. Literature Search

An initial step in the literature search process is publication of a call for information in the Federal Register that invites the public to provide information relevant to the assessment, such as new or recent publications on health or welfare effects of the pollutant. The EPA maintains an ongoing literature search process for identification of 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 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/). 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 have been published (or accepted for publication) are considered for inclusion in the ISA. All relevant epidemiologic, controlled human exposure, toxicological, and ecological and welfare effects studies published since the last review are considered, including those related to exposure-response relationships, mode(s) of action (MOA), and potentially at-risk populations and lifestages. Studies and data anlyses 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).

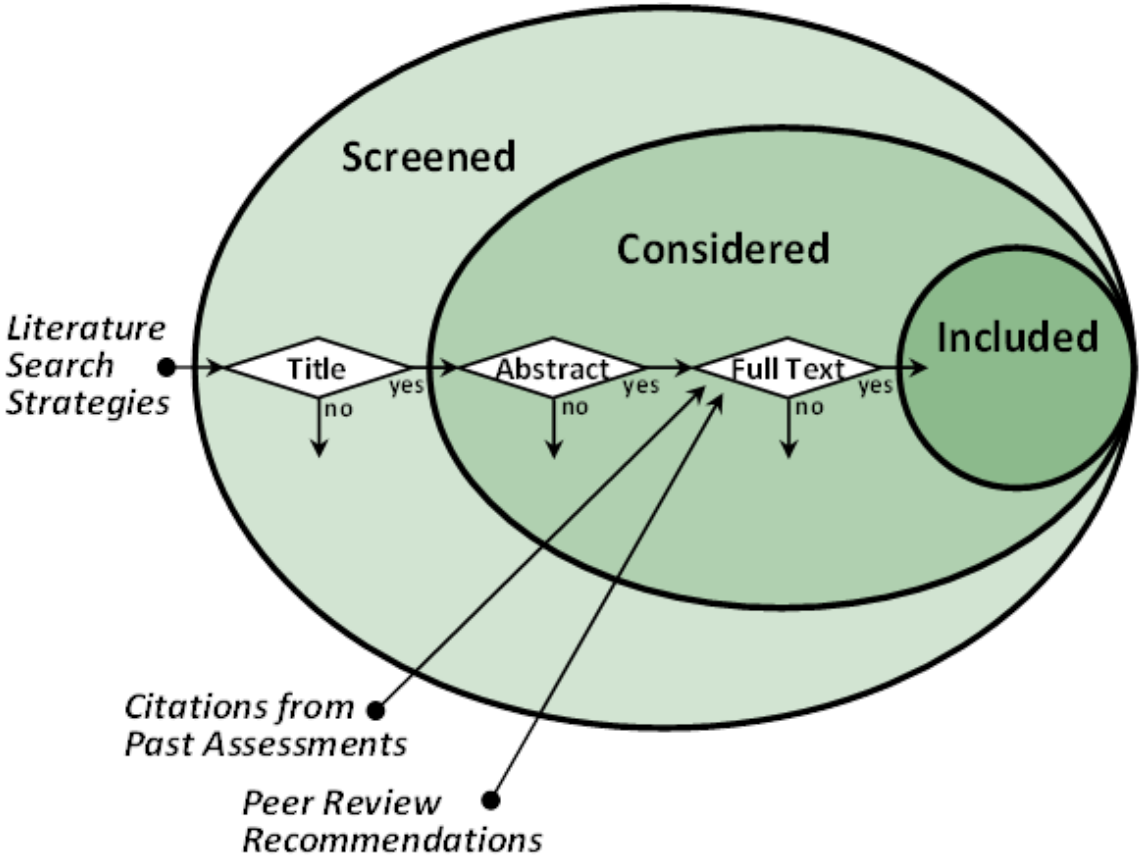

Figure III Illustration of processes for literature search and study selection used for development of ISAs.

Each ISA builds upon the conclusions of previous assessments for the pollutant under review. EPA focuses on peer reviewed literature published following the completion of the previous review 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

Considered references undergo abstract and full-text review to determine if they will be included in the ISA. The selection process is based on the extent to which the study is

informative and policy-relevant. Informative 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 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 whether the results are positive, negative, or null. 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.

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

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 to allow for study evaluation?
- 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 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

Considered atmospheric science and exposure assessment studies 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 high-quality 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 including 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 and metrics.

Exposure measurement error, which refers to the uncertainty associated with the exposure metrics used to represent exposure of an individual or population, can be an important contributor to uncertainty in air pollution epidemiologic study results. Exposure 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, nonambient sources of exposure, topography of the natural and built environment, meteorology, instrument errors, time-activity patterns, and the infiltration into indoor environments. Additional information present in high-quality exposure studies includes location and activity information from diaries, questionnaires, global positioning system

data, or other means, as well as information on commuting patterns. In general, atmospheric science and exposure studies focusing on locations pertinent to the U.S. will have maximum value in informing review of the NAAQS.

b. Epidemiology

In selecting 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 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. 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 if 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 may be acting as a surrogate for an unmeasured or poorly measured pollutant or for a particular mixture of pollutants. In addition, pollutants may independently exert effects on the same system; for example, several pollutants may be

associated with a respiratory effect through either the same or different modes of action. 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 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. These experiments allow investigators to 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 ambient concentrations, which may vary in animal studies depending on dosimetry, toxicokinetics, and biological sensitivity of the species or strain. 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 be included if they provide mechanistic insight or support results demonstrated in vivo.

d. Ecological Effects

In evaluating studies that consider ecological effects, in addition to assessing the general quality considerations discussed previously, emphasis is placed on studies that evaluate effects at or near ambient concentrations of the criteria air pollutants. 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 modes of action and illustrate the wide range of sensitivity to air pollutants across taxa or across biomes and ecoregions. Studies conducted in any country that contribute significantly to the general understanding of air pollutant effects are considered for inclusion. 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.

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

EPA has developed a consistent and transparent basis for integration of scientific evidence and evaluation of the causal nature of air pollution-related health or welfare effects for use in developing ISAs. The framework described below establishes uniform language concerning causality and brings specificity to the conclusions. This standardized language was drawn from sources across the federal government and wider scientific community, especially the U.S. EPA *Guidelines for Carcinogen Risk Assessment (2005)* and National Academy of Sciences (NAS) Institute of Medicine (IOM) document, *Improving the Presumptive Disability Decision-Making Process for Veterans* (2008), a comprehensive report on evaluating causality.

This framework:

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

Approaches to assessing the separate and combined lines of 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 IOM of the NAS (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, 2004). Causal inference criteria have also been described for ecological effects evidence (U.S. EPA, 1998a; 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 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.

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

Moving from association to causation involves the elimination of alternative explanations for the association. The ISA focuses on evaluation of the findings from the body of evidence across disciplines, drawing upon the results of all studies determined to meet 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 body of evidence is based on evaluation of study design and quality. The relative importance of different types of evidence to 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 effects may be drawn from a variety of experimental approaches (e.g., greenhouse, laboratory, field) and numerous disciplines (e.g., community ecology, biogeochemistry and paleontological/historical reconstructions). Other evidence including mechanistic, toxicokinetics, and exposure assessment may be highlighted if it is relevant to the evaluation of health and ecological effects and if it is of sufficient importance to affect the overall evaluation.

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 which is an indication of publication bias in that 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 response modifying 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. Although some controlled human exposure studies have included health-compromised individuals such as those with respiratory or cardiovascular disease, these individuals may also be relatively healthy and may not represent the most sensitive individuals in the population. Thus, observed effects in these studies may underestimate the response in certain populations. 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: e.g., cross-sectional, prospective cohort, panel, and time-series studies, and have 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, which is 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 physiological differences of the experimental species from humans, these studies inform characterization of health effects of concern, exposure-response relationships and MOAs. Further, animal models can inform determinations of response modifying 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 causality 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 they may not reflect responses that would occur in the natural environment. In addition, larger-scale processes are difficult to reproduce in the laboratory.

Field observational studies measure biological changes in uncontrolled situations 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 for assessments of multiple stressors or 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 (i.e., mesocosm studies). This type of study in manipulated natural environments can be considered a hybrid between a field experiment and laboratory study since some sources of response variation are removed through use of control conditions while others are included to mimic natural variation. They make it possible to observe community and/or ecosystem dynamics, and provide strong evidence for causality when combined with findings of studies that have been made under more controlled conditions.

b. Application of Framework for 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.

Table I Aspects to aid in judging causality.

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

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, 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, 2009a).[2] Although these aspects provide a framework for assessing the evidence, they do not lend themselves to being considered in terms of simple formulas or fixed rules of evidence leading to conclusions about causality (Hill, 1965). For example, one cannot simply count the number of studies reporting statistically significant results or statistically nonsignificant results and reach credible conclusions about the relative weight of the evidence and the likelihood of causality. Rather, these aspects provide a framework for systematic appraisal of the body of evidence, informed by peer and public comment and advice, which includes weighing alternative views on controversial issues. In addition, it is important to note that the aspects in Table I cannot be used as a strict checklist, but rather to determine the weight of the evidence for inferring causality. In particular, not meeting one or more of the principles does not automatically preclude a determination of causality [see discussion in (CDC, 2004)].

c. Determination of Causality

In the ISA, EPA assesses the body of relevant literature, building upon evidence available during previous NAAQS reviews, to draw conclusions on the causal relationships between relevant pollutant exposures and health or environmental effects. ISAs use a five-level hierarchy that classifies the weight of evidence for causation[3]. In developing this hierarchy, EPA has drawn on the work of previous evaluations, most prominently the IOM's *Improving the Presumptive Disability Decision-Making Process for Veterans* (IOM, 2008), EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), and the U.S. Surgeon General's smoking report (CDC, 2004). This weight of evidence

[1] The "aspects" described by Sir Austin Bradford Hill (Hill, 1965) have become, in the subsequent literature, more commonly described as "criteria." The original term "aspects" is used here to avoid confusion with "criteria" as it is used, with different meaning, in the Clean Air Act.
[2] The Hill aspects were developed for interpretation of epidemiologic results. They have been modified here for use with a broader array of data, i.e., epidemiologic, controlled human exposure, ecological, and animal toxicological studies, as well as in vitro data, and to be more consistent with the EPA Guidelines for Carcinogen Risk Assessment.
[3] The Center for Disease Control (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.

evaluation is based on integration of findings from various lines of evidence from across the health and environmental effects disciplines. These separate judgments are integrated into a qualitative statement about the overall weight of the evidence and causality. The five descriptors for causal determination are described in Table II.

Determination of causality involves the evaluation and integration of evidence for different types of health, ecological or welfare effects associated with short- and long-term exposure periods. In making determinations of causality, evidence is evaluated for major outcome categories or groups of related endpoints (e.g., respiratory effects, vegetation growth), integrating evidence from across disciplines, and 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 mode of action, interspecies differences, or factors that may increase risk of effects for a population. Thus, a causality determination is based on weight of evidence evaluation for health or welfare effects, focusing on the evidence from exposures or doses generally ranging from current levels to one or two orders of magnitude above current levels.

In addition, EPA evaluates evidence relevant to understand 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 levels at which effects are observed. The ISA also draws scientific conclusions regarding important exposure conditions for effects and populations that may be at greater risk for effects, as described in the following section.

Table II Weight of evidence for causal determination.

	Health Effects	Ecological and Welfare Effects
Causal relationship	Evidence is sufficient to conclude that there is a causal relationship with relevant pollutant exposures (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 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 of a causal relationship	Evidence is suggestive of a causal relationship with relevant pollutant exposures, but is limited. For example, (1) at least one high-quality epidemiologic study shows an association with a given health outcome although inconsistencies remain across other studies that are or are not of comparable quality; or (2) a well-conducted toxicological study, such as those conducted in the National Toxicology Program (NTP), shows effects relevant to humans in animal species.	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.

6. Public Health Impact

Once a determination is made regarding the causal relationship between the pollutant and outcome category, important questions regarding the public health impact include:

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

In order 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 form of relationships between the pollutant and health outcomes and draws conclusions on the shape of these relationships. Animal data may also inform evaluation of concentration-response relationships, particularly relative to MOAs and characteristics of at-risk populations.

An important consideration in characterizing the public health impacts associated with exposure to a pollutant is whether the concentration-response relationship is linear across the range of concentrations or if nonlinear relationships exist along any part of this range. 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 between individuals in susceptibility to air pollution health effects, tend to smooth and “linearize” the concentration-response function and thus can obscure the existence of a threshold or nonlinear relationship. Since individual thresholds vary from person to person due to individual differences such as genetic level susceptibility or pre-existing disease conditions (and even can vary from one time to another for a given person), it can be difficult to demonstrate that a threshold exists in a population study. These sources of variability and uncertainty may explain why the

available human data at ambient concentrations for some environmental pollutants (e.g., particulate matter [PM], O_3, lead [Pb], environmental tobacco smoke [ETS], radiation) do not exhibit population-level thresholds for cancer or noncancer health effects, even though likely mechanisms include nonlinear processes for some key events.

Finally, identification of the population groups or lifestages that may be at greater risk, or in some cases decreased risk, of health effects from air pollutant exposures contributes to an understanding of the public health impact of pollutant exposures. In the ISA, the term "at-risk population" is used to encompass characteristics of populations or lifestages that have a greater, or decreased, likelihood of experiencing health effects related to exposure to an air pollutant due to a variety of risk modifying factors.It should be noted that other terms have often been used in the literature to identify these populations and lifestages, including susceptible, vulnerable, and sensitive.

It is recognized that these factors may be intrinsic due to an increase in risk for an effect through a biological mechanism, such as 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, the factors may be extrinsic due to an increase in risk for an effect through an external, non-biological factor, such as socioeconomic status (SES) (e.g., educational attainment, income, access to healthcare, etc.), activity pattern and exercise level, reduced access to health care, low educational attainment, or increased pollutant exposures (e.g., near roadways). Some groups are at risk of increased internal dose at a given exposure concentration, which includes individuals that have a greater dose of delivered pollutant because of breathing pattern. This category would include 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 placed at increased risk for experiencing a greater exposure by being exposed at a higher concentration. Some factors described above are multifaceted and may influence the risk of an air pollutant related health effect through a combination of avenues. The emphasis is to identify and understand the factors that potentially increase, or in some cases decrease, 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.

7. Approach to Classifying At-Risk Factors

To identify at-risk factors that potentially lead to some populations or lifestages being at increased or decreased risk of air pollution-related health effects, the evidence is

systematically evaluated across relevant scientific disciplines (i.e., exposure sciences, dosimetry, toxicology, and epidemiology). An evaluation of studies first consists of focusing on studies that conducted stratified analyses (i.e., epidemiologic or controlled human exposure) to compare populations or lifestages exposed to similar air pollutant concentrations within the same study design. Experimental studies also provide important lines of evidence in the evaluation of at-risk factors that may lead to increased or decreased risk of an air pollutant related-health effect. Toxicological studies conducted using animal models of disease and controlled human exposure studies that examine individuals with underlying disease or genetic polymorphisms may provide evidence in the absence of stratified epidemiologic analyses. Additionally these studies can provide support for coherence with the health effects observed in epidemiologic studies as well as an understanding of biological plausibility. The potential increased or decreased risk of an air pollutant-related health effect may also be determined from studies that examined at-risk factors that result in differential air pollutant exposures. Building on the causal framework discussed in detail above, conclusions are reached regarding the strength of evidence across scientific disciplines for each at-risk factor that may contribute to increased or decreased 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 Classification 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 or decreased risk of air pollutant-related health effect(s) relative to some reference population or lifestage. Where applicable this includes coherence across disciplines. Evidence includes multiple high-quality studies.
Suggestive evidence	The collective evidence suggests that a factor results in a population or lifestage being at increased or decreased risk of an air pollutant-related health effect relative to some reference population or lifestage, but the evidence is limited due to some inconsistency within a discipline or, where applicable, a lack of coherence across disciplines.
Inadequate evidence	The collective evidence is inadequate to determine if a factor results in a population or lifestage being at increased or decreased risk of an air pollutant-related health effect relative to some reference population or lifestage. The available studies are of insufficient quantity, quality, consistency, and/or statistical power to permit a conclusion to be drawn.
Evidence of no effect	There is substantial, consistent evidence within a discipline to conclude that a factor does not result in a population or lifestage being at increased or decreased risk of air pollutant-related health effect(s) relative to some reference population or lifestage. Where applicable this includes coherence across disciplines. Evidence includes multiple high-quality studies.

8. Quantitative Relationships: Effects on Welfare

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

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

Evaluations of causality generally consider the probability of quantitative changes in 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.

9. Concepts in Evaluating Adversity

a. Evaluating Adversity of Health Effects

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

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 (ATS) in an official statement titled *What Constitutes an Adverse Health Effect of Air Pollution?* (ATS, 2000b). An air pollution-induced shift in the population distribution of a given risk factor for a health outcome was viewed as adverse, even though it may not increase the risk of any one individual to an unacceptable level. For example, a population of asthmatics could have a distribution of lung function such that no identifiable individual has a level associated with significant impairment. Exposure to air pollution could shift the distribution such that no identifiable individual experiences 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.

b. Evaluating Adversity of Ecological Effects

Adversity of ecological effects can be understood in terms ranging in biological level of organization; from the cellular level to the individual organism and to the population, community, and ecosystem levels. In the context of ecology, a population is a group of individuals of the same species, and a community is an assemblage of populations of different species 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 at the regional level, over both long and short terms."

Beyond the level of 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." 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.

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PREFACE

Legislative Requirements for the Primary NAAQS Review

Two sections of the Clean Air Act (CAA) govern the establishment and revisions of the National Ambient Air Quality Standards (NAAQS). Section 108 (42: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 …" (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] 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]). A secondary standard, as defined in Section 109(b)(2), must "specify a level of air quality the attainment and maintenance of which, in the judgment of the Administrator, based on such criteria, is requisite to protect the public welfare from any known or anticipated adverse effects associated with the presence of [the] air pollutant in the ambient air."[2]

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

[2] 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).

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. See *Lead Industries Association v. EPA*, 647 F.2d 1130, 1154 (D.C. Cir 1980); *American Petroleum Institute v. Costle,* 665 F.2d 1176, 1186 (D.C. Cir. 1981); *American Farm Bureau Federation v. EPA*, 559 F. 3d 512, 533 (D.C. Cir. 2009); *Association of Battery Recyclers v. EPA*, 604 F. 3d 613, 617-18 (D.C. Cir. 2010). Both kinds of uncertainty are components of the risk associated with pollution at levels below those at which human health effects can be said to occur with reasonable scientific certainty. Thus, in selecting primary standards that provide an adequate margin of safety, the Administrator is seeking not only to prevent 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, see *Lead Industries Association v. EPA*, 647 F.2d at 1156 n.51, but rather at a level that reduces risk sufficiently so as to protect public health with an adequate margin of safety.

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

In setting standards that are "requisite" to protect public health and welfare as provided in Section 109(b), EPA's task is to establish standards that are neither more nor less stringent than necessary for these purposes. In so doing, EPA may not consider the costs of implementing the standards. See generally, *Whitman v. American Trucking Associations*, 531 U.S. 457, 465-472, 475-476 (2001. Likewise, "[a]ttainability and technological feasibility are not relevant considerations in the promulgation of national ambient air quality standards." *American Petroleum Institute v. Costle,* 665 F. 2d at 1185.

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

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

Introduction to the NAAQS for Nitrogen Dioxide (NO_2)

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

Nitrogen dioxide (NO_2) is the indicator for a broad category of oxides of nitrogen. As specified in Section 108(c) of the CAA (42:U.S.C.21:7408(c)), EPA considers the term oxides of nitrogen to refer to all forms of oxidized nitrogen including multiple gaseous species (e.g., NO_2, nitric oxide [NO]) and particulate species (e.g., nitrates). EPA has evaluated the atmospheric chemistry, exposure, and health effects associated with nitrogen compounds present in particulate matter (PM) in the Agency's review of the NAAQS for particulate matter (PM). Thus, the review of the NAAQS for NO_2 focuses on the gaseous oxides of nitrogen.

History of the Review of Air Quality Criteria for the Oxides of Nitrogen and the NAAQS for NO_2

On April 30, 1971 the EPA initially promulgated primary and secondary NAAQS for NO_2, under section 109 of the Act (36 FR 8186). Both primary and secondary standards were set at 0.053 parts per million (ppm), annual average. The standards were based on scientific information contained the 1971 Air Quality Criteria Document for Nitrogen Oxides (U.S. EPA, 1971). Since then, the Agency has completed multiple reviews of the

[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.

air quality criteria upon which the NAAQS are set and the standards themselves. Table IV provides a brief summary of these reviews.

Table IV History of the National Ambient Air Quality Standards for NO_2 during the Period 1971-2012.

Final Rule	Primary/Secondary	Indicator	Averaging Time	Level	Form
1971 36 FR 8186 Apr, 30, 1971	Primary and Secondary	NO_2	Annual	53 ppb[1]	Annual arithmetic average
1985 50 FR 25532 Jun 19, 1985	Primary and secondary NO_2 standards retained, without revision.				
1996 61 FR 52852 Oct 8, 1996	Primary and secondary NO_2 standards retained, without revision.				
2010 FR 74 34404 Feb 9, 2010	Primary	NO_2	1-hour	100 ppb	98th percentile, averaged over 3 years[2]
			Primary annual NO_2 standard retained, without revision.		
2012 77 FR 20218 Apr 3, 2012	Secondary	Secondary annual standard retained, without revision.			

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 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).

The most recent 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).[3] The Agency's plans for conducting the review were contained the Integrated Review Plan for the Primary National Ambient Air Quality Standard for NO_2

[1] The initial standard level of the annual NO_2 standard was 0.053 ppm, which is equal to 53 ppb.
[2] The form of the 1-hour standard is the 3-year average of the 98th percentile of the yearly distribution of 1-hour daily maximum NO_2 concentrations.
[3] Documents related to reviews completed in 2010 and 1996 are available at: http://www.epa.gov/ttn/naaqs/standards/nox/s_nox_index.html.

(U.S. EPA, 2007a)[1] 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, 2008c), 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, 2008d), multiple drafts of which received CASAC and public review.

In the course of reviewing the second draft REA, 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 review's of the second draft REA, the EPA's Deputy Administrator indicated in a letter to the chair of CASAC chair, addressing earlier CASAC comments on the NAAQS review process, that the risk and 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 REA included a new policy assessment chapter. This policy assessment chapter considered the scientific evidence in the ISA and the exposure and risk characterization results presented in other chapters of the REA as they related to the adequacy of the then current primary NO_2 standard and potential alternative primary standards for consideration.[2] CASAC discussed the final version of the REA, with an emphasis on the policy assessment chapter during a public teleconference on December 5, 2008. Following that teleconference, CASAC offered comments and advice on the primary NO_2 standard in a letter to the Administrator (Samet, 2008).

At the time of the last review, the epidemiological evidence had grown substantially since the completion of the 1995 Staff Paper, with the addition of field and panel studies, intervention studies, and time-series studies of effect (75 FR 6488). After considering an

[1] The EPA conducted a separate review of the secondary NO_2 NAAQS jointly with a review of the secondary sulfur dioxide (SO_2) NAAQS. The Agency retained those secondary standards, without revision, to address the direct effects on vegetation of exposure to gaseous oxides of nitrogen and sulfur (77 FR 20218, April 3, 2012).
[2] Subsequent to the completion of the 2008 NO_2 REA, EPA Administrator Jackson called for 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).

integrative synthesis of the entire body of this evidence on human health effects associated with presence of NO_2 in the air, the Administrator determined that the 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. The Administrator noted a particular concern with the potential for adverse health effects following exposures to elevated NO_2 concentrations that can occur around major roads. *Id* at 6482. On July 15, 2009, the EPA proposed to supplement the existing annual, primary standard for NO_2 by establishing a new short-term standard (75 FR 34404). 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 yearly distribution of 1-hour daily maximum concentrations. The EPA also retained the existing primary annual NO_2 standard with a level of 53 ppb (75 FR 6474).

Revisions to the NAAQS were accompanied by revisions to the data handling procedures, the ambient air monitoring and reporting requirements, and the Air Quality Index (AQI).[1] One aspect of the new monitoring network requirements included requirements for States to locate monitors within 50 meters of major roadways in large urban areas, and in other locations 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 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).

References for Preface

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EXECUTIVE SUMMARY

Purpose of the Integrated Science Assessment

This Integrated Science Assessment (ISA) is a comprehensive evaluation and synthesis of the most policy-relevant science that serves as the scientific foundation for the review of the primary (health-based) National Ambient Air Quality Standards (NAAQS) for nitrogen dioxide (NO_2). The Clean Air Act requires the Environmental Protection Agency (EPA), every five years, to review the NAAQS including the science upon which the NAAQS are based. Scientific information and conclusions presented in the ISA guide the development of quantitative assessments in EPA's Risk and Exposure Assessment (if one is warranted). Information in the ISA, and in the Risk and Exposure Assessment, is integrated and interpreted in the EPA Policy Assessment to frame the broadest range of policy options that can be supported by the available scientific and technical information to guide decisions made by the EPA Administrator on whether to retain or revise the NAAQS (see Figure I, Preamble to the ISA).

The most recent review of the primary NAAQS for NO_2 was completed in 2010. EPA retained the annual standard with a level of 53 parts per billion (ppb), annual average (avg) concentration, to protect against health effects potentially associated with long-term NO_2 exposures. EPA established a new 1-hour (h) standard at a level of 100 ppb, based on the 3-year average of the 98th percentile of yearly 1-h daily maximum (max) concentrations. The 1-hour standard was established to protect against a broad range of respiratory effects associated with short-term NO_2 exposures in potential at-risk populations such as people with asthma and people who spend time on or near major roadways. EPA also set requirements for a monitoring network that includes monitors near major roadways, where maximum NO_2 concentrations are expected to occur.

Scope and Methods

Oxides of nitrogen are one of six criteria pollutants for which EPA has established NAAQS. Oxides of nitrogen include all oxidized nitrogen compounds, including gases such as NO_2 and nitric oxide (NO)[1] and several particle species (Figure 2-1, Section 2.2)[2]. The NAAQS are specified in terms of the indicator NO_2. As this ISA serves as the scientific foundation for the review of the primary NO_2 NAAQS, it evaluates scientific information on the atmospheric chemistry and human exposure to the gaseous forms of oxides of nitrogen and associated health effects only. The particle species (e.g., nitrates)

[1]Gaseous oxides of nitrogen also include NO_X, which refers to the sum of NO_2 and NO.
[2]Section 108(c) of the Clear Air Act refers to oxides of nitrogen as all forms of oxidized nitrogen including multiple gaseous and particulate species. 42. U.S.C. 21 7408(c).

were most recently examined in the 2009 ISA for Particulate Matter (PM) (U.S. EPA, 2009a). The ecological and other welfare effects of oxides of nitrogen are being considered 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, 2013).

EPA uses a structured and robust process for evaluating available scientific information and drawing conclusions about the health effects associated with air pollution exposure. This process includes criteria for identifying and selecting relevant recent peer-reviewed literature published since the previous ISA (i.e., studies published starting in 2008 for this ISA), assessing quality of scientific information, and integrating information. EPA also uses a consistent and transparent framework for drawing conclusions about the causal nature of air pollution-related health effects in the ISA (Section 5, Preamble to the ISA). Evidence is integrated across epidemiologic, controlled human exposure, and toxicological studies and across related outcomes to make a determination about causation, not just association. Conclusions are formed by synthesizing findings from studies reviewed in previous assessments and recent studies. Based on judgments of aspects such as the consistency, coherence, and biological plausibility of observed effects (i.e., evidence for the direct effect on a health outcome or key events that inform the mode of action), the evidence is classified according to a five-level hierarchy:

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

In addition to describing causal determinations, the ISA addresses policy-relevant issues such as: (1) exposure concentrations, durations, and patterns associated with health effects; (2) the concentration-response relationship(s), including information related to identifying thresholds for effects; and (3) lifestages or populations at increased risk for health effects related to exposure to oxides of nitrogen. In the evaluation of the scientific information and policy-relevant issues, the ISA also describes uncertainties and limitations in the scientific evidence base including the potential for NO_2 to serve primarily as an indicator of another ambient air pollutant or mixture.

Sources of Oxides of Nitrogen to Human Exposure

Ambient concentrations of oxides of nitrogen are determined by the types and strength of emissions sources present, the chemical transformations that occur in the atmosphere, weather conditions, deposition to surfaces, and transport to other locations. Based on the 2008 National Emissions Inventory[1], the major emissions source categories for the U.S. as a whole are highway vehicles (39%), off-highway vehicles (19%), fuel combustion by electric utilities (17%), and industrial fuel combustion (8%) (Section 2.3, Figure 2-2). Smaller source categories include other industrial operations and microbial processes in the soil. Specific sources that can affect local air quality include on-road vehicles, airports, railyards, shipping ports, home wood burning, intense industrial and chemical processes, activities for oil and gas development, and wildfires. NO_2 transported from continents outside of North America (i.e., North American Background [NAB]) contributes less than 1% to ambient concentrations in the U.S. (Section 2.5.6).

Direct emissions consist of mostly NO but also include some NO_2. A major chemical transformation in the air is the reaction of NO and ozone (O_3) to form NO_2 (Section 2.2, Figure 2-1). Rather than direct emissions, this reaction is the main source of the ambient air NO_2 concentrations measured in most urban locations. Chemical reactions also remove NO_2 and NO from the atmosphere. For example, reactions between NO_2 and volatile organic compounds (VOCs) form O_3. NO and NO_2 also are transformed into other oxides of nitrogen by reactions with reactive radical species and O_3. Other major mechanisms by which oxides of nitrogen are removed from the atmosphere are deposition including impaction with surfaces, reactions with plants, diffusion into cloud droplets, and washout in falling rain. Chemical transformations and deposition vary according to several environmental factors such as season, time of day, air circulation patterns, and ambient concentrations of other pollutants.

There are differences across locations and time in factors such as the sources present, chemical reactions that occur, atmospheric conditions, and topography. Thus, ambient concentrations of oxides of nitrogen are highly variable. This variability occurs across spatial scales, including national, regional, urban, neighborhood, and microscale environments (Section 2.5). Variability also occurs across time scales such as years, seasons, days of the week, and time of day.

Much of the information on ambient concentrations of oxides of nitrogen in the U.S. is for NO_2 and comes from the State and Local Monitoring Air Stations Network of about 500 sites. This monitoring network serves many purposes: assessing compliance with the NAAQS, providing the public with air pollution data in a timely manner, and supporting

[1]Information on emissions sources will be updated in the Second External Review Draft of the ISA for Oxides of Nitrogen with data from the 2011 National Emissions Inventory, which became available in November 2013.

air pollution research studies. NO and NO_X also are measured at many of these stations, but other oxides of nitrogen are not routinely measured. Across the U.S. during 2009-2011, the mean and 99th percentile for 1-h daily maximum ambient NO_2 concentrations were 20 ppb and 57 ppb, respectively (Table 2-1). During the same time period, the mean and 99th percentile for annual average NO_2 concentrations were 9.4 ppb and 25 ppb, respectively (Table 2-2). NO_2 concentrations are higher in urban areas than in nonurban areas (Figure 2-10 and Figure 2-12, for annual average and seasonal average, respectively). In microscale environments, ambient NO_2, NO, and NO_X concentrations have been shown to be 30% to 200% higher at locations within 15 m of a roadway (averaged over hours to weeks) compared with locations farther away from the road. The nature of the gradient varies according to factors such as traffic volume, time of day, meteorology, and local topography (Section 2.5.3). Concentrations can be higher in urban street canyons or in locations where built or natural topographical features influence air circulation.

With respect to time trends, annual average ambient NO_2 concentrations in the U.S. as a whole decreased by 48% from 1990 to 2012 (Figure 2-16), mainly due to decreases in NO_X emissions from on-road vehicles and electric utilities (Figure 2-3). On shorter time scales, ambient NO_2 concentrations typically are higher in winter than summer, on weekdays than weekends, and during early mornings (corresponding with rush hour) than other times of the day (including evening rush hour) (Section 2.5.4).

The variability in ambient NO_2 concentrations observed across spatial and time scales as specified above are important influences on human exposure to ambient NO_2. Human exposure to ambient NO_2 is determined by the concentrations in various ambient microenvironments, including in vehicles, and the time spent in those microenvironments (Section 2.6.1). Another component of total exposure is indoor NO_2 exposure, which is influenced by indoor sources (e.g., gas stoves, gas heaters, oil furnaces, wood burning stoves, kerosene heaters, smoking). Ambient concentrations penetrate indoors (Section 2.6.3.3). Ventilation characteristics (e.g., air conditioning, open windows) can affect the amount of NO_2 that penetrates indoors and thus contribute to variability in human exposure to ambient NO_2. Understanding human exposure to ambient NO_2 and the relationships between exposure and ambient concentrations is essential for interpreting scientific information on relationships between ambient NO_2 concentrations and health effects and in turn, for informing the review of the primary NO_2 NAAQS.

Many epidemiologic studies assess human exposure to NO_2 using ambient concentrations obtained from central site monitors. The siting of monitors does not cover all locations where people live or spend their time. Ambient concentrations may not be available at the microenvironment of interest (Section 2.6.5), for example near roads. Data from the near-

road monitoring network are not available yet. Thus, the use of ambient concentrations obtained from central site monitors to represent human exposure is associated with measurement error. The extent of exposure measurement error is influenced by the relationships between central site ambient concentrations and personal exposure. Across the population, there is wide variation in personal-ambient NO_2 relationships, indicating that there is heterogeneity among individuals in how well spatial and temporal variability in ambient concentrations correlate to personal exposure, accounting for varying time-activity patterns. Personal-ambient relationships may vary by age, season, and local sources (Sections 2.6.4 and 2.6.5.1).

Exposure measurement error can have important implications for the relationships observed between ambient NO_2 concentrations and health effects. For example, some studies estimated larger respiratory effects in association with more spatially-resolved NO_2 exposure estimates than with NO_2 concentrations obtained from a single central site monitor or averaged over area monitors (Sections 2.6.5.2 and 4.2.4.4). More spatially-resolved estimates included personal exposure measures, outdoor school measurements, and ambient concentrations at the nearest central site monitor. Health effects also are associated with more spatially-resolved measures of long-term NO_2 or NO_X exposure estimated from models. Measurement error is a component of the various exposure assessment methods, and is influenced by the spatial and temporal variability in NO_2 concentrations, time-activity patterns, air exchange characteristics of locations, and accuracy and precision of instrumentation. Measurement methods of regulatory networks are found to overestimate NO_2 concentrations because of interference from other oxides of nitrogen, but the impact typically is less than 10% in urban areas and near sources (Section 2.4.1). New measurement methods are being developed. Exposure measurement error may reduce the magnitude and/or precision (i.e., widen confidence intervals) of health effect associations (Section 2.6.5.3). However, the effect of measurement error resulting from the use of central site NO_2 to represent near-road exposures is not known.

Associations between NO_2 and health effects observed in epidemiologic studies may represent an independent effect of NO_2 or the effect of another air pollutant or mixture that is related to both the health effect being examined and NO_2 concentrations. A wide range of correlations is reported between NO_2 and copollutants such as O_3, SO_2, particulate matter ($PM_{2.5}$, PM_{10})[1], elemental or black carbon (EC or BC), ultrafine particles (UFP), and carbon monoxide (CO) (Table 2-4, Figure 2-19). Correlations generally are higher for EC, UFP, and CO, which like NO_2, are emitted from motor vehicles. The relationship between NO_2 and a given copollutant varies across locations

[1] $PM_{2.5}$: particulate matter with mean aerodynamic diameter less than or equal to a nominal 2.5 µm.
PM_{10}: particulate matter with mean aerodynamic diameter less than or equal to a nominal 10 µm.

and exposure assessment methods, which indicates that there is variability in the potential for a copollutant to bias (i.e., confound) associations between NO_2 and health effects.

Health Effects of Oxides of Nitrogen

This ISA evaluates relationships between oxides of nitrogen and a broad range of outcomes related to respiratory effects, cardiovascular effects, reproductive and developmental effects, total mortality, and cancer as examined in epidemiologic, controlled human exposure, and toxicological studies. For experimental studies, emphasis is placed on studies with exposures that are relevant to human ambient exposures, defined as concentrations no greater than 5,000 ppb, which is about one to two orders of magnitude higher than peak concentrations of NO_2, NO, or NO_X that humans experience on roads (Section 2.5.3). Causal determinations are based on evidence that is integrated across scientific disciplines and related outcomes, including information about potential modes of action. Relevant information on exposure and dosimetry also is considered. Separate causal determinations are made for health effects related to short-term (minutes up to 1 month, Chapter 4) and long-term (more than 1 month to years, Chapter 5) exposures. Although the scope of this ISA includes all gaseous oxides of nitrogen, information on the dosimetry, modes of action, and health effects is available for NO_2, NO, or NO_X exposures. This may be explained, in part, by the little information available on ambient exposures to other oxides of nitrogen. In this and the 2008 ISA for Oxides of Nitrogen, the majority of scientific information is available for health effects related to NO_2 exposure; thus, causal determinations are formed only for NO_2. Because there is at least some biological plausibility for negative health effects resulting from ambient-relevant NO_2 exposures but not from ambient-relevant NO exposures, associations between health effects and ambient NO_X are considered to be reflecting associations with NO_2 and are considered in causal determinations for NO_2.

The causal determinations from the 2008 ISA for Oxides of Nitrogen and this ISA are presented in Table ES-1. Integrated with previous evidence, results from recent studies are the basis for strengthening the causal determinations for all of the evaluated health effect categories. In some cases, recent studies show health effects associated with NO_2 exposure where previous results were inconsistent or showed no association. In other cases, recent studies expand on previous supporting evidence. Most of the recent literature base consists of epidemiologic studies. The previous body of controlled human exposure and toxicological studies and the relatively small body of recent studies inform the biological plausibility for health effects of NO_2 exposure with information on the direct effects on health outcomes or biological processes by which effects may occur. In the 2008 ISA for Oxides of Nitrogen, a major uncertainty noted for the relationships between NO_2 exposure and several health effect categories was the difficulty in

distinguishing whether the epidemiologic associations observed with ambient NO_2 concentrations were independent of the effects of another traffic-related air pollutant or mixture. This uncertainty regarding the potential for copollutant confounding or the potential for NO_2 to serve primarily as an indicator for another traffic-related pollutant or mixture remains for some health effects. For other health effects, evidence from recent studies reduces this uncertainty by indicating the independent effects of NO_2 exposure.

Table ES-1 Causal determinations for NO_2 by exposure duration and health effect category from the 2008 ISA and current draft ISA for oxides of nitrogen.

Exposure Duration and Health Effect Category[a]	Causal Determination[b]: 2008 ISA for Oxides of Nitrogen	Causal Determination[b]: Current Draft ISA for Oxides of Nitrogen
Short-term NO_2 Exposure		
Respiratory Effects Section 4.2, Table 4-23	Sufficient to Infer a Likely Causal Relationship	Causal Relationship
Cardiovascular Effects Section 4.3, Table 4-36	Inadequate to Infer the Presence or Absence of a Causal Relationship	Likely to be a Causal Relationship
Total Mortality Section 4.4, Table 4-41	Suggestive but not Sufficient to Infer a Causal Relationship	Likely to be a Causal Relationship
Long-term NO_2 Exposure		
Respiratory Effects Section 5.2, Table 5-9	Suggestive but not Sufficient to Infer a Causal Relationship	Likely to be a Causal Relationship
Cardiovascular Effects Section 5.3, Table 5-12	Inadequate to Infer the Presence or Absence of a Causal Relationship	Suggestive of a Causal Relationship
Reproductive and Developmental Effects[c] Sections 5.4.2, 5.4.3, 5.4.4, and Table 5-15	Inadequate to Infer the Presence or Absence of a Causal Relationship	Fertility, Reproduction, and Pregnancy Suggestive of a Causal Relationship
		Birth Outcomes Suggestive of a Causal Relationship
		Postnatal Development Suggestive of a Causal Relationship
Total Mortality Section 5.5, Table 5-19	Inadequate to Infer the Presence or Absence of a Causal Relationship	Suggestive of a Causal Relationship
Cancer Section 5.6, Table 5-21	Inadequate to Infer the Presence or Absence of a Causal Relationship	Suggestive of a Causal Relationship

[a]A 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 supports the causal determinations presented in the current draft ISA and the NO_2 concentrations with which health effects have been associated. Summary information also is presented in Table 1-1.

[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 current draft ISA, reproductive and developmental effects are separated into smaller subcategories of outcomes based on varying underlying biological processes and exposure patterns over different lifestages.

Providing insight into the processes by which exposure to NO_2 or NO can lead to health effects are the dosimetry and modes of action of NO_2 and NO. Once inhaled, NO_2 leaves the gas phase and enters the extracellular lining fluid of the lung. In the extracellular lining fluid, NO_2 readily reacts with substances such as antioxidants. The formation of secondary oxidation products and the cascade of events that follow are likely responsible for the health effects associated with NO_2 exposure. These products can initiate oxidative stress, alter permeability of the alveolar capillary barrier, and change the activity of enzymes in the respiratory tract (Section 3.2.2). In the respiratory tract, the biological responses demonstrated to occur with NO_2 exposure include, but are not limited to, initiation of inflammation, enhancement of bronchial smooth muscle reactivity, modification of immune responses, and remodeling of airways (Section 3.3.2). These observations provide support for the respiratory effects observed in relation to NO_2 exposure across disciplines by providing insight into potential underlying biological processes. The processes by which inhaled NO_2 may lead to health effects outside the respiratory system are not well characterized. Some products formed by reactions of inhaled NO_2 have been found in the blood of animal models but with higher than ambient-relevant NO_2 exposure concentrations. Limited evidence suggests that mediators may spillover from the respiratory tract into the blood in response to NO_2 exposure (Section 3.3.2.8), which could explain cardiovascular effects.

Unlike NO_2, NO is not transformed by reactions in the extracellular lining fluid of the respiratory tract and can diffuse into the blood. However, NO is formed naturally in the body from biological processes and from nitrates and nitrites that are present in foods consumed. It is not clear whether ambient-relevant concentrations of inhaled NO alter biological processes that are affected by endogenous or diet-derived NO. Thus, it is not clear by what processes inhaled NO may induce health effects (Section 3.3.3).

Health Effects Associated with Short-term NO_2 Exposure

Across the array of health effects evaluated in this ISA, evidence is more robust for health effects associated with short-term NO_2 exposure than long-term NO_2 exposure. For several outcomes related to respiratory and cardiovascular effects and for total mortality, epidemiologic associations are more consistently observed for short-term than long-term NO_2 exposure. Differences in causal determinations for these health effects relate to the extent to which there is biological plausibility for the effects of NO_2 exposure.

The strongest evidence is for respiratory effects, and it indicates that there is a causal relationship with short-term NO_2 exposure (Section 4.2.9). This conclusion is

based on the consistency, coherence, and biological plausibility of evidence integrated across epidemiologic, controlled human exposure, and animal toxicological studies indicating increases in asthma exacerbations. Epidemiologic studies consistently show associations between short-term increases in ambient NO_2 concentration and increases in hospital admissions and emergency department (ED) visits for asthma. Associations also are found with respiratory symptoms, pulmonary inflammation, and decreases in lung function in children with asthma. Epidemiologic associations are demonstrated in studies conducted in diverse geographical locations and using varied designs, including multicity analyses. Evidence from controlled human exposure and animal toxicological studies for NO_2-induced increases in airway responsiveness in adults with asthma and increases in allergic inflammation and oxidative stress demonstrate that the effects of NO_2 exposure on asthma exacerbations are biologically plausible. NO_2 exposure showed effects on many of the same endpoints in humans and experimental animals, indicating similar mechanisms across species. There also is evidence for NO_2-related increases in respiratory infection and chronic obstructive pulmonary disease (COPD) exacerbations, but inconsistencies are found across scientific disciplines or related outcomes. Recent epidemiologic studies also support the effects of ambient NO_2 exposure on a continuum of respiratory outcomes by demonstrating associations with respiratory mortality.

Much of the evidence described above was available in the 2008 ISA for Oxides of Nitrogen. In the current ISA, the causal determination is strengthened from likely to be a causal relationship to causal relationship because the recent epidemiologic evidence reduces the previously identified uncertainty regarding confounding by other traffic-related pollutants. Recent epidemiologic studies add to the evidence that associations of ambient NO_2 with asthma-related effects and other respiratory effects remain positive in copollutant models that statistically adjust for the effects of another pollutant such as PM_{10}, $PM_{2.5}$, SO_2, O_3, or examined in fewer studies, CO, EC, BC, or UFP (e.g., Figure 4-10 and Figure 4-11). Further, previous and recent studies demonstrate associations of indoor NO_2 with respiratory symptoms in children with asthma. The epidemiologic evidence and the experimental evidence together provide sufficient evidence that short-term NO_2 exposure has an independent, causal relationship with respiratory effects.

For cardiovascular effects, there is likely to be a causal relationship with short-term NO_2 exposure (Section 4.3.9) based strongly on associations consistently found between short-term increases in ambient NO_2 concentration and hospital admissions for ischemic heart disease (IHD) and cardiovascular mortality. The conclusion is supported by the concurrence between findings for cardiovascular mortality and hospital admissions for IHD, which is a leading cause of death. Recent epidemiologic studies reduce previous uncertainty regarding copollutant confounding by demonstrating that NO_2-associated cardiovascular hospital admissions and mortality remain positive in copollutant models

with PM_{10}, SO_2, O_3, or in some but not all locations, $PM_{2.5}$ or CO (e.g., Figure 4-15 and Figure 4-16). Recent findings from epidemiologic and controlled human exposure studies for NO_2-related decreases in heart rate variability and changes in ventricular repolarization provide some biological plausibility. Findings from some experimental studies for NO_2-induced increases in inflammation and oxidative stress describe effects on other key events that inform the modes of action for IHD and cardiovascular mortality. However, since experimental evidence is inconsistent and analysis of confounding does not include the array of potentially correlated copollutants, the collective evidence is not sufficient to conclusively demonstrate the independent cardiovascular effects of NO_2 exposure.

For total mortality, there is likely to be a causal relationship with short-term NO_2 exposure (Section 4.4.8) based on associations consistently observed between short-term increases in ambient NO_2 concentration and increases in mortality from all nonaccidental causes. Recent epidemiologic studies that pool data across various cities demonstrate the robustness of association and reduce previous uncertainty regarding confounding by SO_2, PM_{10}, or O_3. The findings for NO_2-related increases in cardiovascular hospital admissions provide some understanding of the biological processes by which NO_2 exposure may lead to mortality. Cardiovascular diseases account for a large portion of mortality (e.g., 35% in the U.S.). However, the limited findings for effects on measures of cardiovascular physiology produce some uncertainty regarding the spectrum of cardiovascular effects that NO_2 exposure may induce to lead to mortality. The robust evidence for the effects of NO_2 on asthma exacerbations but limited evidence for effects on COPD exacerbations and respiratory infection produces some uncertainty regarding the spectrum of respiratory effects that NO_2 exposure may induce to lead to mortality. The limited biological plausibility and limited analysis of potential copollutant confounding are not sufficient to conclusively demonstrate the independent effects of NO_2 exposure on total mortality.

Policy-relevant Considerations for Evaluating Health Effects Associated with Short-term NO_2 Exposure

Recent epidemiologic studies continue to find respiratory and cardiovascular effects as well as increases in total mortality in association with 24-h avg NO_2 and shorter averaging times, including 1-h max, 3-h max, and 8-h max NO_2 (Section 1.6.1). No consistent difference is demonstrated in the magnitude of health effects associated with 24-h avg ambient NO_2 versus shorter averaging times. However, potential differences in exposure measurement error may obscure differences in health effects associated with various NO_2 exposure metrics. Controlled human exposure studies demonstrate increases

in airway responsiveness of adults with asthma following NO_2 exposures of 30 minutes and 1 hour to concentrations of 200 to 300 ppb, and 100 ppb, respectively. Also, respiratory effects are found in association with ambient NO_2 exposures of 2 to 5 hours in adults with asthma and healthy adults subject to outdoor exposures at various traffic and nontraffic locations. Across health effects, epidemiologic associations are found with ambient NO_2 concentrations lagged 0 to 7 days or averaged over 2 to 7 days (Section 1.6.2). For several respiratory outcomes, larger effects are estimated for multiday averages of NO_2 than single-day averages. NO_2 exposures during time spent outdoors are associated with increases in respiratory effects immediately after exposure that persist to the following day. The shape of the NO_2 concentration-response relationship was formally evaluated mostly for respiratory ED visits and total mortality. These studies found evidence for linear relationships, and results do not identify a threshold for effects (Section 1.6.3).

The public health significance of NO_2-related health effects is supported by the large percentage of the population living near major roads and potentially having elevated exposures to NO_2 (Section 1.6.5). Further, short-term ambient NO_2 exposure is consistently associated with effects that are clearly adverse such as hospital admissions, ED visits, and mortality. NO_2-related increases in effects such as airway responsiveness in adults with asthma and changes in cardiovascular physiology in adults with cardiovascular disease are considered adverse on a population level because a shift in the distribution of the outcome can increase the proportion of individuals with clinically-important effects such as asthma exacerbations. The presence of at-risk lifestages and populations also informs the public health significance, as the NAAQS are intended to protect public health for at-risk populations. There is suggestive evidence that NO_2-related health effects differ by pre-existing asthma, pre-existing COPD, genetic variants for oxidative metabolism enzymes, dietary antioxidant intake, sex, and SES (Chapter 6). There is adequate evidence that children (ages 0-14 years) and older adults (ages 65 years and older) are at increased risk of NO_2-related health effects. The reasons for their increased risk (e.g., higher NO_2 exposure, biological susceptibility) are not clear. However, co-occurring risk factors in children and older adults may magnify the public health impact of NO_2-related health effects. For example, asthma is the leading chronic illness among U.S. children, and cardiovascular disease is prevalent in older adults (Table 6-3). The large potential for elevated exposures to NO_2, the increased risk for children and older adults, and prevalence of asthma in children and other chronic diseases in older adults can translate into a large number of people affected by NO_2 exposure

Health Effects Associated with Long-term NO_2 Exposure

A broad range of health effects has been evaluated for relationships with long-term NO_2 exposure. **The strongest evidence is for respiratory effects, and it indicates that there is likely to be a causal relationship with long-term NO_2 exposure** (Section 5.2.17)**.** The key supporting evidence includes consistent recent epidemiologic findings for associations between long-term ambient NO_2 concentrations and asthma incidence in children. Also, recent epidemiologic studies continue to demonstrate associations with decreases in lung function and partially irreversible decreases in lung function growth in children. The recent evidence reduces previous uncertainty regarding a relationship with asthma incidence. A relationship between NO_2 exposure and asthma incidence also is supported by recent epidemiologic associations observed with related outcomes such as respiratory symptoms in children with asthma and development of allergy in children. Biological plausibility for effects on asthma is provided by previous findings for increases in airway responsiveness and T-derived lymphocyte helper (Th)2 immune responses in guinea pigs induced by long-term NO_2 exposure and Th2 immune responses in humans and guinea pigs induced by short-term NO_2 exposure. Airway responsiveness and Th2 immune responses are key processes involved in the development of asthma and allergy. Previous and recent epidemiologic studies found NO_2-related respiratory effects in copollutant models with O_3, SO_2, PM, or EC. However, the limited examination of potential confounding by copollutants that often are highly correlated with NO_2 and limited experimental evidence are not sufficient to conclusively demonstrate an independent relationship between long-term NO_2 exposure and respiratory effects.

For the other health effects examined, **cardiovascular effects** (Section 5.3.6), **reproductive and developmental effects** (Section 5.4.5), **total mortality** (Section 5.5.3), **and cancer** (Section 5.6.12), **evidence is suggestive of a causal relationship with long-term NO_2 exposure.** The evidence base and uncertainties informing each of these causal determinations share common characteristics. The causal determination for each health effect category is strengthened from that made in the 2008 ISA for Oxides of Nitrogen based on some recent epidemiologic studies showing associations with long-term NO_2 or NO_X exposure. However, for each of the health effect categories, some epidemiologic studies show no association. For cardiovascular effects, some recent studies showed associations between long-term NO_2 or NO_X concentrations and heart failure, myocardial infarction, stroke, or cardiovascular mortality. Reproductive and developmental effects are evaluated as three subcategories that likely occur by different biological processes and exposure patterns over different lifestages. There is limited recent evidence for NO_2-related effects on fertility, reproduction, and pregnancy indicated as increases in pre-eclampsia. There is limited recent evidence for NO_2-related effects on birth outcomes indicated as fetal growth restriction. For postnatal development,

the strongest evidence is for partially irreversible decreases in lung function growth in children with less consistent evidence for decreases in cognitive function in children. Some recent epidemiologic studies also show associations of long-term NO_2 or NO_X exposure with total mortality and lung cancer incidence and mortality. For each of these health effect categories, there also is uncertainty in the relationship with long-term NO_2 exposure because of limited or no supporting evidence in experimental animals to provide biological plausibility.

Policy Relevant Considerations for Evaluating Health Effects Associated with Long-term NO_2 Exposure

Asthma incidence, decreases in lung function, and partially irreversible decreases in lung function growth in children are found in association with various long-term averages of NO_2, including 6-month average and NO_2 averaged over 1 to 10 (representing lifetime exposure) years. Relationships between long-term NO_2 exposure and outcomes such as asthma incidence and decreases in lung function growth in children or COPD hospital admissions in adults appear to be linear (Section 1.6.3). Most studies did not conduct analyses to formally evaluate the shape of the concentration-response relationship or evaluate whether there is evidence for a threshold for effects related to long-term NO_2 exposure. A U.S. study did not find strong regional heterogeneity in the association between NO_2 exposure and asthma evaluated in several U.S. cities, New York, NY; Chicago, IL; Houston, TX; and San Francisco, CA, as well as Puerto Rico (Section 1.6.4). The public health significance of the effects of long-term NO_2 exposure is supported by the evidence for increases in asthma incidence in children (Section 1.6.5). Asthma is a leading cause of missed school days and hospital admissions in children. Also, the NO_2-related decreases in lung function growth found in children could have implications for higher risk of mortality and cardiovascular morbidity in adulthood.

Regarding at-risk lifestages and populations, evidence indicates that the risk of developing asthma or allergy and the magnitude of decreases in lung function growth may be larger for long-term NO_2 exposure around birth or infancy compared with exposure later in childhood. Results for associations of long-term NO_2 exposure with asthma and lung function growth also informed the conclusion that there is suggestive evidence for genetic variants for oxidative metabolizing enzymes increasing risk of NO_2-related health effects.

Conclusions

Based on the 2008 National Emissions Inventory, the major NO_X emissions source categories in the U.S. are highway and off-highway vehicles and fuel combustion by electric utilities. Ambient concentrations of NO_2, NO, and NO_X show spatial and temporal heterogeneity at multiple scales and have been shown to be 30% to 200% higher at locations within 15 m of a roadway (averaged over hours to weeks) compared with locations farther away from the road. Emissions of NO_X and ambient concentrations of NO_2 have decreased over the past 20 years in the U.S. Relationships between NO_2 concentrations obtained from ambient monitors and personal exposures vary in the population, and exposure measurement error resulting from the use of ambient concentrations has been shown to reduce epidemiologic associations observed with health effects.

Recent studies, most of which are epidemiologic, expand on findings reported in the 2008 ISA for Oxides of Nitrogen and previous assessments. The consistency, coherence, and biological plausibility of evidence integrated across scientific disciplines and outcomes related to asthma exacerbations indicate that there is **a causal relationship between short-term exposure to NO_2 and respiratory effects**. Evidence indicates there is **likely to be a causal relationship between short-term exposure to NO_2 and cardiovascular effects as well as total mortality**. There is **likely to be a causal relationship between long-term NO_2 exposure and respiratory effects** based strongly on findings in children for asthma incidence and decreases in lung function. **Evidence is suggestive of a causal relationship between long-term NO_2 exposure and cardiovascular effects, reproductive and developmental effects, total mortality, and cancer**.

A major uncertainty in the 2008 ISA for Oxides of Nitrogen was the extent to which evidence indicated that NO_2 has effects on health that are independent of effects of another traffic-related pollutant or mixture. For respiratory effects, cardiovascular effects, and total mortality related to short-term exposure and respiratory effects related to long-term exposure, recent epidemiologic studies reduce this uncertainty with additional results for associations with NO_2 that remain positive in copollutant models. However, analysis of confounding by the array of potentially correlated copollutants, in particular CO, UFP, EC, and BC, which also are emitted from vehicles, is limited. Therefore, other lines of evidence that inform biological plausibility are key in addressing limitations of the epidemiologic evidence. For cardiovascular effects and total mortality related to short-term exposure and respiratory effects related to long-term exposure, biological plausibility is limited, and evidence is not sufficient to conclusively demonstrate effects of NO_2 exposure that are independent of those of other traffic-related pollutants.

There is adequate evidence that children (ages 0-14 years) and older adults (ages 65 years and older) have increased risk for NO_2-related health effects. A large proportion of the population lives near major roads; thus, the potential for elevated exposures to oxides of nitrogen, relative to people living 500 meters or more from roads, is large. There is suggestive evidence that the risk of NO_2-related health effects differs by pre-existing asthma, pre-existing COPD, genetic variants for oxidative metabolism enzymes, dietary antioxidant intake, sex, and SES. Daily average and 1-h max NO_2 concentrations as well as concentrations averaged over 30 minutes to a few hours are associated with health effects. For many respiratory outcomes, larger effects are estimated for multiday averages of ambient NO_2 concentrations than single-day concentrations. For long-term exposure, respiratory effects are associated with 6-month average NO_2 and NO_2 averaged over 1 year to 10 (representing lifetime exposure) years. The concentration-response relationship for associations of short-term ambient NO_2 exposure with respiratory-related ED visits and total mortality is found to be linear, and results do not identify a threshold for effects.

References for Executive Summary

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CHAPTER 1 INTEGRATED SUMMARY

The Integrated Science Assessment (ISA) is a comprehensive evaluation and synthesis of the most 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, 1990a). This ISA serves as the scientific foundation for the review of the health criteria for a broad category of oxides of nitrogen, which includes nitrogen dioxide (NO_2). As such, it communicates critical science judgments to inform the review of the current primary (health-based) National Ambient Air Quality Standards (NAAQS) for NO_2. This ISA incorporates key information and judgments contained in the 1993 Air Quality Criteria Document (AQCD) and the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c, 1993) to provide the foundation for the review of recent studies. Additional details of the relevant scientific literature published since the previous ISA, as well as key studies from previous assessments, are included. Thus, this ISA serves to update the evaluation of the scientific evidence that was available at the time of completion of the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c).

The most recent review of the primary NO_2 NAAQS was completed in 2010. EPA retained the annual standard with a level of 53 parts per billion (ppb) NO_2, annual average (avg) concentration, to protect against health effects potentially associated with long-term exposure. EPA established a new 1-hour (h) standard at a level of 100 ppb NO_2 based on the 3-year average of the 98th percentile of the yearly distribution of 1-h daily maximum (max) concentrations. The 1-hour 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 major roads. In 2010, EPA also established requirements for a monitoring network that includes monitors near major roads in urban areas, locations where maximum NO_2 concentrations are expected to occur (U.S. EPA, 2010c). Additional information on the legislative requirements and historical background for the NO_2 NAAQS is contained in the Preface to this ISA.

This chapter provides a summary and synthesis of the scientific evidence reviewed in the ISA and the conclusions and findings that best inform many policy-relevant questions that frame the review of the NO_2 NAAQS as identified in "The Integrated Review Plan for the Primary National Ambient Air Quality Standard for Nitrogen Dioxide." To that end, this chapter includes:

- An evaluation of 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 based on the integration of findings across various scientific disciplines and across related health outcomes as well as a discussion of important uncertainties identified in the interpretation of the scientific evidence, including the role of NO_2 within the broader ambient mixture of pollutants.

- Discussion of policy-relevant considerations such as: exposure averaging times and lags associated with health effects; concentration-response relationships and thresholds below which effects do not occur; and lifestages and populations potentially with increased exposure to oxides of nitrogen and/or risk of associated health effects.

1.1 ISA Development and Scope

EPA uses a structured and transparent process for evaluating the scientific evidence and forming conclusions and causal determinations for the relationships between air pollution exposures and health effects. The ISA development process describes approaches for literature searches, criteria for selecting and evaluating relevant studies, guidelines for evaluating the weight of the evidence, and a framework for forming causal determinations. As part of this process, the ISA is reviewed by the Clean Air Scientific Advisory Committee, a formal independent panel of scientific experts, and by the public. The ISA development process and causal framework are described in detail in the Preamble to the ISA and are summarized below.

This ISA evaluates scientific information for gaseous species of oxides of nitrogen[1]. Oxides of nitrogen consist of all forms of oxidized nitrogen compounds, including gases such as NO_2 and nitric oxide (NO) as well as their gaseous and particulate reaction products (e.g., organic and inorganic nitrates and nitrites, nitro-polycyclic aromatic hydrocarbons) (Section 2.2, Figure 2-1)[2]. The particle species (e.g., nitrates, nitro-polycyclic aromatic hydrocarbons) are not the focus of this ISA and were reviewed most recently in the 2009 ISA for PM (U.S. EPA, 2009a). When referring to the group of gaseous oxidized nitrogen compounds as a whole, the ISA uses the term oxides of nitrogen. Based on the definition commonly used in the scientific literature, this ISA uses the abbreviation NO_X to refer specifically to the sum of NO_2 and NO concentrations.

[1] The other criteria pollutants are ozone (O_3), particulate matter (PM), oxides of sulfur (SO_X and SO_2), carbon monoxide (CO), and lead (Pb).
[2] Section 108(c) of the Clear Air Act refers to oxides of nitrogen as all forms of oxidized nitrogen including multiple gaseous and particulate species. 42. U.S.C. 21 7408(c).

As this ISA informs the review of the primary NO_2 NAAQS, it evaluates information on potential relationships of oxides of nitrogen with health effects as reported in epidemiologic, controlled human exposure, and toxicological studies. Relevant studies also include those informing concentration-response relationships, modes of action, and potential at-risk lifestages and populations for exposure to oxides of nitrogen and/or related health effects. Also relevant to this ISA are studies on atmospheric chemistry and fate of emissions as well as EPA analyses of air quality and emissions data. The ecological and other 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, 2013).

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). From that time, literature searches were conducted routinely to identify peer-reviewed studies published since the previous ISA (i.e., studies published starting in 2008). Multiple search methods were used (Section 2, Preamble), and relevant studies were also identified by referrals from the public and scientific experts. Some studies were judged to be irrelevant (see preceding paragraph) based on title and were excluded. Studies that were judged as potentially relevant based on review beyond the title 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 (http://hero.epa.gov/oxides-of-nitrogen) for the ISA for Oxides of Nitrogen lists the references that are cited in the ISA as well as the references that were considered for inclusion but not cited, and also contains electronic links to bibliographic information and abstracts.

Health effects were considered for evaluation in this ISA if examined in previous assessments for oxides of nitrogen or if examined in multiple recent studies in more than one location (e.g., neurodevelopmental effects). Literature searches identified one or two recently published epidemiologic studies each on outcomes such as epilepsy, headache, depression, ocular effects, gastrointestinal effects, and bone density [Supplemental Table S1-1 (U.S. EPA, 2013e)]. These health effects are not evaluated in the current draft ISA because of the large potential for publication bias. These studies were conducted in areas and populations for which associations between oxides of nitrogen and other health effects have been demonstrated. Thus, the exclusion of these studies does not exclude the assessment of particular geographic locations, potential at-risk lifestages or populations, or range of ambient concentrations of oxides of nitrogen.

The Preamble describes the general framework for evaluating scientific information, including criteria for assessing study quality and findings and developing scientific conclusions. Greater weight is placed on those studies most relevant to the review of the

NAAQS. For epidemiologic studies, this includes high-quality studies that can be characterized as: (1) studies that provide understanding of the quantitative relationships between varying concentrations of oxides of nitrogen and health effects; (2) studies that examine oxides of nitrogen as a component of a complex mixture of air pollutants and consider other potential confounding factors; (3) studies with a priori aims to examine potential at-risk lifestages and populations; or (4) multicity studies that employ standardized analytical methods for evaluating health effects of oxides of nitrogen across locations and provide overall estimates for effects by pooling 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 concentrations of oxides of nitrogen that are relevant to human ambient exposures. Ambient-relevant exposures are defined as those no greater than 5,000 ppb, which is one to two orders of magnitude higher than peak concentrations of NO_2, NO, or NO_X that humans experience on roads (Section 2.5.3). Studies with higher exposure concentrations are included in cases where results inform potential modes of action. For the evaluation of human exposure to ambient oxides of nitrogen, 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.

The ISA uses a formal causal framework to classify the weight of evidence according to a five-level hierarchy (Table II of the Preamble). Conclusions are drawn based on information integrated across scientific disciplines and related endpoints and the synthesis of evidence from previous and recent studies. Determinations are made for 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 the direct effect of a pollutant on a health outcome or key events that inform the mode of action) as well as related uncertainties.

- **Causal relationship**: the consistency and coherence of evidence integrated across scientific disciplines and related outcomes are sufficient to rule out chance, confounding, and other biases with reasonable confidence.

- **Likely to be a causal relationship**: several studies show effects that do not appear to be explained by chance, confounding, and other biases, but uncertainty remains in the evidence base. For example, there may be some uncertainty regarding potential confounding by other pollutants in the ambient mixture or biological plausibility because of some inconsistency of evidence among different disciplines.

- **Suggestive of a causal relationship**: evidence overall is limited, for example, to a high-quality epidemiologic or animal toxicological study with effects relevant to humans. Or, effects are found in some high-quality studies but not in other studies that are or are not of comparable quality.

- 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 lifestages and populations, consistently show no effect.

Beyond forming causal determinations for relationships between pollutant exposures and health effects, the ISA aims to address questions relevant to quantifying health risks using information on the quantitative relationships between pollutant exposures and health effects. These questions include:

- What is the nature of the concentration-response, exposure-response, or dose-response relationship?

- Under what exposure conditions (dose or concentration, duration, and pattern) are effects observed?

- What lifestages or populations appear to have increased exposure to oxides of nitrogen and/or risk of associated health effects?

1.2 Organization of the ISA

The ISA comprises the Preamble, Preface (with Legislative Requirements and History of the NAAQS for NO_2), Executive Summary, and six chapters. Subsequent sections of Chapter 1 synthesize the scientific evidence that best informs policy-relevant questions that frame this review of the primary NO_2 NAAQS. Section 1.3 summarizes information on the sources, atmospheric chemistry, ambient concentrations, and human exposure to oxides of nitrogen. Section 1.4 summarizes the causal determinations for health effects associated with short-term and long-term NO_2 exposure and the key contributing evidence, including information on the dosimetry of NO_2 and NO and the key events that inform the modes of action underlying health effects. Section 1.5 is an integrated discussion of the scientific information addressing the independent effects of exposure to oxides of nitrogen on various health outcomes, which was an important uncertainty identified in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c). The discussion addresses potential confounding by copollutants and other factors and whether oxides of

nitrogen have independent effects or serve primarily as an indicator of other traffic-related air pollutants. Section 1.6 presents a discussion of policy-relevant considerations, such as exposure metrics associated with health effects, concentration-response relationships for NO_2; and potential at-risk lifestages and populations.

Chapter 2 characterizes the sources, atmospheric chemistry, and fate of oxides of nitrogen in the environment; trends in ambient concentrations; and factors influencing human exposure to oxides of nitrogen. Chapter 3 describes the dosimetry and modes of action for health effects related to NO_2 and NO. Chapter 4 and Chapter 5 evaluate and integrate epidemiologic, controlled human exposure, and toxicological evidence for the health effects related to short-term and long-term exposure to oxides of nitrogen, respectively. Chapter 6 evaluates the evidence for potential at-risk lifestages and populations.

1.3 Sources of Oxides of Nitrogen to Human Exposure

1.3.1 Sources of Oxides of Nitrogen

Direct emissions of oxides of nitrogen from sources comprise a mix of NO and NO_2, with a ratio in favor of NO. Based on the 2008 National Emissions Inventory[1], the major NO_X emissions categories in the U.S. are related to combustion processes, dominated by highway vehicles (39%) and followed by off-highway vehicles (19%), fuel combustion by electric utilities (17%), and industrial fuel combustion (8%) (Section 2.3, Figure 2-2). Smaller source categories (each less than 5% of the inventory) include other industrial operations and microbial processes in soil. Specific NO_X emissions sources that can affect local air quality include on-road vehicles, airports, railyards, shipping ports, home wood burning, intense industrial and chemical processes, activities for oil and gas development, and wildfires (Section 2.3). Some of these specific sources can emit large, transient peaks of NO_X. Specific locations vary in both the presence and the mix of specific emissions sources that contribute to total emissions.

Emissions from natural and anthropogenic sources from continents other than North America contribute to North American Background NO_2 concentrations. Seasonal mean background concentrations are estimated to be less than 0.3 ppb over most of the continental U.S. and account for a small fraction of ambient concentrations in the U.S. For example, in the Eastern U.S. where ambient NO_2 concentrations are the highest,

[1]The data presented on emissions sources will be updated in the Second External Review Draft of the ISA for Oxides of Nitrogen using the 2011 National Emissions Inventory, which became available in November 2013.

North American Background accounts for less than 1% of the total concentration (Section 2.5.6).

1.3.2 Atmospheric Chemistry and Fate of Oxides of Nitrogen

In addition to emissions sources, ambient concentrations of oxides of nitrogen are determined by chemical transformations, transport to other locations, meteorology, and deposition to surfaces. A major transformation is the rapid (i.e., minutes) reaction that occurs between direct NO emissions and ozone (O_3) to form NO_2 (Section 2.2, Figure 2-1). Rather than direct emissions, the reaction of NO with O_3 is the main source of NO_2 concentrations obtained from most ambient air monitors in U.S. urban locations.

NO and NO_2 also are transformed into other oxides of nitrogen. Reactions with gas phase hydroxyl radicals, hydroperoxy radicals, organic peroxyl radicals, and O_3 form compounds such as nitric acid (HNO_3), peroxyacetyl nitrate (PAN), nitrous acid (HONO), and particulate nitrates. NO and NO_2 also are involved in reaction cycles with radicals produced from volatile organic compounds (VOCs) to form O_3 (Section 2.2). The major gas-phase products of the reactions of NO and NO_2 are PAN and HNO_3. Morning rush hour NO and NO_2 emissions can be completely converted to HNO_3 and other products by late afternoon under warm, sunny conditions via reaction with highly abundant hydroxyl radicals. The abundance of nitrate radicals at night favors formation of gas phase organic nitrates, secondary organic aerosols, and dinitrogen pentoxide. PAN and isoprene nitrates can be transported to distant locations, where they decompose to release NO_2, which is then available to participate in O_3 formation. HNO_3 acts similarly but is highly soluble and has a high deposition rate. The transformations of NO and NO_2 into other oxides of nitrogen followed by dry deposition (i.e., impaction with surfaces or gas exchange with plants), and wet deposition (i.e., diffusion into cloud droplets, washout by impaction with falling rain drops) are the major processes by which oxides of nitrogen are removed from the atmosphere.

1.3.3 Ambient Concentrations – Temporal and Spatial Trends

Information on ambient concentrations of NO_2, NO, and NO_X in the U.S. is provided primarily by the State and Local Air Monitoring Stations (SLAMS) Network of about 500 sites (Section 2.4.5), and most information is for NO_2. This network serves many objectives: determining compliance with the NAAQS, providing the public with air pollution data in a timely manner, and providing estimates of human ambient exposure for many epidemiologic studies of health effects. The regulatory network monitors use

chemiluminescence techniques that directly measure NO and NO_X and then report a calculated NO_2 as the difference between NO_X and NO. Monitoring sites are located within U.S. Metropolitan Statistical Areas or urban areas. The near-road network promulgated as part of the 2010 decision on the primary NO_2 NAAQS is being phased in, with the first of three phases of monitoring scheduled to begin in January 2014. Data from this network are not available yet.

Across U.S. SLAMS, the mean and 99th percentile for 1-h daily maximum ambient NO_2 concentrations for 2009-2011 were 20 ppb and 57 ppb, respectively (Table 2-1). During the same time period, the mean and 99th percentile for annual average NO_2 concentrations were 9.4 ppb and 25 ppb, respectively (Table 2-2). Although ambient concentrations of species such as HNO_3 and HONO can be higher than those of NO_2 far downwind of sources, the limited available data indicate that with typical ambient concentrations of NO_2, ambient concentrations of HNO_3 and HONO range from less than 0.1 ppb to a few ppb. With respect to long-term temporal trends, U.S.-wide annual average NO_2 concentrations decreased by 48% from 1990 to 2012 (Figure 2-16). This decrease is attributed to a decrease in NO_X emissions, which declined by more than 50% in the U.S. from 1990 to 2012 (Figure 2-3). Emissions were reduced for on-road vehicles and electric utilities due, in part, to the use of pollution control technologies (Sections 2.3.2 and 2.3.7). In addition to long-term trends, ambient NO_2 concentrations show seasonal trends, with higher concentrations measured in the winter than summer. In urban areas, ambient NO and NO_2 concentrations rise during the night when atmospheric mixing is reduced because of low wind speeds and low mixing layer heights. Ambient NO and NO_2 concentrations peak in early mornings corresponding with morning rush hours, decrease until late afternoon, then increase again in early evening. Ambient concentrations at most urban sites are higher on weekdays versus weekends.

Ambient NO_2 concentrations vary on multiple spatial scales, including regional, urban, neighborhood, and microscale environments (Section 2.5). Corroborating previous data, data from monitoring networks for 2009-2011 indicate higher NO_2 concentrations in urban locations than less populated nonurban locations (Figure 2-10 and Figure 2-12 for annual average and seasonal average, respectively). There are more monitors in urban areas, which may make it difficult to assess regional trends in ambient NO_2 concentrations. Several lines of evidence demonstrate the large variability in ambient NO_2 concentrations with distance to roads. Across geographic areas, ambient NO, NO_2, and NO_X concentrations decrease exponentially with increasing distance from the roadway, reaching background concentrations within 100 to 500 meters downwind of the roadway (Section 2.5.3). LUR models show that at the urban scale, ambient NO_2 concentrations can be predicted well by roadway proximity and in some cases, proximity to industrial sources, which are other major sources of NO_2 (Section 2.6.2.3).

Neighborhood-scale and smaller near-road scale ambient concentrations of oxides of nitrogen can be affected by temporal variation in emissions sources, atmospheric conditions, and characteristics of the built environment. Characteristics of the built environment such as vehicle direction and speed, roadway structure (barriers versus open terrain), slopes of roadways, and height of buildings adjacent to roadways can affect ambient concentrations by affecting movement of air.

1.3.4 Human Exposure to Oxides of Nitrogen

The spatial and temporal variability in ambient concentrations measured at various scales are important influences on human exposure to ambient oxides of nitrogen. Human exposure is determined by concentrations in specific ambient, indoor, and in-vehicle, microenvironments and time spent in those microenvironments (Section 2.6.1). Characterizing human exposure to ambient oxides of nitrogen is of primary importance to the review of the NO_2 NAAQS. Among oxides of nitrogen, human exposure to ambient NO_2 is the most well characterized, and key findings described below are supported by recent studies and those reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c). A recent analysis found that a time-weighted average of NO_2 concentrations in various microenvironments corresponded well with total personal NO_2 exposures (Section 2.6.5.2). Such findings indicate that variability in ambient concentrations and time-activity patterns affect how exposure varies within individuals as they move across locations in time and how exposure varies among individuals in the population who are in different locations. Human exposure also is determined by indoor NO_2 concentrations. Indoor concentrations not only are affected by indoor sources such as gas stoves and heaters, oil furnaces, wood burning stoves, kerosene heaters, and smoking (Section 2.6.3.3) but also the penetration of ambient concentrations indoors and in turn, human exposure, can vary according to ventilation characteristics (e.g., open windows, air conditioning, building air exchange rate). The relative contribution of NO_2 exposure in specific microenvironments, including in vehicles, to peak or total exposure is not widely characterized. However, a recent study in the U.K. found that indoor exposures made up the majority of total NO_2 exposure in adults, with smaller contributions coming from in-vehicle and outdoor exposures.

In many epidemiologic studies, human exposure to ambient NO_2 is estimated using ambient concentrations obtained from central site monitors. The siting of monitors indicates that ambient NO_2 concentrations likely represent area-wide exposures at the regional, urban, or neighborhood scale. Thus, central site monitors do not cover all locations where people live or spend at least part of their time. Ambient concentrations may not be available for the microenvironment of interest, for example near roads. Data

from the near-road monitoring network are not available yet. Hence, the use of ambient concentrations obtained from central site monitors to represent human exposure to ambient NO_2 is associated with measurement error. The extent of exposure measurement error is influenced by the relationship between personal and central site ambient concentrations. These relationships are not consistently characterized for NO_2, particularly for long-term NO_2 exposures. Further, examination generally is limited to relationships between ambient NO_2 concentrations and total personal NO_2 exposure, rather than the ambient component of personal exposure (Section 2.6.5.1). In some cases, personal exposure measures are comparable to corresponding central site monitor concentrations. In other cases, the two exposure metrics vary considerably. An analysis combining several studies found personal and ambient NO_2 concentrations to have low to moderate correlation from 0.16 to 0.45 (Table 2-11). Thus, there is heterogeneity among individuals in how well the spatial or temporal variability in ambient concentrations obtained from central monitors correlate with personal exposure, taking into account varying time-activity patterns. A recent meta-analysis found that personal-ambient NO_2 relationships may be influenced by season, age, and local sources (Section 2.6.5.2). Higher personal-ambient correlations are reported for adults than for children.

Several studies have aimed to characterize the exposure error that is due to the spatial or temporal variability in ambient NO_2 concentrations. A study of cardiovascular-related emergency department (ED) visits added an error term to a time-series health effects model that accounted for correlations of concentrations across monitor sites and found that associations with NO_2 were attenuated toward the null. Some studies estimated larger respiratory effects in association with more spatially-resolved estimates of short-term NO_2 exposure than with NO_2 concentrations obtained from a single city central site monitor or averaged over area monitors (Sections 2.6.5.3 and 4.2.4.5). Exposure estimates with higher spatial resolution included personal NO_2 measures, outdoor school measurements, and ambient concentrations at the nearest central site monitor. Health effects also are associated with measures of long-term NO_2 or NO_X exposure that are more spatially resolved than central site concentrations. For example, LUR or dispersion models and spatial interpolation methods have been used to estimate ambient NO_2 concentrations at the neighborhood scale or at the level of an individual's residence.

Measurement error is a component of each NO_2 exposure assessment method. Error can depend on the accuracy and precision of the instrumentation. For example, the chemiluminescence method used by regulatory networks 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 and near sources, and new measurement methods are being developed (Section 2.4.1). Spatially-resolved estimates of NO_2 exposure may include measurement error because they do not incorporate

information on exposure in the range of potentially important microenvironments. Modeled residential ambient NO_2 may account better for differences among individuals in distance to sources but may estimate total ambient exposure with error because they may not account for time-activity patterns (i.e., movement of individuals across areas). On the other hand, concentrations averaged across multiple monitors may represent the mean ambient exposures of the population moving within the area during a given time period especially if there are few or well-dispersed local sources of NO_2.

Exposure measurement error can have important implications for the relationships observed between NO_2 concentrations and health effects. Study design can influence the nature of the effect of exposure measurement error (Section 2.6.5.3). Repeated measures time-series or panel studies examine relationships between temporal patterns in exposures and outcomes. If the temporal variation in ambient NO_2 concentrations is the same as the temporal variation in true ambient exposure, there may be little effect on associations with health. But, differences in the correlation across people and time, for example due to varying nonambient exposures, may decrease the magnitude or precision of associations. Cohort or cross-sectional studies tend to compare exposures and outcomes among people who live in different locations. In these studies, estimating exposure with ambient concentrations from central site monitors can decrease the magnitude and/or precision of effect estimates if the monitor is not located close to the study population and/or if local emission sources are not well dispersed. The impact on epidemiologic associations of exposure measurement error resulting from the use of central site ambient NO_2 concentrations to represent near-road exposures is not well characterized. However, the contribution of near-road exposure to total ambient NO_2 exposure or to the health effects found in association with ambient NO_2 concentrations is not well characterized either.

1.4 Health Effects of Oxides of Nitrogen

This ISA evaluates relationships between a broad range of health effects and short-term (Chapter 4) and long-term (Chapter 5) exposures to oxides of nitrogen 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. Most of these studies examine effects related to exposures in the range of 1 hour to 1 week; a few controlled human exposure and animal toxicological studies examine exposures of less than 1 hour. Long-term exposures are defined as those with durations of more than 1 month to years, with 4 weeks used as the cut-off in most toxicological studies. The evidence informing the causal determinations is described in detail in Chapter 4 and Chapter 5, and is summarized in this section and in Table 1-1. Across disciplines, studies

examined health effects primarily in relation to NO_2; information is limited for NO or NO_X. Thus, causal determinations are formed only for NO_2.

An uncertainty noted in the 2008 ISA for Oxides of Nitrogen for the relationships between NO_2 exposure and health effects was the potential for the effects observed with NO_2 to be biased (i.e., confounded) by the effects of another traffic-related pollutant or mixture that is highly correlated with NO_2 concentrations. Section 1.5 presents an integrated evaluation of confounding, and the discussions of the health effects evidence in the sections that follow also describe the extent to which recent studies inform this uncertainty. The copollutants most frequently examined include PM with aerodynamic diameter less than or equal to a nominal 2.5 µm ($PM_{2.5}$), PM with aerodynamic diameter less than or equal to a nominal 10 µm (PM_{10}), SO_2, and O_3. Examination of potential confounding by VOCs, carbon monoxide (CO), black or elemental carbon (BC or EC), and ultrafine particles (UFPs) is more limited and varies across health effects.

1.4.1 Dosimetry and Modes of Action Informing Respiratory Effects

Linking NO_2, NO, or NO_X exposure to the occurrence of health effects are the dosimetry (Section 3.2) and modes of action (Section 3.3) of these pollutants. Relevant to all health effects, inhaled NO_2 at ambient-relevant concentrations is unlikely to penetrate the extracellular lining fluid (ELF) to reach underlying sites in the respiratory tract epithelium. During absorption into the ELF, NO_2 becomes a solute and rapidly reacts with antioxidants and other ELF constituents (Section 3.2.2). The formation of secondary oxidation products likely is the initiating event in the sequence of key events comprising the mode of action for NO_2. These products can mediate oxidation of cell membrane lipids which may lead to alterations in permeability of the alveolar capillary barrier. They also can mediate thiol oxidation which can alter enzyme activity and the antioxidant-oxidant balance. Subsequent responses at the cellular, tissue, or organ level likely lead to the health effects associated with NO_2 exposure. Key events that pertain to specific health effects are described in the sections that follow. NO_2 is produced endogenously by enzymatic and nonenzymatic pathways that are enhanced during inflammation and other immune responses. It is not clear how ambient-relevant NO_2 exposures compare with endogenous concentrations or rate of production (Section 3.2.2.4).

Although not as extensively examined as NO_2, ambient NO or NO_X exposures also are found to be associated with health effects. Inhaled NO is present in the respiratory tract in the gas phase and is not transformed by reactions in the ELF. Ambient NO concentrations generally are in the range of endogenous NO concentrations found in the respiratory tract. Thus, it is not clear whether inhaled NO at ambient-relevant concentrations significantly

affects the absorption, metabolism, or downstream biological processes of endogenous NO (Section 3.2.3). Because there is biological plausibility for negative respiratory effects occurring with ambient-relevant NO_2 exposures but not with ambient-relevant NO exposures, respiratory effects associated with ambient NO_X are considered to be reflecting associations with NO_2 and are considered in causal determinations for NO_2.

1.4.2 Respiratory Effects

The strongest evidence for a relationship between NO_2 exposure and respiratory effects is for asthma exacerbations in children and adults related to short-term exposure and for asthma development in children related to long-term exposure. Biological plausibility is provided by findings for NO_2-induced increases in airway responsiveness and effects on other key events informing the mode of action such as inflammation and oxidative stress. NO_2 showed effects on many of the same endpoints in humans and experimental animals, indicating similar biological processes across species. The respiratory effects of NO_2 exposure also are supported by the characterization of the uptake of inhaled NO_2 in the respiratory tract and reactions to form secondary oxidation products (Section 1.4.1).

This insight into potential biological processes by which the inhalation of NO_2 may lead to asthma exacerbations and asthma development supports the causal determinations made for relationships of respiratory effects with both short-term and long-term NO_2 exposure (Table 1-1). Each of the causal determinations is strengthened from the 2008 ISA for Oxides of Nitrogen based on evidence from recent epidemiologic studies that reduces previously identified uncertainties. For short-term exposure, the causal determination is strengthened from likely to be a causal relationship to a causal relationship because recent epidemiologic studies demonstrate that associations of ambient NO_2 concentrations with asthma and other respiratory effects remain positive with adjustment for various copollutants. For long-term exposure, the causal determination is strengthened from suggestive of a causal relationship to likely to be a causal relationship based on recent epidemiologic evidence consistently demonstrating NO_2-related increases in asthma incidence in children. Previous studies did not consistently find NO_2-related increases in asthma incidence in children. A difference in the evidence bases for respiratory effects related to short-term and long-term NO_2 exposure is the extent to which evidence supports the independent effects of NO_2, including findings from experimental studies and findings from epidemiologic studies that examine associations with NO_2 in models with a copollutant.

Respiratory Effects Associated with Short-term NO_2 Exposure

The strongest evidence in support of a causal relationship between short-term NO_2 exposure and respiratory effects comprises results across scientific disciplines indicating effects of NO_2 exposure on asthma exacerbations (Section 4.2.9, Table 4-23). Recent epidemiologic studies in diverse geographic locations and of varied study designs expand evidence for NO_2-related increases in hospital admissions and ED visits for asthma as well as increases in respiratory symptoms in children with asthma. As uncontrolled symptoms are a major reason for seeking medical treatment for asthma, the coherence of findings for these outcomes further supports a relationship between NO_2 exposure and respiratory effects. These studies are considered to be high quality based on analyses that apply a consistent statistical model to data pooled across multiple cities and across several years, thus minimizing the potential for publication bias. Several panel studies finding NO_2-associated respiratory effects in children with asthma are noteworthy for spatially-resolved estimates of exposure, including personal exposure, modeled outdoor concentrations, and outdoor school concentrations. Both time-series and panel studies adjust for potential confounding by temporal factors such as meteorology and long-term time trends. Recent studies of hospital admissions, ED visits, and respiratory symptoms reduce previous uncertainty regarding copollutant confounding with additional findings that associations of NO_2 remain positive with adjustment for a copollutant among $PM_{2.5}$, PM_{10}, SO_2, O_3, or as examined in fewer studies, CO, EC, BC, or UFP. These copollutant-adjusted associations were found across geographic locations that vary from each other in correlations between NO_2 and copollutants, pointing to varied air pollution mixtures. Associations are found in studies with mean 24-h avg NO_2 concentrations 18 to 29 ppb and maximum concentrations 48 to 106 ppb. Associations are found in studies with mean 1-h max NO_2 concentrations 22to 66 ppb and maximum concentrations 59 to 298 ppb.

Biological plausibility for the independent effects of NO_2 exposure on asthma exacerbations is demonstrated by findings from previous controlled human exposure studies that NO_2 exposures of 200 to 300 ppb for 30 minutes, and 100 ppb for 1 hour induced increases in airway responsiveness in adults with asthma. Airway hyper-responsiveness is a key pathophysiological characteristic of asthma and can lead to respiratory symptoms and asthma exacerbations. Studies across disciplines also characterized key events informing the modes of action for airway responsiveness and respiratory symptoms, including allergic inflammation and oxidative stress. Some but not all controlled human exposure and animal studies found NO_2-induced oxidative stress, and results are inconsistent for the effects of ambient-relevant NO_2 exposures on lung permeability. However, in experimental studies of both humans and rats, NO_2 induced increases in indicators of allergic inflammation such as T-derived lymphocyte helper

(Th)2 cytokines, immunoglobulin E, activated eosinophils, and neutrophils. Epidemiologic studies found ambient NO_2-related increases in pulmonary inflammation in children with asthma, and several study populations had high prevalence of allergy. These epidemiologic associations were found in several studies characterized as having strong exposure assessment with school or personal monitoring or time-weighted estimates of outdoor exposure. The observations of NO_2-related increases in allergic inflammation support the findings of NO_2-induced increases in airway responsiveness in adults with asthma and increases in respiratory symptoms found in association with ambient NO_2 in epidemiologic studies of children with asthma and allergy.

NO_2-related decreases in lung function were not found consistently in adults with asthma in controlled human exposure studies but were found in recent epidemiologic studies of children with asthma. Several of the study populations of children with asthma had high prevalence of allergy. The epidemiologic findings are supported by observations that NO_2-induced lung function decrements may be mediated by increases in mast cell degranulation and airway obstruction. The cascade of events leading from NO_2-related increases in allergic inflammation and airway obstruction to decreases in lung function may provide an additional explanation for NO_2-related increases in respiratory symptoms and hospital admissions and ED visits for asthma.

A causal relationship between short-term NO_2 exposure and respiratory effects is supported by evidence for other specific respiratory effects such as impaired host defense and exacerbations of chronic obstructive pulmonary disease (COPD) as well as hospital admissions, ED visits, and mortality for all respiratory causes combined. There is clear evidence for impaired host defense demonstrated by NO_2-induced (1,500 to 5,000 ppb for 1 to 8 hours) mortality in animal models following bacterial or viral infection, and support from associations observed between ambient NO_2 concentrations and respiratory infections in children. Some experimental evidence describes effects of NO_2 exposure on key events informing the mode of action, including NO_2-induced decreases in alveolar macrophage function and increases in pulmonary inflammation. Effects on pulmonary clearance were more variable. Evidence also supports associations of ambient NO_2 concentrations with COPD hospital admissions and ED visits. However, NO_2 was not consistently associated with increases in respiratory symptoms or decreases in lung function in epidemiologic or controlled human exposure studies of adults with COPD. Recent epidemiologic studies consistently indicate ambient NO_2-associated increases in respiratory mortality, which demonstrates the effects of NO_2 exposure on a continuum of respiratory effects. However, it is not entirely clear what changes in respiratory morbidity NO_2 exposure may induce to lead to increases in respiratory mortality. Strong evidence demonstrates NO_2-related effects on asthma exacerbations, but asthma is not a leading cause of mortality. There is limited coherence among lines of evidence indicating

NO_2-related effects on COPD exacerbations and respiratory infections, which are larger causes of mortality.

In conclusion, evidence indicates that there is a causal relationship between short-term NO_2 exposure and respiratory effects, based strongly on the consistency, coherence, and biological plausibility of findings for the effects on exacerbations of asthma. There is some evidence for relationships of NO_2 exposure with impaired host defense, COPD exacerbations, and respiratory mortality but limited coherence among outcomes or disciplines. Previous uncertainty regarding copollutant confounding is reduced with additional recent epidemiologic results showing that associations between ambient NO_2 concentrations and respiratory effects remain positive in copollutant models with $PM_{2.5}$, PM_{10}, SO_2, O_3, or as examined in fewer studies, BC, EC, UFP, or CO. Copollutant models have limitations (Section 1.5) and were not analyzed for every potentially correlated copollutant or study. Therefore, evidence for NO_2-induced increases in airway responsiveness in adults with asthma from controlled human exposure studies is key in providing biological plausibility for effects on asthma exacerbations. Further, evidence for NO_2-related oxidative stress and inflammation (including allergic inflammation) describes other biological processes informing the modes of action for exacerbations of asthma. Thus, the epidemiologic and experimental evidence together demonstrate the independent respiratory effects of short-term NO_2 exposure and together are the basis of concluding a causal relationship.

Respiratory Effects Associated with Long-term NO_2 Exposure

The strongest evidence indicating that there is likely to be a causal relationship between long-term NO_2 exposure and respiratory effects comprises the associations consistently found between ambient NO_2 concentrations and asthma incidence in children in diverse geographical locations (Section 5.2.17, Table 5-9). This recent evidence is provided by several high-quality single- and multi-city studies characterized by prospective follow-up of children, in several cases from birth to ages 8-12 years. Associations were found with adjustment for potential confounding by socioeconomic status (SES), smoking exposure, housing characteristics, and gas stove use. Associations were found with NO_2 obtained from central site monitors and more spatially-resolved residential outdoor NO_2 estimated with LUR or dispersion models. These models predicted ambient concentrations that correlated well with measured concentrations (R^2 = 0.42 to 0.69). Asthma incidence was associated with the average NO_2 from the first year of life and NO_2 averaged over multiple years (study mean concentrations: 14 to 21 ppb). A relationship between long-term NO_2 exposure and asthma is supported by evidence for respiratory effects associated with short-term exposure. Several epidemiologic found increases in respiratory symptoms and pulmonary inflammation in children in the general population associated

with short-term increases in ambient NO_2 concentrations. Recurrent episodes of inflammation and respiratory symptoms are diagnostic indicators of asthma. An effect on asthma incidence also is supported by evidence from prospective studies showing increases in respiratory symptoms in children with asthma in association with long-term NO_2 exposure.

There is limited biological plausibility for NO_2-related increases in asthma incidence, provided by the small body of results showing increased airway responsiveness in guinea pigs in response to short- and long-term NO_2 exposure (1,000 to 4,000 ppb). NO_2 exposure (2,000 to 4,000 ppb) also induced Th2 immune responses with short-term exposure in a controlled human exposure study of healthy adults and with short- and long-term NO_2 exposure in guinea pigs. Enhanced Th2 immune responses can contribute to the development of asthma. This evidence also provides biological plausibility for the NO_2-associated increases in allergic sensitization found in a few prospective studies of children. Results from a few toxicological studies also describe other key events that inform modes of action for NO_2-related asthma development. NO_2 induced airway responsiveness with increased airway resistance suggesting the involvement of airway obstruction and airway remodeling. A few animal studies found increased oxidative stress and inflammation with long-term NO_2 exposure but not consistently across studies.

Continued evidence in children for decreases in lung function and partially irreversible decreases in lung function growth with long-term NO_2 exposure also supports a likely to be a causal relationship. Single- and multi-city studies found associations with 6-month and annual average NO_2 before lung function measurement, the average NO_2 in the first year of life, and 10-year lifetime average NO_2 (study mean concentrations: 14 to 34 ppb). Changes in lung function may represent the effect of short-term exposure occurring at the time of measurement. Some studies reduced the potential for bias from the effects of short-term exposure with observations that long-term NO_2-associated decreases in lung function persisted with adjustment for short-term NO_2 exposure. Limited biological plausibility for decreases in lung function resulting from long-term NO_2 exposure is provided by findings for increases in airway responsiveness in guinea pigs induced by short- and long-term NO_2 exposure (1,000 to 4,000 ppb). NO_2 exposure did not alter lung function in experimental animals, and the morphological effects induced by NO_2 exposure in adult animals such as hyperproliferation of lung epithelial cells and fibrosis are not directly related to the effects on lung function described in studies of children.

Recent epidemiologic studies also provide new evidence for associations between long-term NO_2 exposure and respiratory effects in adults, with the most consistent findings for asthma. Associations are less consistent for hospital admissions for COPD or asthma, and studies did not adjust for effects of short-term exposure. Results also are inconsistent for

respiratory mortality associated with long-term NO_2 exposure. A recent case-control study found higher NO_2 exposures among adults with hospital admissions or ED visits for pneumonia, but the most robust evidence for impaired host defense consists of the findings in experimental animals for NO_2-induced increases in mortality following bacterial or viral infection and changes in alveolar macrophage function.

For any given respiratory effect, copollutant-adjusted results are available for one or two epidemiologic studies. These studies of lung function, respiratory symptoms, and asthma generally found associations with long-term NO_2 exposure to change little when adjusted for copollutants such as O_3, SO_2, PM_{10}, $PM_{2.5}$, or EC. However, because of the limited examination of copollutant confounding and the limited biological plausibility, there remains some uncertainty regarding the independent effects of long-term NO_2 exposure.

In conclusion, evidence indicates that there is likely to be a causal relationship between long-term NO_2 exposure and respiratory effects based primarily on recent epidemiologic findings in children for increases in asthma incidence and collective results for decreases in lung function and partially irreversible decreases in lung function growth. Supporting evidence includes NO_2-related increases in respiratory symptoms in children with asthma, allergic sensitization in children, asthma in adults, and impaired host defense in animal models. NO_2 associations with respiratory effects remain positive in copollutant models, and findings for NO_2-induced airway responsiveness and development of Th2 immune responses in experimental studies provide biological plausibility. However, the limited nature of such evidence does not conclusively demonstrate that respiratory effects of long-term NO_2 exposure are independent of other traffic-related pollutants.

1.4.3 Dosimetry and Modes of Action Informing Extrapulmonary Effects

Health effects in various organ systems have been found in relation with NO_2, NO, and NO_X exposure in epidemiologic studies and NO_2 exposure in experimental studies. However, in contrast with the respiratory tract, there is weak characterization of how ambient-relevant exposures to NO_2 or NO affect processes that may underlie the health effects observed beyond the respiratory system (Section 3.2.2). As described in Section 1.4.1, NO_2 is transformed by reactions in the ELF. A major reaction product, nitrite, can gain access to the blood, where it can react with red blood cell hemoglobin to form nitrosylhemoglobin, methemoglobin, and nitrate. Some of these reaction products of NO_2 have been found in the blood of experimental animals but with higher than ambient-relevant NO_2 exposure concentrations (Section 3.2.2.4). Further, nitrite has not been shown to have negative health effects. Another process that could mediate extrapulmonary effects of inhaled NO_2 is the spillover of mediators from the respiratory

tract into the blood. For example, a recent controlled human exposure study found an increase in a vasoactive mediator in the plasma of NO_2-exposed adults (Section 3.3.5).

Inhaled NO can diffuse across the alveolar capillary barrier into the blood and bind with hemoglobin in red blood cells to form nitrosylhemoglobin, methemoglobin, and nitrate (Section 1.4.1). Methemoglobin has been linked with health effects, but increases in blood levels of methemoglobin and nitrosylhemoglobin are not consistently found with inhalation of ambient-relevant concentrations of NO (Section 3.3.3). NO is produced endogenously from nitrates and nitrites derived from diet and enzymatic pathways that are enhanced during inflammation. Endogenous NO can affect diverse physiological processes through interactions with heme proteins, other transition metal-containing proteins, and radical species. However, it is not clear if ambient-relevant concentrations of inhaled NO alter physiological processes that are affected by endogenous NO, in part, because endogenous concentrations of NO in the respiratory tract are similar to those found in ambient air. Because there is at least some biological plausibility for negative effects resulting from ambient-relevant NO_2 exposure but not from ambient-relevant NO exposure, extrapulmonary health effects associated with ambient NO_X are considered to be reflecting associations with NO_2 and are considered in causal determinations for NO_2.

1.4.4 Cardiovascular Effects

Although the biological processes mediating the extrapulmonary effects of ambient-relevant NO_2 exposures are not well understood (Section 1.4.3), other lines of evidence support relationships between both short-term and long-term NO_2 exposure and cardiovascular effects. Results from recent epidemiologic studies are the primary basis for strengthening the causal determinations from the 2008 ISA for Oxides of Nitrogen (Table 1-1). For short-term exposure, the causal determination is strengthened from inadequate to infer a causal relationship to likely to be a causal relationship because recent epidemiologic studies consistently find cardiovascular hospital admissions and mortality and changes in measures of cardiovascular physiology in association with ambient NO_2 concentrations and reduce previous uncertainty about copollutant confounding. For long-term exposure, the conclusion is strengthened from inadequate to infer a causal relationship to suggestive of a causal relationship because of associations with heart failure, myocardial infarction, stroke, and cardiovascular mortality found in some recent epidemiologic studies. The single previous long-term exposure study did not find NO_2-associated increases in cardiovascular events. A common uncertainty in the relationships with cardiovascular effects is the limited biological plausibility, including the effects of inhaled NO_2 on changes in mediators in the blood and subsequent events that inform the modes of action for cardiovascular effects. Epidemiologic evidence is

more consistent for effects associated with short-term NO_2 exposure than with long-term exposure, and more copollutant-adjusted results indicate independent NO_2 associations.

Cardiovascular Effects Associated with Short-term NO_2 Exposure

Evidence indicates that there is likely to be a causal relationship between short-term NO_2 exposure and cardiovascular effects based strongly on recent epidemiologic studies consistently indicating associations of NO_2 with increases in cardiovascular hospital admissions and mortality in diverse geographic locations (Section 4.3.9, Table 4-36). The evidence for cardiovascular hospital admissions and mortality is substantiated by the associations found in high-quality studies that were conducted over several years and adjusted for potential confounding by weather and long-term time trends. Particularly for cardiovascular-related mortality, but also for hospital admissions, there are multicity studies demonstrating the robustness of association with short-term increases in ambient NO_2 in data pooled across cities. Recent studies of cardiovascular hospital admissions and mortality reduce an important uncertainty identified in the 2008 ISA regarding the potential for copollutant confounding. Several studies showed that associations with NO_2 remain positive with adjustment for copollutants such as PM_{10}, SO_2, O_3, or in some but not all locations, $PM_{2.5}$ or CO. In studies of cardiovascular hospital admissions and mortality, city-specific mean 24-h avg NO_2 were 12 to 41 ppb, 90th percentiles were 22 to 100 ppb, and maximum concentrations were 19 to 132 ppb. For 1-h max NO_2, overall study mean concentrations were 43 and 46 ppb, and 90th percentiles were 66 and 68 ppb.

The coherence between evidence for increases in cardiovascular mortality and hospital admissions further supports a relationship between cardiovascular effects and short-term NO_2 exposure. The strongest evidence for cardiovascular hospital admissions is for ischemic heart disease (IHD), the leading cause of death in the world (Finegold et al., 2013). Limited biological plausibility is provided by results showing that NO_2 exposure is related to changes in cardiovascular physiology that may lead to cardiovascular hospital admissions or mortality. Decreases in heart rate variability (HRV) have been linked with premature mortality and are found in association with increases in ambient NO_2 in recent epidemiologic studies of adults with cardiovascular disease and in a recent controlled human exposure study of healthy adults. Consistent with hospital admissions for IHD, a few recent epidemiologic studies in adults with coronary artery disease found NO_2-associated changes in ventricular repolarization, which are markers of myocardial ischemia. However, neither epidemiologic nor controlled human exposure studies consistently demonstrate effects on cerebrovascular diseases, arrhythmia, or blood pressure. Some experimental and epidemiologic studies indicate NO_2-related increases in inflammation and oxidative stress. Different mediators were examined in humans and rodents, but these results indicate effects on other key events that inform the modes of

action for IHD and cardiovascular mortality. However, because cardiovascular effects are not consistently demonstrated in experimental studies, the available evidence does not conclusively demonstrate the independent cardiovascular effects of NO_2 exposure.

In conclusion, evidence indicates that there is likely to be a causal relationship between short-term NO_2 exposure and cardiovascular effects, based strongly on epidemiologic evidence from single- and multi-city studies for NO_2-related increases in cardiovascular hospital admissions, particularly for IHD, and mortality. Previous uncertainty regarding copollutant confounding is reduced by recent results showing that NO_2-associated increases in cardiovascular hospital admissions and mortality remain positive with adjustment for PM_{10}, SO_2, O_3, or in some but not all locations, $PM_{2.5}$ or CO. Biological plausibility is provided by some findings for NO_2-associated changes in ventricular repolarization and HRV. However, because experimental evidence is inconsistent and epidemiologic studies did not clearly exclude confounding by the array of potentially correlated traffic-related copollutants, some uncertainty remains as to whether the cardiovascular effects of NO_2 exposure are independent of other traffic-related pollutants.

Cardiovascular Effects Associated with Long-term NO_2 Exposure

Evidence is suggestive of a causal relationship between long-term NO_2 exposure and cardiovascular effects based on some recent epidemiologic studies indicating associations of NO_2 or NO_X with myocardial infarction, heart failure, and stroke but other studies showing no associations (Section 5.3.6, Table 5-12). The recent epidemiologic studies contributing to the evidence base are considered to be high quality based on their large sample sizes, prospective follow up of subjects (up to 9 years), and adjustment for potential confounding by age, sex, SES, cardiovascular disease, and other comorbid factors. Associations were found with ambient NO_2 or NO_X averaged over 1 to 9 years (study mean concentrations: 12 and 34 ppb NO_2 and 96 ppb NO_X). From the small number of studies, it is difficult to assess whether the studies finding no association clearly differed in NO_2 or NO_X concentrations, duration of follow up, or other factors. The cardiovascular morbidity findings have limited support from mortality results. IHD includes myocardial infarction and can lead to heart failure, and some but not all studies found associations between long-term NO_2 exposure and mortality from cardiovascular causes, including IHD. Support is provided by the associations observed between short-term increases in ambient NO_2 concentration and increases in IHD hospital admissions.

In addition to the uncertainty due to inconsistent epidemiologic evidence for cardiovascular effects associated with long-term NO_2 exposure, there is uncertainty in the relationship because of limited biological plausibility. The few available recent epidemiologic studies indicate NO_2-associated increases in arterial stiffness and

decreases in HRV (study mean concentrations: 18 or 23 ppb for annual average), which are related to myocardial infarction and mortality, respectively. Long-term NO_2 exposure of rats induced dyslipidemia, a risk factor for IHD. There is weak evidence describing other key events that inform modes of action for NO_2-related cardiovascular effects. Epidemiologic evidence is inconsistent for associations between long-term NO_2 exposure and increases in inflammation in adults, and short-term NO_2 exposure induced increases in indicators of inflammation and oxidative stress in some but not all experimental studies.

In summary, evidence is suggestive of a causal relationship between long-term NO_2 exposure and cardiovascular effects based on associations between NO_2 or NO_X and myocardial infarction, heart failure, and stroke found in some recent epidemiologic studies but no association found in other studies. There also is uncertainty because there is inconsistent evidence across disciplines for effects on an array of key events that inform modes of action for cardiovascular effects to provide biological plausibility.

1.4.5 Total Mortality

The causal determinations for relationships of total mortality with both short-term and long-term NO_2 exposure are strengthened from the 2008 ISA based on evidence from recent epidemiologic studies. Conclusions are drawn by evaluating mortality from all nonaccidental causes. For short-term exposure, the conclusion for total mortality is strengthened from suggestive of a causal relationship to likely to be a causal relationship because recent high-quality epidemiologic studies add evidence for associations with NO_2 and reduce previous uncertainty regarding copollutant confounding (Table 1-1). For long-term exposure, the conclusion is strengthened from inadequate to infer a causal relationship to suggestive of a causal relationship because some recent high-quality studies reported associations with NO_2 or NO_X, whereas the previous evidence was more limited and inconsistent. A common uncertainty in the relationships for short-term and long-term NO_2 exposure is the limited biological plausibility, which is informed by the extent to which evidence indicates effects on a spectrum of cardiovascular and respiratory morbidity and mortality outcomes. The limited coherence of findings across scientific disciplines and across the array of respiratory (Section 1.4.2) and cardiovascular (Section 1.4.4) morbidity and mortality effects produces some uncertainty as to what spectrum of effects NO_2 exposure may induce to lead to increases in mortality. Evidence is more consistent for total mortality associated with short-term NO_2 exposure than with long-term exposure, and more results indicate NO_2 associations with copollutant adjustment.

Total Mortality Associated with Short-term NO_2 Exposure

Evidence indicates that there is likely to be a causal relationship between short-term NO_2 exposure and total mortality based on consistent evidence from several recent high-quality epidemiologic studies conducted in diverse geographic locations (Section 4.4.8, Table 4-41). These studies are considered to be high quality based on multicity analyses that apply a consistent statistical model to data pooled across cities, thus minimizing the potential for publication bias. These studies also adequately adjust for potential confounding by weather and long-term time trends. Individual cities had mean 24-h avg NO_2 concentrations 9.2 to 55 ppb and maximum concentrations 55 to 161 ppb. City-specific 1-h max NO_2 mean concentrations were 16 to 81 ppb, 90th percentile concentrations were 33 to 133 ppb, and maximum concentrations were 55 to 161 ppb. Recent studies reduce the previously identified uncertainty regarding copollutant confounding by showing that NO_2-related mortality effect estimates are similar with adjustment for PM_{10}, SO_2, or O_3. A relationship between short-term NO_2 exposure and total mortality also is supported by the consistent evidence for NO_2-associated increases in hospital admissions for cardiovascular diseases, which are the leading cause of deaths in the U.S. (35% as cited in Hoyert and Xu, 2012). However, evidence is inconsistent for NO_2-related changes in measures of cardiovascular physiology such as HRV, arrhythmia, and blood pressure (Section 1.4.4). Respiratory causes comprise a smaller fraction of mortality (9%); however, COPD and respiratory infections are among the leading causes of mortality in the world. There is limited coherence among lines of evidence indicating NO_2-related effects on COPD and respiratory infection (Section 1.4.2). Strong evidence demonstrates NO_2-related exacerbations of asthma, but asthma is not a leading cause of mortality. Thus, is not entirely clear what spectrum of cardiovascular and respiratory effects NO_2 exposure may induce to lead to mortality.

In summary, evidence indicates that there is likely to be a causal relationship between short-term NO_2 exposure and total mortality based on consistent evidence of association in previous and recent multicity studies. Results show robust NO_2 associations with adjustment for PM_{10}, SO_2, or O_3. Because the available evidence does not conclusively identify an independent effect of NO_2 from those of other measured or unmeasured traffic-related pollutants or clearly characterize the biological processes by which NO_2 exposure may lead to total mortality, there remains some uncertainty in the relationship between short-term NO_2 exposure and total mortality.

Total Mortality Associated with Long-term NO_2 Exposure

Evidence is suggestive of a causal relationship between long-term NO_2 exposure and total mortality based on some high-quality recent studies in the U.S. and Europe showing

associations with NO_2 or NO_X but other high-quality studies showing no association (Section 5.5). Studies are considered to be high quality based on large sample sizes; long periods of follow-up up to 26 years; and adjustment for potential confounding by age, sex, smoking, education, comorbid factors, and in some cases, community-level characteristics. Results are inconsistent between the Harvard Six Cities and the American Cancer Society cohorts, both of which are considered to be seminal air pollution studies of multiple U.S. cities. Increases in total mortality were 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. Study mean ambient concentrations were 14 to 34 ppb. There is no clear indication that the mean ambient NO_2 concentrations or exposure period examined differed in the studies finding no association. As in the 2008 ISA, associations were found with copollutants such as $PM_{2.5}$, and copollutant models generally were not analyzed. However, some recent studies found associations with NO_2 with adjustment for traffic, indicating that NO_2 was not only serving as an indicator of traffic. Similar to mortality related to short-term NO_2 exposure, there is limited evidence to explain the biological processes by which long-term NO_2 exposure may lead to mortality. As described in Section 1.4.4, there is inconsistent epidemiologic evidence for associations between long-term NO_2 or NO_X exposure and cardiovascular morbidity and limited biological plausibility. There is limited available evidence for associations between long-term NO_2 exposure and increases in asthma and hospital admissions for COPD in adults to support associations found between long-term exposure and total mortality.

In summary, evidence is suggestive of a causal relationship between long-term NO_2 exposure and total mortality based on associations found with ambient NO_2 or NO_X concentrations in some previous and recent high-quality studies but not in other studies of comparable quality. The limited coherence of findings across a spectrum of cardiovascular and respiratory morbidity outcomes also produces uncertainty regarding the biological processes by which long-term NO_2 exposure may lead to mortality.

1.4.6 Reproductive and Developmental Effects

The 2008 ISA for Oxides of Nitrogen formed a single causal determination for the heterogeneous group of reproductive and developmental effects. The current draft ISA presents separate conclusions for more defined subcategories of outcomes that are likely to occur by different biological processes and exposure patterns over different lifestages: (1) fertility, reproduction, and pregnancy (Section 5.4.2); (2) birth outcomes (Section 5.4.3); and (3) postnatal development (Section 5.4.4). The causal determination is strengthened from inadequate to infer a causal relationship for the broad category to

suggestive of a causal relationship with long-term NO_2 exposure for each of the three subcategories, based on some high-quality studies finding associations with monitored or modeled ambient NO_2 or NO_X concentrations (Table 1-1). Previous epidemiologic evidence was limited and inconsistent for effects on birth outcomes and there was weak evidence in experimental animals to provide biological plausibility. This weak biological plausibility remains an uncertainty for all three subcategories of reproductive and developmental effects, as there are no recent animal toxicological studies to consider. Another uncertainty that pertains to all three subcategories is the lack of association found in some recent high-quality epidemiologic studies.

Fertility, Reproduction, and Pregnancy

Evidence is suggestive of a causal relationship between long-term NO_2 exposure and effects on fertility, reproduction, and pregnancy based primarily on limited but consistent epidemiologic evidence for associations of pregnancy NO_2 or NO_X exposure with pre-eclampsia (Section 5.4.5, Table 5-15), a pregnancy complication related to hypertension and protein in the urine of pregnant women. Single- and multi-city studies found pre-eclampsia in association with NO_2 (mean 23 ppb for entire pregnancy) and NO_X (means: 7.2 for entire pregnancy, 7.5 ppb for 3rd trimester) modeled for residential locations and with adjustment for potential confounding by maternal age, smoking, SES, diabetes, and parity. There are no toxicological studies on effects related to pre-eclampsia to inform biological plausibility, and epidemiologic evidence for pregnancy-induced hypertension is inconsistent. Reduced fertility was found in association with short- and long-term NO_2 exposure in a recent epidemiologic study of women undergoing in vitro fertilization, but a study in rats found no effect on fertility. Epidemiologic evidence does not consistently indicate associations with gestational diabetes or reduced placental growth and function, and epidemiologic and toxicological evidence does not indicate effects on sperm quality.

Birth Outcomes

Evidence is suggestive of a causal relationship between NO_2 exposure and effects on birth outcomes because while epidemiologic studies found associations of higher prenatal NO_2 exposure with fetal growth restriction, associations with other outcomes are not consistent (Section 5.4.5, Table 5-15). The studies of fetal growth restriction are considered to be high quality because they adjust for potential confounding by maternal age, SES, smoking, alcohol use, and season of conception and assess fetal growth restriction with fetal or neonatal physical measurements. Recent epidemiologic studies found fetal growth restriction in association with ambient residential NO_2 (means: 7.8 to

21 ppb) estimated from LUR or dispersion models. Associations were stronger among children with mothers who spent more time at home and less outdoor time in locations other than home. Consideration of time activity patterns may have improved the relationship of modeled exposure estimates to personal exposures. Associations were found with early pregnancy, 3rd trimester, and entire pregnancy NO_2 exposure, without a clear indication of risk differing among exposure periods. Birth weight was inconsistently associated with NO_2 exposure estimated using LUR and central site concentrations, but there is limited biological plausibility provided by findings of decreased birth weight in a study of rats. Effects on other key events informing modes of action are unclear. Prenatal ambient NO_2 exposure was associated with an indicator of inflammation in human cord blood, but its role in influencing birth outcomes is not clear. Epidemiologic evidence for effects on other birth outcomes such as preterm birth and birth defects is inconsistent.

Postnatal Development

The evidence is suggestive of a causal relationship between NO_2 exposure and effects on postnatal development based mostly on previous and recent epidemiologic associations observed between NO_2 exposure (means: 34 ppb for 6-mo avg, 14 to 21 ppb for annual avg) and partially irreversible decreases in lung function growth in children (Section 5.4.5, Table 5-15). Studies adjusted for potential confounding by age, body mass index, and smoking exposure, but SES was not examined. Copollutants also were associated with decreases in lung function growth, and few studies adjusted for copollutants. Because the changes in lung morphology induced by NO_2 exposure in experimental animals do not inform the changes observed in children, uncertainty remains regarding the independent effect of NO_2 exposure. Another line of evidence contributing to the causal determination consists of decreases in cognitive function in children found in association with prenatal (mean: 16 ppb) or concurrent outdoor school annual average (mean: 17 ppb) NO_2 in some recent epidemiologic studies. Other studies with comparable mean NO_2 concentrations did not find NO_2-associated decreases in cognitive function, and evidence was absent or inconsistent for other neurodevelopmental effects such as attention, motor function, and psychological distress. Associations with decreases in cognitive function were found with adjustment for SES, and in one study, noise. However, potential confounding by copollutants including lead or PM was not examined. Results from animal toxicological studies do not clearly indicate the effects of NO_2 exposure on neurodevelopmental outcomes, and it is not clear how comparable the endpoints examined in children and rodents are. Effects of prenatal or infancy NO_2 exposure on other postnatal development effects also are variable. Both epidemiologic and toxicological evidence for infant mortality is inconsistent. Physical development was

not affected by NO_2 exposure in all animal toxicological studies and not examined in epidemiologic studies.

1.4.7 Cancer

For cancer, the causal determination is strengthened from inadequate to infer a causal relationship in the 2008 ISA for Oxides of Nitrogen to suggestive of a causal relationship with NO_2 exposure in the current draft ISA because among the several recent epidemiologic studies that examined lung cancer incidence or mortality, some high-quality studies found associations with monitored or modeled ambient NO_2 or NO_X concentrations (Table 1-1, Table 5-21, Section 5.6.12). Previous epidemiologic evidence was more limited, and experimental evidence that NO_2 is a complete carcinogen was lacking. There is uncertainty regarding a relationship with NO_2 because some recent high-quality epidemiologic studies did not find associations with lung cancer incidence or mortality. Studies are considered to be high quality based on the large numbers of cancer cases examined, the follow-up of adults over 7-30 years, and adjustment for several potential confounding factors such as SES, smoking, diet, and occupational exposures. Associations were found with a wide range of exposure durations: NO_2 or NO_X averaged over 1 year at the beginning of follow-up to a 30-year average before the outcome (means: 14- to 23 ppb for NO_2 and 11 to 42 $\mu g/m^3$ for NO_X). Models used to estimate exposure were validated to ensure that the data were of sufficient quality. Studies not finding associations did not differ in mean NO_2 or NO_X concentrations, exposure duration examined, or exposure assessment method (central site monitors or modeled estimates of exposure at residences). Several studies finding associations of NO_2 or NO_X with lung cancer incidence or mortality also reported associations with copollutants such as $PM_{2.5}$, PM_{10}, or CO. Another uncertainty in the relationship between NO_2 exposure and cancer is the limited biological plausibility. NO_2 did not independently induce lung tumor formation in various animal models, but 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. A few findings of formation of secondary oxidation products in the respiratory tract (Section 1.4.1) and NO_2-induced increases in hyperplasia of the lung epithelium of rodents describe plausible biological processes mediating NO_2-related lung cancer effects. While NO_2 exposure impaired 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 were not studied.

A few recent epidemiologic studies indicate associations between NO_2 exposure and leukemia, bladder cancer, prostate cancer, and breast cancer. However, there is a lack of biological plausibility for these findings. There are inconsistent findings for NO_2-induced (higher than ambient-relevant concentrations) mutagenicity and carcinogenicity in bone marrow, spermatocytes, and lymphocytes. Further, the effects of inhaled NO_2 on transforming other chemicals in the body into mutagens or carcinogens are found only with higher than ambient-relevant NO_2 exposure concentrations, i.e., 16,500 to 20,000 ppb.

In conclusion, evidence is suggestive of a causal relationship between long-term NO_2 exposure and cancer based primarily on associations between ambient NO_2 or NO_X concentrations and lung cancer incidence and mortality found in some previous and recent high-quality epidemiologic studies but not in other studies of comparable quality. There also is uncertainty in the relationship between NO_2 exposure and lung cancer because biological plausibility is limited. There are findings for lung tumor promotion and hyperplasia of lung epithelial cells with NO_2 exposure, some at higher than ambient-relevant concentrations, but no evidence for direct effects on carcinogenesis

Table 1-1 Key evidence contributing to causal determinations for NO_2 exposure and health effects evaluated in the current draft ISA for Oxides of Nitrogen.

Health Effect Category[a]	Causal Determination[b]	NO_2 Concentrations Associated with Effects[c]
Respiratory Effects	**Short-term Exposure (Section 4.2)** 2008 ISA – Sufficient to Infer a Likely Causal Relationship Current draft ISA – Causal Relationship	
Key Evidence: (Table 4-23)	Strongest evidence is for NO_2-related increases in asthma exacerbations indicated as increases in asthma hospital admissions and ED visits in single- and multi-city studies in diverse populations as well as increases in respiratory symptoms and pulmonary inflammation and decreases in lung function in children with asthma. Associations found with adjustment for weather, time trends and in copollutant models for PM_{10}, $PM_{2.5}$, SO_2, or O_3, or examined in fewer locations, EC, BC, CO, or UFP. Biological plausibility demonstrated by NO_2-induced increases in airway responsiveness of adults with asthma in controlled human exposure studies and effects on other key events informing modes of action including initiation of inflammation, allergic inflammation and oxidative stress. Clear evidence for impaired host defense in experimental animals, with some epidemiologic associations with respiratory infections in children. Some evidence for effects on COPD indicated as NO_2-related increases in hospital admissions and ED visits. Inconsistent findings for respiratory symptoms or decreases in lung function in adults with COPD. Consistent epidemiologic evidence for increases in respiratory symptoms and pulmonary inflammation in children in the general population. Consistent NO_2-related increases in respiratory mortality.	Overall study ambient maximums: 24-h avg: 52 to 80 ppb 1-h max: 59 to 298 ppb 24-h avg personal maximums: 48, 106 ppb Airway responsiveness: 200 to 300 ppb for 30 min, 100 ppb for 1 hour Mortality from infection in animals: 1,500 to 5,000 ppb
Recent studies add:	Additional epidemiologic evidence that associations for NO_2 are independent of many traffic-related copollutants; new epidemiologic evidence for associations with decreases in lung function in children with asthma.	

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

Health Effect Category[a]	Causal Determination[b]	NO_2 Concentrations Associated with Effects[c]
Respiratory Effects	**Long-term Exposure (Section 5.2)** 2008 ISA – Suggestive but not Sufficient to Infer a Causal Relationship Current draft ISA - Likely to be a Causal Relationship	
Key Evidence: (Table 5-9)	Strongest evidence is for associations of ambient NO_2 averaged over 1-3 yr with asthma incidence in several diverse cohorts of children, some followed from birth. Supporting evidence for increases in respiratory symptoms in children with asthma and allergic sensitization in children. Associations found with adjustment for SES, smoking exposure and housing characteristics. Supporting evidence for asthma, respiratory symptoms and COPD hospital admissions in adults. Limited biological plausibility demonstrated by increases in airway responsiveness and Th2 responses in guinea pigs with NO_2 long-term exposure; development of Th2 allergic phenotype with short-term exposure in humans and guinea pigs. Consistent evidence in children for decreases in lung function and partially irreversible decreases in lung function growth. Lung edema, hypertrophy, fibrotic changes found in adult experimental animals not related to changes in children. Clear animal toxicological evidence for impaired host defense. NO_2 associations for lung function, bronchitic symptoms and asthma remain robust in the few copollutant models analyzed with PM_{10}, $PM_{2.5}$, O_3, SO_2, or EC.	Overall study ambient means: Children: 14 to 21 ppb annual avg, 34 ppb 6-mo avg Adults: 8 to 20 ppb annual avg Th2 responses: 2,000 ppb in humans, 4 days and 3,000 ppb in guinea pigs, 2 weeks Airway responsiveness: 1,000 to 4,000 ppb in guinea pigs, 6, 12 weeks Impaired host defense in animals: 500, 5,000 ppb, 200 ppb base + 800 ppb spike
Recent studies add:	New evidence for asthma incidence and respiratory symptoms in children; respiratory effects in adults.	
Uncertainty/Limitation:	Independent effect of NO_2 from copollutants not widely characterized; limited biological plausibility, i.e., characterization of spectrum of key events informing mode of action.	
Cardiovascular Effects	**Short-term Exposure (Section 4.3)** 2008 ISA - Inadequate to Infer the Presence or Absence of a Causal Relationship Current draft ISA - Likely to be a Causal Relationship	
Key Evidence: (Table 4-36)	Consistent evidence for increases in cardiovascular hospital admissions and ED visits, particularly for IHD, as well as cardiovascular mortality in single- and multi-city studies in diverse populations. Associations found with adjustment for weather, time trends and in copollutant models for PM_{10}, SO_2, O_3, or in some but not all locations, $PM_{2.5}$ or CO. Limited biological plausibility demonstrated by decreases in HRV and changes in ventricular repolarization in epidemiologic studies of adults with cardiovascular disease but inconsistent changes in HRV in controlled human exposure studies. Changes in other cardiovascular effects generally not found in epidemiologic or experimental studies. Weak evidence to describe other key events informing mode of action with observations of oxidative stress and endothelial inflammation in some experimental studies and associations with indicators of inflammation in some but not all epidemiologic studies.	Individual city ambient 24-h avg: 90th: 22 to 100 ppb, Maximum: 19 to 132 ppb Overall study ambient 1-h max: 90th: 66 and 68 ppb Oxidative stress in rats: 5,320 ppb, Inflammation in rats: 2,660 and 5,320 ppb
Recent studies add:	Epidemiologic associations of NO_2 with cardiovascular hospital admissions, ED visits, and mortality that are independent of many copollutants, consistent epidemiologic evidence for decreases in HRV.	Inflammation in human cells exposed to human plasma,

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

Health Effect Category[a]	Causal Determination[b]	NO_2 Concentrations Associated with Effects[c]
Uncertainty/Limitation:	Limited biological plausibility, i.e., characterization of spectrum of key events informing mode of action.	oxidative stress in human plasma: 500 ppb
Cardiovascular Effects	**Long-term Exposure (Section 5.3)** 2008 ISA - Inadequate to Infer the Presence or Absence of a Causal Relationship Current draft ISA – Suggestive of a Causal Relationship	
Key Evidence: (Table 5-12)	Evidence from some recent high-quality, large cohort studies for associations of NO_2 or NO_X averaged over approximately 1 to 9 yr with myocardial infarction, heart failure and stroke. Associations found with adjustment for age, sex, SES, cardiovascular disease and other comorbid factors. Limited support from increases in cardiovascular mortality. No association found with myocardial infarction in some recent studies of comparable quality or with cardiovascular events in a previous study. Limited biological plausibility demonstrated by findings for arterial stiffness or decrease in HRV in a few epidemiologic studies and dyslipidemia in a study of rats. Weak evidence to describe other key events informing mode of action. Increased oxidative stress and endothelial inflammation found in some but not all experimental studies with short-term exposure. Inconsistent epidemiologic evidence for long-term NO_2-associated increases in indicators of systemic inflammation in adults.	Overall study ambient means: Annual avg: 12 to 23 ppb 9.5-yr avg: 34 ppb NO_2 and 96 ppb NO_X in cases Dyslipidemia in rats: 160 ppb Oxidative stress in rats: 5,320 ppb, Inflammation in rats: 2,660 and 5,320 ppb Inflammation in human cells exposed to human plasma, oxidative stress in human plasma: 500 ppb
Recent studies add:	New evidence for myocardial infarction and heart failure in some recent high-quality cohort studies.	
Uncertainty/Limitation:	Inconsistent epidemiologic evidence, limited biological plausibility, i.e., characterization of spectrum of key events informing mode of action.	
Total Mortality	**Short-term Exposure (Section 4.4)** 2008 ISA - Suggestive but not Sufficient to Infer a Causal Relationship Current draft ISA - Likely to be a Causal Relationship	
Key Evidence: (Table 4-41)	Consistent evidence for NO_2-related increases in total mortality from multicity studies in diverse locations. Associations found with adjustment for weather, time trends and are robust to adjustment using various methods and varying degrees of freedom to specify temporal trends. Several studies found robust NO_2 associations in copollutant models with PM_{10}, SO_2, or O_3. Biological processes leading to NO_2-related mortality not entirely clear. Large percentage of mortality is due to cardiovascular causes, but there is limited coherence of evidence across the spectrum of cardiovascular morbidity outcomes. 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 161 ppb Individual city ambient 1-h max: 90th: 33 to 133 ppb, Maximums: 96 to 112 ppb
Recent studies add:	Additional evidence from multicity studies and additional evidence for associations of NO_2 that are independent of the few copollutants that are examined.	
Uncertainty/Limitation:	Biological processes (i.e., effects on morbidity) by which NO_2 exposure leads to mortality not well characterized.	

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

Health Effect Category[a]	Causal Determination[b]	NO_2 Concentrations Associated with Effects[c]
Total Mortality	**Long-term Exposure (Section 5.5)** 2008 ISA - Inadequate to Infer the Presence or Absence of a Causal Relationship Current draft ISA – Suggestive of a Causal Relationship	
Key Evidence: (Table 5-19)	NO_2-related mortality found in the Harvard Six Cities cohort but not full American Cancer Society cohort, seminal studies of multiple U.S. cities. Consistent evidence in single-city studies in diverse locations but inconsistent evidence among other large cohorts of multiple U.S. locations. Associations found with NO_2 averaged over 1 to 16 years for periods 0 to 20 yr before death. Similar results found with extended follow-up of cohorts up to 26 years. Associations found with adjustment for age, sex, smoking, education, comorbid factors and in some cases, neighborhood-level SES. A few studies adjust for a copollutant or traffic and generally find positive NO_2 associations. Biological processes by which NO_2 exposure leads to mortality not entirely clear because of limited coherence among findings for respiratory and cardiovascular morbidity outcomes.	Overall study ambient means: 14 to 34 ppb
Recent studies add:	Evidence from additional cohorts and similar results with extended follow-up of previous cohorts.	
Uncertainty/Limitation:	Lack of association in some high-quality U.S. cohort studies; biological processes (i.e., effects on morbidity) by which NO_2 exposure leads to mortality not well characterized.	
Reproductive and Developmental Effects Long-term Exposure[d] 2008 ISA - Inadequate to Infer the Presence or Absence of a Causal Relationship for broad category		
Fertility, Reproduction, and Pregnancy (Section 5.4.2) Current draft ISA – Suggestive of a Causal Relationship		
Key Evidence: (Table 5-15)	Consistent but limited evidence for increases in pre-eclampsia in association with pregnancy NO_2 exposure in a few epidemiologic studies with adjustment for maternal age, smoking, SES, diabetes, parity. Lack of toxicological studies to inform biological plausibility. Decreased odds of live birth found in an in vitro fertilization study in association with short-term NO_2 exposure in periods leading up to in vitro fertilization and long-term exposure up to birth. Lack of biological plausibility with no effect on fertility found in a rat study. Inconsistent epidemiologic evidence for changes in blood pressure in pregnancy, gestational diabetes, effects on placenta. No effects found on sperm count or quality in epidemiologic or animal toxicological studies.	Overall study ambient means: NO_2: 12- to 14-day avg: 19 ppb Entire pregnancy: 23 ppb NO_X: Entire pregnancy mean: 7.2 ppb 3rd trimester median: 7.5 ppb
Recent Studies add:	New epidemiologic investigation of these outcomes with some supporting evidence.	
Uncertainty/Limitation:	Inconsistent and limited evidence for several outcomes, weak biological plausibility, i.e., characterization of spectrum of key events informing mode of action.	

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

Health Effect Category[a]	Causal Determination[b]	NO_2 Concentrations Associated with Effects[c]
Birth outcomes (Section 5.4.3) Current draft ISA – Suggestive of a Causal Relationship		
Key Evidence: (Table 5-15)	Consistent evidence from high-quality studies for associations of prenatal NO_2 exposure with fetal growth restriction particularly, as assessed with fetal or neonatal physical measurements. Associations found with adjustment for maternal age, SES, smoking, alcohol use, season of conception. Associations found with copollutants, with examination in only a few studies. Key events to inform mode of action not clearly characterized. Decreased birth weight found in a few high-quality epidemiologic studies but not in other studies. Limited biological plausibility provided by findings of decreased birth weight in a rat study. Inconsistent epidemiologic evidence for associations with preterm birth, birth defects. Findings for decreased litter size in rodents are inconsistent and do not directly inform epidemiologic observations.	Overall study ambient means: Entire pregnancy: 16 to 20 ppb Specific trimesters: 7.8 to 21 ppb Decreased birth weight in rats: 1,300 to 5,300 ppb
Recent studies add:	Large body of epidemiologic investigation of birth outcomes, with new evidence for fetal growth restriction.	
Uncertainty/Limitation:	Inconsistent evidence for some outcomes, lack of clear biological plausibility, i.e., characterization of spectrum of key events informing mode of action.	
Postnatal development (Section 5.4.4) Current draft ISA – Suggestive of a Causal Relationship		
Key Evidence: (Table 5-15)	Evidence for partially irreversible decreases in lung function growth in a few cohorts of children in association with NO_2 averaged over 6 mo, 1 or 8 yr. Associations found with adjustment for age, body mass index, smoking exposure. SES not examined. Associations also found with copollutants. Impairments in lung morphology found in experimental animals not related to changes in children. Some evidence for decreases in cognitive function in association with concurrent annual avg or prenatal NO_2 exposure. Inconsistencies found across the various neurodevelopmental effects examined. Associations found with adjustment for SES and in one study, noise, but potential confounding by lead or other pollutants not examined. Weak biological plausibility due to inconsistent findings in rodents for effects on emotional responses and motor function. Relationship between outcomes examined in children and rodents not clear. Inconsistent epidemiologic and toxicological evidence for postnatal mortality. Limited and inconsistent evidence for impaired physical development in rats and no analogous epidemiologic investigation. Across outcomes, effects on key events to inform mode of action not characterized.	Overall study ambient means: Lung function growth Annual avg: 14 to 21 ppb 6-mo avg: 34 ppb Cognitive function Concurrent annual avg: 17 ppb Prenatal: 15 ppb
Recent studies add:	New epidemiologic investigation of neurodevelopmental effects with some supporting evidence.	
Uncertainty/Limitation:	Inconsistent evidence for some outcomes; weak biological plausibility, i.e., characterization of the spectrum of key events informing mode of action.	

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

Health Effect Category[a]	Causal Determination[b]	NO_2 Concentrations Associated with Effects[c]
Cancer	**Long-term Exposure (Section 5.6)** 2008 ISA - Inadequate to Infer the Presence or Absence of a Causal Relationship Current draft ISA – Suggestive of a Causal Relationship	
Key Evidence: (Table 5-21)	Evidence from some high-quality studies for increases in lung cancer incidence and mortality, but no association in other studies of comparable quality. Cohorts followed for 7-30 yr, with NO_2 or NO_X exposures assessed for 1- to 30-yr periods. Associations found with adjustment for smoking, diet, SES and occupational exposures. Associations also found with copollutants. Lack of evidence in experimental animals for direct effect of NO_2 in lung tumor induction, but limited biological plausibility provided by findings that high NO_2 exposures promote lung tumors with co-exposure to diesel exhaust particles or known carcinogens. Limited evidence for other key events informing mode of action with findings of hyperplasia of lung epithelium and formation of secondary oxidation products in the respiratory tract. Limited epidemiologic evidence for associations with cancers of other sites. Weak evidence to describe key events informing mode of action with mixed findings for mutagenic and genotoxic effects in experimental animals and in vitro.	Overall study ambient means: Lung cancer NO_X: 11 µg/m^3 for 5-yr avg in all subjects, 32 and 42 µg/m^3 averaged over follow-up of 9.6 or 6.7 yr in cases NO_2: 14 to 23 ppb for 1-yr or 5-y avg Lung tumor promotion in rodents: 4,000 to10,000 ppb
Recent studies add:	Evidence in some studies for lung cancer incidence and mortality.	
Uncertainty/Limitation:	Lack of evidence that NO_2 acts as a direct carcinogen, weak evidence for key events informing mode of action.	

[a]A 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 supports 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]The concentrations refer to NO_2 unless otherwise specified.

[d]In the current draft ISA, separate causal determinations are formed for smaller subcategories of reproductive and developmental effects based on varying underlying biological processes and exposure patterns over different lifestages.

1.5 Evaluation of the Independent Effects of NO_2

As described in the preceding section, a key consideration in the causal determinations is the extent to which the evidence demonstrates that NO_2 exposure has an independent effect on health outcomes. The evaluation of the independent effect of NO_2 exposure has two major components. One is the extent to which epidemiologic studies account for potential confounding factors. The other is the extent to which controlled human exposure and animal toxicological studies demonstrate a direct effect of NO_2 exposure on the key events that inform the mode of action for a health outcome. In the 2008 and this ISA for Oxides of Nitrogen, potential confounding by other traffic-related pollutants is identified as an uncertainty in characterizing relationships between NO_2 exposure and many health effects. Other factors can potentially confound associations between ambient NO_2 concentrations and health effects. Across the health effects examined, epidemiologic studies are noted for consistently adjusting for potential confounding by factors such as meteorology and time trends in analyses of short-term NO_2 exposure, and for factors such as SES and smoking exposure in analyses of long-term NO_2 exposure. This section assesses the epidemiologic evidence for the independent effect of NO_2 exposure on health outcomes by integrating information across various lines of investigation.

In epidemiologic studies evaluated in this ISA, confounding was assessed primarily using multivariable models that include NO_2 concentrations and potential confounders 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 and precision of the effect estimate for NO_2 in multivariable models, not a change in statistical significance. There are limitations to multivariable models. 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., O_3), or if meteorology affects formation of both pollutants. Adding correlated but noncausal variables can produce models that fit the data poorly, and residual confounding is possible if confounders are excluded or poorly measured.

1.5.1 Potential confounding by time-varying factors and individual- or population-level characteristics

Most epidemiologic studies of short-term NO_2 exposure evaluated in the ISA are repeated measures population-level time series or panel studies that examine correlation between time-varying patterns in NO_2 and outcome. Using linear terms, interaction terms, and splines, these studies found associations with health effects with adjustment for factors such as temperature, humidity, day of the week, season, and long-term time trends, which can have similar temporal patterns as both ambient NO_2 concentrations and health outcomes. Time-series and panel studies accounted for serially correlated errors that can arise with repeated measures of ambient NO_2 and outcomes. Studies reported adjusting for temperature and humidity using splines with 3 or 4 degrees of freedom, and associations between NO_2 and respiratory hospital admissions in 8 Korean cities were robust to using 3 to 6 degrees of freedom for adjustment (Son et al., 2013). Studies reported adjusting for time trends using natural splines with 4 to 12 degrees of freedom per year. Effect estimates were robust to using 6 to 10 degrees of freedom to control for time trends for NO_2-associated respiratory hospital admissions (Son et al., 2013) and 4 to 14 degrees of freedom for NO_2-associated total mortality (Wong et al., 2010; Stieb et al., 2008). Evidence indicates adequate control for confounding by weather or time trends.

Most epidemiologic studies of long-term exposure compare individuals living in varying locations. These studies found associations with health effects with adjustment for factors that vary among individuals including SES, smoking, and other health conditions. Confounding also can occur by factors that vary among locations and affect the spatial pattern of health effects. Many studies of asthma and lung function in children adjusted for community of residence or accounted for spatial variation in outcome. A few of studies of respiratory effects and mortality found associations with NO_2 exposure with adjustment for community-level SES factors such as the percentage of individuals with low or high education. Neither noise nor stress, which like NO_2 concentrations are found to be higher near traffic, was widely examined as a potential confounder. A recent study found that the association between residential ambient NO_2 and asthma in children was limited to those with higher exposure to violence (Clougherty et al., 2007). If exposure to violence is only a confounder, the association for NO_2 would tend to be similar by level of exposure to violence. Individual-level stress was not examined as a potential confounder, but results from the analysis of SES and violence provide some support for respiratory effects of NO_2 exposure being independent of psychosocial stress. Although associations for NO_2 were inconsistent overall, one study each found associations of ambient NO_2 with poorer memory or decreased fetal growth with adjustment for aircraft or road traffic noise (van den Hooven et al., 2012b; van Kempen et al., 2012).

1.5.2 Potential confounding by copollutant exposures

The near-road environment is characterized as having higher concentrations of NO_2 and other traffic-related pollutants such as EC, CO, and UFP (Section 2.6.4.1). Studies examining the near-road environment found spatial gradients in NO_2 concentrations that are correlated with gradients in pollutants such as UFP, VOCs such as BTEX (sum of benzene, toluene, ethylbenzene, xylene), and polycyclic aromatic hydrocarbons (Section 2.6.4.1). These observations indicate the potential for NO_2-related health effects to be confounded by other traffic-related pollutants or NO_2 to serve as a surrogate for a mixture of traffic-related pollution. NO_2 concentrations decrease less sharply with increasing distance from the roadway than concentrations of UFP and CO, suggesting that pollutants other than NO_2 may serve as better indicators of near-road pollution gradients. However, NO_2 may capture spatial and temporal trends in traffic pollution better than $PM_{2.5}$ concentrations (Section 2.6.4.3). Gradients of traffic-related pollutants are influenced by factors other than distance to roadway including traffic volumes, local topography, meteorology, and conditions affecting chemical transformations.

These differences in spatial patterns among pollutants may explain the wide range of correlations found between ambient concentrations of NO_2 and copollutants (Table 2-4). In studies of health effects (Chapter 4 and Chapter 5), moderate correlations often were observed for SO_2 (Spearman or Pearson r = 0.12 to 0.74 for 25th-75th percentiles), $PM_{2.5}$ (r = 0.38 to 0.65), and PM_{10} (r = 0.26 to 0.62), whereas higher correlations were found for CO (r = 0.66 to 0.82) and EC (r = 0.25 to 0.92) (Figure 2-19). Ozone generally is poorly or inversely correlated with NO_2 (r = -0.16 to 0.41 for 25th-75th percentiles) even during the summer, when O_3 concentrations are higher (Section 2.6.4.2) (Table 2-4). The data described above also indicate that the magnitude of correlation varies by location. The few available studies show weaker correlations between personal exposures of NO_2 and $PM_{2.5}$ or EC (Pearson r = 0.06 to 0.49) (Table 2-7). Thus, the potential for copollutant confounding may vary by pollutant, location, and exposure assessment method.

Epidemiologic studies evaluated in this ISA examined copollutant confounding primarily with copollutant (i.e., two pollutants) models. The ISA does not consider multipollutant (i.e., three or more pollutants) models because multicollinearity among three or more pollutants can produce less reliable and precise effect estimates. Respiratory effects were consistently associated with short-term increases in ambient NO_2 concentration across locations with adjustment for copollutants such as PM_{10}, $PM_{2.5}$, SO_2, or O_3, or as examined in fewer studies, CO, EC, BC, UFP, or VOCs [Figure 4-10, Figure 4-11, and S4-1 (U.S. EPA, 2013d)]. An independent effect of NO_2 exposure also is supported by findings from some studies that NO_2 but not copollutants such as $PM_{2.5}$, PM_{10}, EC, CO, or SO_2 were associated with decreases in lung function or increases in pulmonary

inflammation in single-pollutant models (Sarnat et al., 2012; Oftedal et al., 2008; Holguin et al., 2007; Lagorio et al., 2006). In these studies, NO_2 concentrations were weakly to moderately correlated with copollutants (Spearman r = 0.05 to 0.51).

As described above, NO_2 is more highly correlated with CO and EC than other pollutants. Studies of respiratory effects provide limited evidence that associations observed with NO_2 are independent of CO, EC, or BC. In studies conducted in Atlanta, GA, Canada, and Australia, associations between NO_2 and increases in respiratory ED visits remained positive with adjustment for CO (Jalaludin et al., 2008; Tolbert et al., 2007; Villeneuve et al., 2007). In the Australian study, NO_2 showed a stronger association in the warm season, when no association was found for CO (Jalaludin et al., 2008). Associations between NO_2 and cardiovascular hospital admissions were robust to adjustment for CO in studies conducted in Taiwan but not other locations (Figure 4-16). In some cases, effect estimates for CO were attenuated with adjustment for NO_2, indicating confounding by NO_2. NO_2 and CO were moderately to highly correlated (Spearman or Pearson r = 0.55 to 0.74), which may limit the implications of some of the copollutant model results.

Several panel studies characterized as having strong exposure assessment with personal exposure monitoring or measurement of ambient NO_2 and copollutants at the location and time of subjects' exposures in outdoor locations found associations of NO_2 with respiratory effects with adjustment for EC or BC (Strak et al., 2012; McCreanor et al., 2007; Delfino et al., 2006). Among children with asthma in Southern California, personal NO_2 but not EC was associated with decreases in lung function (Delfino et al., 2008a). The correlations of NO_2 with EC or BC varied widely among these studies from the weak correlations typically found with personal exposures (r = 0.21, 0.38) to the higher correlations typically found with near-road exposure (r = 0.58 to 0.67). In Delfino et al. (2006), the effect estimate for central site NO_2 was reduced with adjustment for central site EC but remained positive. In the Children's Health Study, long-term NO_2 concentrations were associated with bronchitic symptoms in children with asthma with adjustment for EC (McConnell et al., 2003). In most studies, effect estimates for EC or BC were robust to adjustment for NO_2. However, in a few cases, effect estimates for EC were reduced with adjustment for NO_2 (Strak et al., 2012; McConnell et al., 2003). Among children in Beijing, China, the association between NO_2 and pulmonary inflammation was reduced with adjustment for BC but remained positive (Lin et al., 2011), indicating that BC may explain some but not all of the effects of NO_2.

Similar to NO_2, UFP and VOCs show gradients with distance from roads. Limited available evidence indicates that health effects are associated with NO_2 independently of UFP or VOCs. Also, a recent review indicated a lack of consistent association between

UFP and health effects, although deficiencies in data and heterogeneity in study designs were noted as limitations to drawing conclusions (HEI Review Panel on Ultrafine Particles, 2013). Panel studies found NO_2-associated decreases in lung function and increases in asthma medication use with adjustment for UFP (Figure 4-11). This evidence is substantiated by the strong exposure assessment of studies with measurement of NO_2 and UFP (Spearman r = 0.56, 0.58) at the time and location of subjects' outdoor exposures in traffic and other locations (Strak et al., 2012; McCreanor et al., 2007). Some studies did indicate that UFP confounded associations of NO_2 with particular outcomes, for example, lung function but not exhaled nitric oxide (eNO), the pro-inflammatory cytokine IL-6, or protein in nasal lavage fluid (Steenhof et al., 2013; Strak et al., 2012) and wheeze but not asthma medication use (von Klot et al., 2002). Some effect estimates for UFP were robust to adjustment for NO_2; others were attenuated. In Copenhagen, Denmark, the association between NO_X and cardiovascular hospital admissions largely decreased and became imprecise with adjustment for UFP (Figure 4-15). Potential confounding of NO_2-associated health effects by VOCs has been little examined. NO_2, BTEX, and individual VOCs were associated with pulmonary inflammation and lung function in children with asthma or wheeze (Greenwald et al., 2013; Martins et al., 2012). NO_2 concentrations showed a wide range of correlations with VOCs (r = -0.43 to 0.77). Martins et al. (2012) examined copollutant models and found that associations of NO_2 with pulmonary inflammation were robust to VOC adjustment. The association between NO_2 and FEV_1 was attenuated with adjustment for benzene but not ethylbenzene.

Correlations for NO_2 with PM_{10}, $PM_{2.5}$, and SO_2 are variable across locations and are low or inverse for O_3. A few results indicate that these copollutants confound associations with NO_2. For example, NO_2 was not associated with asthma hospital admissions in Greece (Samoli et al., 2011) or with lung function in children with asthma in Canada (Liu et al., 2009b) with adjustment for PM_{10}, $PM_{2.5}$, or SO_2. Most evidence shows that associations of short-term NO_2 exposure with respiratory effects, cardiovascular hospital admissions, and total mortality remain positive with adjustment for PM_{10}, SO_2, or O_3 (Figure 4-10 and Figure 4-15, and Section 4.4.4). NO_2 associations with respiratory effects, and to a more limited extent, cardiovascular effects remain positive with adjustment for $PM_{2.5}$. The few studies of long-term exposure that adjusted for these copollutants found robust associations between NO_2 and bronchitic symptoms and lung function growth (Hwang and Lee, 2010; Rojas-Martinez et al., 2007a; McConnell et al., 2003). Limited available results do not indicate that associations of short-term NO_2 exposure with wheeze or pulmonary inflammation (Strak et al., 2012; Mann et al., 2010) or long-term NO_2 exposure with bronchitic symptoms are confounded by $PM_{10-2.5}$ (McConnell et al., 2003). Adjustment for NO_2 had varying effects on the associations for PM_{10}, $PM_{2.5}$, SO_2, and O_3. Across the aforementioned studies, copollutant associations did not change with adjustment for NO_2 in some cases but were

attenuated in other cases. For respiratory and cardiovascular hospital admissions and mortality, several multicity studies found robust associations for NO_2 with adjustment for PM_{10}, SO_2, or O_3. These results pooled across cities add to the evidence for the independent effects of NO_2 exposure by indicating an effect of NO_2 across locations that vary from each other in the correlations between NO_2 and copollutants (Faustini et al., 2013; Chen et al., 2012b; Wong et al., 2010).

In some locations, ambient concentrations of gases are associated more strongly with personal PM than personal exposures to gases, suggesting that ambient gases may serve as a surrogate for personal PM exposure (Sarnat et al., 2001). However, limited recent data show weak to moderate correlations between personal NO_2 and ambient copollutants and between ambient NO_2 and personal copollutant exposures (r = -0.30 to 0.44) (Table 2-5 and Table 2-6). This is true of Suh and Zanobetti (2010b) in Atlanta, GA where several studies link ambient NO_2 with asthma and respiratory ED visits (Section 4.2.7.4). A recent meta-analysis found that in some cases, ambient NO_2 concentrations were more strongly related with personal $PM_{2.5}$ than personal NO_2 concentrations. In other cases, ambient and personal NO_2 were well correlated. Among children with asthma in Southern California, the association of personal NO_2 with decreases in lung function was reduced with adjustment for central site $PM_{2.5}$; however, there still was evidence for association with ambient NO_2 (Delfino et al., 2008a). The collective data do not indicate that ambient NO_2 concentrations serve only as surrogates for personal exposures to PM.

Analyses other than copollutant modeling provide support for the independent effects of NO_2 from PM. Studies of respiratory-related ED visits and mortality conducted in the U.S., Canada, Australia, Europe, and Asia showed stronger associations with NO_2 in the warm season than cold season (Figure 4-9, Section 4.4.6). Although these results may point to lower exposure measurement error because of more time outdoors, they also could support the independent effects of NO_2 from $PM_{2.5}$ or PM_{10} since lower correlations are reported for the warm season (Section 2.6.4.1). In the multicontinent APHENA study, PM_{10}-total mortality risk estimates were higher with higher (75th versus 25th percentile) mean ambient NO_2 concentration (Katsouyanni et al., 2009). If PM_{10} and NO_2 were only confounders of each other, risk estimates would tend to be similar by NO_2 concentration.

Indoor NO_2

Indoor NO_2 exposures (averaged over 3 to 7 days or 4 weeks) are consistently associated with respiratory symptoms in children (Section 4.2.6.1). In the 2008 ISA for Oxides of Nitrogen, results indicating reductions in respiratory symptoms after an intervention in classrooms to reduce NO_2 concentrations with use of flued gas heaters (Pilotto et al.,

2004) were used as support for the independent effects of NO_2 exposure. Few studies examined other indoor pollutants. In children with asthma, pulmonary inflammation was associated with both indoor school NO_2 and various PM metrics (Sarnat et al., 2012). In another study, indoor home NO_2 was associated with respiratory symptoms with adjustment for indoor $PM_{2.5}$ (Hansel et al., 2008). Sarnat et al. (2012) found correlations between NO_2 and copollutants such as BC and PM to differ in magnitude or direction between the indoor and outdoor school environments, suggesting that NO_2 may exist as part of a different pollutant mixture in indoor and outdoor environments.

NO_2 as an Indicator for Traffic-related Pollution or Proximity to Traffic

Vehicles make up the largest single emission source of NO_X, and vehicle NO_X emissions or factors such as distance to major roadway or roadway density are important inputs into LUR models that predict ambient NO_2 or NO_X concentrations (Sections 2.6.2.2 and 2.6.2.3). A recent review of near-road studies concluded that residence near busy roads is consistently associated with risk of development of asthma in children and risk of asthma exacerbations (HEI, 2010). Some evidence also was reported for lung function, mortality, and cardiovascular effects. However, several lines of evidence indicate that NO_2 may not serve only as an indicator of traffic pollution. NO_2 concentrations display different gradients with distance from the roadway from other pollutants, so using NO_2 concentrations as indicator for traffic may misrepresent concentration gradients for UFP, CO, and $PM_{2.5}$ (Sections 2.5.3 and 2.6.4.3). Further, since other sources contribute to ambient NO_2 concentrations, including electric utilities, airports, wildfires, and shipping ports (Section 2.3), NO_2 is not unique to vehicle emissions. The relative contributions of various sources to ambient concentrations of NO_2 are difficult to distinguish.

Several studies evaluated in this ISA found associations of effects such as lung function, pulmonary inflammation, asthma, and mortality with both NO_2 and traffic indicators such as roadway proximity or density and traffic density. However, other studies reported associations with outcomes such as decreases in lung function in children with asthma or hospital admissions in adults with COPD with NO_2 but not roadway proximity or density (Andersen et al., 2011; Holguin et al., 2007). Andersen et al. (2011) reported low to moderate correlations between traffic variables and 1-year or 25-year averages of NO_2 (Spearman r = 0.30 to 0.49). Studies also found associations of ambient (Gauderman et al., 2007) or indoor (Hansel et al., 2008) NO_2 concentrations with respiratory effects to persist with adjustment for traffic variables such as distance to freeway, distance to curb, or type of street in front of the home. McConnell et al. (2010) found that the association between ambient NO_2 and asthma in children was attenuated with adjustment for modeled NO_X, which was highly correlated with NO_2 and other pollutants. The association between modeled NO_X and asthma was robust to adjustment for NO_2

concentrations. These results suggest that associations for modeled NO_X may reflect effects of NO_2 and other traffic-related pollutants. Although long-term NO_2 exposure was not consistently associated with total mortality, a few studies indicated associations with NO_2 with adjustment for traffic proximity (Jerrett et al., 2009) or modification of the NO_2 association by traffic density (Lipfert et al., 2009). Source apportionment models often combine NO_2 with traffic-related PM species in a single factor (Section 2.6.4.3); however, there is support for an effect of NO_2 distinct from that of a mixture of traffic-related pollution. Among children in southern New England, NO_2 and a source apportionment factor of EC, zinc, lead, copper, and selenium (labeled a motor vehicle source) were moderately correlated (Pearson r = 0.49), and each was associated with asthma symptoms in a copollutant model (Gent et al., 2003). The observations from several studies indicating that NO_2 associations are independent of those for measures of roadway or traffic proximity and density or a mixture of traffic-related pollutants provide evidence that NO_2 does not serve only as a surrogate for traffic-related pollution.

1.5.3 Summary of Evaluation of the Independent Effects of NO_2 Exposure

Several lines of epidemiologic evidence indicate that associations of NO_2 exposure with health outcomes are independent of other correlated factors such as meteorology, season, and factors associated with traffic and other emission sources including SES and exposure to other pollutants. Multivariable models indicate health effects in association with short-term NO_2 exposure that are independent of temperature, humidity, day of the week, season, and long-term time trends. Associations between long-term NO_2 exposure and health effects are found to be independent of individual- and community-level SES measures, smoking exposure, and other health conditions. Potential confounding by stress or noise was not widely examined, particularly for health effects consistently associated with NO_2. The array of potential confounders were measured with methods widely used in the literature, but residual confounding is possible if factors are measured with error. NO_2 and copollutants such as PM_{10}, $PM_{2.5}$, SO_2, O_3, EC or BC, UFP, CO, and VOCs do not always show similar trends in ambient concentrations or associations with health effects. Examination of potential confounding by EC, BC, UFP, CO, and VOCs is limited overall, particularly for cardiovascular effects, and is absent for mortality. Epidemiologic studies consistently report associations between short-term increases in ambient NO_2 concentration and an array of respiratory effects, cardiovascular hospital admissions, and total mortality with copollutant adjustment across locations with varying correlations between NO_2 and copollutants. Although examined in fewer studies, associations between long-term NO_2 exposure and respiratory effects were found with copollutant

adjustment. There also is evidence for health effects associated with long-term NO_2 exposure but not measures of traffic or with adjustment for traffic proximity.

Epidemiology is limited in its ability to demonstrate the independent effects of NO_2 exposure because not all potential confounding factors are examined, including the full array of traffic-related pollutants potentially correlated with NO_2. Further, multivariable models can produce biased or unreliable effect estimates, including those used to adjust for multiple copollutants together. Thus, the evidence from experimental studies is key for informing the independent effect of a pollutant. Differences in determinations of a causal versus likely to be a causal relationship relate to the extent to which there is coherence among various lines of evidence to provide biological plausibility for the effects of NO_2 exposure. For cardiovascular effects and total mortality related to short-term NO_2 exposure and respiratory effects related to long-term NO_2 exposure, there remains some uncertainty regarding an independent effect of NO_2 exposure because there is limited or inconsistent evidence from experimental studies or across a spectrum of related outcomes. For respiratory effects of short-term NO_2 exposure, epidemiologic evidence for copollutant-adjusted results and indoor NO_2 together with experimental evidence provide sufficient evidence for the independent effects of NO_2 exposure.

1.6 Policy-Relevant Considerations

A key policy-relevant issue that frames the review of the NAAQS as described in detail in the Integrated Review Plan is how the available scientific evidence informs decisions on the basic elements of the NAAQS: indicator, averaging time, level, and form (Preface to the ISA, Section 1.1). The NAAQS are required to provide an adequate margin of safety, and thus understanding of the adverse nature of health effects and of at-risk lifestages and populations also is a key policy-relevant consideration. This ISA addresses the key policy-relevant considerations with the health effects for which the evidence indicates there is a causal or likely to be a causal relationship with NO_2 exposure. The discussion focuses on respiratory effects, cardiovascular effects, and total mortality of short-term NO_2 exposure and respiratory effects of long-term NO_2 exposure.

1.6.1 NO_2 Exposure Metrics

The primary short-term and long-term NO_2 NAAQS are based on 1-h daily max concentrations and annual average concentrations, respectively (Preface to the ISA). These standards were set to protect against a broad range of respiratory effects associated with short-term NO_2 exposures and health effects potentially associated with long-term

exposure. Thus, an important consideration in the review of the NAAQS is evaluation of the health effects evidence for various averaging times of NO_2 exposure.

For short-term exposure, the majority of previous and recent evidence is for health effects associated with 24-h avg ambient NO_2, but the 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 to 10 hours. These subdaily averages of NO_2 concentrations were associated with a spectrum of effects related to asthma exacerbations, measures of cardiovascular physiology and hospital admissions, as well as total mortality. NO_2 exposures occurring over 2 to 5 hours in outdoor traffic and nontraffic locations were associated with decreases in lung function and pulmonary inflammation in adults (Strak et al., 2012; McCreanor et al., 2007). This evidence is substantiated by strong exposure assessment with measurement of NO_2 at the locations of adults' outdoor exposures. Biological plausibility is provided by demonstrations of airway responsiveness (Section 4.2.2.2) and allergic inflammation (Section 4.2.4.3) in adults with asthma or animal models of allergic disease induced by NO_2 exposures in the range of 30 minutes to 6 hours.

Across epidemiologic studies, the robustness of evidence for associations with respiratory effects, cardiovascular effects, and total mortality is similar for 24-h avg and 1-h max NO_2 concentrations. Based on the few within-study comparisons, the magnitude of association with health effects did not clearly differ between 24-h avg and 1-h max NO_2. In some cases, associations with respiratory or cardiovascular effects were larger for 1-h max NO_2 than 24-h avg NO_2 (Carlsen et al., 2012; Ballester et al., 2006). In other cases, associations were stronger for 24-h avg NO_2 than 1-h max NO_2 (Rodriguez et al., 2007; Morgan et al., 1998). For asthma-related ED visits in Atlanta, GA, associations were similar for 1-h max and 24-h avg NO_2 with a 1-day lag, and slightly larger for 6-h nighttime avg NO_2 (12 a.m.-6 a.m.) (Darrow et al., 2011a). The NO_2 averaging times varied in the distribution of concentrations and spatial heterogeneity, which may account for differences in associations with asthma ED visits. Nighttime avg NO_2 had a wider distribution of concentrations than 24-h avg NO_2. Nighttime avg NO_2 was similarly spatially heterogeneous as 1-h max NO_2 but was lower in concentrations. The spatial heterogeneity in ambient NO_2 concentrations within urban areas (Section 2.5.2) and with distance to roadways (Section 2.5.3) and diurnal trends with higher concentrations measured during morning rush hours (Section 2.5.4) are not unique to Atlanta, GA. This heterogeneity in ambient NO_2 concentrations along with diurnal variation in time-activity patterns suggest that exposure measurement error can vary among different NO_2 averaging times, which could obscure true differences in association with health effects.

Various long-term NO_2 exposure metrics were associated with respiratory effects in children, including 6-month, 1-year, 3-year, 4-year, and 10-year lifetime average ambient

NO_2 concentrations (Section 5.2). Associations did not consistently differ among long-term exposure metrics. However, stronger associations were found with 1-year NO_2 averages that corresponded with exposure in the first year of life (Gruzieva et al., 2013; Gruzieva et al., 2012; Schultz et al., 2012).

1.6.2 NO_2 Lag Structure in Epidemiologic Studies

Characterization of the NO_2 exposure durations and lags associated with health effects can increase the understanding of the nature of relationships between NO_2 exposure and health effects. The lag structure for NO_2 exposure may vary among health effects depending on differences in the time course by which various biological processes occur. Identifying important lag structures can depend on whether the lag structure varies within the population according to differences in time activity patterns, pre-existing disease, or other factors that influence exposure and responses to exposure. Associations among exposure lags, particularly single-day and multiday averages of NO_2, may vary because the spatial and temporal heterogeneity in ambient NO_2 concentrations can result in differences in exposure measurement error. The lag structure was examined in studies of short-term NO_2 exposure for an array of respiratory effects, cardiovascular effects, and total mortality. While no particular lag of NO_2 exposure was more strongly associated with cardiovascular effects, evidence indicates that NO_2-associated respiratory effects and total mortality generally are larger for multiday exposures than single-day exposures.

Epidemiologic panel studies of children with asthma found increases in pulmonary inflammation and respiratory symptoms and decreases in lung function in association with increases in NO_2 lagged 0 or 1 day and multiday averages of 2 to 7 days. Increases in respiratory symptoms also were associated with NO_2 lagged 2 to 7 days. Consistent with these findings, increases in respiratory hospital admissions and ED visits were found in association with NO_2 lagged 0 or 1 day or averaged over 2 to 7 days. Whereas no particular lag of exposure was more strongly associated with decreases in lung function, several studies indicated larger increases in pulmonary inflammation, respiratory symptoms, and respiratory hospital admissions and ED visits for increases in multiday averages of NO_2 than single-day lags. Multiple studies indicate the largest increases in total mortality occurring with a lag of 1 day; however, several studies found associations with NO_2 averaged over 2 to 7 days.

Studies in which adults with asthma and healthy adults were exposed for 2 to 5 hours in outdoor traffic and nontraffic locations indicated decreases in lung function and increases in pulmonary inflammation immediately or 2 hours after exposures (Strak et al., 2012; McCreanor et al., 2007). 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 (Strak et al., 2012). These data based on strong exposure assessment 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 people with and without asthma. NO_2 exposure appears to affect the biological processes underlying the 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 (Sections 4.2.2.2 and 4.2.2.3). In experimental studies, NO_2 induced allergic inflammation 30 minutes up to 19 hours after a single or 2-day exposure in humans and 7 days after exposure in rats.

1.6.3 Concentration-Response Relationships and Thresholds

Characterizing the shape of the concentration-response relationship aids in understanding the public health impacts of NO_2 exposure. Of particular interest for the review of the NO_2 NAAQS 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-hour standard of 100 ppb and annual average standard of 53 ppb. The true concentration-response relationship may be obscured by fewer observations and greater exposure measurement error in the lower than upper range of the ambient concentration distribution, the influence of other determinants or risk factors for the health effect, and heterogeneity among individuals in the population in their response to air pollution exposures.

The shape of the concentration-response relationship for NO_2-associated health effects was examined in a limited number of epidemiologic studies and is better characterized for respiratory hospital admissions and ED visits and total mortality than other outcomes. Consistent with evidence reported in the 2008 ISA for Oxides of Nitrogen, results from recent studies indicate a linear concentration-response relationship for respiratory hospital admissions and ED visits and total mortality using various methods, including analysis of splines, higher order terms for NO_2 (e.g., quadratic, cubic), and quantiles of NO_2. The collective evidence for short-term exposure does not identify a threshold for the effects of NO_2 exposure.

Recent studies continue to support a linear relationship between short-term NO_2 exposure and asthma ED visits in U.S. cities. For 1-h max NO_2 (lag 0-2 day avg) combined across urban monitors by population-weighting, a linear association was indicated with asthma ED visits in Atlanta, GA during 1993-2004 (Strickland et al., 2010). Relative risks

increased across quintiles of NO_2 between 28 and 181 ppb (with concentrations less than 28 ppb as the reference), and models with nonparametric smoothing showed increasing risk with increasing 1-h max NO_2 between the 5th and 95th percentile of concentrations (11 to 37 ppb) (Strickland, 2013). The concentration-response relationship was not examined for 1-h max NO_2 concentrations less than 11 ppb. For the relationship between 24-h avg NO_2 and pediatric asthma ED visits in Detroit, MI, there was not evidence for the association differing below and above a threshold set by the investigators at 23 ppb NO_2 (between the 82nd and 85th percentiles) in conditional logistic regression models. The risk was not assumed to be zero below 23 ppb, and the threshold model did not fit the data better than the linear model did (Li et al., 2011b).

Linear concentration-response relationships also are indicated for mortality associated with short-term NO_2 exposure (lag 1 day or 0-1 day avg) in the U.S., Canada, and Asia based on comparisons of linear and various nonlinear models with natural (Moolgavkar et al., 2013; Wong et al., 2008b) and cubic (Chen et al., 2012b) splines or quadratic and cubic terms for NO_2 (Stieb et al., 2008). Most results are for 24-h avg NO_2, with limited evidence for 3-h max NO_2 (Stieb et al., 2008). The results do not identify a threshold for NO_2-related mortality. The analysis of 85 U.S. cities indicated less certainty in the shape of the concentration-response at 24-h avg NO_2 concentrations less than 20 ppb, where the density of data was low and 95% CIs were wide (Moolgavkar et al., 2013).

A few previous results point to nonlinear concentration-response relationships but for outcomes for which the concentration-response relationship has not been widely examined, including cough in children or cardiovascular hospital admissions in adults. The studies tended to find NO_2-related increases in effects that were larger in magnitude per increment in NO_2 in the lower range of NO_2 concentrations than in the upper range of concentrations.

The shape of the concentration-response relationship was not formally evaluated in previous or recent studies of long-term NO_2 exposure and is not well characterized. Limited available evidence from Europe indicates increasing respiratory effects with increasing ambient NO_2 concentration in analyses of tertiles (concentrations not reported) of modeled ambient residential NO_2 for asthma incidence in children (Modig et al., 2009) and a cubic spline of ambient NO_2 concentrations for asthma hospital admissions in adults (Andersen et al., 2012) (Section 5.2.12). These studies reported annual average NO_2 concentrations with ranges 1.8 to 24 ppb and 5.3 to 21 ppb.

In summary, the shape of the concentration-response relationship is better characterized for short-term NO_2 exposure than for long-term exposure. Previous and recent evidence indicates a linear relationship between short-term NO_2 exposure and respiratory hospital admissions or ED visits and mortality. Evidence is available primarily for 24-h avg NO_2

but also 1-h and 3-h max NO_2 and for NO_2 averaged over 2 to 5 days or lagged 1 day. There is uncertainty in the shape of the concentration-response in the low range of the distribution of NO_2 concentrations where data density is low. However, results do not identify a threshold for respiratory hospital admissions or ED visits or mortality. Results from U.S. studies indicate uncertainty in the shape of the concentration-response relationship for asthma ED visits at 1-h max NO_2 concentrations less than 11 ppb and for total mortality at 24-h avg ambient NO_2 concentrations less than 20 ppb.

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 the concentration-response varies across geographical regions. Such information is limited largely to European and Asian cities. In the only U.S. study, a test for heterogeneity was not statistically significant for the association between ambient NO_2 concentrations (for the first year or first 3 years of life) and asthma among Latino and African American individuals ages 8-21 years in Chicago, Houston, San Francisco, New York, and Puerto Rico (Nishimura et al., 2013a). Comparisons of odds ratios do indicate differences among cities, namely a larger association in the San Francisco cohort comprising only African American children, an imprecise association in New York for NO_2 in the first 3 years of life and no association in Houston, which had a much smaller sample size. San Francisco had lower ambient NO_2 and SO_2 concentrations than New York. $PM_{2.5}$ and SO_2 were associated with asthma in Houston but not New York or San Francisco. There was not strong indication of regional heterogeneity in associations of short-term or long-term NO_2 exposure with respiratory effects among European or Korean cities (Jacquemin et al., 2009b; Moon et al., 2009; Timonen et al., 2004).

Regional heterogeneity is indicated among European and Asian cities in the relationship between short-term NO_2 exposure and total mortality. Larger risk estimates were found for European cities with lower prevalence of smoking and greater household use of gas (Samoli et al., 2006). Larger risk estimates were found for Asian cities with a larger population of older adults and higher concentrations of PM_{10} and cities with higher temperature, populations with more time outdoors, less air conditioning use, higher mortality from infection, more deaths among younger people, and lower mean ambient concentrations of NO_2 and other pollutants (Wong et al., 2008b).

Studies also did not clearly show heterogeneity in NO_2-related respiratory effects between neighboring communities. Higher NO_2 concentrations are found in urban areas than nonurban areas; however, NO_2-related respiratory effects do not consistently differ

between urban and suburban communities in Europe (Section 6.5.4). A recent study found larger NO_2-related increases in pulmonary inflammation among children with asthma in Ciudad Juarez, Mexico schools than nearby El Paso, TX schools (Sarnat et al., 2012). The reasons for the heterogeneity were not explicitly analyzed.

In summary, there is not clear evidence for regional heterogeneity in the relationship between short-term or long-term NO_2 exposure and respiratory effects, including the only U.S. study, which examined asthma. Studies of short-term NO_2 exposure and mortality found heterogeneity across cities in Europe and cities in Asia and indicated that heterogeneity may be due to differences among cities in factors that influence exposure to air pollution such as time outdoors or air conditioning use, differences in exposure to other pollutants, or differences in the distribution of other indicators of health.

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 air pollution or affected by the health outcome, and the presence of populations or lifestages with higher exposure or greater risk of air pollution-related health effects.

Evaluating Adversity of Health Effects

Both the World Health Organization (WHO) and the American Thoracic Society (ATS) have addressed what health effects may be considered adverse. In defining health as "the state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity" (WHO, 1948), WHO acknowledges that changes in health outcomes that are not severe enough to result in a diagnosis of a clinical outcome can be adverse if they affect the well-being of an individual. ATS also considered a wide range of health outcomes in defining adverse effects. Distinguishing between individual and population risk, ATS indicated that small air pollution-related changes in an outcome observed in individuals can be considered adverse on a population level since a shift in the distribution of population responses due to higher air pollution exposure can increase the proportion of the population with clinically important effects or at increased risk of a clinically important effect that can be caused by another risk factor (ATS, 2000b).

Increases in ambient NO_2 concentrations are associated with a broad spectrum of health effects, including those characterized as adverse by ATS such as mortality, the development of asthma, and asthma exacerbations (ATS, 2000b). Ambient NO_2 exposure also is associated with more subtle changes in function such as increases in airway

responsiveness and pulmonary inflammation and decreases in lung function (Section 1.4.2). Increases in airway responsiveness and pulmonary inflammation are key events informing the mode of action for acute asthma exacerbations and asthma development. While evidence is not consistent across cardiovascular endpoints, ambient NO_2 concentrations are associated with increases in cardiovascular mortality and hospital admissions as well as decreases in HRV, and decreases in HRV have been linked with increased risk of life-threatening cardiovascular events (Section 1.4.4). These physiological measures show a distribution within populations, and NO_2-associated changes in airway responsiveness, pulmonary inflammation, or HRV 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 and cardiovascular events, respectively.

At-risk Lifestages or Populations for Exposure of Oxides of Nitrogen or Related Health Effects

The NAAQS are intended to protect public health with an adequate margin of safety, and protection is provided for the population as a whole and groups at increased risk for health effects from exposure to the 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 having higher NO_2 exposure or NO_2-related health effects. The 2009 American Housing Survey reports that 17.5% of occupied housing units in the U.S. are within 90 meters of NO_X emissions sources such as a four-lane highway, railroad, or airport (U.S. Census Bureau, 2009). In Los Angeles, CA, 44% of the population was found to live within 100 meters of a major road (HEI, 2010). Such proximity to roadways can be characterized by higher concentrations of NO_2 than background (Section 2.5.3). Thus, a large proportion of the U.S. population has the potential for elevated ambient NO_2 exposures and for increased risk of health effects that are related to higher NO_2 exposure.

At-risk lifestages or populations 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 air pollution-related health effects or the magnitude of physiological responses to air pollution exposure (Table III of the Preamble). Similar to the causal framework, conclusions about at-risk factors are based on judgments of the consistency and coherence of evidence within and across disciplines (Chapter 6). Conclusions on at-risk factors are based on NO_2 exposure since other oxides of nitrogen were examined in few studies. Briefly, the evaluation includes analysis of studies that

compared exposure or health effect relationships among different groups (e.g., people in different age categories, people with and without asthma) and 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 included to assess coherence with evidence for health effects and inform how a particular factor may modify NO_2-related risk of health effects or physiological responses (e.g., exposure differences, differences in biological effect for a given dose). Because the framework for at-risk factors was not available for the 2008 ISA for Oxides of Nitrogen, previous and recent studies are considered in the current conclusions.

There is adequate evidence to indicate that children (ages 0-14 years) and older adults (ages $\geq$ 65 years) are at increased risk for NO_2-related health effects. There is suggestive evidence that genetic variants (primarily in antioxidant genes), asthma, COPD, SES, sex, and diet modify health effects associated with NO_2 exposure based on a limited evidence base or inconsistent results within a discipline. In most cases, there is coherence with evidence from another discipline. Because of insufficient consistency and quantity of evidence within a discipline and lack of information from another discipline, there is inadequate evidence to determine whether cardiovascular disease, diabetes, obesity, race/ethnicity, smoking, or urban/nonurban residence modifies NO_2-related health effects.

Children are identified as an at-risk lifestage based on consistent evidence for larger risks of asthma hospital admissions and ED visits associated with short-term NO_2 exposure in children (Section 6.4.1.1). Most studies compared children ages 0-14 years with people of all ages or adults. Compared with people ages 15-64 years, children ages 0-14 years had 2- to 3-fold higher risk of asthma hospital admissions or ED visits for the same increase in 24-h avg NO_2 concentrations. Substantiating the evidence for NO_2 specifically, evidence indicates that NO_2 exposure has independent effects on asthma exacerbations (Section 1.4.2). The reasons for the increased risk for children are not clear. Compared with adults, children have developing respiratory systems, oronasal breathing, higher ventilation rates (Section 3.2.2.3), and different time activity patterns characterized by more time outdoors and more vigorous activity (Section 6.4.1). However, it is not clear whether these physiological and behavioral characteristics result in differences in NO_2 exposure, uptake in the respiratory tract, or exposure measurement error for children (Sections 2.6.5.2 and 3.2.5). Limited data do not clearly indicate higher personal NO_2 exposures in children (Table 2-9). Many studies reported a higher number of asthma ED visits or hospital admissions among children than other age groups. Thus, higher incidence of asthma exacerbations in children may be a reason for their increased risk.

Among children, several studies found that associations with asthma, allergic sensitization, and decreases in lung function in individuals ages 4-21 years (mostly children) were larger for NO_2 exposure around birth or infancy compared with NO_2 exposure in the first four years of life, year before diagnosis, or lifetime average NO_2 exposure (Sections 5.2 and 6.4.1) These results suggest that the prenatal period or infancy may represent a critical time window of exposure for NO_2-related respiratory effects in children. Lung development begins during the prenatal period and continues throughout childhood; however, it is not clear whether differences in lung development contribute to higher risk associated with NO_2 exposure during the prenatal period or infancy.

Children ages 18 years and younger 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 versus 7.0 per 100 persons with asthma)[1]. Further, asthma is the leading chronic illness (9.5% prevalence) and reason for school absenteeism in children in the U.S. Although there is only suggestive evidence for people with asthma having increased risk for NO_2-related health effects because of inconsistent epidemiologic evidence, there are some studies showing larger NO_2-related increases in respiratory symptoms or decreases in lung function in children with asthma than without asthma (Section 6.3.1). Based on the large number of children in the U.S. population and the high prevalence of asthma morbidity among children, even slightly higher risks of asthma exacerbations for children compared with adults can translate into large numbers affected, magnifying the potential public health impact of NO_2 exposure.

There is adequate evidence that older adults, i.e., those ages 65 years and older, have increased risk for NO_2-related health effects compared with younger adults (Section 6.4.1.2). This conclusion is based mainly on evidence for hospital admissions for asthma or COPD and for mortality. Substantiating this conclusion, evidence indicates that NO_2 exposure has independent effects on asthma exacerbations. Studies showed a wide range in difference in magnitude of risk for older adults, from <1- to 3-fold higher risk of NO_2-related respiratory hospital admissions to 2- to 6-fold higher for NO_2-related mortality. As with children, the reasons for the increased risk for older adults are not well understood. Older adults did not consistently have higher absolute numbers of respiratory hospital admissions compared with younger adults, so higher incidence of the health effect does not seem to explain their higher NO_2-related risk estimates. Studies show different time activity patterns (Section 6.4.1) and rates of ventilation in older than younger adults; however, it is not known whether these factors contribute to differential exposure and uptake of NO_2 in older adults. Regardless of the reasons for increased risk of NO_2-related mortality, the higher incidence of mortality in older adults than other age

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

groups, and the growing proportion of older adults in the U.S. magnify the public health impact of increased risk of mortality from NO_2 exposure.

At-risk lifestages and populations likely are not characterized by a single factor. Cardiovascular diseases and diabetes are more prevalent in adults ages 65 years and older (Table 6-3), and comorbid factors potentially could contribute to their higher risk of NO_2-related health effects. Compared with younger adults, older adults had larger risks of NO_2-associated COPD hospital admissions in the studies reviewed in this ISA. A controlled human exposure study of older adults found a larger NO_2-induced decrease in lung function among adults with COPD (mean age 60 yr) than among healthy older adults (mean age 61 yr) (Section 6.3.2). While pre-existing cardiovascular disease did not consistently modify all NO_2-related health effects, there is evidence for cardiovascular disease increasing the risk of NO_2-associated mortality. These various lines of evidence suggest that comorbidities in older adults could contribute to their higher risk of NO_2-related health effects. In children, there is some evidence for larger NO_2-related respiratory effects in lower SES groups than higher SES groups (Section 6.4.2). Lower SES also is associated with higher asthma prevalence and exacerbations as well as higher NO_2 or NO_X exposure. Co-occurring risk factors in a lifestage or population may influence their risk of NO_2-related health effects. Such inter-relationships among potential risk factors have not been well examined for NO_2-related health effects.

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 major roads, resulting in a large number of people potentially with elevated ambient NO_2 exposure. There is evidence for relationships with effects that are clearly adverse such as premature mortality, hospital admissions, ED visits, and asthma incidence. More subtle NO_2-related effects such as increases in airway responsiveness and pulmonary inflammation or decreases in lung function and lung function growth can be considered adverse on a population level because higher NO_2 exposure can lead to an increase in the proportion of the population with clinically important effects. The public health significance of NO_2-related health effects also is supported by the increased risk for children (ages 0-14 years) compared with adults and increased risk for older adults (ages 65 years and older) compared with younger adults. Children and older adults differ from other lifestages in behavior, physiology, and comorbidities; however, it is not clear whether these characteristics contribute to their increased risk of NO_2-related health effects. The large proportions of children and older adults in the U.S. population and the higher prevalence of co-occurring factors such as COPD and cardiovascular disease in older adults and asthma in children can translate into a large number of people affected by ambient NO_2 exposure and thus magnify the public health impact of ambient NO_2 exposure.

1.7 Conclusions

The major emissions sources of NO_X in the U.S. based on the 2008 National Emissions Inventory are motor vehicles and electric utilities. The distribution of emissions sources and chemical transformation, transport, and deposition of these emissions contribute to spatial and temporal heterogeneity in ambient concentrations and human exposure to NO_2, NO, and NO_X. In the U.S., NO_X emissions and ambient NO_2 concentrations have decreased over the past 20 years. Ambient concentrations have been shown to be 30% to 200% higher at locations within 15 m of a roadway (averaged over hours to weeks) compared with locations farther away from the road. Relationships between ambient NO_2 concentrations and personal exposures vary in the population, and exposure measurement error resulting from the use of ambient concentrations can reduce epidemiologic associations observed with health effects. Once inhaled, NO_2 is transformed in the respiratory tract to secondary oxidation products, which can initiate a cascade of events, including inflammation, bronchial smooth muscle reactivity, and modification of immune responses. The processes by which ambient-relevant NO_2 exposures lead to effects outside of the respiratory system are not well characterized.

Recent studies, most of which are epidemiologic, expand on findings reported in the 2008 ISA for Oxides of Nitrogen and earlier assessments. The consistency, coherence, and biological plausibility of evidence integrated across disciplines and outcomes related to asthma exacerbations indicate that there is a causal relationship between short-term NO_2 exposure and respiratory effects. Evidence indicates there is likely to be a causal relationship between short-term NO_2 exposure and cardiovascular effects as well as total mortality. There is likely to be a causal relationship between long-term NO_2 exposure and respiratory effects based strongly on findings in children for asthma incidence and decreases in lung function. Epidemiologic studies provide compelling evidence for the independent effects of NO_2 exposure with associations of health effects with NO_2 in copollutant models across locations that vary in copollutant relationships and across exposure assessment methods, associations with indoor NO_2, or differences in effects with traffic proximity or intensity. However, the extent of examination of potential copollutant confounding varies across health effects, particularly confounding of the relationships of short-term NO_2 exposure with cardiovascular effects and total mortality by CO, UFP, EC, and BC. For these relationships, the limited biological plausibility does not conclusively demonstrate that NO_2 exposure has an effect independent of the effects of another traffic-related pollutant or mixture. Evidence is suggestive of a causal relationship between long-term NO_2 exposure and cardiovascular effects, reproductive and developmental effects, total mortality, and cancer.

There is adequate evidence that children (ages 0-14 years) and older adults (ages 65 years and older) have increased risk for NO_2-related health effects. Large numbers of people in the U.S. live near major roads and potentially have elevated exposures to ambient NO_2 compared with people living 500 meters or more from roads. There is suggestive evidence that risk of NO_2-related health effects differs by pre-existing asthma, pre-existing COPD, genetic variants for oxidative metabolism enzymes, dietary antioxidant intake, SES, and sex. Daily average and 1-hour maximum NO_2 as well as concentrations averaged over 30 minutes to a few hours are associated with health effects. For many respiratory outcomes, larger effects are estimated for multiday averages of ambient NO_2 concentrations than single-day concentrations. Respiratory effects are associated with long-term NO_2 concentrations averaged over 6 months and 1 year to 10 (representing lifetime exposure) years. Evidence indicates that the concentration-response relationship for relationships of short-term ambient NO_2 exposure with respiratory hospital admissions and ED visits and mortality is linear, and results do not identify a threshold for effects.

References for Chapter 1

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CHAPTER 2 ATMOSPHERIC CHEMISTRY AND EXPOSURE TO OXIDES OF NITROGEN

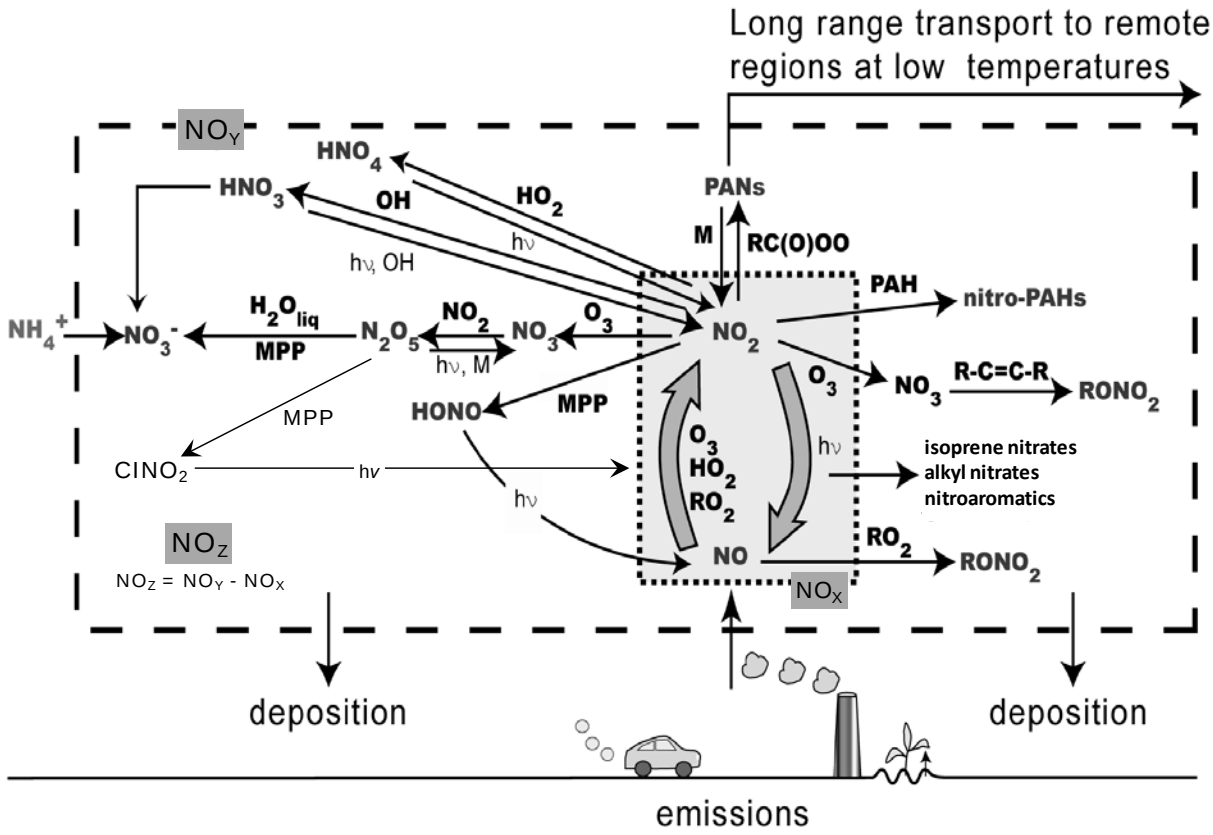
2.1 Introduction

This chapter presents concepts and findings relating to emissions sources, atmospheric science, and human exposure assessment. It is intended as a prologue for detailed discussions on the evidence for health effects 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 and exposures.

In the ISA, "oxides of nitrogen" (NO_Y) refer to all forms of oxidized nitrogen (N) compounds, including NO, 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, including organic nitrates ($RONO_2$) such as the peroxyacyl nitrates (PANs). NO_2 can also react with a variety of atmospheric species, resulting in organic and inorganic nitrates, and making substantial contributions to the mass of atmospheric particulate matter (PM) and the acidity of cloud, fog, and rainwater, as well as PM. The abbreviation NO_X refers specifically to the sum of NO and NO_2. This chapter describes origins, distribution, fate, and exposure of gaseous oxides of nitrogen, while aspects of particulate nitrogen species (such as pNO_3) were addressed in the 2009 PM ISA (U.S. EPA, 2009a).

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 (U.S. EPA, 2008c). The role of NO_X in O_3 formation was reviewed in Chapter 3 of the 2013 ISA for Ozone and other 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.



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, hν 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: NCEA

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

The overall chemistry of reactive, oxidized nitrogen compounds in the atmosphere is summarized in Figure 2-1. There are a large number of oxidized nitrogen species in the atmosphere including nitric oxide (NO), nitrogen dioxide (NO_2) nitrate radical (NO_3), nitrous acid (HONO), nitric acid (HNO_3), dinitrogen pentoxide (N_2O_5), peroxynitric acid (HNO_4), peroxyacetyl nitrate (PAN) and its homologues (PANs), other organic nitrates, such as alkyl nitrates (including isoprene nitrates), and particulate nitrate (pNO_3). Collectively these species are referred to as NO_Y. Oxidized nitrogen compounds are emitted to the atmosphere mainly as NO, which rapidly interconverts with NO_2 and so NO and NO_2 are often "lumped" together into their own group or family, which in the atmospheric sciences community are referred to as NO_X (shown in the inner box in

Figure 2-1). All the other species mentioned above in the definition of NO_Y are products of reactions of NO or NO_2 and collectively are referred to as NO_Z (shown in the outer box in Figure 2-1). The totality of all the species shown in Figure 2-1 is referred to as NO_Y, such that $NO_Y = NO_X + NO_Z$. 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., Na^+, 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.

Inorganic NO_Z species include HONO, HNO_3, HNO_4, and pNO_3. Mollner et al. (2010) identified pernitrous acid (HOONO), an unstable isomer of nitric acid, as a product of the major gas phase reaction forming HNO_3. However, since pernitrous acid is unstable, it is not a substantial reservoir for NO_X. While a broad range of organic nitrogen compounds are emitted by combustion sources (e.g., nitrosamines and nitro-PAHs), they are also formed in the atmosphere from reactions of NO, NO_2, and NO_3. These include peroxyacyl and alkyl nitrates, other nitro-PAHs, and the more recently identified nitrated organic compounds in the quinone family. Most of the mass of products shown in the outer box of Figure 2-1 is in the form of PAN and HNO_3, although other organic nitrates (e.g., isoprene nitrates) can be important at locations closer to biogenic sources (Horowitz et al., 2007; Singh et al., 2007). The concentrations and atmospheric lifetimes of inorganic and organic products from reactions of NO and NO_2 vary widely in space and time.

Sources of NO_X include naturally occurring processes associated with fires, lightning and microbial processes occurring in soils. Anthropogenic sources are dominated by combustion processes from electricity generating units and wide spread transportation sources. Sources are distributed with height with some occurring at or near ground level and others aloft as indicated in Figure 2-1. 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).

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 form 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 represents a major sink for NO_Y.

Many species, including particulate nitrate 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. 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. N_2O_5 is the acid anhydride of HNO_3, and its uptake on aqueous aerosol represents a major sink for NO_X. Macintyre and Evans (2010) showed that the sensitivity of key tropospheric species such as O_3 varies from very small to significant over the range of uptake coefficients (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, in large part because of 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. Organic aerosol and soot can reduce $\gamma(N_2O_5)$ by two orders of magnitude or more. The uptake of N_2O_5 by aqueous aerosols containing chloride (Cl^-) and bromide (Br^-) has also been associated with the release of gaseous nitryl chloride ($ClNO_2$) from marine (sea-spray) aerosol (Osthoff et al., 2008). $ClNO_2$ readily photolyzes to yield Cl and NO_2. Although gas phase $ClNO_2$ can be a major sources of reactive Cl, capable of initiating the oxidation of hydrocarbons (as do OH radicals), $ClNO_2$ causes only modest ozone increases (e.g., ~1 to 1.5 ppb for nominal O_3 concentrations between 60 and 85 ppb in the Houston airshed) (Simon et al., 2009). Nitryl chloride is found not only in coastal and marine environments. 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.

The lifetimes of PANs are strongly temperature dependent but they are stable enough at low temperatures to be transported long distances before decomposing to release NO_2, which can then participate in O_3 formation in regions remote from the original NO_X source. Nitric acid acts similarly to some extent, but its high solubility and high deposition rate imply that it is removed from the gas phase faster than PAN and thus would not be as important as a source of NO_X in remote regions.

The time scale for reactions of NO_X to form products shown in the outer box of Figure 2-1 typically ranges from a few hours during summer to about a day during winter. 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, 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 (e.g., Valin et al., 2013; Hameed et al., 1979). Because the time required for mixing of emissions to the surface is similar or longer than the time for oxidation of NO_X, emissions of NO_X from elevated sources tend to be transformed to more oxidized NO_Z products (such as particulate nitrate and HNO_3) before they reach the surface. It should be noted that O_3 can still be formed aloft in the remnants of power plant plumes. However, because people live in closer proximity 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 inventories.

The oxidation of many 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), it is 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 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). 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 ICARTT (International Consortium for atmospheric research on Transport and Transformation) 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, these radicals favor reaction with HO_2 radicals to produce mainly 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' isoprene nitrates (INs). Lifetimes of 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 first generation INs can undergo reactions with OH radicals and O_2 and 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. The second generation organic nitrates are more stable than the first generation INs because they lack a double 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 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 yield of 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 organic nitrates. They 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 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 isoprene nitrates (INs). Regional or global models that use 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 used 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 ozone production by 10% over the U.S. and that uncertainties in the NO_X

recycling efficiency had a larger affect than the isoprene nitrate 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 the concentrations of its precursors (NO_X and VOCs), it is unlike many other atmospheric species whose rates of formation 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 (c.f., Figure 2-12, low-NO_X regime), 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 a 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 ozone 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.

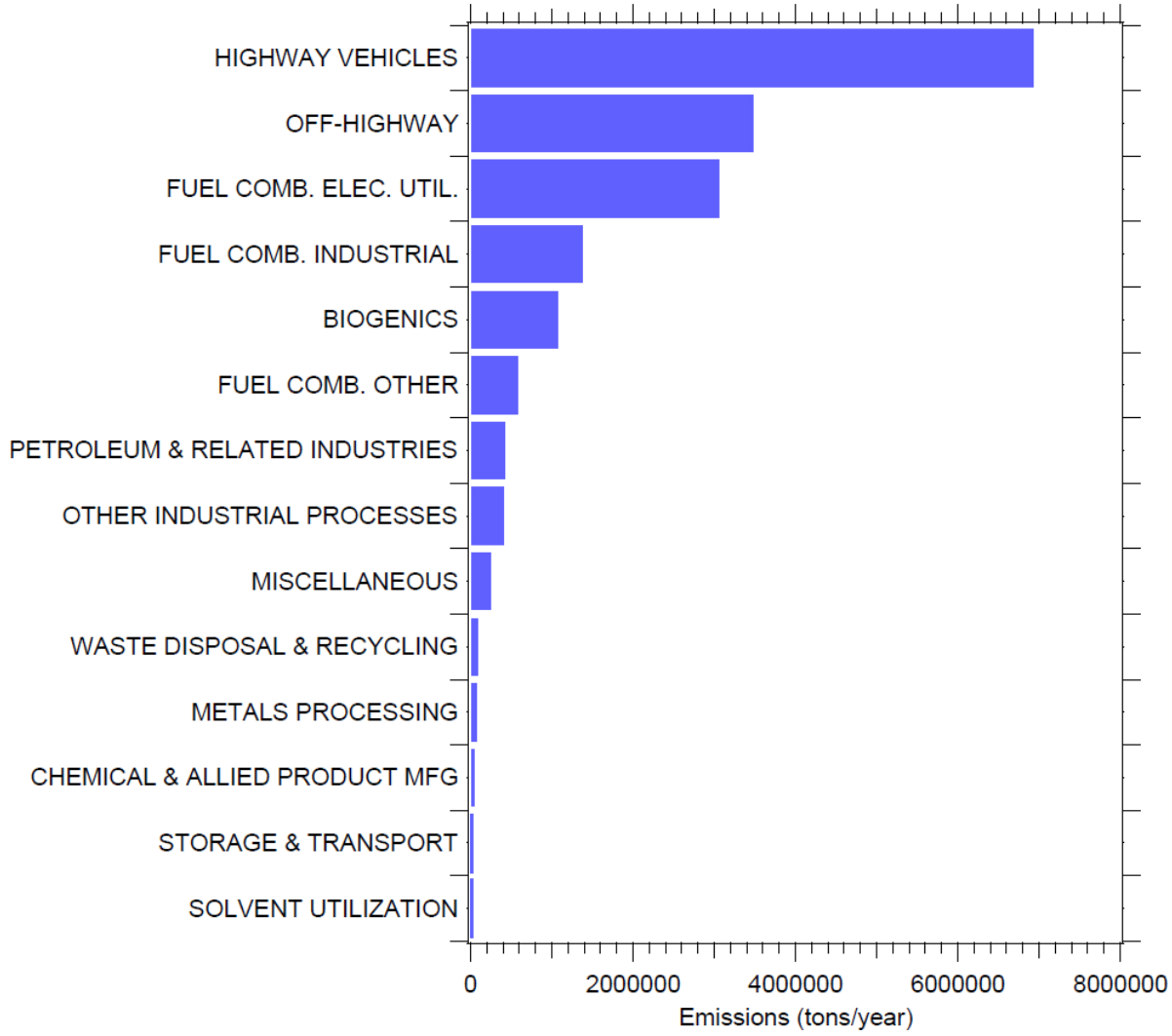
Brown et al. (2012) conducted a field study comparing nighttime chemistry in the plumes of power plants, one with selective catalytic reduction (SCR) NO_X emissions controls and one without these controls, in Texas. They noted that the plume from the power plant with SCR controls did not have enough 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, and 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

The major sources of NO_X in the U.S. identified from the 2008 National Emission Inventory U.S. EPA (2011) are described in Figure 2-2.[1] The values shown are nationwide averages and may not reflect an individual person's exposures to NO_2. Highway vehicles are the largest source of NO_X, contributing 39% of total emissions. Off-highway vehicles account for 19% of emissions, fuel combustion by electric utilities makes up 17% of emissions, and industrial fuel combustion accounts for 8% of emissions. Other sources listed in Figure 2-2 account for less than 5% of national emissions each. Sources that are not listed in Figure 2-2 can still be important for exposure. For example, intense industrial operations including cement plants are not listed as nationally important sources, but they are subject to variable emissions with high peaks (Walters et al., 1999). Note that lightning emissions of NO are not in included in Figure 2-2. Estimates of emissions of NO from lightning found in the literature are given in Section 2.3.9.

[1] This section currently discusses data from the 2008 National Emissions Inventory, version 3 U.S. EPA (2011). The 2011 National Emissions Inventory became available to the public in November 2013 and will be incorporated into the Second External Release Draft of the Integrated Science Assessment for Oxides of Nitrogen.

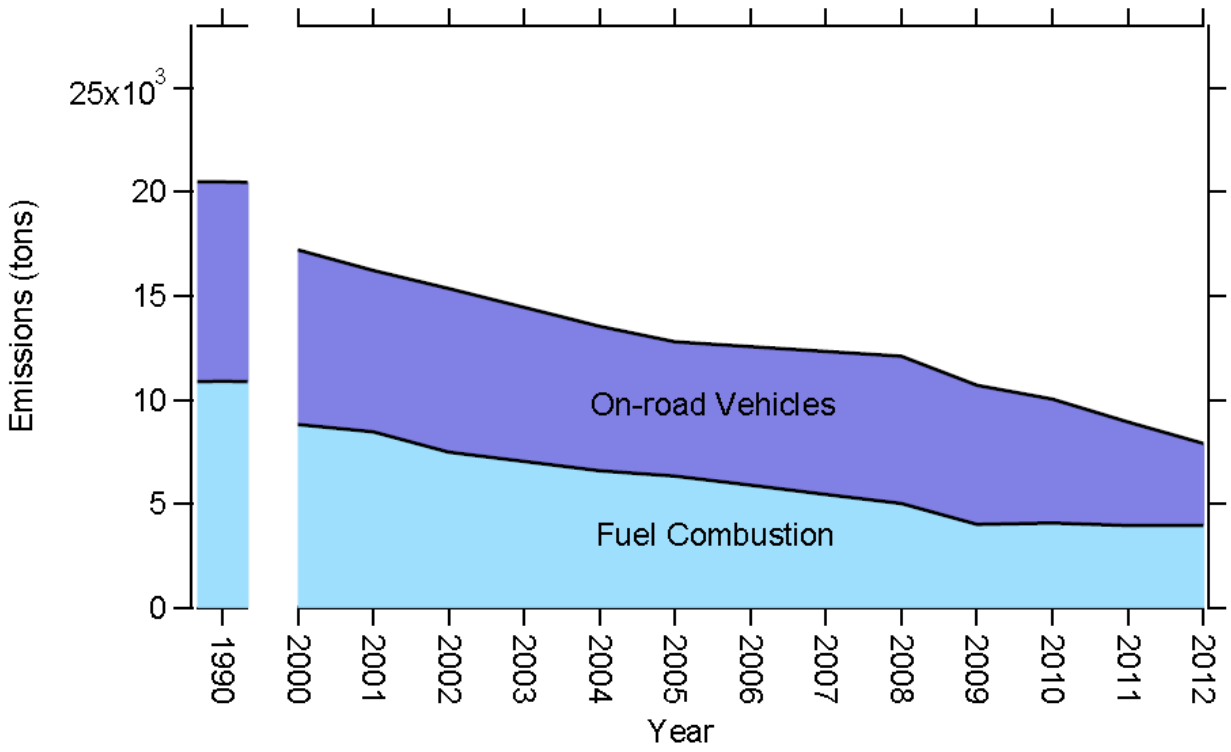


Note: Lightning is not included.

Source: U.S. EPA (2011)

Figure 2-2 Major sources of NO_X averaged over the United States, 2008.

Figure 2-3 describes the decrease in NO_X emissions in the U.S. for two major sources over the period from 1990 to 2012. Overall emissions decreased by more than 50% over this period, with substantial declines for both on-road vehicles and fuel combustion. It is possible to describe emissions through 2012 using the 2008 National Emissions Inventory because estimates later than 2008 are posted as updates, with methods for estimating sector emissions in these later years clearly described in the inventory documentation. These updates are still considered as versions of the 2008 inventory.



Source: U.S. EPA (2011)

Figure 2-3 National average NO_X emissions attributed to on-road vehicles and fuel combustion, from 1990 to 2012.

The composition of NO_X, specifically the ratio of NO_2 to total NO_X, can vary considerably among source types or between sources of the same type. Emission height can also have a substantial influence on resulting NO_X concentrations at ground level where most exposure occurs, with some sources emitted at or near ground level (e.g., highway vehicles) and others aloft from tall stacks (e.g., electric utility fuel combustion). These considerations were described in detail in the 2008 ISA for Oxides of Nitrogen. There have been some new developments with regard to composition as new control technologies have become available for mobile sources. As described in the 2008 ISA for Oxides of Nitrogen, the fraction of NO_2 in total NO_X emissions from the exhaust of gasoline vehicles has generally been reported as only a few percent, but catalyzed diesel particle filters (CDPFs) can increase the NO_2 fraction to 30-70% (U.S. EPA, 2008c).

Improvement of NO_X emission control technology is an active area of research. A number of new advances have been reported for electric utility and motor vehicle engine emission abatement. For electric utilities, emission control strategies fall into three broad categories: (1) pre-combustion modification through fuel purification or fuel choice to

reduce the amount of nitrogen introduced, (2) combustion modification by reducing combustion temperature, creating oxygen deficient conditions, or varying residence time within different parts of the combustion zone; or (3) post-combustion treatment. Emission control technologies were thoroughly reviewed recently (Skalska et al., 2010). The most widely used control technology is selective catalytic reduction (SCR) with ammonia in the presence of oxygen (Bruggemann and Keil, 2008).

For gasoline powered vehicles, emission control is usually achieved with a three-way catalyst for simultaneous control of NO_X, CO, and hydrocarbons (Heeb et al., 2008), in which NO is reduced to N_2 by CO (Roy and Baiker, 2009). However, this approach is not as effective for diesel or lean burning gasoline engines because oxygen levels are too high (Brandenberger et al., 2008; Takahashi et al., 2007).

New technologies developed for diesel are NO_X storage reduction (NSR) and selective NO_X recirculation (SNR) (Roy and Baiker, 2009). These and other methods of NO_X emission control applied to diesel engines before 2010 were recently reviewed by Skalska et al. (2010). Since 2010, SCR, which was originally developed for electric utility emission controls, has been applied to diesel emission control to achieve even lower emissions. SCR with ammonia proved unfeasible for diesel emission control because of slip, manipulation, storage, and corrosion problems (Skalska et al., 2010), but replacing ammonia with urea led to successful application of SCR control technology to diesel emissions control (Johnson et al., 2009).

2.3.2 Highway Vehicles

Highway vehicles account for a large fraction of NO_X emissions in high traffic areas. 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).

The relative importance of diesel and gasoline engine related NO_X emissions varies considerably among airsheds. Mcdonald et al. (2012) estimated that diesel engines were the dominant on-road NO_X sources in the San Joaquin Valley, accounting for up 70% of NO_X emissions. In contrast in Fulton County, Georgia 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.

NO_X emissions from on-road diesel engines in the U.S. have decreased substantially as the result of stricter emission standards. Emission standards for heavy duty diesel trucks were first established at 10.7 g/bhp-h in 1988 and decreased to 2.0 g/bhp-h for the 2004 model year and after (66 FR 5002). Standards were achieved mainly through development of selective catalytic reduction (SCR) with urea (Johnson et al., 2009). A NO_X emission 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 have been reduced by more than a factor of 50 between 1988 and 2010.

As discussed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), increases in the NO_2/total NO_X emission ratio have been observed in exhaust from diesel engines equipped with catalyzed diesel particulate filters (CDPFs) and diesel oxidation catalysts (DOCs). Both CDPF and DOC control techniques involve oxidation of exhaust pollutants through catalysis reactions for regeneration of filter media and effective removal of targeted species, respectively. During catalysis, combustion-derived NO may be oxidized to NO_2, potentially increasing the amount of NO_X emitted as NO_2. The SCR control process also intentionally generates NO_2 to manipulate NO_2/NO_X ratios in order to optimize performance, and integrated NO_X/PM control systems are currently being developed (Johnson et al., 2009). However, the SCR control system is also designed to eliminate the excess NO_2 generated.

The HEI ACES study sampled NO_X emissions from a variety of engines equipped with exhaust gas recirculation (EGR) and DOC/CDPF, which represent the typical control device configuration used in 2007-2009 EPA compliant diesel engines (Khalek et al., 2011). NO_X emissions averaged 1.09 ± 0.15 g/bhp-h, which is 73% and 9% lower than the 1998 and 2007 EPA standards, respectively. However, average NO_2 emissions from 2007-2009 compliant engines were 0.73 ± 0.12 g/bhp-h, indicating that NO_2 from newer, 2007 engines is 33% higher than emissions from 1998 EPA compliant engines. NO_X emissions from 2010 EPA compliant engines designed to meet the 0.20 g/bhp-h standard are also being evaluated in the HEI ACES study, but results are not yet published.

2.3.3 Off-Highway Vehicles

Off-highway sources include aviation, marine, and railroad engines as well as agricultural and industrial equipment, all of which emit NO_X through combustion processes. Aviation, marine, and railroad engines are treated in the following section. In this section, other off-highway engines are considered. A few examples of these engines include farm tractors, excavators, bulldozers, and wheel loaders. Emissions from the nonroad source sector can also significantly contribute to local and national air quality. On a national

scale, Zhu et al. (2011) estimated that nonroad diesel engines contribute 12% of total NO_X emissions from mobile sources.

Though limited measurement data exists on non-road diesel engine emissions, studies show that these emissions are widely variable across engine type, engine operating mode, engine age, and ambient temperature, with most studies focusing on differences in emissions during different operation modes (Fu et al., 2012b; Zhu et al., 2011; Abolhasani et al., 2008). Fu et al. (2012b) studied NO_X emissions from a variety of construction equipment in Beijing, China, including 12 excavators and 8 wheel loaders. They found that NO_X emissions factors during working mode were 3.66 and 1.36 times higher than emission factors during idling mode and moving mode, respectively. Similar to Fu et al. (2012b); Abolhasani et al. (2008) observed large differences in NO_X emissions from three different excavators during various operating modes. These differences in emissions were larger than the variation in emissions among different engines. Together these studies emphasize the importance of considering intercycle operating modes when estimating non-road diesel engine emissions using modeling approaches.

EPA has set a series of standards to reduce non-road diesel NO_X emissions, referred to as Tier 1-4 standards. The most recent standard, Tier 4, was introduced in May 2004, and the fleet turnover is currently underway, covering a time period between 2008 and 2015. In most cases, advanced diesel engine design has been used to comply with these standards.

2.3.4 Aviation Emissions

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 NO_X in the background air. 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., NH_3, CO, SO_2, VOC), airport NO_X emissions had the largest contribution to regional air quality in Atlanta, GA.

Different aircraft operations and ground-level airport activities impact NO_X emissions. Higher NO_X emissions are typically observed during more power-intensive operations such as landing and take-off cycles (LTO) compared to taxiing and idling (Klapmeyer and Marr, 2012; Mazaheri et al., 2011, 2009). Klapmeyer and Marr (2012) showed that emission indices (mass of pollutant per mass of fuel used) for LTO cycles can be 3 to 7

times higher than taxiing operations at a regional airport in Virginia. Engine type and aircraft/cargo weight also impact the amount of NO_X emitted from an aircraft (Carslaw et al., 2008). Ground-level support activities and auxiliary power units are also important sources of NO_X emissions at airports. In some cases, these NO_X emissions from ground level activities can be comparable to, or more than, NO_X emissions from aircraft operations (LTO cycles) (Klapmeyer and Marr, 2012).

2.3.5 Shipping Emissions

Globally, shipping emissions are a significant source of nitrogen 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., 2011b; Williams et al., 2009; Vutukuru and Dabdub, 2008). In Los Angeles, CA Vutukuru and Dabdub (2008) estimated that commercial shipping contributed approximately 4.2% to total NO_X emissions in 2002. Using the NEI-05, Kim et al. (2011b) estimated that roughly 50% of NO_X concentration near the Houston Shipping Channel is associated with commercial shipping emissions. However, this estimation is much higher than observed in satellite and aircraft measurements.

NO_X emissions vary among different ship types and operation modes. NO_X emissions are typically lower in docked ships compared to underway ships. In the Houston Shipping Channel, Williams et al. (2009) calculated NO_2 emission factors, and they found that they were highest for bulk freight carriers (87.0 ± 29.6 g NO_2/kg fuel), followed by underway tanker ships (79 ± 23 g NO_2/kg fuel) and other smaller underway vessels (stationary vessels) (60 g NO_2/kg fuel). Despite the large variability in shipping emissions, no clear trend in emissions could be drawn from differences in engine load and speed. Though these studies report unique findings, uncertainties exist in current shipping emission inventories, making it challenging to accurately estimate air quality impacts of shipping emissions (Kim et al., 2011b; Williams et al., 2009; Corbett et al., 1999).

2.3.6 Locomotive Emissions

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 non-road 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 non-attainment areas (U.S. EPA, 2010a). 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, 2009a). Cahill et al. (2011) measured gaseous and PM pollutants during a two-week period near the Roseville Rail Yard in Placer County, California. They observed several transient NO_X emission events, where NO levels of 100s of ppb were observed downwind of the Rail Yard, which was roughly 7 times larger than the observed urban background NO.

Limited measurement data exist on emissions from locomotives. Among these studies, there is evidence that NO_X levels from locomotives vary spatially and temporally depending upon train activity. On a per fuel basis, engine idling is associated with the highest NO_X emission factor compared to activities at higher engine loads (or notches) (Sawant et al., 2007). Sawant et al. (2007) emphasized the importance of this trend since many types of locomotives (switching yard locomotives) spend a significant amount of time idling (U.S. EPA, 2010a; Sawant et al., 2007). However, most of these studies measured emissions from locomotives equipped with older emission control technology and may not entirely reflect emissions from locomotives that are currently operating.

2.3.7 Fuel Combustion for Electrical Utilities and Industrial Use

NO_X from coal combustion is emitted primarily as NO, increases with decreasing temperature, and also increases with increasing nitrogen content of the coal (Kim et al., 2011a). Emission control strategies, including widely used SCR controls described in Section 2.3.1, have been thoroughly reviewed by Skalska et al. (2010). Decreased NO_X emissions as well as decreases in predicted ozone concentrations have been attributed to power generation controls Gégo et al. (2008). Satellite data consistent with decreasing power plant emissions over time are presented in detail in Section 2.4.3.

NO_X emissions from electric utility power plants have decreased considerably since the Clean Air Act Amendments of 1990 (CAA, 1990b). The Acid Rain Program (described by Title IV of the Amendments) targeted SO_2 and NO_X emissions from coal-fired power plants and other major stationary sources. Title I addressed regional transport of ground level O_3, aiming to reduce transport of O_3 across state boundaries in the eastern U.S. Title I led to the formation of the Ozone Transport Assessment Group (OTAG), a partnership between the U.S. EPA, the Environmental Council of the States and various industry and environmental groups to assess long-range transport of O_3 and O_3

precursors, and their efforts eventually resulted in the U.S. EPA's publication of the NO_X SIP Call (63 FR 57356) to control NO_X concentrations in order to reduce O_3 concentrations.

Title IV set NO_X emission limitations for coal-fired electric utility boilers to be implemented by 1995. The first phase set targets for 239 older coal-fired generating units. Emission targets were achieved mainly by retrofitting with low-NO_X burners or similar modifications that control fuel and air mixing to limit NO_X formation. Average NO_X emission rates from these units decreased by 40% between 1990 and 1996.

This first phase was followed in 1998 by the NO_X SIP Call which required 22 eastern states (later amended to 20 states) and the District of Columbia to set statewide O_3-season NO_X budgets, with emission reduction measures to be in place by 2003. As a result, summertime NO_X emission rates from the electric power generating units, which were subject to control, decreased by approximately 50% between 1999 and 2003, as measured by Continuous Emission Monitoring System (CEMS) measurements at a subset of these power generating units (Frost et al., 2006). The NO_X SIP Call target emission rates were considerably lower than those established by the Title IV Acid Rain Program, and were achieved primarily through more advanced controls, such as selective catalytic reduction (SCR) and selective non-catalytic reduction (SNCR).

Satellite based observations confirm these reductions in power plant NO_X emissions. van der A et al. (2008) found a consistent decline of NO_2 concentrations at 7% per year for the period from 1996–2006 over the eastern United States. Stavrakou et al. (2008) found a decrease in emissions of 4.3% per year over the Ohio River Valley from 1997 to 2006 from the tropospheric NO_2 column based on decreases in NO_2 column measurements in locations where electric utility power plant emissions predominate. National scale spatial variability is further explored in Section 2.5.1.

2.3.8 Biogenics and Wildfires

Major biogenic sources of NO_X in the U.S. include controlled biomass burning, vegetation, and soil. Uncertainties in natural NO_X emissions are much larger than for anthropogenic NO_X emissions.

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 ranging 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 can be more than double per amount of energy consumed for forests than for shrubs (Mebust et al., 2011).

NO_X emissions increase with increasing fuel nitrogen content (Burling et al., 2010). Burning conditions also play an important role, with biomass emissions higher during flaming than during smoldering conditions (Burling et al., 2010), but fuel nitrogen content is more important than burning conditions (Burling et al., 2010; McMeeking et al., 2009). Biomass burning also produces HONO in both laboratory (Roberts et al., 2010; Keene et al., 2006) and field conditions (Yokelson et al., 2009; Yokelson et al., 2007).

NO_X concentrations in plumes from boreal forest fires as well as tropical biomass burning for agricultural purposes decay with time due to the formation of HNO_3, and NO_X reservoir species such as PAN (e.g., Alvarado et al., 2010; Leung et al., 2007; Real et al., 2007; Mauzerall et al., 1998; Jacob et al., 1992). Rapid conversions of NO_X to PAN and pNO_3 have been observed in wildfire plumes (Akagi et al., 2012).

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. Nationwide, about 60% of the total NO_X emitted by soils is estimated to occur in the central corn belt of the United.States. Spatial and temporal variability in NO_X emissions from soil leads to considerable variability in emission estimates. However, these emissions are relatively low, comprising only about 6% of total anthropogenic NO_X emissions. Additionally, these emissions occur mainly during summer when O_3 concentrations are highest across the entire country, including areas where anthropogenic emissions are low.

2.3.9 Lightning

Lightning is not included in the emission inventory of Figure 2-2, but it is an important source that varies with season, region, and altitude. It has been well established that lightning produces NO, which influences atmospheric chemistry (Chameides and Walker, 1973; Crutzen, 1973; Crutzen, 1970). For example, Noxon (1976) and Noxon (1978) first reported direct observations of NO_2 concentrations up to 100 ppb near lightning flashes. Lightning is usually a minor contributor to urban ground-level NO_X concentration, but it can be important on a regional or national scale. For example, lightning has been widely recognized as a particularly important source over the U.S. in the summer, especially in the Southeast (Hudman et al., 2007; Bond et al., 2001; Biazar and McNider, 1995). Kaynak et al. (2008) estimated that nearly 30% of all U.S.-wide NO_X generation during

July could be attributed to lightning. However, lightning-generated NO_X generally deposits in the free troposphere Pickering et al. (1998), and several recent studies concluded that an even greater fraction ends up in the free troposphere than previously thought (Fang et al., 2010; Ott et al., 2010; Kaynak et al., 2008). NO_X depositing in the free troposhere has been observed to occur in part because intracloud flashes are more productive relative to cloud-to-ground flashes than previously considered (Ott et al., 2007; DeCaria et al., 2005). For example, Fang et al. (2010) estimated that lightning-generated NO_X over the U.S. for July 2004 was ~40% of the anthropogenic emissions for the same period, but the authors estimated that ~98% is formed in the free troposphere; therefore, contributions to the ground-level NO_X burden are low because most of this NO_X is oxidized to NO_3^- -containing species during downward transport into the planetary boundary layer. The remaining 2% is formed within the planetary boundary layer itself.

There is greater uncertainty in NO_X production from lightning than from other sources, with recent global estimates ranging from 2 to 8 Tg/year (Schumann and Huntrieser, 2007). Recent research has advanced our understanding of NO_X production from lightning, with results generally suggesting that lightning is a somewhat more important source than previously thought. Recent research suggests that the amount of NO_X produced per flash of lightning (a widely used modeling parameter) was previously substantially underestimated (Jourdain et al., 2010). Ott et al. (2007) and Ott et al. (2010) recommended a mean value of 500 moles NO_X per flash for both cloud-to-ground and intracloud flashes based on field observations. Peterson and Beasley (2011) investigated ice crystals as a catalyst for production of NO_X by lightning, and they estimated 2.7 times more NO is produced when ice crystals are present. Finally, enhancement of lightning by urban aerosols, first suggested by Westcott (1995), has been further documented (Kar et al., 2009), and its impact on increasing lightning generated NO_X is an active area of research (Yuan et al., 2012; Wang et al., 2011).

2.3.10 Oil and Gas Development

The oil and gas production sector is an increasing source of NO_X, with 2008 emission estimates of 400,000 tons nationally. A number of operational activities contribute to emissions from oil and gas production facilities. Pacsi et al. (2013) estimated that routine operating activities from the Barnett Shale production facility near Dallas, Texas can emit roughly 46 to 30 tons of NO_X/day, depending on the demand for natural gas electricity generation. Non-routine gas flares can also result in episodic peaks of large NO_X emissions, affecting local air quality (Olaguer, 2012). While a majority of production facilities are located in remote areas, emissions can impact regional air quality, including

major urban centers (Olaguer, 2012) and national parks (Rodriguez et al., 2009) downwind of these facilities.

2.4 Measurement Methods

2.4.1 Federal Reference and Equivalent Methods

Nitric oxide (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. 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).

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 (measuring total oxides of nitrogen) 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. Hence, the chemiluminescence analyzers could be subject to unknown and varying interference. This interference by NO_Z compounds has long been recognized based on intercomparisons of measurements using the FRM and other techniques for measuring NO_2 (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 five week period during the spring of 2004 was due to interference

from NO_Z compounds. Comparable levels of NO_2 are found in many locations in the U.S. However, similar comparisons have not been carried out under conditions typical for AQS monitoring sites in the United States. 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 rural study in 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. Since 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 (in 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.

To summarize the discussion of NO_2 measurements by the FRM: 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, chiefly HNO_3 and PAN as well as other oxidized *N*-containing compounds. Note, though, the magnitude of this positive bias is largely unknown and can change rapidly. Measurements of these oxidation products in urban areas are sparse. Concentrations of these oxidation products are expected to peak in the afternoon because of the continued oxidation of NO_2 emitted during the morning rush hours during conditions conducive to photochemistry in areas well downwind of sources, particularly during summer.

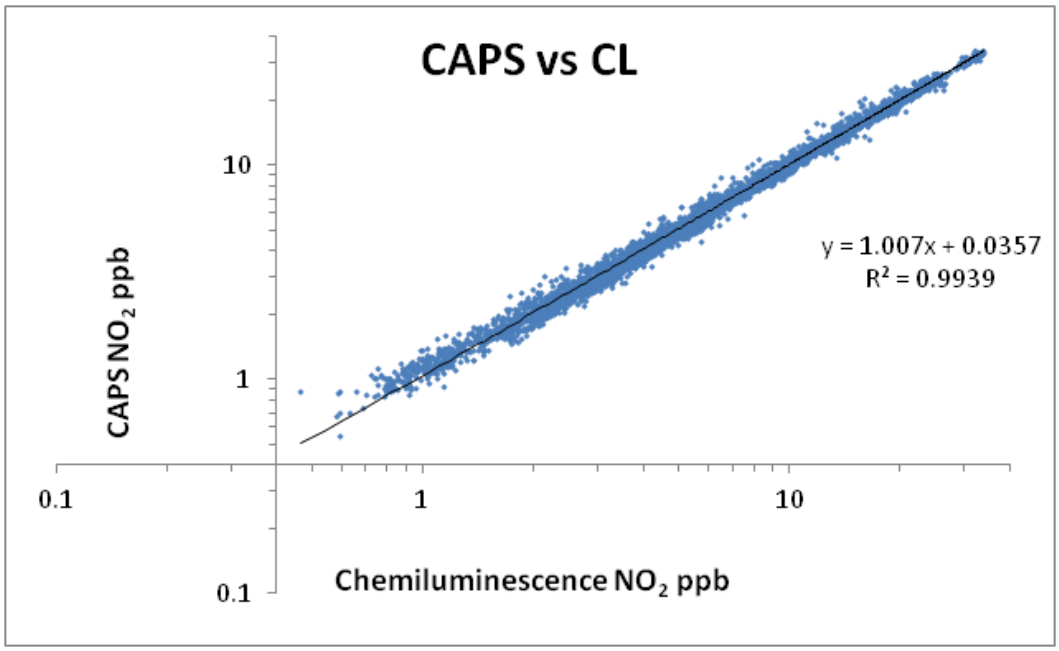
Within the urban core of metropolitan areas, where many of the ambient monitors are sited close to strong NO_X sources such as motor vehicles on busy streets and highways (i.e., where NO_2 concentrations are highest), the positive artifacts due to the NO_2 oxidation products are much smaller on a relative basis, typically <~10%. Conversely, the positive artifacts are larger in locations more distant from NO_X sources (i.e., where NO_2 concentrations are lowest) and could exceed 50%.

2.4.2 Other Methods for Measuring NO_2

Optical methods such as those using differential optical absorption spectroscopy (DOAS) or laser induced fluorescence (LIF) are also available. However, these particular methods are even more expensive than either the FRM monitors or photolytic reduction technique and require specialized expertise to operate as well; 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 60s time scale.

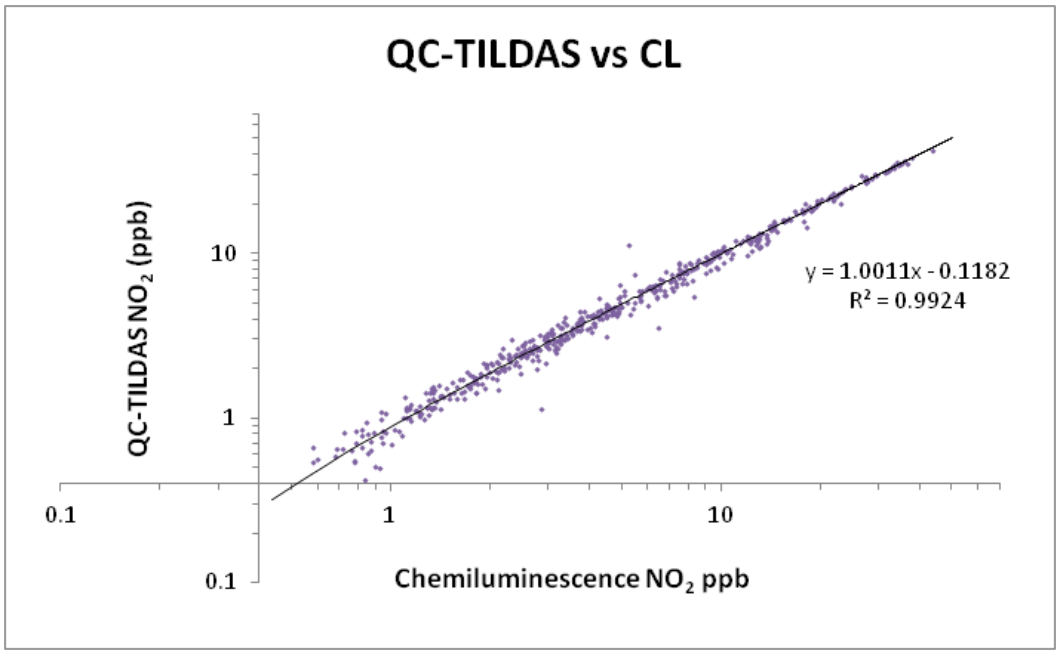
Lee et al. (2011a) describe the development of a dual continuous – wave mode quantum cascade – tunable infrared laser differential absorption spectrometer or QC-TILDAS to measure NO_2 and HONO simultaneously. The one-second detection limit (S/N = 3) is 30 ppt. Field comparisons of measurements of NO_2 by CAPS and QC-TILDAS to NO_2 measured by chemiluminescence monitors with MoO_X converters (CL/MC) in Houston,

TX, are shown in Figure 2-4 and Figure 2-5. Both figures show very high R^2 and close agreement over concentrations ranging from <1 ppb to >30 ppb and both comparisons are characterized by small non-zero intercepts. For the CAPS instrument (see Figure 2-4), slightly higher values than those reported by the CL/MC monitor are seen at concentrations < ~2 ppb. The CAPS – CL/MC (Thermo Electron 42I) data were obtained over 4 days in a parking lot located ~200 meters from a major arterial highway (Route 3 in Billerica, MA). Figure 2-5 shows that the QC-TILDAS obtains slightly lower concentrations than reported by CL/MC at concentrations < ~1 ppb. The measurements shown in Figure 2-5 were made under rather highly polluted conditions in Houston, TX, over a period of 4 weeks during the SHARP (Study of Houston Atmospheric Radical Precursors) campaign (Olaguer et al., In Press). Under polluted conditions such as these, the possibility of interference by NO_Z species in the measurements by CL/MC should be considered. Interference 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.



Source: NCEA, using data from Kebabian et al. (2008)

Figure 2-4 Comparison of NO_2 measured by CAPS (Cavity Attenuated Phase Shift) spectroscopy to NO_2 measured by chemiluminescence.



Source: NCEA, using data from Lee et al. (2013)

Figure 2-5 Comparison of NO_2 measured by QC-TILDAS (Quantum Cascade-Tunable Infrared Differential Absorption Spectroscopy) to NO_2 measured by chemiluminescence with photolytic converter.

Villena et al. (2011) describe the development of a long path absorption photometer, or 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 absorption in the visible is measured by long path photometry. This reaction was the basis for a much earlier manual method for measuring NO (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 chemiluminescence monitor with photolytic converter (CL/PC) conducted 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

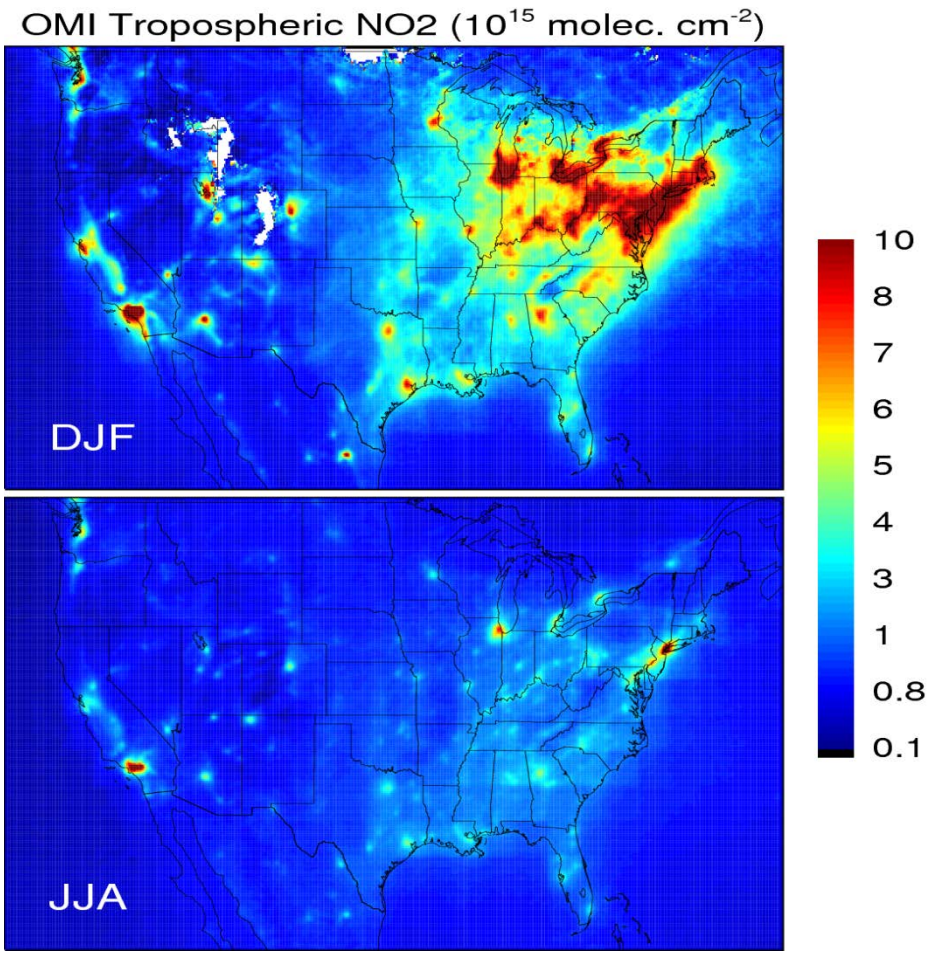
chemiluminescence monitors that measure NO_2 indirectly based on direct measurement of NO, NO_2 (formed by reaction of NO with excess O_3) is directly measured in CRDS. NO is then determined by subtracting NO_2 measured in the first cavity from the sum of NO_2 and NO (i.e., NO_X) measured in the second 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 quantitative than relying on the reduction of NO_2 and NO_Z species with variable efficiency on a Mo converter. Again, it should be noted 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 in general, concentrations for these potentially interfering species are generally lower than for NO_2. Note that 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.

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).

2.4.3 Satellite Measurements of NO_2

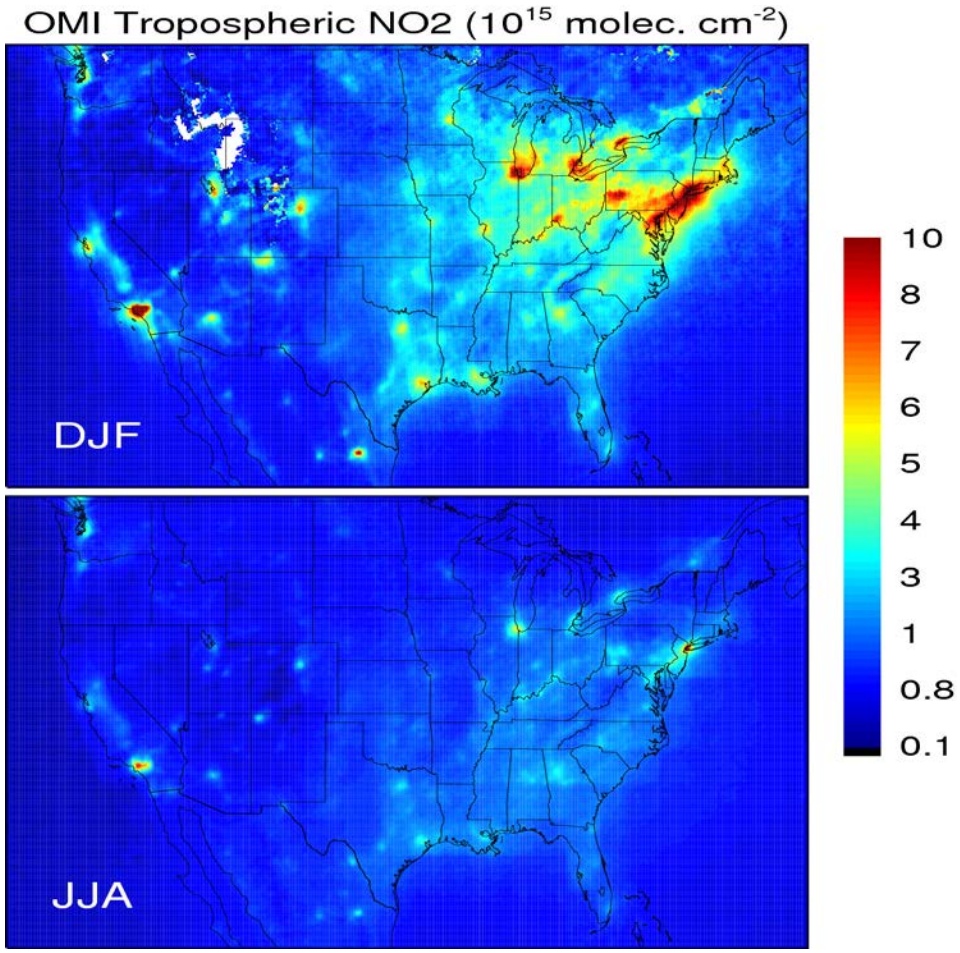
Remote sensing by satellites is an approach that could be especially useful in areas where surface monitors are sparse. The retrieval involves three steps: (1) determining the total NO_2 integrated line-of-sight (slant) abundance by spectral fitting of solar backscatter measurements, (2) removing the stratospheric contribution by using data from remote regions where the tropospheric column abundance[1] is small, and (3) applying an air mass factor (AMF) for the scattering atmosphere 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). Retrievals are largely limited to cloud fractions <20%. The current algorithm used 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 2-6 for 2005 to 2007 and in Figure 2-7 for 2010 to 2012 showing seasonal average NO_2 columns obtained by the Ozone Monitoring Instrument (OMI) on the AURA satellite.

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



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/instruments/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 2-6 Seasonal average tropospheric column abundances for NO_2 (10^{15} molecules/cm^2) derived by 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/instruments/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 2-7 Seasonal average tropospheric column abundances for NO_2 (10^{15} molecules/cm^2) derived by 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 periods shown in Figure 2-6 and Figure 2-7. 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 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 and since NO_2 is mainly a near surface pollutant, its

concentration will be sensitive to mixing layer heights, which are lower in winter than in summer. As a result, column abundances of NO_2 are lower in summer than during winter during both periods shown in Figure 2-6 and Figure 2-7.

However, since 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) used satellite data for column NO_2 from OMI combined with results from the GEOS-Chem global scale, chemistry-transport model to derive surface concentrations-to-NO_2 columns and by a factor accounting for feedbacks of NO_2 on its lifetime calculated by the GEOS-Chem global scale. GEOS-Chem is a chemical transport model to derive surface NO_2 concentrations (see Figure 2-12 for an example of seasonally averaged surface NO_2 concentrations derived by this method). Note though, this approach is based on data collected during the daily satellite overpass in early afternoon and thus is applicable only for time of satellite overpass in early afternoon.

Over the past decade, satellite measurements have shown appreciable reductions in NO_X power plant emissions across the U.S. as a result of emission abatement strategies (Stavrakou et al., 2008; Kim et al., 2006b). For instance, Kim et al. (2006b) observed a 34% reduction in NO_X emission over the Ohio River Valley from 1999-2006 due to such strategies. Based on these results, less than 25% of anthropogenic NO_X emissions were expected to originate from power plants in this region. Uncertainty in NO_X satellite measurements are impacted by several factors, such as cloud and aerosol properties, surface albedo, stratospheric NO_X concentration, and solar zenith angle. Boersma et al. (2004) estimated an overall uncertainty between 35-60% for satellite-retrieved NO_X measurements in urban, polluted regions. Although trends in satellite-retrieved NO_X power plant emissions reported by Kim et al. (2006b) are uncertain to some extent, similar reductions were reported by region-wide power plant measurements (e.g., Continuous Emission Monitoring System observations, CEMS).

2.4.4 Measurements of Total Oxidized Nitrogen Compounds (NO_Y) 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, close to sources of fresh combustion emissions, such as highways during rush hour, most of the NO_Y is present as NO_X. 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. However, it is worth reiterating that the direct measurements of NO are still the most reliable of all. 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.

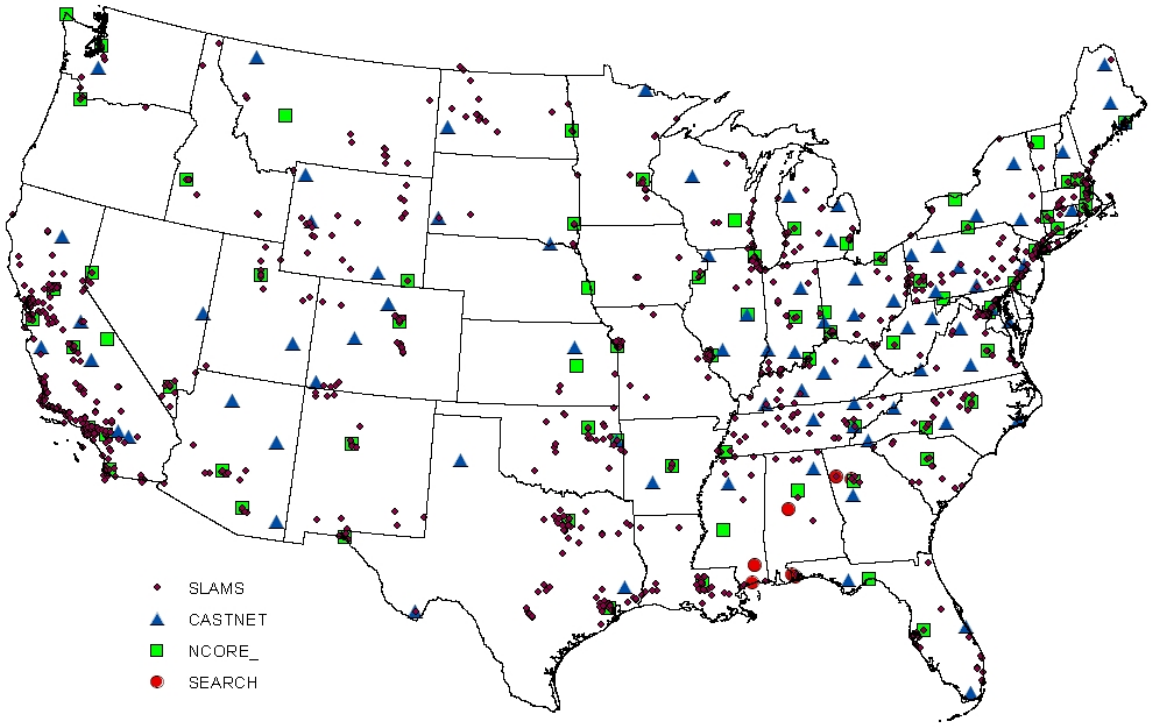
2.4.5 Ambient Sampling Network Design

Figure 2-8 shows routinely operating monitoring sites for approximately 500 ambient air oxidized nitrogen measurement sites across the U.S. Four networks are highlighted: (1) regulatory based State and Local Air Monitoring Stations (SLAMS) designed to determine NAAQS compliance, (2) National Core (NCORE) network of approximately 70 stations designed to capture area representative multiple-pollutant concentrations that provides routinely measured NO_Y, (3) 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), and (4) Clean Air Status and Trends Network (CASTNET) which provides weekly averaged values of total nitrate (HNO_3 and pNO_3) in rural locations.

With the exception of 4-6 sites in the Southeast Aerosol Research Characterization (SEARCH), 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 SLAMS/NAMS/PAMS Network Review Guidance (U.S. EPA, 1998b). NO_2 monitors are meant to be representative of several scales: microscale (in close proximity, up to 100 meters from the source), middle (several city blocks, 100 to 500 meters), neighborhood (0.5 to 4 km), and urban (4 to 50 km) (40 CFR Park 58, Appendix D). Micro-scale to neighborhood-scale monitors are used to determine highest concentrations and source impacts, while neighborhood- and urban-scale monitors are used for monitoring population exposures.

In recognition that roadway-associated exposures account for a majority of ambient exposures to peak NO_2 concentrations, EPA promulgated new minimum monitoring requirements in February of 2010 for state and local air monitoring agencies to install near-road NO_2 monitoring stations at locations where peak hourly NO_2 concentrations are expected to occur 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 Core Based Statistical Area (CBSA) with a population of 500,000 or more persons, 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, with the first of three phases scheduled to be operational in January of 2014.



Source: U.S. EPA (2013p)

Figure 2-8 Map of oxides of nitrogen monitoring sites in the U.S., from four networks (SLAMS, Castnet, NCore, and SEARCH).

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.

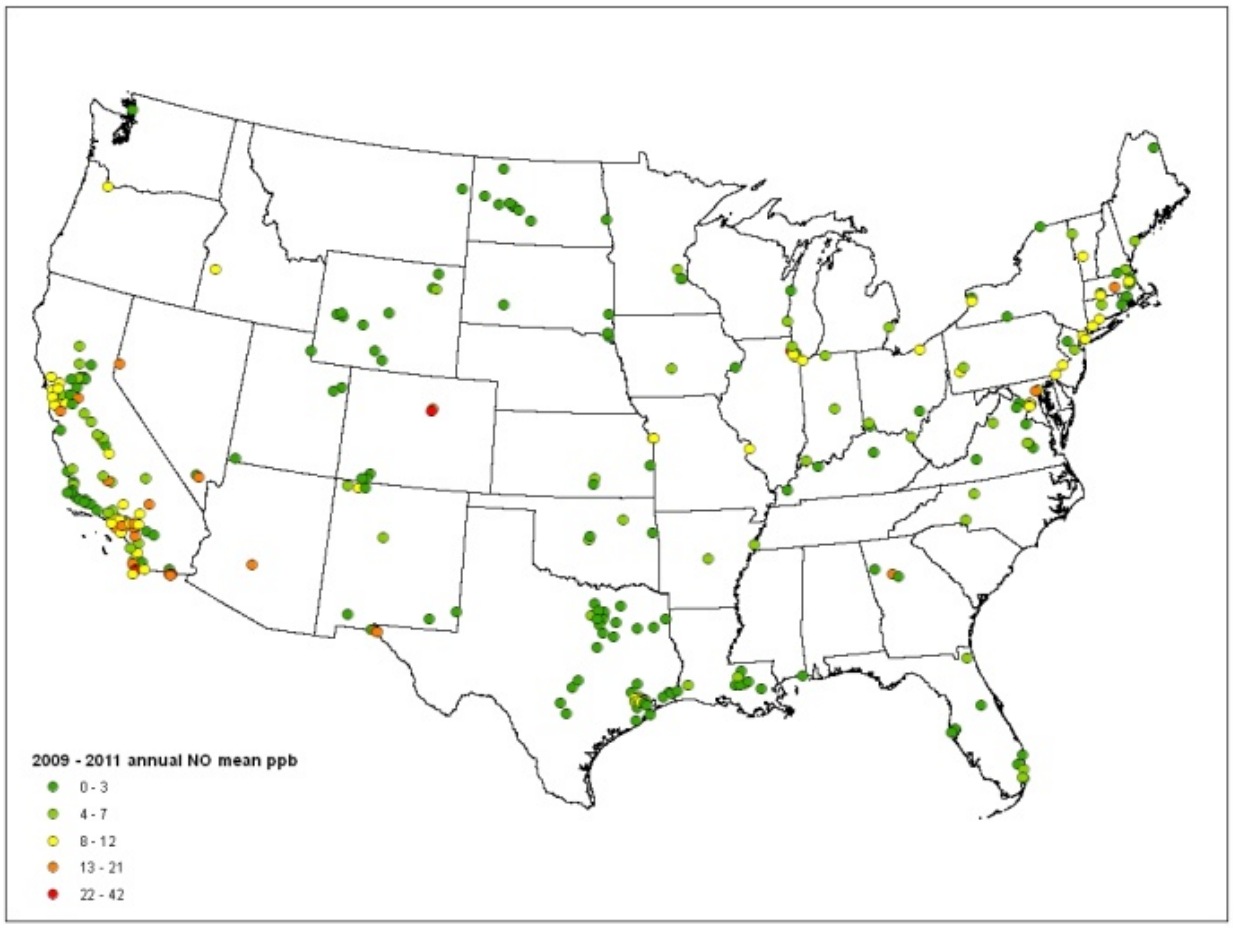
The 2008 ISA for Oxides of Nitrogen summarized NO_2 concentrations by explaining that the annual avg NO_2 concentrations of ~15 ppb reported by the regulatory monitoring networks are well below the level of the current NAAQS (53 ppb), but that the daily

maximum 1-h avg concentrations can be greater than 100 ppb in some locations, especially in areas with heavy traffic (U.S. EPA, 2008c).

2.5.1 National Scale Spatial Variability

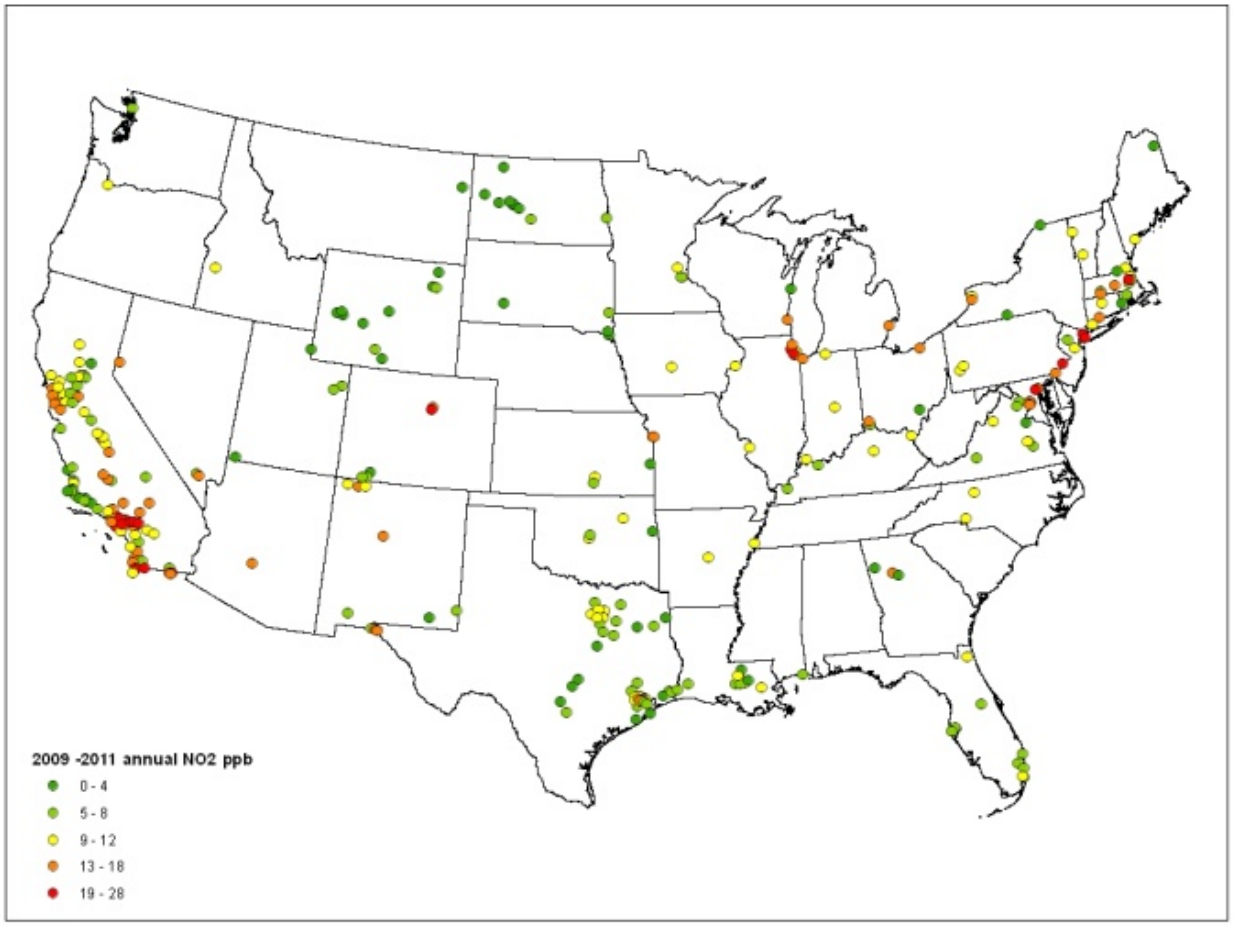
In the 2008 ISA for Oxides of Nitrogen, urban data were analyzed in several Consolidated Metropolitan Statistical Areas (CMSAs), with NO_2 measured at all monitoring sites located within MSAs or urbanized areas in the U.S. (U.S. EPA, 2008c). NO_2 concentrations were ~15 ppb for averaging periods ranging from a day to a year and average daily 1-h max NO_2 concentration was ~30 ppb, about twice as high as the 24-h avg. The highest maximum hourly concentration (~200 ppb) found was more than a factor of ten greater than the overall mean 24-h concentrations. Data on NO_Z concentrations were very limited in the 2008 ISA for Oxides of Nitrogen, with HNO_3 and HONO concentrations indicating that they were considerably lower than NO_2 concentrations. HNO_3 concentrations in one study 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 and HONO concentrations were several percent of those of NO_2 in traffic (U.S. EPA, 2008c). Field study results indicating much higher NO_Z concentrations than NO_X concentrations in relatively unpolluted rural air were also described (U.S. EPA, 2008c).

Figure 2-9, Figure 2-10, Figure 2-11, Table 2-1, and Table 2-2 present monitoring data for 2009-2011. Note that several mid-sized urban areas do not have data records capturing this three-year period. Table 2-1 and Table 2-2 present data from selected urban areas that are examined in recent epidemiological studies on the health effects of NO_2 (Chapter 4 and Chapter 5). The highest concentrations are evident in the Northeast Corridor and other urbanized regions, and the lowest concentrations are in sparsely populated regions, notably in the west. These observations are consistent with those described in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c).



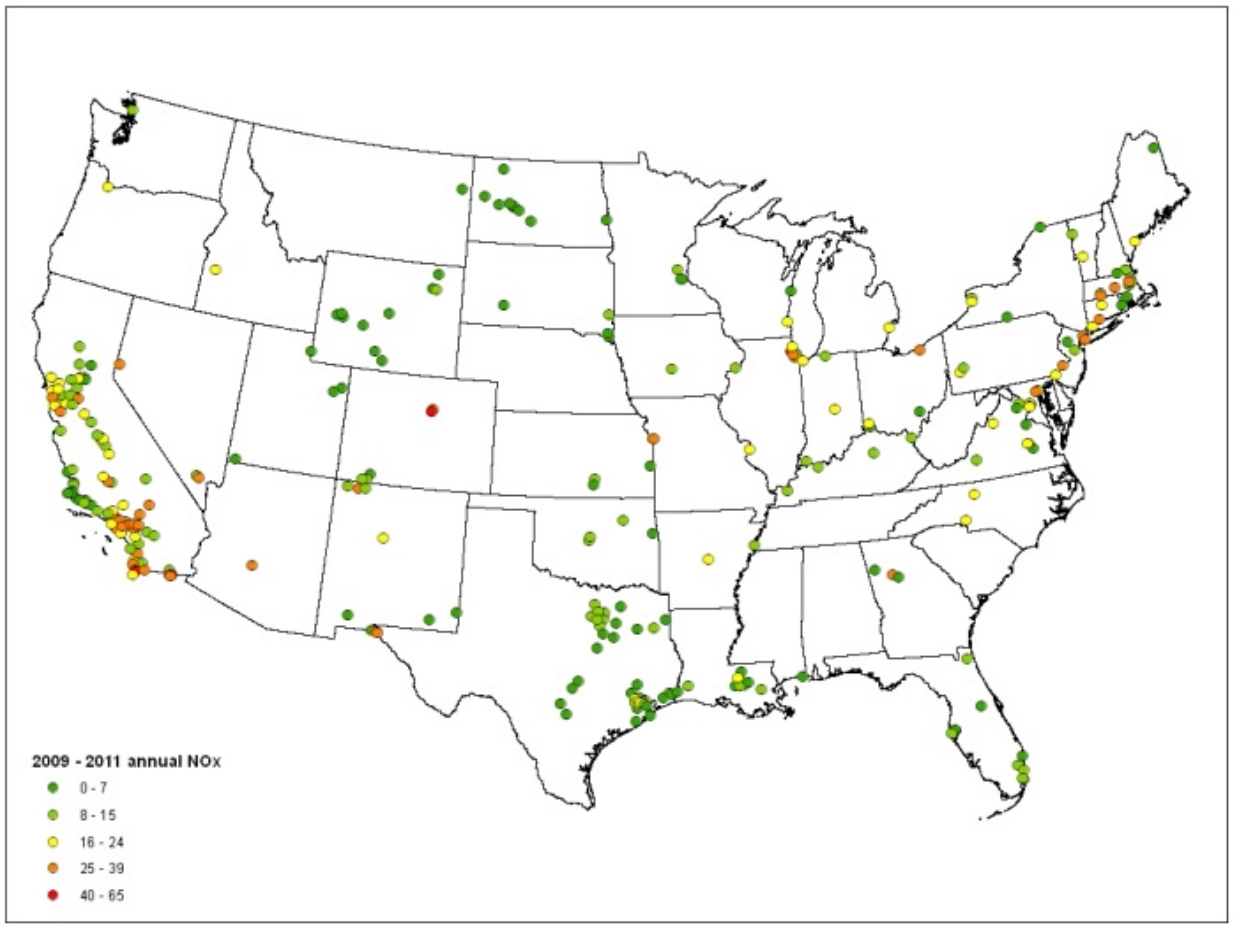
Source: OAQPS and NCEA analysis of AQS network data.

Figure 2-9 Annual average ambient NO concentrations for 2009-2011 at the site level for the SLAMS regulatory monitors.



Source: OAQPS and NCEA analysis of AQS network data.

Figure 2-10 Annual average ambient NO_2 concentrations for 2009-2011 at the site level for the SLAMS regulatory monitors.



Source: OAQPS and NCEA analysis of AQS network data.

Figure 2-11 Annual average ambient NO_X concentrations for 2009-2011 at the site level for the SLAMS regulatory monitors.

Table 2-1 Summary statistics for 1-hour daily maximum NO_2 concentrations based on 295 SLAMS monitoring sites (ppb).

	Year	N	Mean	Min	1	5	10	25	50	75	90	95	99	Max
Pollutant														
NO_2	2009-2011	389,946	20	0.1	1	2	4	8	17	29	39	45	57	360
NO_2	2009	130,681	20	0.1	1	3	4	9	18	29	40	46	58	215
NO_2	2010	131,308	20	0.1	1	2	4	8	17	28	39	45	57	141
NO_2	2011	127,957	19	0.1	1	2	4	8	16	28	39	45	57	360
NO_2	1st Quarter	95,266	23	0.1	1	3	5	11	22	34	43	49	60	215
NO_2	2nd Quarter	97,947	17	0.1	1	2	3	7	14	24	35	41	53	360
NO_2	3rd Quarter	99,500	17	0.1	1	2	3	7	14	23	34	41	54	229
NO_2	4th Quarter	97,223	22	0.1	1	3	5	10	21	23	41	47	60	137
City														
Atlanta[a]	2009-2011	3,237	14	1	1	2	3	4	8	20	36	44	54	77
Atlanta-all[b]	2009-2011	3,354	15	1	1	2	3	4	9	22	38	44	55	77
Boston[a]	2009-2011	7,210	23	0	4	7	9	13	21	30	36	44	53	197
Boston-all[b]	2009-2011	11,150	20	0	1	3	5	10	18	28	38	43	52	197
Denver[a]	2009-2011	1,739	39	0	5	14	21	30	39	47	55	61	72	94
Denver-all[b]	2009-2011	1,739	39	0	5	14	21	30	39	47	55	61	72	94
Houston[a]	2009-2011	11,932	20	0	1	3	5	10	17	29	36	45	55	82
Houston-all[b]	2009-2011	17,148	19	0	1	3	5	8	15	26	38	43	55	118
Los Angeles[a]	2009-2011	14,850	28	0	4	7	10	17	28	39	47	53	64	137
Los Angeles-all[b]	2009-2011	34,266	30	0	4	8	11	19	30	40	49	54	67	137
New York[a]	2009-2011	7,598	30	0	3	6	9	18	30	40	49	55	69	108
New York-all[b]	2009-2011	14,279	28	0	2	5	8	16	28	38	48	54	69	153
Seattle[a]	2009-2011	804	11	3	4	5	6	8	10	13	19	23	30	54
Seattle-all[b]	2009-2011	1,333	12	0	3	4	6	8	10	14	19	23	42	88

[a]City name only rows meet 75% completeness criteria.
[b]City-all rows report data regardless of whether completeness criteria are met.

Source: OAQPS and NCEA analysis of AQS network data.

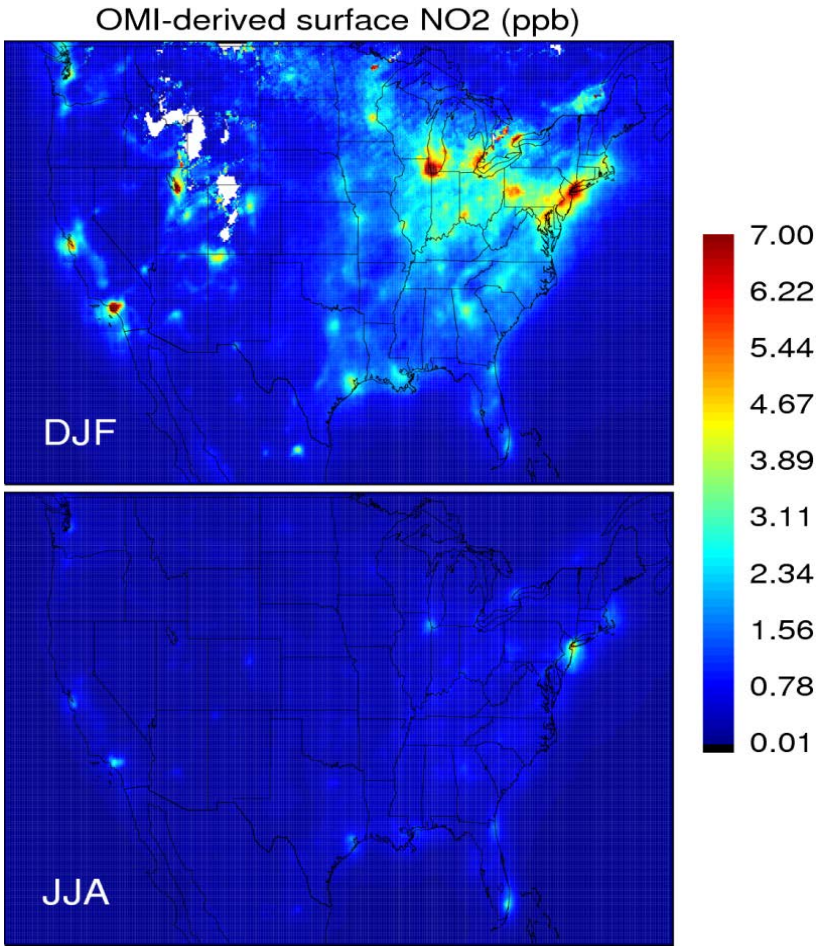
Table 2-2 Summary statistics for NO_2, NO and NO_X annual average concentrations based on 295 SLAMS monitoring sites (ppb).

Pollutant	Year	N	Mean	Min	1	5	10	25	50	75	90	95	99	Max
NO_2														
NO_2	2009-2011	295	9.42	0.25	0.63	1.83	2.71	5.00	8.63	12.95	17.17	20.62	24.56	27.66
NO_2	2009	295	9.75	0.23	0.50	1.82	2.64	5.06	8.65	13.42	18.18	21.51	27.35	30.84
NO_2	2010	295	9.33	0.24	0.51	1.83	2.76	4.92	8.56	12.87	17.14	20.02	24.87	27.70
NO_2	2011	295	9.19	0.19	0.60	2.02	2.68	5.05	8.35	12.67	16.78	20.23	23.73	24.66
NO														
NO	2009-2011	295	5.78	0.02	0.04	0.23	0.42	1.34	3.68	8.37	14.06	15.98	30.76	42.35
NO	2009	295	6.41	0.02	0.03	0.19	0.44	1.34	3.87	8.85	14.58	18.78	35.93	49.53
NO	2010	295	5.42	0.00	0.03	0.20	0.38	1.24	3.51	7.69	13.86	16.12	27.13	37.60
NO	2011	295	5.52	0.01	0.03	0.23	0.45	1.27	3.41	8.13	13.51	15.86	27.02	50.94
NO_X														
NO_X	2009-2011	295	15.20	0.27	0.66	2.15	3.3	6.28	12.67	20.81	30.46	34.25	54.29	65.16
NO_X	2009	295	16.15	0.27	0.54	2.05	3.26	6.52	12.39	21.78	33.05	38.22	59.91	74.65
NO_X	2010	295	14.75	0.27	0.52	2.11	3.31	6.36	12.37	20.42	29.18	35.99	50.22	58.29
NO_X	2011	295	14.71	0.20	0.70	2.38	3.45	6.15	11.83	19.72	30.04	33.80	50.46	75.59

Source: OAQPS and NCEA analysis of AQS network data.

Because of the short lifetime of NO_X due to oxidation from PANs and HNO_3, NO_X concentrations are highly spatially and temporally variable. Average concentrations range from tens of ppt in remote areas of the globe to tens of ppb in urban cores, i.e., by three orders of magnitude. Because ambient NO_2 monitoring data are so sparse across the U.S., especially in rural areas (see Figure 2-8 for location of monitoring sites), measurement data on NO_X concentrations across the continental U.S. are incomplete. The short lifetime of NO_2 with respect to conversion to NO_Z species and the concentrated nature of NO_2 emissions result in steep gradients and low concentrations away from major sources that are not adequately captured by the existing monitoring networks. 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 2-12. 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 CTMs is given in the O_3 ISA (U.S. EPA, 2013b).

Large variability in NO_2 concentrations is apparent in Figure 2-12. As expected, the highest NO_2 concentrations are seen in large urban regions, such as the Northeast Corridor, and lowest values are found in sparsely populated regions located mainly in the West. As can be seen, minimum hourly values are of the order of ~10 ppt, leading to a range between maximum and minimum concentrations of over a factor of a thousand. NO_2 concentrations tend to be higher in January than in July reflecting lower planetary boundary layer heights in winter and more widespread emissions from residential heating during 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/instruments/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 2-12 Seasonal average surface NO_2 concentrations in ppb for winter (upper panel) and summer (lower panel) derived by OMI/GEOS-Chem, for 2009-2011.

2.5.2 Urban Scale Spatial Variability

In the past decade considerable urban scale NO_X spatial variability was observed in several studies (Monn, 2001; Fischer et al., 2000; Kingham et al., 2000; Lebret et al., 2000). More recently, spatial variability has been further characterized, especially through determining factors influencing NO_2 spatial variations and refining methods for estimating urban scale concentrations.

This high spatial variability makes it impractical to rely solely on measurements to obtain urban scale concentrations. Instead, interpolation and modeling methods are often used, and these were recently reviewed (Briggs, 2005; Jerrett and Finkelstein, 2005). Of these methods, land use regression has emerged as a widely used tool for estimating urban scale air pollutant concentrations, particularly for NO_2. Land-use regression combines monitoring of air pollution at a small number of locations and development of stochastic models using predictor variables usually obtained through geographic information systems (GIS). A critical review of 25 recent applications of land use regression, many of them specifically targeting NO_2, concluded that land use regression models have generally been applied successfully in a variety of North American and European cities, and that its performance in predicting measured concentrations in urban areas is typically better or equivalent to geostatistical methods such as kriging and dispersion models (Hoek et al., 2008).

Jerrett et al. (2007) pioneered the use of land use regression modeling in North America by applying it to NO_2 in Toronto. They noted elevated NO_2 concentrations within 1,500 meters of roadways and concluded that small area variations probably due to traffic were captured by land use regression methods. In a highly industrialized urban area in Sarnia, Ontario, land use regression methods were used to determine that the factors responsible for elevated concentrations included proximity to the region's industrial core, placement within 1,600 meters of industrial areas, placement 400 meters from highways, and dwelling counts within 2,400 meters (Atari et al., 2008).

Hart et al. (2009) developed generalized additive models to spatially model NO_2 concentrations in the continental U.S., and concluded that distance to road, population density, elevation, land use, and distance to emissions of the nearest oxide of nitrogen-emitting power plant were all statistically significant predictors of measured NO_2.

Applications of land use regression for assessing exposure to NO_2 are described in detail in Section 2.6.2.3.

2.5.3 Micro-to-neighborhood Scale Spatial Variability, Including Near Roads

Complex spatial gradients in NO, NO_2, and NO_X concentrations have been observed in near road environments. Several factors that impact near-road NO_X concentrations, such as distance from roadway, traffic volume, and season, were discussed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c). These studies reported a sharp decline in NO_X concentration within 350 meters downwind of roadways (Gilbert et al., 2007; Singer et al., 2004) and several meters above street canyons (Restrepo et al., 2004). Another study by Monn (2001) reported the influence of meteorology and enhanced photochemical

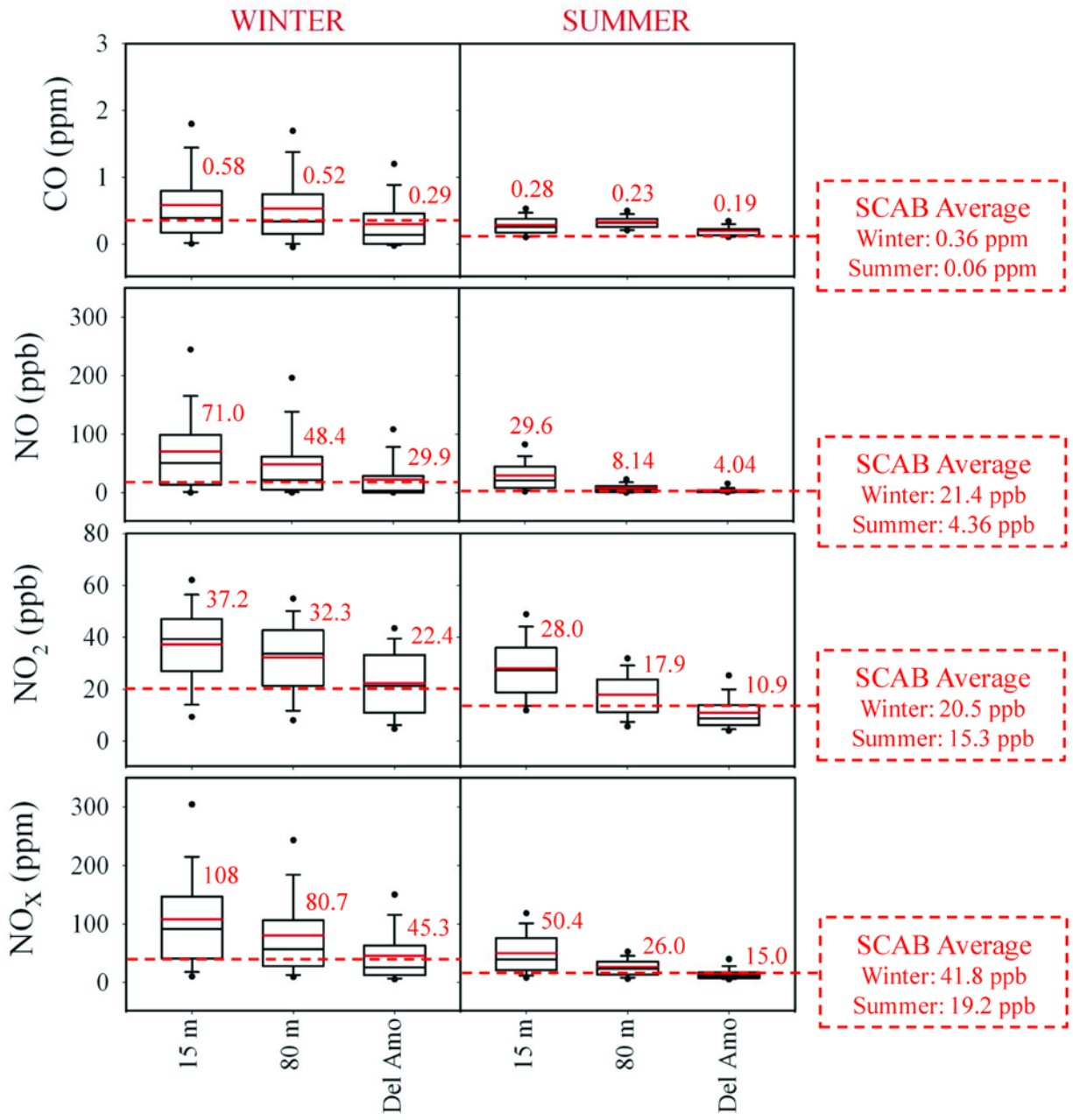
activity in the spring and summer on NO_2 spatial gradients was also discussed (Monn, 2001). This section discusses the characteristics and factors impacting near-road spatial gradients in NO_X concentrations.

Concentrations of NO, NO_2, and NO_X measured on or near roads have varied substantially depending on measurement location, fleet mix, and time of day. For example, Zhu et al. (2008) used a mobile monitoring station to measure NO_X on two freeways in Los Angeles, CA: I-405, which predominantly carries automobile traffic, and I-710, which carries the majority of diesel truck traffic for the area (~25,000 trucks per day) (Fruin et al., 2008). On I-405, average NO_X concentrations of unfiltered air measured 267 ppb with a maximum concentration around 850 ppb, while on I-710 unfiltered NO_X concentrations averaged 432 ppb with a maximum concentration around 950 ppb. Similarly, Fruin et al. (2008) measured on-road NO concentrations on three Los Angeles highways and five arterial roads. They observed average NO concentrations ranging from 170-390 ppb on the freeways, with the highest average concentration corresponding to the highest diesel truck traffic on I-710. On-road concentrations on arterial roads averaged 17-79 ppb. Baldauf et al. (2008a) presented time-series of near road pollutants measured 5 meters 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 rush hour period spanned 6:00 a.m. - 9:00 a.m., and exhibited a distinct peak during that time period. During the evening rush hour between 4:00 p.m. and 7:00 p.m., NO concentrations fluctuated between 20-150 ppb. Clements et al. (2009) measured concentrations of NO, NO_2, and NO_X, 5 meters 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. Taken together, these results suggest that NO_2 represented 10-38% of freshly emitted NO_X.

More recent studies show similar spatial gradients in NO, NO_2, and NO_X concentrations near roadways and also provide more fine-scale temporal and spatial information. In a pilot study to better understand issues involved in meeting new monitoring requirements for the 1-hour NO_2 NAAQS promulgated in 2010, state and local air agencies collected NO_2 and NO_X concentration data with passive sampling devices (PSDs) near heavily trafficked roads within five Core Based Statistical Areas (CBSAs): Albuquerque, New Mexico; Baltimore, Maryland; Boise, Idaho; and Miami–Broward County and Tampa-Hillsborough County in Florida (STi, 2011). The study confirmed that near-road NO_2 concentrations were generally highest at locations nearest the roadway and near those roads with the highest daily traffic. Deviations from this pattern could be explained by roadway configuration or other considerations (e.g., higher NO_2 observed because of

accelerating truck traffic on an on-ramp, site placement on a toll booth near a port, tunnel entrance/exit, and rail activities). Horizontal concentration gradients were relatively gradual, with concentrations decreasing slightly from 7 to 45 meters. Vertical concentration gradients were also evaluated, and the highest concentrations were typically at the sampling height closest to the roadway (typically closest to ground level), although concentration differences were relatively small.

Additional studies show that NO, NO_2, and NO_X concentrations decrease exponentially with greater horizontal distance from the roadway. Average concentrations near the roadway are typically 30% to 200% of urban background concentrations, and average concentrations fall to background levels within 100-500 meters of the roadway (Polidori and Fine, 2012; Karner et al., 2010; Beckerman et al., 2008; Zhou and Levy, 2007). This behavior is illustrated in Figure 2-13, which describes measurements from two monitoring stations in southern California, located 15 meters and 80 meters east and downwind of the I-710 freeway ("near" and "far" site, respectively), near the intersection with North Long Beach Boulevard. It also includes results from a monitoring site far from the influence of the I-710 and representative of background conditions was operated in Carson, CA, next to Del Amo Elementary School (labeled 'Del Amo' in Figure 2-13).



Note: The corresponding average levels for the South Coast Air Basin calculate for similar time periods are also included for comparison.

Source: Reprinted from South Coast Air Quality Management District; Polidori and Fine (2012)

Figure 2-13 Spatial distributions of CO, NO, NO_2, and NO_X concentrations at the "near" (15 meters), "far" (80 meters), and Del Amo (background) sites during winter and summer in southern California.

While levels of all NO, NO_2, and NO_X concentrations decrease from the roadway, different species have different decay profiles which are influenced by chemistry and atmospheric factors. NO typically decays more rapidly than NO_2 or CO, typically reaching background levels within the first 100 meters of the roadway. This trend suggests that chemical processing by O_3 as well as dilution is an important factor in determining NO concentrations in near road environments (Clements et al., 2009). This titration mechanism likely explains the more pronounced spatial gradient observed in NO during the summer when O_3 is prevalent due to enhanced photochemical activity (Polidori and Fine, 2012; Monn, 2001). This is consistent with results by Polidori and Fine (2012) (from a near-road field campaign in Los Angeles, CA) showing that roadside NO was 7 times higher than the background during the summer compared to 3 times higher during the winter.

The decrease in NO_2 concentrations from the road is more gradual than that of most other traffic related air pollutants (including NO) (Gordon et al., 2012; Beckerman et al., 2008), or does not even decrease with distance (Massoli et al., 2012). Quantitative reviews of near-roadway pollutant impacts demonstrate that the NO_2 spatial gradient extends further from the roadway (200-500 meters) than pollutants rapidly removed by chemical reaction (Karner et al., 2010; Zhou and Levy, 2007). Massoli et al. (2012) described another characteristic of NO_2 concentrations near roads, an abrupt increase after sunrise due to conversion of abundant NO near roads to quickly reach a NO_2 concentration "ceiling" that is limited by background O_3 levels. They concluded that over short temporal scales (shorter than the time scale for replenishment of background O_3) less than 500 meters from a source, NO_2 alone was not a good indicator of traffic related emissions. Production of other traffic related pollutants is not limited by available background O_3 concentrations.

In addition, near road profiles of NO_X concentration are largely influenced by meteorological parameters and atmospheric stability. Wind direction dictates the spatial profile of NO, NO_2, and NO_X concentrations (as well as other traffic pollutants) with more gradual gradients on the downwind side of the roadway (Durant et al., 2010; Clements et al., 2009; Hu et al., 2009; Beckerman et al., 2008). Wind speed and atmospheric stability also impact roadway NO_X concentrations. Peak roadway concentrations are often observed during pre-sunrise hours when winds are weak and atmospheric inversions are present (Gordon et al., 2012; Durant et al., 2010; Hu et al., 2009). During these pre-sunrise hours, the spatial impact of roadway NO_X concentrations may also extend, resulting in a more gradual decay from the roadway. 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 meters downwind of the roadway during pre-sunrise hours, which is much larger than the expected spatial extent of NO (100-300 meters) (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 near-background levels 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 because near-roadway concentrations are as low as background levels due to dilution (Gordon et al., 2012; Durant et al., 2010). However, the exact response of the concentration gradient to boundary layer expansion 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 may 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 concentrations 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. Under stable atmospheric conditions, Hu et al. (2009) reported elevated NO extended as far as 1,200 meters downwind of a roadway, which is much further than the expected spatial extent of NO in the daytime with lower

atmospheric stability (Karner et al., 2010). 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) also 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) also 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 turbulence kinetic energy from two-way traffic was roughly 20% higher than for one-way traffic, and they found that the turbulence 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, Gaussian dispersion caused vertical dispersion with the plume spread. 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., 2012a). 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., 2009b). 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.

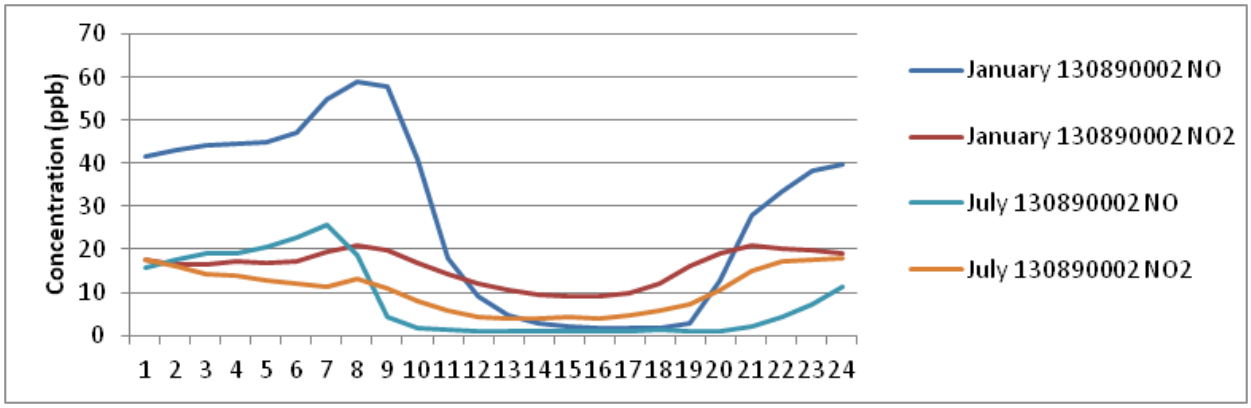
2.5.4 Seasonal, Weekday/Weekend and Diurnal Trends

Month-to-month variability in 24-h avg NO_2 concentrations was described in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c). 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. Differences between weekdays and weekends were also noted, with lower concentrations and a more compressed cycle on weekends, with more pronounced differences at sites more influenced by traffic. Observations of lower NO_2 concentrations on weekends are consistent with other recent results. Summer satellite data indicated 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).

Recent data presented in Table 2-1 continue to show similar seasonal trends for average seasonal concentrations across 3 years. Mean concentrations are highest in the first and fourth quarters, but maximum concentrations are highest in the second and third quarters.

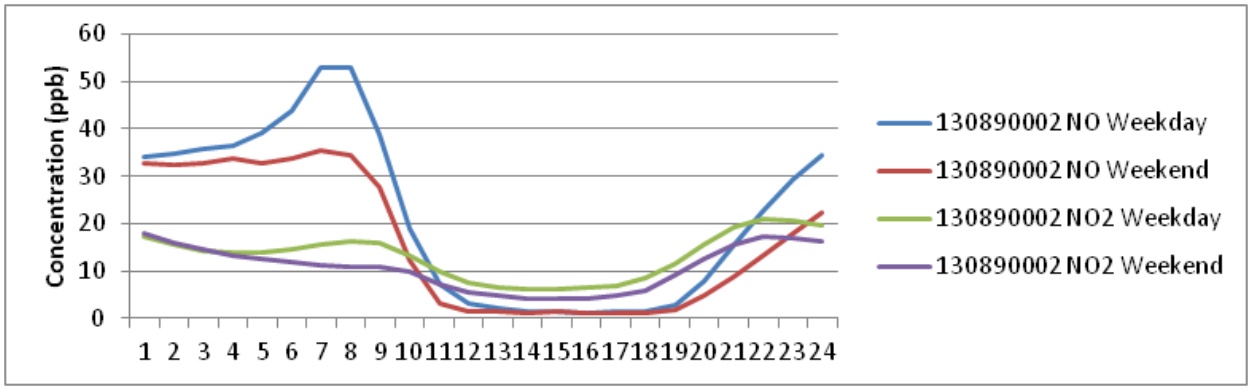
Recent data also indicate concentration patterns of NO and NO_2 are affected strongly by emissions and meteorology as concentrations peak during early morning hours and seasonally (higher in winter) when planetary boundary layer heights are lowest (Figure 2-14). NO_2 exhibits flatter profiles relative to NO as secondary formation processes influence concentration patterns.

Figure 2-15 shows a typical diurnal cycle for NO and NO_2. As described in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), the NO_2 typically exhibits a daily maximum during morning rush hour, although it can occur at other times of day. This pattern is shown for Atlanta, GA, in Figure 2-15, but it is also typical for other urban sites.



Source: EPA Analysis of AQS Data.

Figure 2-14 January and July hourly profiles of NO and NO_2 (ppb) for Atlanta, Georgia (site with maximum NO_2 levels).



Source: EPA Analysis of AQS Data.

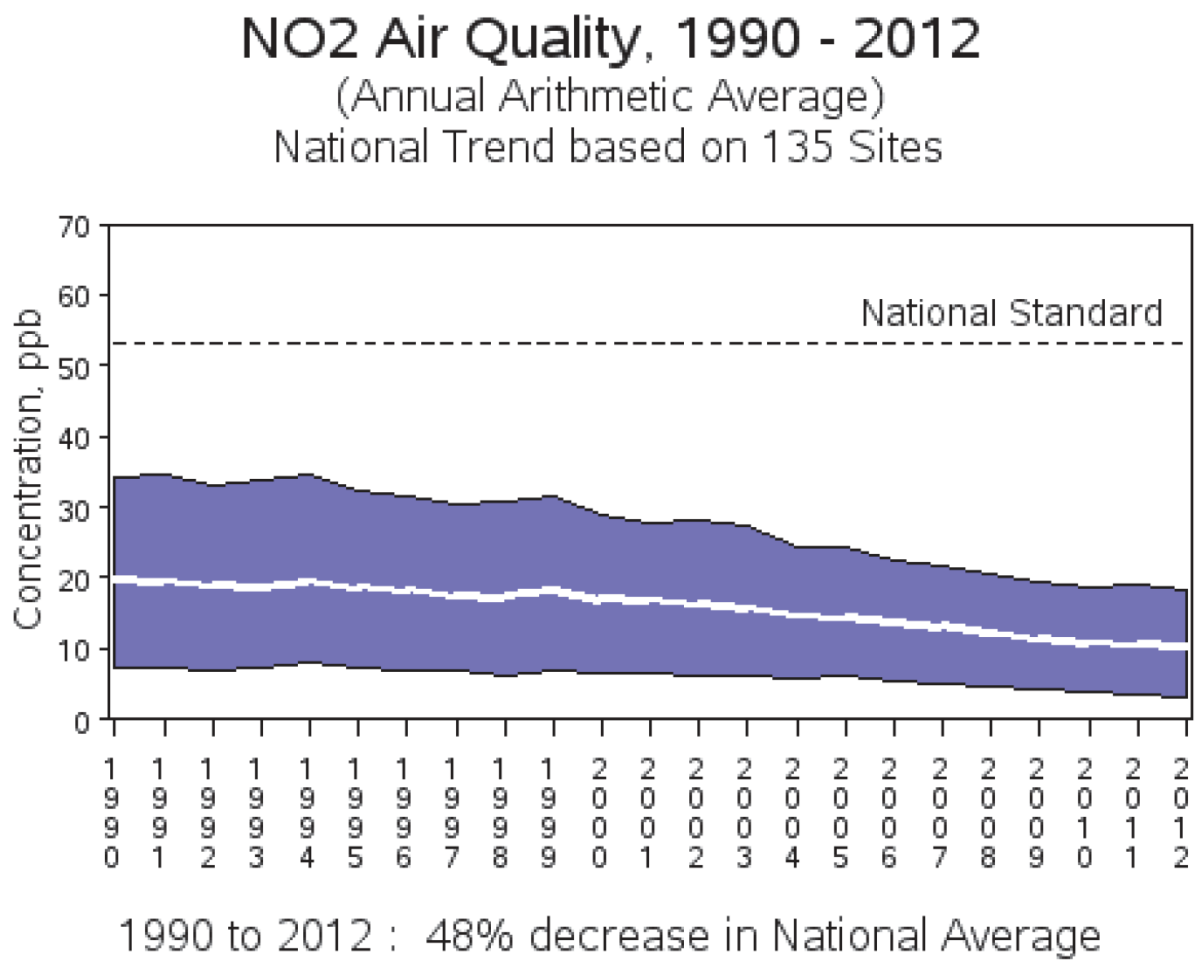
Figure 2-15 Weekend/Weekday hourly profiles of NO and NO_2 (ppb) for Atlanta, Georgia (site with maximum NO_2 levels).

Differences between weekdays and weekends are also observed. Typically, weekday levels of NO_X, particularly NO, exceed weekend levels and diurnal cycles are more compressed on weekends. The weekend effect for NO was first observed by Cleveland et al. (1974) and it is a general characteristic of urban NO and NO_X levels observed in many locations (Tonse et al., 2008; Pun et al., 2003; Marr and Harley, 2002). The lower concentrations of NO_X on weekends can have a profound but complex effect on urban atmospheric chemistry. The lower concentrations of NO_X on weekends can also substantially increase or decrease O_3 concentrations, depending on whether O_3 production is limited by VOC or NO_X concentrations (Tonse et al., 2008; Heuss et al., 2003). It can also lead to differences the composition of NO_Z between weekdays and weekends. For example, Pollack et al. (2012) observed greater production of HNO_3, a radical termination product, on weekdays and greater production of PAN, a VOC-NO_X oxidation product, on weekends in southern California.

2.5.5 Multi-year Trends in Oxides of Nitrogen

The annual average NO_2 concentration across the U.S. decreased by 48% from 1990 to 2012, as shown in Figure 2-16. 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 (cf. Figure 2-3).

In Atlanta, 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).



Source: U.S. EPA (2013)

Figure 2-16 National annual average ambient NO_2 concentration trends, 1990-2012.

2.5.6 Background Concentrations

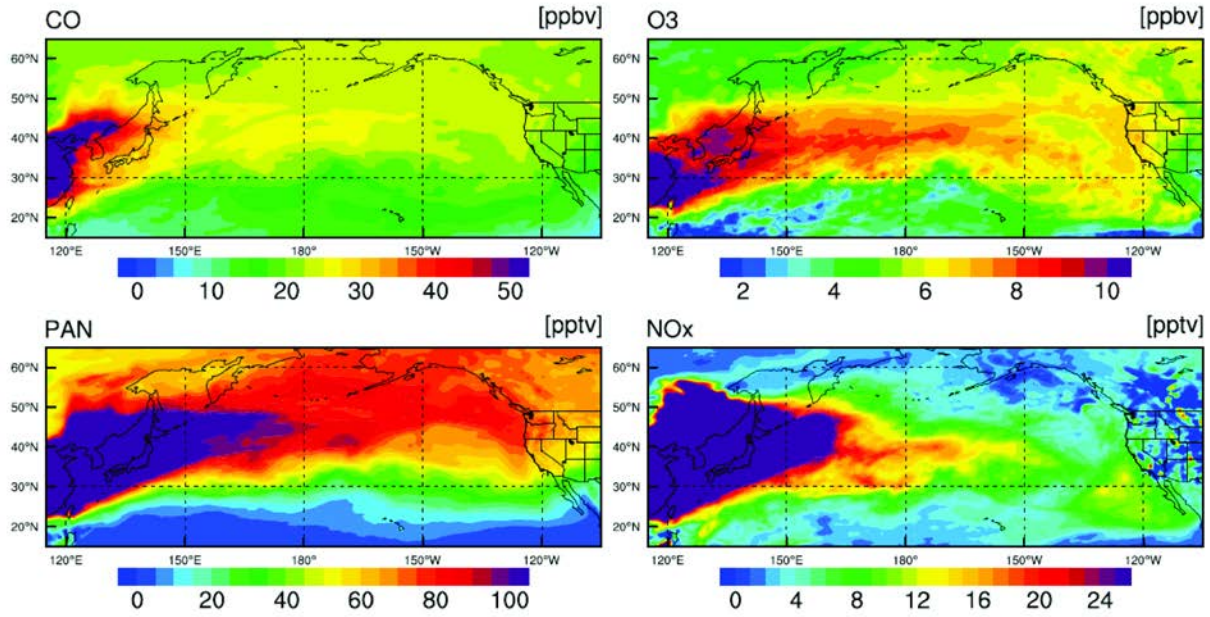
In the context of a review of the NAAQS, EPA generally defines "background concentrations" in a way that distinguishes between concentrations that result from precursor emissions that are relatively less controllable from those that are relatively more controllable through U.S. policies or through international agreements. The most commonly used form in the past 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 (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 2-12, maximum 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, background 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 the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c). The distribution of background concentrations largely reflects the distribution of soil NO emissions, with some local enhancements 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.

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 so they can be transported to the U.S. Figure 2-17 shows monthly average contributions for April 2010 to concentrations of PAN, and NO_X, in addition to CO and O_3, resulting from the transport of Asian anthropogenic emissions. The contribution from Asian anthropogenic emissions was estimated by taking the difference between two model simulations, one with emissions from all continents globally (see Figure 2-18) and the other omitting Asian emissions. The results from the second simulation were subtracted from the first and the contribution from Asia was taken to be the difference between the two simulations. As opposed to simply zeroing out anthropogenic emissions outside of Asia, this approach allows for the effects of global scale chemistry to be felt on emissions from Asia. These simulations were carried out using the AM3, global scale, three-dimensional chemical tracer model described by Lin et al. (2012). As noted in the O_3 ISA (U.S. EPA, 2013b), spring is the dominant season for effects of intercontinental transport of pollution to be detected in the United States. Results for April are shown because it is the month in which maximum springtime contributions are found. As can be seen from Figure 2-17, transported PAN concentrations over the western U.S. can be larger than those for NO_X by a factor of ten or more. These values refer to the 800 hPa level and are likely to be lower at the surface because of dilution and deposition. Corresponding model calculations of total monthly average concentrations for the sum of PAN and NO_X concentrations over the western U.S. are less than ~1 ppb (see Figure 2-18). These values are broadly consistent with those given in Figure 2-12, which shows seasonal average NO_2 concentrations typically less than 1 ppb across broad areas of the U.S., and with

modeling results and data from field studies given in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c). All of these results indicate that background levels of NO_2 are well beneath the level of the current NO_2 NAAQS.



Note: The contribution from Asian anthropogenic emissions was estimated by taking the difference between two model simulations using the modeling approaches described in Lin et al. (2012). One simulation included emissions from all continents globally (see Figure 2-18) and the other omitted Asian emissions. The results from the second simulation were subtracted from the first and the contribution from Asia was taken to be the difference between the two simulations.

Figure 2-17 Simulated Asian contributions to CO, O_3, PAN, and NO_X concentrations at 800 hPa in April, 2010.

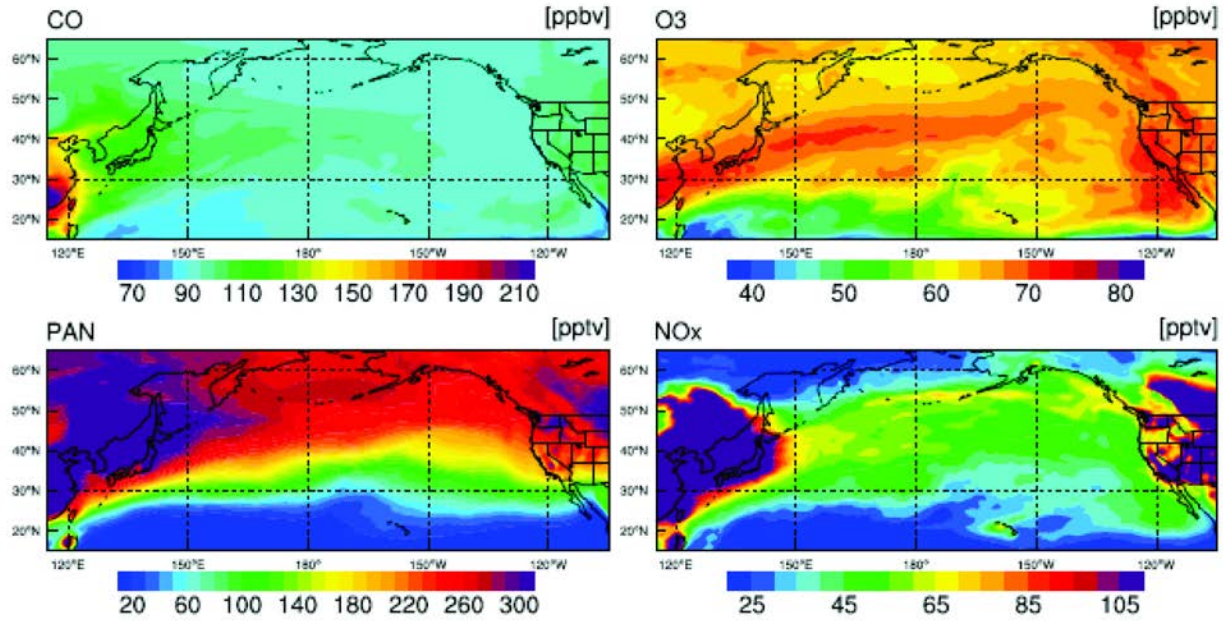


Figure 2-18 Simulated total CO, O_3, PAN, and NO_X concentrations at 800 hPa in April, 2010.

2.6 Exposure Assessment

2.6.1 Conceptual Model

Personal exposure to an ambient air pollutant, such as NO_2, is the concentration of the air pollutant encountered by an individual over a given time. In addition to time-activity (i.e., time spent in different microenvironments), personal exposure to ambient NO_2 is associated with climate (including weather and season), housing characteristics (e.g., window openings, draftiness, air conditioning), and microenvironmental sources (e.g., roadways, construction equipment, indoor sources).

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:

$$E_T = \sum_{i=1}^{n} C_i t_i$$

Equation 2-1

where C_i = average NO_2 concentration in the *i*th microenvironment, t_i = fraction of total time spent in the *i*th microenvironment, and n = total number of microenvironments which the individual has encountered (U.S. EPA, 2008c; Klepeis et al., 2001). Hence, both the microenvironmental NO_2 concentration and time activity aspects of total exposure must be considered. Note that if NO_X concentration is measured, then it can be used in lieu of NO_2 concentration.

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 non-ambient NO_2 exposure, E_{na}, components (U.S. EPA, 2008c; Wilson and Brauer, 2006):

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

Equation 2-2

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 2-1 and the component formulation presented in Equation 2-2 can be rectified by recognizing that E_a and E_{na} can both be expressed in terms of microenvironmental concentrations and time spent in outdoor and indoor microenvironments. During the fraction of a day spent outdoors, y_o, it is often presumed that an individual is exposed to the ambient concentration of NO_2.

Outdoor microenvironmental NO_2 exposures, E_o, can then be expressed simply as the product of the fraction of the time spent outdoors, y_o and ambient NO_2 concentration, C_a:

$$E_o = y_o C_a$$

Equation 2-3

Indoor NO_2 exposures in the ith microenvironment, E_i, are more complicated, because some part of indoor exposure emanates from nonambient sources, and some part of indoor exposure infiltrates from outdoors. Indoor exposures from nonambient sources are already given as E_{na}. Indoor exposures from ambient sources are also influenced by infiltration of outdoor NO_2, INF, time spent indoors, y_i, and C_a:

$$E_i = y_i INF \cdot C_a + E_{na}$$

Equation 2-4

Infiltration is a function of the ith microenvironment's air exchange rate, a_i, air pollutant penetration, P_i, and decay rate, k_i:

$$INF = P_i a_i/(a_i + k_i)$$

Equation 2-5

Hence, indoor NO_2 exposure for microenvironment i is the sum of an ambient and a nonambient component:

$$E_i = y_i[P_i a_i/(a_i + k_i)]C_a + E_{an}$$

Equation 2-6

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 *i* indoor microenvironments (U.S. EPA, 2008c; Wilson and Brauer, 2006; Wilson et al., 2000):

$$E_a = y_o C_a + \sum_i y_i [P_i a_i/(a_i + k_i)]\, C_a = \left\{ y_o + \sum_i y_i [P_i a_i/(a_i + k_i)] \right\} C_a$$

Equation 2-7

If it is further assumed that the individual occupies only one indoor and one outdoor microenvironment, then the infiltration term simplifies to $y_i[Pa/(a + k)]$, and since $y_o + y_i = 1$, 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 2-8

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

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

Equation 2-9

Comparison of Equation 2-2, Equation 2-7, and Equation 2-9 reveals that α can also be defined as the ratio E_a/C_a. This assessment focuses on the ambient component of NO_2 exposure, E_a, because this is more relevant to the review of the NAAQS compared with E_{na}. As such, subsequent sections examine how E_a and the factors α and C_a of the E_a term are modeled or measured.

2.6.2 Spatially Resolved Models for Use in Exposure Assessment

Computational models are employed to provide estimates of exposure when measurements are not available at locations and/or times needed to estimate spatial and temporal variability in concentration within communities. 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 2.6.2.4. 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, 2008a) and Section 3.8 of the 2009 ISA for PM (U.S. EPA, 2009a). Methods include chemical transport models (CTM), land use regression (LUR) models, spatial interpolation through statistical techniques, and dispersion models.

2.6.2.1 Chemical Transport Models

CTMs can be used to develop estimates of human exposure to NO_X, as tested in Marshall et al. (2008). CTMs 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 (AQM). Work to integrate meteorology and chemistry was done in the mid-1990s by Lu et al. (1997a, b) 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/).

CTMs provide regional concentration estimates, and they are typically run with surface grid resolutions of 4 km, 12 km, or 36 km. Temporal resolution of CTMs can be as fine as one hour, although larger temporal aggregation often occurs for the purpose of maintaining reasonable data file size. Hence, substantial uncertainties at the subgrid scale remain (U.S. EPA, 2008a). In densely populated regions of the country, monitor density may be finer than CTM surface grid resolution. Moreover, Community Multiscale Air Quality (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 combination (Fuentes and

Raftery, 2005) and downscaling (Berrocal et al., 2010a, b) have recently been developed to improve spatial resolution and provide bias correction. Isakov et al. (2009) developed a methodology to model subgrid spatial variability within CMAQ using the American Meteorological Society/Environmental Protection Agency Regulatory Model (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 to NO_2 exposure assessment.

2.6.2.2 Dispersion Models

Dispersion models, or Gaussian plume models, predict 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. 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; 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 usually too coarse to resolve spatial variations on the neighborhood scale. More finely resolved spatial scales that better represent human exposure scales are provided by smaller scale dispersion models. Several models could be used to simulate concentration fields near roads, each with its own set of strengths and weaknesses. The California Department of Transportation's most recent line dispersion model is CALINE4; see http://www.dot.ca.gov/hq/env/air/software/caline4/calinesw.htm. 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.

In addition, AERMOD (http://www.epa.gov/scram001/dispersion_prefrec.htm) is a steady state plume model formulated as a replacement to the ISC3 dispersion model. In the stable boundary layer (SBL), it assumes the concentration distribution to be Gaussian in both the vertical and horizontal dimensions. In the convective boundary layer (CBL), 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 (PBL) 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 are usually not in steady state. Moreover, most simple dispersion models including AERMOD are designed without explicit chemical mechanisms but do have non-default options to estimate conversion of NO to NO_2 based on a NO_X/O_3 titration model.

There are also non-steady 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 non-steady-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. 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 could be unimportant for studying health effects where long exposure time scales are relevant; however, when short exposure time scales are of interest, it may be more important to capture the temporal variability in emissions.

2.6.2.3 Land-use Regression Models

Empirical 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 (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., 2008a). LUR fits a statistical model of concentration based on land use data and then applies that model to locations without monitors to improve the spatial resolution of the concentration field. LUR methods are used frequently, because they offer improved spatial variability over other methods, with spatial resolutions of 300 meters for NO and 1 km for NO_2 (Marshall et al., 2008). Recently, nationwide LUR has been implemented to examine local-scale estimates across a nation (Hystad et al., 2011; Novotny et al., 2011; Hart et al., 2009). 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 or weeks of data and hence do not account for temporal variability well.

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_X concentration in a city is represented.

More finely resolved spatial resolution of calibration points can improve goodness of fit of the model for the city in which it was fit, although generalizability of LUR results to other cities may be independent of these factors. 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. Parenteau and Sawada (2012) examined LUR model performance when basing the model on successively finer spatial resolution from 2 km down to 50 meters, 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 validation 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 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.

Studies have evaluated LUR model performance when the LUR was fit with different numbers of NO_2 measurement sites and observed that the number of measurement sites needed is sensitive to the LUR model design. Basagaña et al. (2012) evaluated LUR model 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 in 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.

Selection of predictor variables, such as meteorology, traffic, land use, and population density, influences the ability of the LUR model to predict NO_X concentrations 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 street canyon aspect ratio as an LUR predictor variable to account for retention of pollutants in street canyons. They observed that, upon adding 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. 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. In this study, statistically significant correlations ($p < 0.005$) were observed between NO_2, NO, and NO_X concentrations and distance to road, traffic volume, concentrations from dispersion models, population density, elevation of the neighborhood relative to the community, local standard deviation of the elevation, transportation land use, commercial land use, and residential land use. Su et al. (2009a) developed a method to optimize the 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 is added to the model if its addition produces 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-h 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.

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 ($\beta = 0.9998$; $R^2 = 0.89$). Similarly, Wang et al. (2013) 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. (2013) 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.

LUR comparison with other models varies substantially among studies and depends on the validation algorithm as well as the model conditions. A recent study of LUR application in twenty European study areas, in which Wang et al. (In Press) 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 entails repeatedly withholding a fraction of the monitoring sites from the fitting process for validation and then computing an ensemble R^2, whereas HEV entails prediction with the LUR at locations not fit by the model. Mercer et al. (2011) compared ten-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 (UK: R^2 = 0.75, 0.72, and 0.74; LUR: R^2 = 0.74, 0.60, 0.67). Li et al. (2012b) developed a new formulation for LUR using generalized additive models (GAM) and cokriging to boost the performance of LUR over LUR methods using linear 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). Beelen et al. (2010) compared LUR with a dispersion model incorporating a near road module for modeling NO_2 concentrations in a Rotterdam, the Netherlands neighborhood. The dispersion model agreed better (R = 0.77) compared with LUR (R = 0.47) with NO_2 measurements from 18 validation 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). Mölter et al. (2010a) used dispersion modeling data in lieu of measurement data in an LUR for Greater Manchester, U.K. and found reasonable agreement of NO_2 predictions with validation monitoring data (R^2 = 0.86). Marshall et al. (2008) compared LUR with inverse distance-weighted spatial interpolation of NO and NO_2 measurements, nearest NO and NO_2 measurements, and a 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 bias in the

concentration compared with measured 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% bias for both pollutants and methods.

2.6.2.4 Stochastic Population Exposure Models

Stochastic population exposure models combine measured or modeled ambient NO_2 concentration data with population-level statistical distributions of time-activity data, air exchange rate for residences and other buildings, meteorology, physiological parameters, and other relevant data to simulate microenvironmental NO_2 concentrations and individuals' exposures to NO_2, which is then summarized through descriptive statistics for the simulated population. 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, 2008c). Examples of stochastic population exposure models include the Air Pollution Exposure (APEX), Stochastic Human Exposure and Dose Simulation (SHEDS), and EXPOLIS (exposure in polis, or cities) models, 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_2 tends to be comparable in spatial variability 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. Zidek et al. (2007) examined uncertainty and variability in ambient and microenvironmental concentrations, time-activity data, intrinsic variables (e.g., age, sex, exercise), meteorology, and geographical position. 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 parameters 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_2.

Sarnat et al. (2013b) recently compared risks of cardiovascular and respiratory morbidity with 24-h average NO_X concentration 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 its exposure estimates. Epidemiologic analyses for emergency department admission 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 negligible difference among the APEX and hybrid results for emergency department admission for all respiratory or cardiovascular diseases.

2.6.3 Personal Sampling Considerations

The following sections outline personal NO_2 and NO_X exposure sampling techniques, penetration, and indoor sources, sinks, and chemistry. This information is provided for context about exposure to oxides of nitrogen infiltrating indoors, since total personal exposure consists of ambient and nonambient oxides of nitrogen.

2.6.3.1 Personal Sampling Techniques

Personal sampling for NO_2 was described in detail in Annex 3.3 to the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c). 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 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 NO_2 sampling in a badge, tube, or radial manifold. Passive sampling results are integrated over the time period during which the sorbent material is exposed. The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) 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 relative humidity (Centro di Ricerche Ambientali, 2006).

Recent work has been performed to evaluate passive sampling device performance. Sather et al. (2007) compared Ogawa passive samplers with an NO_2 FRM monitor over a four-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, 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. 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.

Triethanolamine (TEA) is used as an alternative to activated charcoal sorbent material, because it can be applied in an even coating. However, sampling efficiency is sensitive to sampler flow rate (Vichi and De Santis, 2012) and relative humidity (Poddubny and Yushketova, 2013; Šerevičienė and Paliulis, 2012; Vardoulakis et al., 2009). 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.

2.6.3.2 Sources, Sinks, and Penetration

The general understanding production of oxide of nitrogen indoors has not changed since the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c). Indoor sources of oxides of nitrogen are combustion-based, including gas stoves, gas heating, oil furnaces, coal stoves, wood burning stoves, kerosene heaters, smoking, and to a lesser extent, electric cooking. The magnitude of indoor oxides of nitrogen depends on ventilation of the indoor space and appliances as well as source strength. Recent studies show associations between indoor NO_2 levels and indoor combustion (Vrijheid et al., 2012; Kornartit et al., 2010; Park et al., 2008). HONO can also be emitted directly during combustion or through surface reactions, as described in Section 2.6.3.3. 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 2-3. This review 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, 2008c). 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 (N.D. – 2 $\times 10^{-6}$ m/sec) were reported for NO, PAN, and organic NO_3 species.

Table 2-3 Indoor NO_2 and 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	-	-	-	1-12
Spengler et al. (1993)[d]	Gas range, stove, furnace	24-h	-	60 (24-115)	-	4.7 (2-8)
Simon and Dasgupta (1995)[e]	Kerosene heater	8 min	-	-	5-10	-
Leaderer et al. (1999)[f]	No source[f]	24-h	-	-	-	0.8 (0.0-2.9)
	Gas stoves[f]	24-h	-	-	-	4.0 (0.0-11.3)
	Kerosene heaters[f]	24-h	-	-	-	6.8 (0.2-35.9)
	No source[f]	24-h	-	-	-	2.4 (0.1-20.1)
	Gas stoves[f]	24-h	-	-	-	5.5 (0.4-20.1)
Khoder (2002)[g]	Gas appliances (summer)	24-h	-	39 (20-73)	-	3.7 (1.3-7.3)
	Gas appliances (winter)	24-h	-	65 (27-120)	-	6.8 (1.6-12.5)
Lee et al. (2002)[h]	Gas range, etc.	6-day	-	28 (4.3-52.0)	-	4.6 (0.1-21.1)
Jarvis et al. (2005)[i]	Gas hob		-	12.8	-	4.1
	Gas oven		-	12.8	-	5.0
Hong et al. (2007)[j]	Gas range	4 min	81.1	-	9.3	-
Park et al. (2008)	Gas range	4 min	189.3	19.4	15.2	2.1

[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 2785 kcal/h).

[b]Location: Maryland research home, unvented combustion condition; Gas range operation hours: 1 h (with one burner and 2320 kcal/h).

[c]Location: 11 Boston, MA, homes (winter).

[d]Location: 10 homes in Albuquerque, NM (winter).

[e]Location: four different home environments with small kerosene heater (2270 kcal/h).

[f]Location: 58 homes (summer) and 223 homes (winter) in southwest Virginia and Connecticut, U.S.; 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).

Source: Reprinted with permission of Elsevier, Park et al. (2008).

2.6.3.3 Indoor Chemistry

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) 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 identification of potential sources of uncertainty in estimates of indoor exposure to ambient oxides of nitrogen. 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 volatile organic compounds 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 organic nitrates ($RONO_2$) and peroxynitrates ($RC(=O)OONO_2$). 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 reaction increases with increased relative humidity. 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 ($r = 0.64$) 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).

Recent gas-phase indoor chemistry work has shed light on processes involving organic compounds and/or secondary organic aerosols (SOA). Carslaw et al. (2012) 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. 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)· radicals and HONO were both observed to be products of these reactions.

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 (260 m^3) chamber and observed little N_2O_5 uptake by solid organic aerosols, more efficient uptake by liquid aerosols, and uptake that increased with increasing relative humidity (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.

2.6.4 Oxides of Nitrogen in a Multipollutant Context

Correlations between ambient or personal NO_2 and other copollutants can help reveal information on source emissions, exposures, and health outcomes of NO_2. Studies and analyses reported in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) demonstrated that ambient NO_2 was moderately correlated with several traffic-related

pollutants (e.g., $PM_{2.5}$, CO, and EC) in urban and suburban areas, suggesting that in some cases NO_2 can be a surrogate for traffic pollution. Compared to other traffic-related pollutants, EC generally had the strongest correlations with NO_2. In contrast, O_3 was generally poorly or negatively correlated with NO_2. A limited number of studies reported in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) 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). In most cases, personal NO_2 was moderately correlated with these pollutants. More recent studies expand upon these findings and are discussed below.

2.6.4.1 Ambient Relationships between NO_2 and Copollutants

Numerous air quality, exposure and epidemiologic studies have 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 NAAQS 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, ultrafine particulate matter (UFP), and volatile organic compounds (VOCs). Data for criteria pollutants are summarized in Table 2-4; VOCs are not included in this table due to a limited amount of data.

Figure 2-19 shows the range of NO_2 copollutant correlation coefficients among the studies in Table 2-4. Existing studies indicate that NO_2 is, in general, moderately correlated with other NAAQS and traffic-related pollutants. Similar to findings in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), the strongest correlations are typically observed for NO_2 with primary traffic-related pollutants, such as CO, BC and UFP. Correlations between NO_2 and CO are highest on average, with most correlations exceeding 0.6. A wide range of correlations is observed for NO_2 with $PM_{2.5}$, PM_{10}, and SO_2. The lowest correlations are typically observed between NO_2 and O_3, with correlations typically being negative or very low (r = -0.71-0.66, median r = 0.15).

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 personal, indoor, and 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 small (r = 0.09) between NO_2 and $PM_{2.5}$, whereas during the winter the correlation was slightly stronger (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).

The correlation between NO_2 and O_3 may also have seasonal patterns, although limited seasonal data exists between these two pollutants. In the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), ambient concentrations of NO_2 and O_3 from several sites across Los Angeles, CA were compared during a multi-year period. Slightly positive correlations between these two pollutants were observed during the summer (r = 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. Only one study in Table 2-4 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 correlations across different regions in the U.S. 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 one city 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 moderate correlation in Denver (r = 0.64) to

low correlations in Baltimore and Boston (r = 0.26 for both cities). Other multicity studies conducted outside of the U.S. show that NO_2 copollutant correlations are widely variable (Faustini et al., 2011; Dales et al., 2010, 2009b; Stieb et al., 2008; Timonen et al., 2006).

A small subset of studies investigated correlations between NO_2 and traffic-related VOCs (volatile organic compounds), such as benzene, toluene, ethene, and xylene (BTEX). In these studies, correlations between NO_2 and VOCs are variable. Beckerman et al. (2008) observed a strong correlation between NO_2 and BTEX in a near-road field campaign. In a panel study, Greenwald et al. (2013) compared ambient concentrations of traffic pollutants monitored at two schools in El Paso, Texas, including one school within close proximity to a major roadway with heavy diesel truck traffic. A moderately strong correlation (r = 0.77) was observed between NO_2 and BTEX (presented as the sum of benzene, toluene, ethene, and xylene), suggesting that both pollutants are related to traffic sources. Another study by Martins et al. (2012) estimated personal NO_2 and BTEX exposure during four one-week periods using a microenvironment approach that combined outdoor and indoor concentrations with time activity patterns. In contrast to Beckerman et al. (2008) and Greenwald et al. (2013), Martins et al. (2012) consistently observed poor correlations (r=-0.423-0.138) between NO_2 and BTEX during different periods. 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 roadway and time spent in various indoor and outdoor microenvironments can impact the relationship between NO_2 and traffic-related VOCs.

Table 2-4 Synthesis of NO_2 ambient-ambient copollutant correlations reported in the literature.

Study	Time	Location	Correlation Measure	Pollutant				
				CO	O_3	SO_2	$PM_{2.5}$	PM_{10}
Faustini et al. (2011)	24-h avg	6 Italian Cities	Pearson	NR	NR	NR	NR	0.19-0.79
Samoli et al. (2011)	24-h avg	Athens, Greece	NR	NR	NR	0.55	NR	NR
Ko et al. (2007a)	24-h avg	Hong Kong	Pearson	NR	0.34	0.66	0.44	0.4
Mehta et al. (2013)	24-h avg	Ho Chi Minh City, Vietnam (dry season)	NR	NR	0.44	0.29	NR	0.78
		Ho Chi Minh City, Vietnam (wet season)	NR	NR	0.17	0.01	NR	0.18
Andersen et al. (2008a)	24-h avg	Copenhagen, Denmark	Spearman	NR	-0.58	NR	0.41 ($PM_{2.5}$) 0.67 (UFP)	0.43
Mannes et al. (2005)	24-h avg	Sydney, Australia	Pearson	0.57	0.29	NR	0.66	0.47
Schildcrout et al. (2006)	24-h avg	Albuquerque, NM	NR	0.76	0.04	NR	NR	0.26
		Baltimore, MD	NR	0.69	0.44	0.49	NR	0.62
		Boston, MA	NR	0.80	0.47	0.68	NR	0.48
		Denver, CO	NR	0.85	0.24	0.56	NR	0.64
		San Diego, CA	NR	0.92	0.39	0.23	NR	0.55
		St. Louis, MO	NR	0.71	0.42	0.58	NR	0.45
		Toronto, Canada	NR	0.63	0.40	0.63	NR	0.64
Liu et al. (2009b)	24-h avg	Ontario, Canada	Spearman	NR	-0.51	0.18	0.71	NR
Strak et al. (2013)	24-h avg	Locations across the Netherlands	Spearman	NR	-0.62	NR	0.45 ($PM_{2.5}$) 0.56 (PNC)	0.49
O'Connor et al. (2008)	24-h avg	Inner-cities across the U.S.	NR	0.54	-0.31	0.59	0.59	NR
Timonen et al. (2006)	24-h avg	Amsterdam, the Netherlands	Spearman	0.76	NR	NR	0.49	NR
		Erfurt, Germany	Spearman	0.86	NR	NR	0.82	NR
		Helsinki, Finland	Spearman	0.32	NR	NR	0.35	NR
Guo et al. (2009)	24-h avg	Beijing, China	Pearson	NR	NR	0.53	0.67	NR

Table 2-4 (Continued): Synthesis of NO_2 ambient-ambient copollutant correlations reported in the literature.

Study	Time	Location	Correlation Measure	Pollutant				
				CO	O_3	SO_2	$PM_{2.5}$	PM_{10}
Dales et al. (2010)	24-h avg	Santiago Province, Chile	NR	NR	NR	NR	0.73-0.92	NR
Dales et al. (2009b)	24-h avg	7 Chilean Cities	Pearson	0.79 - 0.84	-0.34 - -0.09	0.42 - 0.80	0.72 – 0.82	0.61 - 0.79
Rojas-Martinez et al. (2007a)	24-h avg	Mexico City, Mexico	Pearson	NR	0.17	NR	NR	0.25
Sarnat et al. (2001)	24-h avg	Baltimore, MD (summer)	Spearman	0.75	0.02	NR	0.37	NR
		Baltimore, MD (winter)	Spearman	0.76	-0.71	-0.17	0.75	NR
Sarnat et al. (2005)	24-h avg	Baltimore, MD (summer)	Spearman	NR	NR	NR	0.44	NR
		Baltimore, MD (winter)	Spearman	NR	NR	NR	0.64	NR
Kim et al. (2006a)	24-h avg	Toronto, Canada	Spearman	0.72	NR	NR	0.44	NR
Roberts and Martin (2006)	24-h avg	Cleveland, OH	NR- Pairwise	0.67	0.36	0.56	NR	0.63
		Nashville, TN	NR-Pairwise	0.36	0.26	0.08	NR	0.44
Andersen et al. (2007)	24-h avg	Copenhagen, Denmark	Spearman	0.74	NR	NR	NR	0.42
Chen et al. (2008)	24-h avg	Shanghai, China	NR	NR	NR	0.73	NR	0.71
Arhami et al. (2009)	24-h avg	San Gabriel Valley, CA (Summer and Fall)	Spearman	NR	NR	NR	0.10	0.31
		San Gabriel Valley, CA (Fall and Winter)	Spearman	NR	NR	NR	0.44	0.34
		Riverside, CA (Summer and Fall)	Spearman	NR	NR	NR	0.07	0.21
		Riverside, CA (Fall and Winter)	Spearman	NR	NR	NR	0.56	0.64
Baxter et al. (2013)	24-h avg	Boston, MA	Spearman	NR	NR	NR	0.41	NR
		Pittsburgh, PA	Spearman	NR	NR	NR	0.46	NR
		Memphis, TN	Spearman	NR	NR	NR	0.27	NR
		Detroit, MI	Spearman	NR	NR	NR	0.59	NR
		Milwaukee, WI	Spearman	NR	NR	NR	0.55	NR
		San Diego, CA	Spearman	NR	NR	NR	0.57	NR
		Riverside, CA	Spearman	NR	NR	NR	0.37	NR
Williams et al. (2012a)	24-h avg	Research Triangle Park, NC	Spearman	NR	-0.12	NR	0.03 0.25 (BC)	NR

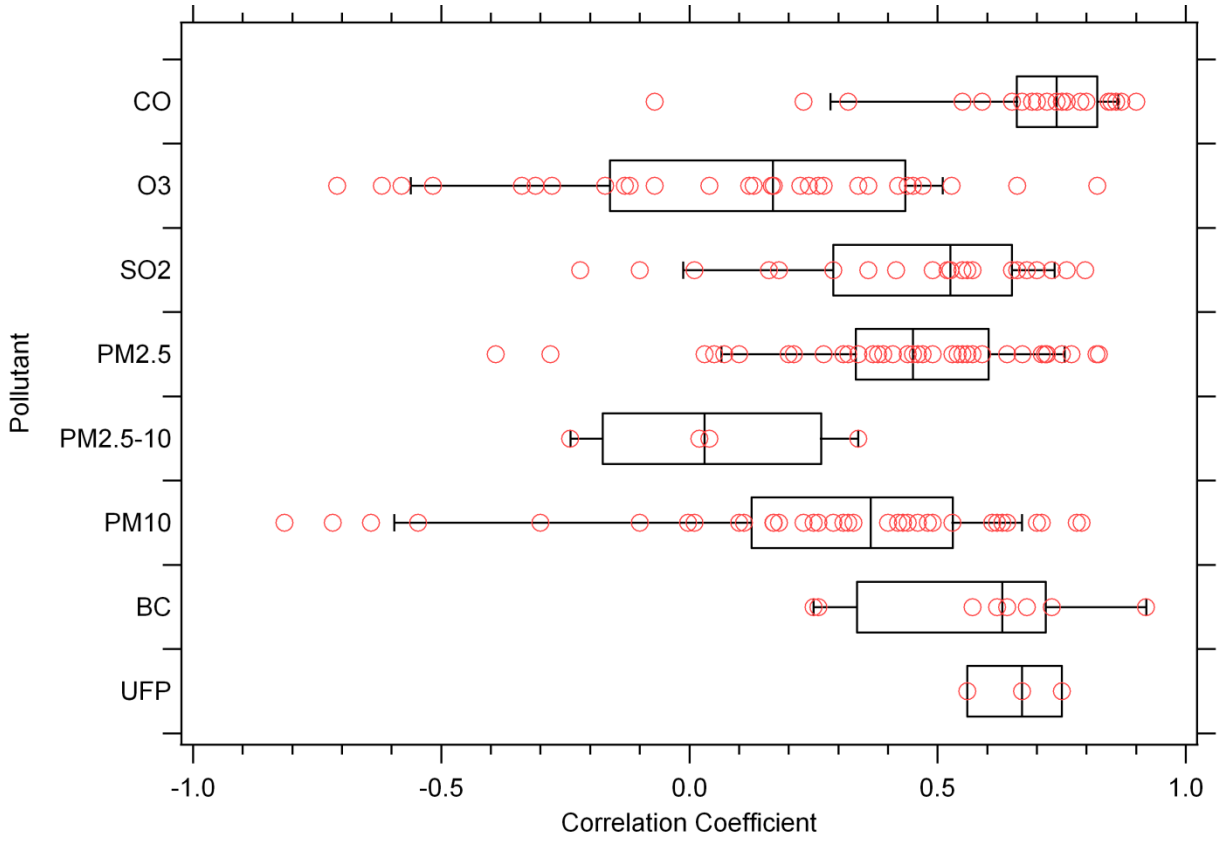
Table 2-4 (Continued): Synthesis of NO_2 ambient-ambient copollutant correlations reported in the literature.

Study	Time	Location	Correlation Measure	Pollutant				
				CO	O_3	SO_2	$PM_{2.5}$	PM_{10}
Delfino et al. (2008a)	24-h avg	Los Angeles, CA	Spearman	NR	NR	NR	0.36 ($PM_{2.5}$) 0.61 (EC)	NR
Suh and Zanobetti (2010b)	24-h avg	Atlanta, GA	Spearman	NR	NR	NR	0.47 ($PM_{2.5}$) 0.58 (EC)	NR
Schembari et al. (2013)	24-h avg	Barcelona, Spain	Spearman	NR	NR	NR	0.41 ($PM_{2.5}$) 0.6 (EC)	NR
Chuang et al. (2008)	Hourly	Boston, MA	Pearson	NR	NR	NR	0.38	0.33
Strickland et al. (2010)	Daily 1-h max	Atlanta, GA (Cold Season)	Spearman	0.59	0.11	0.36	0.37	0.46
		Atlanta, GA (Warm Season)	Spearman	0.54	0.42	0.37	0.36	0.44
Villeneuve et al. (2007)	Daily 1-h max	Edmonton, Canada	Pearson	0.74	NR	NR	NR	NR
Jalaludin et al. (2007)	Daily 1-h max	Sydney, Australia	NR	0.60	0.25	0.46	0.65	0.48
Mortimer et al. (2002)	Daily 1-h max	8 US Cities	NR	NR	0.27	NR	NR	NR
Burnett et al. (2000)	Daily 1-h max	8 Canadian Cities	NR	0.65	0.12	0.49	0.53	0.53
Mar et al. (2000)	Daily 1-h max	Phoenix, AZ	NR	0.87	NR	0.57	0.77	0.53
Tolbert et al. (2007)	Daily 1-h max	Atlanta, GA	Spearman	0.7	0.44	0.36	0.47 ($PM_{2.5}$) 0.64 (EC) 0.62 (OC)	0.53
Moshammer et al. (2006)	8-h avg	Linz, Austria	Pearson	NR	NR	NR	0.54	0.62

Table 2-4 (Continued): Synthesis of NO_2 ambient-ambient copollutant correlations reported in the literature.

Study	Time	Location	Correlation Measure	Pollutant				
				CO	O_3	SO_2	$PM_{2.5}$	PM_{10}
Sarnat et al. (2012)	96-h avg	El Paso, TX (Site A)	Spearman	NR	NR	NR	-0.39 ($PM_{2.5}$) -0.24 ($PM_{10-2.5}$)	-0.3
		El Paso, TX (Site B)	Spearman	NR	NR	NR	-0.28 ($PM_{2.5}$) 0.04 ($PM_{10-2.5}$)	-0.1
		Ciudad Juarez, Mexico (Site A)	Spearman	NR	NR	NR	-0.28 ($PM_{2.5}$) 0.04 ($PM_{10-2.5}$)	-0.1
		Ciudad Juarez, Mexico (Site B)	Spearman	NR	NR	NR	0 ($PM_{2.5}$) 0.34 ($PM_{10-2.5}$)	0.11
Greenwald et al. (2013)	96-h avg	2 sites in El Paso, TX	Pearson	NR	NR	NR	0.05 ($PM_{2.5}$) 0.02 ($PM_{10-2.5}$)	0.01
Katanoda et al. (2011)	1-yr mean	Japanese Cities	Pearson	NR	NR	0.76	NR	NR
Dong et al. (2011)	1-yr avg	7 Cities across China	NR	0.23	0.66	0.52	NR	0.70
Hwang and Lee (2010)	1-yr avg	14 Taiwanese Communities	NR	0.86	-0.07	0.55	0.37	NR
McConnell et al. (2003)	4-yr avg	12 communities in southern California	Pearson	NR	0.59	NR	0.54	0.20

BC = Black carbon; EC = Elemental carbon; OC = Organic level; TC = Total carbon; UFP = Ultrafine particles; PNC = Particle Number Concentration



Note: 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. Original data are plotted as red markers.

Source: NCEA analysis of data from studies referenced in Table 2-4.

Figure 2-19 Summary of copollutant correlation coefficients reported in studies in Table 2-4.

2.6.4.2 Personal and Indoor Relationships between NO_2 and Copollutants

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. Table 2-5, Table 2-6, Table 2-7, and Table 2-8 present correlations of ambient, personal, or indoor NO_2 with similar measurements of

copollutants. Similar to the results in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), moderate correlations were generally 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 due to complex chemistry. More recent studies report indoor NO_2 copollutant correlations and observe moderate correlations between NO_2 and EC.

Table 2-5 Pearson correlation coefficients between ambient NO_2 and personal copollutants.

Study	Location	N	Averaging times	$PM_{2.5}$	EC	OC	O_3
Delfino et al. (2008a)	Los Angeles, CA	≤ 170	All: 24-h	0.32	0.2	0.16	-
Suh and Zanobetti (2010b)	Atlanta, GA	≤ 277	All: 24-h	0.25	0.33	-	-0.09
Williams et al. (2012a)	Chapel Hill, NC	≤ 357	All: 24-h	-0.19	-0.17	-	-0.01
Schembari et al. (2013)	Barcelona, Spain	≤ 65	NO_2: 7-days; $PM_{2.5}$/EC: 2-days	0.21	0.44	-	-

Table 2-6 Pearson correlation coefficients between personal NO_2 and ambient copollutants.

Study	Location	N	Averaging times	$PM_{2.5}$	EC	OC	O_3
Delfino et al. (2008a)	Los Angeles, CA	≤ 170	All: 24-h	0.21	0.2	0.18	-
Suh and Zanobetti (2010b)	Atlanta, GA	≤ 277	All: 24-h	0.2	0.22	-	-
Williams et al. (2012a)	Chapel Hill, NC	≤ 326	All: 24-h	0.33	-0.3	-	-0.26
Schembari et al. (2013)	Barcelona, Spain	≤ 65	NO_2: 7-days; $PM_{2.5}$/EC: 2-days	0.28	0.22	-	-

Table 2-7 Pearson correlation coefficients between personal NO_2 and personal copollutants.

Study	Location	N	Averaging times	$PM_{2.5}$	EC	OC	O_3
Delfino et al. (2008a)	Los Angeles, CA	≤ 486	All: 24-h	0.38	0.22	0.2	-
Suh and Zanobetti (2010b)	Atlanta, GA	≤ 277	All: 24-h	0.29	0.49	-	-0.03
Williams et al. (2012a)	Chapel Hill, NC	≤ 326	All: 24-h	0.06	0.33	-	-0.11
Schembari et al. (2013)	Barcelona, Spain	≤ 65	NO_2: 7-days; $PM_{2.5}$/EC: 2-days	0.11	0.3	-	-

Table 2-8 Correlation coefficients between indoor NO_2 and indoor copollutants.

Study	Location	N	Averaging times	$PM_{2.5}$	EC	OC	O_3
Sarnat et al. (2012)[a]	El Paso, TX (Site A)	15	NO_2: 4-days; $PM_{2.5}$/EC:2-days	-0.35 ($PM_{2.5}$) -0.26 ($PM_{10-2.5}$) -0.19 (PM_{10})	0.58	-	-
	El Paso, TX (Site B)	15	NO_2: 4-days; $PM_{2.5}$/EC:2-days	0.06 ($PM_{2.5}$) 0.28 ($PM_{10-2.5}$) 0.12 (PM_{10})	-0.37	-	-
	Ciudad Juarez, Mexico (Site A)	15	NO_2: 4-days; $PM_{2.5}$/EC:2-days	-0.29 ($PM_{2.5}$) -0.58 ($PM_{10-2.5}$) -0.5 (PM_{10})	0.66	-	-
	Ciudad Juarez, Mexico (Site B)	15	NO_2: 4-days; $PM_{2.5}$/EC:2-days	-0.04 ($PM_{2.5}$) -0.5 ($PM_{10-2.5}$) -0.34 (PM_{10})	0.45	-	-
Greenwald et al. (2013)[b]	2 sites in El Paso, TX	18-26	All: 4-days	0.76 ($PM_{2.5}$) 0.83 (PM_{10})	0.45	-	-

[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 2.6.4.1) compared to personal measurements (e.g., correlations among personal exposure measurements in Table 2-7) (Schembari et al., 2013; Williams et al., 2012; Suh and Zanobetti, 2010b; Delfino et al., 2008a). For example, Suh and Zanobetti (2010b) 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. (2008a) 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 moderate (r = 0.61), lower correlations were observed for personal NO_2-EC (r = 0.22). 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 non-traffic emissions (e.g., gas stoves, residential wood burning, biomass burning). 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 is discussed in more detail in Section 2.6.5.

Few studies have reported indoor NO_2 copollutant correlations, focusing on correlations between NO_2 and PM in different size fractions as well as NO_2 and BC. In these studies, moderate correlations are typically observed between indoor NO_2 and BC; however, less consistent correlations are observed for indoor NO_2 and PM. Sarnat et al. (2012) measured indoor concentrations of NO_2, BC, $PM_{2.5}$, $PM_{10-2.5}$, and PM_{10} at four elementary schools in two cities near the US-Mexico border: El Paso, TX and Ciudad Juarez, Mexico. While correlations between NO_2 and BC were generally moderate (r = -0.37-0.66), NO_2 and PM showed weaker, inverse correlations at all four elementary schools (r = -0.58-0.12). 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 Texas. Although Greenwald et al. (2013) reported similar NO_2/BC correlations to those reported in Sarnat et al. (2012), stronger correlations were observed between NO_2 and $PM_{2.5}$ (r = 0.76) and 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.

A small number of studies have used NO_2 in receptor models to relate health effects to sources/factors. Mar et al. (2000) used factor analysis to apportion PM mass collected in Phoenix, AZ and found high NO_2 loadings on the motor vehicle exhaust factor. Cakmak et al. (2009) applied factor analysis to apportion $PM_{2.5}$ in Santiago, Chile and found a motor vehicle exhaust factor with high loadings of NO_2. Halonen et al. (2009) applied the EPA positive matrix factorization (PMF) method (http://intranet.epa.gov/heasd/products/pmf/pmf.htm) on $PM_{2.5}$ data from Helsinki, Finland and reported a traffic emissions factors with high loadings of NO_2. Similarly, Baxter et al. (2013) conducted PCA analysis using $PM_{2.5}$ data from eight U.S. cities and found NO_2 associated with a traffic related factor in Boston, MA only.

2.6.4.3 NO_2 Concentration as an Indicator of Source-Based Mixtures

Health studies often use NO_2 concentration as a surrogate for exposure to traffic pollution mixtures when measurements of other pollutants are not available, because NO_2 concentration is routinely measured at sampling sites nationwide and is a prevalent component of vehicle exhaust. Section 2.5.2 concluded that NO_2 generally correlates spatially with other traffic-related pollutants in urban areas.

Several recent studies have evaluated the use of central-site NO_X or NO_2 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), polycyclic aromatic hydrocarbons (PAHs) (Brook et al., 2007), BTEX (Beckerman et al., 2008), and EC (Minguillón et al., 2012). The moderate-strong correlation between CO, NO_X, and EC 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 as a weighted average of their concentrations by the ratio of mobile source to total emissions for each pollutant (Pachon et al., 2012):

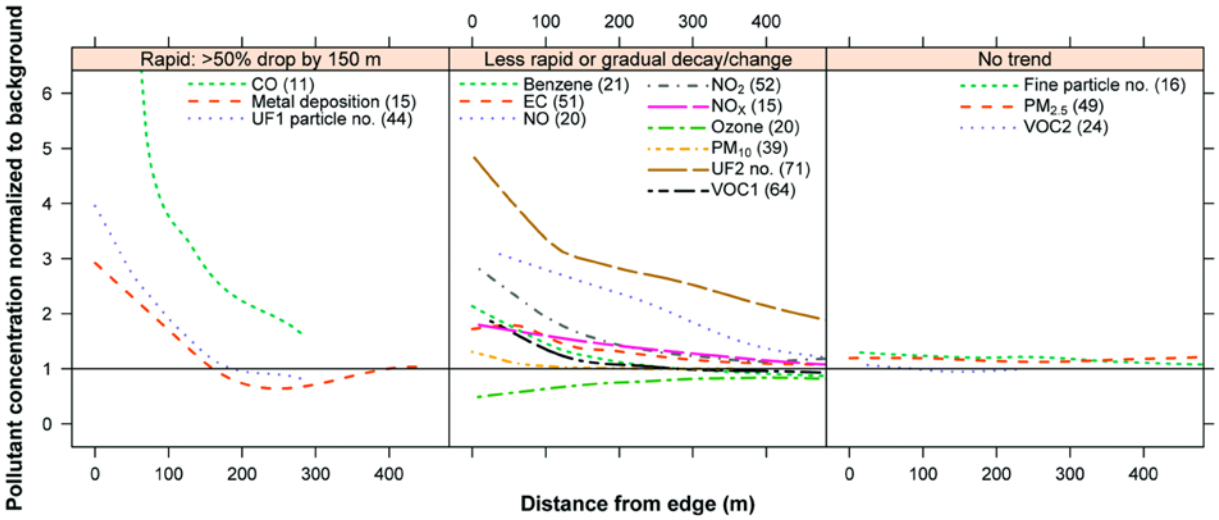
$$IMSI_{EB} = \frac{\frac{Emission_{EC,mobile}}{Emission_{EC,total}} \times C'_{EC} + \frac{Emission_{NOx,mobile}}{Emission_{NOx,total}} \times C'_{NOx} + \frac{Emission_{CO,mobile}}{Emission_{CO,total}} \times C'_{CO}}{\frac{Emission_{EC,mobile}}{Emission_{EC,total}} + \frac{Emission_{NOx,mobile}}{Emission_{NOx,total}}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

Equation 2-10

Compared to other common traffic surrogates ($PM_{2.5}$ and CO), NO_X concentration may better capture spatial and temporal trends of traffic pollution. Wheeler et al. (2008), Beckerman et al. (2008), and Karner et al. (2010) reported strong correlations among NO_2 and several traffic-related pollutants, including benzene and toluene, at various distances from a roadway. These studies concluded that gradients in NO_2 concentrations were spatially correlated with gradients in traffic-related pollution. Brook et al. (2007) demonstrated that benzo(e)pyrene and hopanes, specific mobile source tracers, were more strongly correlated with NO_2 (r = 0.27-0.80) compared to $PM_{2.5}$ (r = 0.26-0.62) at several urban sites in Canada. Sánchez Jiménez et al. (2012) observed similar findings in a spatial variability study in Glasgow and London, which included measurements of several criteria pollutant traffic surrogates (e.g., CO, PM_{10}, $PM_{2.5}$) at roadside and background sites. Of all the surrogates, NO_X and NO_2 concentrations showed the strongest intra-site and inter-site (roadside site versus background site) correlations with particle number count and some water-soluble metal species (Cu and Ni).

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). For example in Beckerman et al. (2008), peak near-road NO_2 concentrations were only 2 times higher than the urban background concentration, defined here as the lowest concentration measured upwind of the road. In contrast, peak near-road UFP counts were 23 times higher than the urban

background concentration. 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 2-20 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).



Note: NO_2, NO, and NO_X concentration gradients are presented in the center panel.
Data presented from Karner et al. (2010) were synthesized from 41 peer reviewed references.

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

Figure 2-20 Spatial variability in concentrations of near-road pollutants, including NO_2, NO_X, CO, $PM_{2.5}$, and UFP. NO_2, NO, and NO_X concentration gradients are presented in the center panel.

As described in Section 2.3, other sources contributing to ambient NO_X concentrations include non-road mobile sources, electric generating units, industrial sources, and wildfires. Non-road mobile sources, such as airports, shipping ports, and rail yards, can contribute substantially to local and regional ambient NO_X concentrations (Kim et al., 2011b; Williams et al., 2009; Vutukuru and Dabdub, 2008; Carslaw et al., 2006; Unal et al., 2005). 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., 2011b). 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.

2.6.5 Considerations for Use of Exposure Metrics in Epidemiology

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) 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, 2008c) concluded that errors associated with the use of NO_2 concentrations measured at central site monitors as exposure metrics for epidemiology studies tended to bias the health effect estimate towards the null. The following sections provide evidence from recent studies to support that conclusion.

2.6.5.1 Personal-Ambient Relationships

In most epidemiologic studies of the health effects of NO_2, the health effect endpoint is modeled as a function of ambient exposure, E_a. Equation 2-2, Equation 2-3, Equation 2-4, Equation 2-5, and Equation 2-6 define E_a as the product of ambient concentration, C_a, and α, a term encompassing time-weighted averaging and infiltration of NO_2 (Section 2.6.1). 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). When averaging across individuals, $\overline{\alpha}$ can quantify the bias introduced by substituting $C_{a,csm}$ for the average exposure to ambient NO_2, $\overline{E_a}$. Personal measurements typically capture both ambient and nonambient exposure contributions; for the purpose of this document, these are referred to as "total personal exposure" measurements, even though time activity data is not always incorporated into their computation. The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) concluded that literature relating ambient NO_2 concentrations measured by a central site monitor to personal NO_2 exposures was mixed, with some studies finding statistically significant associations and other studies finding no statistically significant association. These inconsistencies reflected various factors that influence exposure in respective studies, including proximity and strength of sources of NO_X, spatiotemporal variability of NO_2 concentrations, and time-activity behavior of the exposed sample population. See Table 2-9 and Table 2-10 for data from recent studies including ambient, outdoor, and indoor NO_2 concentration data; total personal NO_2 exposure concentration data; and correlations among measurements. In some cases,

median or average personal exposure measures were comparable to median or average central site monitor concentrations, but in other cases, median or average total personal NO_2 exposures and median or average ambient NO_2 concentrations varied considerably. Personal NO_2 concentration measurements tended to be more highly correlated with indoor concentrations compared with outdoor or ambient concentrations. Personal-outdoor correlations were also higher for summer compared with winter; this is not surprising, because open windows during summer likely increase exposure to outdoor NO_2. Implications for use of central site monitoring data in epidemiologic studies are discussed in Section 2.6.5.3.

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 non-smoking adults and found that total personal NO_2 exposure was not statistically significantly associated with NO_2 measured at central site monitors. Likewise, Suh and Zanobetti (2010b) measured low correlation between total personal exposure and central site NO_2 measurements among an Atlanta panel of 30 adults. Together, these results indicate that most of the total personal NO_2 exposure measurements for these studies were influenced by either spatially variable NO_2 not well detected by the central site monitor or by nonambient sources.

Table 2-9 Ambient, outdoor, transport, indoor, and personal NO_2 measurements (ppb) across studies.

Study	Location	Time Period	Sampling Interval	Ambient	Outdoor	Transport	Indoor	Personal
Sarnat et al. (2012)	El Paso, TX (large city)	January-May, 2008	96-h	14.0-20.6[b]	4.5-14.2[b]	-	4.0-8.1[b]	-
	Ciudad Juarez, Mexico (large city)				18.7-27.2[b]		23.1-120.8[b]	
Williams et al. (2012b); Meng et al. (2012a)	Detroit, MI (large city)	Summer, 2004-2007	24-h	Williams: 22.0[a]; Meng: 22.0[a]; 22.7[b]	-	-	-	Total: Williams: 25.5[b]; Meng: 25.4[b] Ambient: 16.0[a]; 21.0[b]
		Winter, 2004-2007		24.0[a]; 23.9[b]				Total: 24.0[a]; 35.6[b] Ambient: 18.0[a]; 20.4[b]
Suh and Zanobetti (2010b)	Metropolitan Atlanta, GA (large city)	Fall, 1999-Spring, 2000	24-h	17.96[a]; 17.13[b]				8.08[a]; 11.60[b]
Brown et al. (2009)	Metropolitan Boston, MA (large city)	November, 1999-January, 2000	24-h	25.8[c]; 26.8[b]				10.4[c]; 12.9[b]
		June-July, 2000		22.0[c]; 22.8[b]				13.9[c]; 17.4[b]
Delfino et al. (2008a)	Riverside, CA; Whittier, CA (SoCAB) (large city)	July-Dec, 2003 (Riverside); July-Dec, 2004 (Whittier)	24-h	25.3[a]; 25.0[b]				26.7[a]; 28.6[b]

Table 2-9 (Continued): Ambient, outdoor, transport, indoor, and personal NO_2 measurements (ppb) across studies.

Study	Location	Time Period	Sampling Interval	Ambient	Outdoor	Transport	Indoor	Personal
Delgado-Saborit (2012)	Birmingham, U.K. (large city)	July-October, 2011	5-min	47[b]	64[b]	Car: 40[b] Bus: 71[b] Bike: 125[b] Train: 58[b]	Office: 14[b] Home: 17[b]	All: 23[b] Gas oven: 31[b] Electric oven: 19[b]
Kornartit et al. (2010)	Hertfordshire, U.K. (greater London area) (large city)	Winter, 2000	7-days	-	-	-	Electric oven: Bedroom: 7.8[b] Living room: 7.9[b] Kitchen: 7.1[b] Gas oven: Bedroom: 10.8[b] Living room: 13.7[b] Kitchen: 20.6[b]	Electric oven: 8.1[b] Gas oven: 11.2[b]
		Summer, 2001					Electric oven: Bedroom: 12.7[b] Living room: 13.1[b] Kitchen: 11.0[b] Gas oven: Bedroom: 14.3[b] Living room: 14.7[b] Kitchen: 14.2[b]	Electric oven: 13.3[b] Gas oven: 14.6[b]

Table 2-9 (Continued): Ambient, outdoor, transport, indoor, and personal NO_2 measurements (ppb) across studies.

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

Table 2-9 (Continued): Ambient, outdoor, transport, indoor, and personal NO_2 measurements (ppb) across studies.

Study	Location	Time Period	Sampling Interval	Ambient	Outdoor	Transport	Indoor	Personal
Lee et al. (2013) (Continued)	Suncheon, Korea (rural)	July, 2008	NR	15.0[c]; 15.9[b]	-	-	Home: 13.0[c]; 14.3[b] Work: 12.0[c]; 14.5[b]	14.0[c]; 16.3[b]
		January, 2009	NR	12.5[c]; 15.2[b]			Home: 15.9[c]; 20.4[b] Work: 9.3[c]; 12.9[b]	12.9[c]; 15.7[b]
	Total	July, 2008	NR	21.7[c]; 23.7[b]	-	-	Home: 19.5[c]; 21.2[b] Work: 18.4[c]; 21.4[b]	20.5[c]; 22.6[b]
		January, 2009	NR	20.6[c]; 23.6[b]			Home: 19.9[c]; 23.3[b] Work: 16.4[c]; 21.1[b]	18.6[c]; 21.0[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[a]; 18.7[b] 8:00 a.m. to 6:00 p.m.: 20.3[a]; 21.2[b]			Home: 17.2[a]; 16.8[b] Work: 21.6[a]; 21.7[b]	Total: 12.2[f] Home: 8.2[f] Work: 14.7[f] Transit: 23.4[f] Other: 17.4[f]

Table 2-9 (Continued): Ambient, outdoor, transport, indoor, and personal NO_2 measurements (ppb) across studies.

Study	Location	Time Period	Sampling Interval	Ambient	Outdoor	Transport	Indoor	Personal
Sahsuvaroglu et al. (2009)	Hamilton, Canada (mid-sized city)	October, 2003	72-h	-	All: 32.0[b] Non-ETS: 31.7[b]	-	All: 22.4[b] Non-ETS: 21.9[b]	All: 23.3[b] Non-ETS: 22.4[b]
		May, 2004			All: 17.6[b] Non-ETS: 16.8[b]		All: 13.5[b] Non-ETS: 12.3[b]	All: 14.4[b] Non-ETS: 14.0[b]
		August, 2004			All: 9.7[b] Non-ETS: 9.6[b]		All: 8.2[b] Non-ETS: 7.4[b]	All: 8.8[b] Non-ETS: 8.2[b]
		Total			All: 19.3[b] Non-ETS: 18.9[b]		All: 14.4[b] Non-ETS: 13.6[b]	All: 15.2[b] Non-ETS: 14.6[b]
Schembari et al. (2013)	Barcelona, Spain (large city)	November, 2008 and October, 2009	7-day	-	18.7[c,e]; 19.4[b,e]	-	19.2[c,e]; 20.6[b,e]	17.7[c,e]; 18.6[b,e]
Molloy et al. (2012)	Melbourne, Australia (large city)	August, 2008-December, 2008; January, 2009-April, 2009	7-day	-	9.5[a]; 10.0[b]	-	7.9[a]; 8.4[b]	-
Pegas et al. (2012)	Aveiro, Portugal (small city center, suburb)	April-June, 2010	7-day	-	City center: 10.5[b,e]; Suburb: 10.1[b,e]	-	City center: 7.4[b,d,e]; Suburb: 6.9[b,d,e]	-

[a]med

[b]avg

[c]geo. mean

[d]Averaged over 4 classrooms and 2 weeks.

[e]Reported in $\mu g/m^3$ and converted to ppb assuming 25 °C and 760 mmHg.

[f]Data provided by the authors for Figure 1 of Physick et al. (2011).

Table 2-10 Correlations between measured NO_2 concentrations from personal, indoor, outdoor, and ambient monitors.

Study	Location	Personal-Ambient	Outdoor-Personal	Indoor-Personal	Indoor-Outdoor
Sarnat et al. (2012)[a]	Ciudad Juarez, Mexico and El Paso, Texas	---	---	---	CJ-A: 0.36 CJ-B: 0.92 EP-A: 0.66 EP-B: 0.01
Williams et al. (2012a)[a]	Wayne County, Michigan	All Subjects: 0.11 Vest-Compliant (>60%)[c]: 0.14	---	---	---
Suh and Zanobetti (2010b)[a]	Atlanta, GA	0.12	---	---	---
Brown et al. (2009)	Boston, MA	Winter: 0.00 Summer: 0.03			
Delfino et al. (2008a)	2 Southern California Cities	0.43	---	---	---
Delgado-Saborit (2012)	Birmingham, U.K.	1-h NO_2: 0.024 Sampling event NO_2: 0.15	---	---	---
Lee et al. (2013)[b]	Seoul, South Korea	---	Summer: 0.39 Winter: 0.47	Summer: 0.50 Winter: 0.55	Summer: 0.71 Winter: 0.22
	Daegu, South Korea	---	Summer: 0.43 Winter: 0.47	Summer: 0.32 Winter: 0.59	Summer: 0.65 Winter: 0.57
	Asan, South Korea	---	Summer: 0.62 Winter: 0.11	Summer: 0.63 Winter: 0.37	Summer: 0.67 Winter: 0.37
	Suncheon, South Korea	---	Summer: 0.46 Winter: 0.56	Summer: 0.46 Winter: 0.60	Summer: 0.77 Winter: 0.80
	All 4 Cities	---	Summer: 0.58 Winter: 0.53	Summer: 0.60 Winter: 0.55	Summer: 0.78 Winter: 0.55
Physick et al. (2011)	Melbourne, Australia	---	---	---	---

Table 2-10 (Continued): Correlations between measured NO_2 concentrations from personal, indoor, outdoor, and ambient monitors.

Study	Location	Personal-Ambient	Outdoor-Personal	Indoor-Personal	Indoor-Outdoor
Sahsuvaroglu et al. (2009)[b]	Lake Ontario, Canada (Winter)	---	All Subjects: 0.002 Non-ETS: 0.020	All Subjects: 0.430 Non-ETS: 0.283	---
	Lake Ontario, Canada (Spring)	---	All Subjects: 0.233 Non-ETS: 0.187	All Subjects: 0.589 Non-ETS: 0.599	---
	Lake Ontario, Canada (Summer)	---	All Subjects: 0.067 Non-ETS: 0.011	All Subjects: 0.822 Non-ETS: 0.783	---
	Lake Ontario, Canada (All Seasons)	---	All Subjects: 0.517 Non-ETS: 0.540	All Subjects: 0.729 Non-ETS: 0.693	---
Schembari et al. (2013)[a]	Barcelona, Spain	---	0.58	0.78	0.53

[a]Spearman coefficient.
[b]Pearson coefficient.
[c]Subjects wore the sampling vests at least 60% of the sampling period.

2.6.5.2 Factors Influencing Exposure Measurement Error

Estimates of NO_2 exposures are subject to errors that can vary in nature. Classical error is defined as error scattered around the true personal exposure and independent of the measurement. Classical error results in bias of the true E_t. Berkson error is defined as error scattered around the exposure surrogate (i.e., central site monitor measurement) and independent of the true value (Goldman et al., 2011; Reeves et al., 1998).When an epidemiology study is performed, nonambient contributions are thought to introduce Berkson error into the E_t term that does not bias epidemiologic effect estimates for ambient NO_2 assuming that nonambient NO_2 sources are independent of ambient sources but does cause the confidence intervals around effect estimates to widen (Sheppard, 2005; Wilson et al., 2000).

Recent studies suggest that exposure error is a combination of Berkson-like and classical-like errors and depend on how exposure metrics are averaged across space. Goldman et al. (2011) simulated the effect of classical-like and Berkson-like errors due to spatiotemporal variability among ambient or outdoor air pollutant concentrations over a large urban area on estimates of ED visits for cardiovascular disease. The relative risk (RR) per ppm was negatively biased in the case of classical-like error (1-h max NO_2: -1.3%; 1-h max NO_X: 1.1%) and negligibly positively biased in the case of Berkson-like error (1-h max NO_2: 0.0042%; 1-h max NO_X: 0.0030%). Conversely, the 95% confidence interval range for RR per ppm was wider for Berkson-like error (1-h max NO_2: 0.028; 1-h max NO_X: 0.023) compared with classical-like error (1-h max NO_2: 0.0025; 1-h max NO_X: 0.0043). This is in agreement with previous findings (e.g.,Sheppard et al., 2005; Zeger et al., 2000), that Berkson error tends to widen the confidence interval of the effect estimate, while classical error tends to bias the health effect estimate.

Several studies have investigated factors that influence the relationship between personal exposure measurements and ambient concentrations. 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 ambient NO_2 concentrations for 32 sample populations, of which 17 were from pooled analyses, 8 were from longitudinal analyses, and 7 were from daily average analyses. Meta-regression results are shown in Table 2-11 and were reported to be statistically significant. Meng et al. (2012b) found that the associations depended on several factors, including season, age, pre-existing disease, and potentially, sampling artifacts and local sources. Bellander et al. (2012) measured personal NO_2 exposure and modeled it as a function of NO_2 concentrations measured at an urban area, at a rural area, at a 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) with all models being statistically significant except that for rural NO_2 (p = 0.18). Factors influencing the association between $C_{a,csm}$ and E_a include spatiotemporal gradients in ambient NO_2 concentrations, instrument error, housing characteristics such as air conditioning usage (Sheppard et al., 2005), and uncertainty in time-activity data (Isaacs et al., 2013). These factors are described in the following subsections.

Table 2-11 Meta-regression results from 15 studies examining the relationship between personal exposure measurements and ambient concentrations.

	Slope	Correlation	Slope	Correlation
Study design	**Based on original studies**		**Corrected for publication bias**	
Pooled[a]	0.40	0.42	0.30	0.37
Longitudinal[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. (2008a)

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

Spatial Variability of Ambient NO_2 Concentrations

Recent studies have explored the effect of spatial exposure measurement error on health effect estimates. Goldman et al. (2010) simulated spatial exposure measurement error based on a semivariogram function across monitor sites with and without temporal autocorrelation at one- and two-day lags incorporated into the analysis to analyze the influence of spatiotemporal variability among ambient or outdoor concentrations over a large urban area on ED visits for cardiovascular disease. A random error term was calculated from the semivariogram and added to a base case time series to simulate estimates of population exposure to NO_2 concentrations subject to spatial error in 1,000 Monte Carlo simulations. For the analysis with autocorrelation considered, RR per ppm for 1-h max NO_2 dropped to 1.0046 (p = 0.10), and RR per ppm for 1-h max NO_X dropped to 1.0079 (p = 0.026) in comparison with the base case RR per ppm = 1.0139 ($p = 9 \times 10^{-6}$). When autocorrelation was not considered, RR per ppm dropped to 1.0044 (p = 0.12) for 1-h max NO_2 and 1.0074 (p = 0.032) for 1-h max NO_X. Goldman et al.

(2010) results suggest that spatial exposure measurement error results in biasing the health effect estimate towards the null. 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 $PM_{2.5}$.

Spatial resolution of the personal exposure estimates has been evaluated in recent studies. Szpiro et al. (2011) explored the effect of specification of spatial conditions in a health model by comparing bias and prediction accuracy for the health effect estimate using correctly specified and misspecified exposure simulation conditions. The Szpiro et al. (2011) simulations were for a generic air pollutant, but the results are relevant to NO_2 exposure. Land use regression (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 covariate. Although prediction accuracy was higher for the correctly specified model (R^2 = 0.73 to 0.74 versus R^2 = 0.49 to 0.50 for the misspecified model), magnitude of bias in the effects estimate was also slightly higher for the correctly specified model (bias = -0.007 to -0.035 compared with bias = -0.001 to 0.001 for the misspecified model). The results of Szpiro et al. (2011) suggested that use of more spatially resolved personal exposure metrics does not necessarily decrease the magnitude of bias in effect estimates. However, given the small magnitude of bias noted in either case, it is possible that the spatial error was primarily Berkson in nature.

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; see Table 2-12. Specifically, Goldman et al. (2012) examined the correlations between exposure measurement errors with measured and true exposures, where true exposures were estimated from chemical transport modeling (CTM) for a cohort living in the 20-county Atlanta metropolitan area; see Table 2-12. Here, they compared exposure measurement error metrics among exposure estimates obtained from using a central site monitor, an average of monitors distributed throughout the region where the study population lives, a population-weighted average, an area-weighted average, and population-weighted average computed with concentrations modeled using CTM, at 5 km resolution.

Exposure error was simulated in Goldman et al. (2010). They 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 anti-correlated with the true value but had relatively higher positive correlation with the measured value. For the other sites, the exposure measurement errors were anti-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 measurement 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 and more classical-like error in the central site monitor; accounting for spatial variation in NO_X concentrations across the population likely resulted in improved accuracy of the exposure metric. Correlations between measured values at the central site monitor and the CTM were typically lower than for the monitoring average techniques.

Table 2-12 Exposure measurement error metrics for comparing central site monitoring data and various monitor averages compared with values computed from a CTM.

Pollutant	Bias[(Z – Z*)/Z][a]	R^2(Z, Z*)[b]	R[(Z – Z*),Z*]	R[(Z – Z*),Z]
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
Chemical transport 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
Chemical transport model – population-weighted average	N/A	0.52	-0.80	-0.00042

Nore: 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).

Source: Goldman et al. (2012).

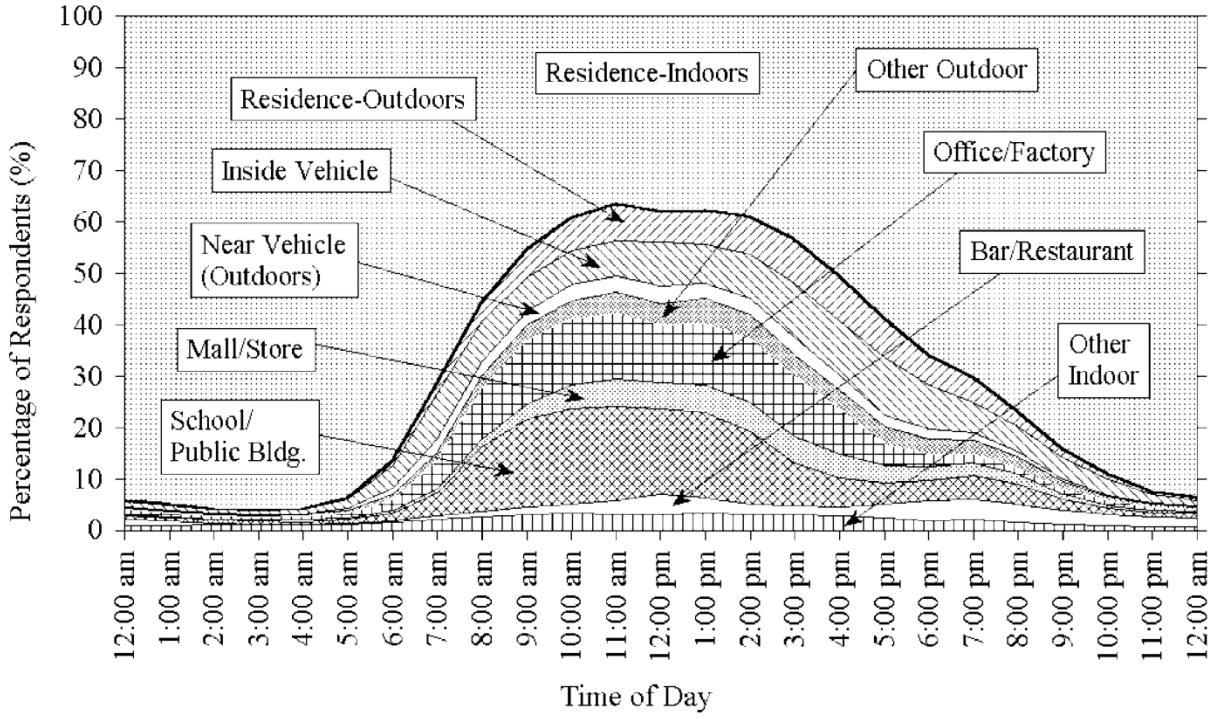
Uncertainty in Time-Activity Data

Large-scale human activity databases, such as those developed for the Consolidated Human Activity Database (CHAD) including the National Human Activity Pattern Survey (NHAPS), have been designed to characterize exposure patterns among much larger population subsets than can be examined during individual panel studies (Klepeis et al., 2001). CHAD consists of a consolidation of human activity data obtained during several panel studies in which diary or retrospective activity data were obtained for metropolitan, state-wide, or nationwide samples (Graham and McCurdy, 2004). CHAD is intended to provide data to reproduce particular study conditions but does not contain a distribution that mimics the U.S. population given that it contains multiple location-specific panel studies; NHAPS by itself does contain a sufficient data distribution to provide for national inference (Graham and McCurdy, 2004).

The complex human activity patterns across the population (all ages) for NHAPS are illustrated in Figure 2-21 (Klepeis et al., 2001). This figure is presented to illustrate the

diversity of daily activities among the entire population as well as the proportion of time spent in each microenvironment. 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 communities with larger percentages of non-whites (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 versus 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 studies have included working adults (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 exposed in each microenvironment.

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 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. 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. The authors concluded that the time-weighting approach provided a reasonable approximation of personal exposure but sometimes underestimated it.



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

Figure 2-21 Distribution of time sample population spends in various environments, from the U.S. National Human Activity Pattern Survey (all ages).

Variability in time-activity data also presents a source of uncertainty in the exposure model, particularly for the effect of exposure misclassification on the α term. Isaacs et al. (2013) performed a time-activity study of a panel of eight adults (four men and four women living around Research Triangle Park, NC) of similar demographic and socioeconomic groups and observed statistically significant inter- and intra-individual variability that could potentially add uncertainty to exposure predictions.

Error and Uncertainty Related to Infiltration

Given that people spend the majority of their time indoors, building air exchange rates influence exposure to ambient NO_2. In an analysis of NO_2 data from the Detroit Exposure and Aerosol Research Study (DEARS), Meng et al. (2012a) observed seasonal differences, with statistically significant slopes of 0.24 ($p < 0.001$) for E_T versus $C_{a,csm}$ and of 0.13 ($p = 0.033$) for E_a versus $C_{a,csm}$ for summer measurements. For winter measurements, the slopes were not statistically significant (E_T versus $C_{a,csm}$: slope = 0.08,

p = 0.10; E_a versus $C_{a,csm}$: slope = 0.07, p = 0.33). Meng et al. (2012a) found that high air exchange rate (>1.3 air changes per hour), no central air conditioning, use and non-use of window fans, and presence of old carpeting were statistically significant determinants of α for NO_2 (p <0.05) in summer; none of these factors were statistically significant determinants of α for NO_2 in winter. 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. and observed that time spent in transit was positively and statistically significantly associated with prediction error of a microenvironmental model of personal NO_2 exposure (p = 0.01). In Mölter et al. (2012), outdoor exposures were calculated with LUR, while indoor exposures were calculated using the 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). Sarnat et al. (2013a) tested if air exchange rate acted as an effect modifier of NO_X exposure on asthma emergency department visits and observed an effect in both interaction and stratified models. Because the Sarnat et al. (2013a) paper treated air exchange as an effect modifier rather than a source of error, it is discussed further in Section 2.6.5.3.

Instrument Error

Exposure measurement error related to instrument precision has a smaller effect compared with error related to spatial gradients in the concentration. Goldman et al. (2010) investigated instrument precision error at locations where ambient monitors were co-located. Instrument precision error increased with increasing concentration and was observed to exhibit some autocorrelation at one- and two-day lags. A random error term based on observations from co-located monitors was added to a base case time series to simulate population estimates for ambient air concentrations subject to instrument precision error in 1,000 Monte Carlo simulations. Very little change in risk ratios and significance levels was observed for 1-h max NO_2 and 1-h max NO_X concentrations. For 1-h max NO_2 concentration, the RR per ppm of NO_2 concentration with simulated instrument precision error was 1.0133 ($p = 2.1 \times 10^{-5}$) compared with RR per ppm = 1.0139 ($p = 9 \times 10^{-6}$) for the base case. For 1-h max NO_X concentration with simulated instrument precision error, RR per ppm = 1.0132 ($p = 1.8 \times 10^{-5}$) compared with the base case of 1.0139 ($p = 9.0 \times 10^{-6}$). Although statistically significant, the amount of exposure measurement bias related to instrument precision was very small.

2.6.5.3 Implications for Epidemiology

The model of human health effects related to ambient NO_2 exposure is of the form:

$$Y = \beta_0 + \beta_1 E_a + \beta_Z Z + \varepsilon$$

Equation 2-11

Where β_0 = model intercept, β_1 = effect estimate for the ambient exposure, E_a = ambient exposure, Z = covariate vector, β_Z = vector of slope related to each covariate, and ε = random error.

Recognizing the relationship between E_a and C_a described in Section 2.6.1, Equation 2-11 can also be written as:

$$Y = \beta_0 + \beta_1 \alpha C_{a,csm} + \beta_Z Z + \varepsilon = \beta_0 + \beta_{1a} \alpha C_{a,csm} + \beta_{1b} \alpha + \beta_{1c} C_{a,csm} + \beta_Z Z + \varepsilon$$

Equation 2-12

Here, α = exposure factor, as described in Section 2.6.1 and $C_{a,csm}$ = ambient concentration measured at a central site monitor, as defined in Section 2.6.5.1. Hence, two metrics of interest for exposure assessment studies can be considered: E_a, personal exposure to ambient NO_2, and $C_{a,csm}$, NO_2 concentration measured at a central site monitor to represent E_a.

For long-term epidemiologic studies of human exposure to NO_2, where the magnitude of the concentration is of most interest, E_a may be the most appropriate metric. If $C_{a,csm}$ is then used as a surrogate for E_a, then α can be considered to encompass the exposure measurement error related to uncertainties in the spatial distribution of NO_2, time activity data, air exchange rate, or instrument precision, as described in detail in Section 2.6.5.2. $C_{a,csm}$ may be an acceptable (i.e., minimally biased) surrogate for E_a if the central site monitor is located in close proximity to the entire study population (e.g., in a dense urban setting) or if there are either few or well-dispersed localized NO_2 sources such that the influence of spatial variability is minimized. There is limited information regarding the influence of near-road exposures on exposure measurement error and if $C_{a,csm}$ may be a biased exposure surrogate when representing people's exposure in the near-road environment for epidemiologic studies of long-term exposure.

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). Magnitude of the concentration is less important in this case, so $C_{a,csm}$ can be an acceptable surrogate if the central site monitor captures the temporal variability of the true air pollutant concentration even if the magnitude of concentration is biased. Additionally, for studies involving thousands of participants, it is not feasible to measure personal exposures. For this reason, $C_{a,csm}$ is typically employed to represent the community average concentration as measured at the central site monitor. Typically, it is assumed that $C_{a,csm}$ is a surrogate for E_a; see U.S. EPA (2008c) and studies cited therein as well as studies cited throughout this section.

Consideration of errors in use of $C_{a,csm}$ from central site monitoring data as a surrogate for E_a is for the purpose of assessing the impact of this substitution on health effect estimates in community time-series studies of short-term exposure. Recently, Setton et al. (2011) investigated how spatial variability and unaccounted study participant mobility bias effect estimates in short-term epidemiologic models of NO_2 exposure in southern California and Vancouver, British Columbia. In this case, a monitor was placed at each participant's home, and bias 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), the effect estimate in the monitor-based approach was more biased towards the null. Similarly, Van Roosbroeck et al. (2008) evaluated effect estimates for the influence of NO_2 on four respiratory outcomes among children obtained with the NO_2 data from a single monitor located at the children's school in an epidemiologic study of short-term exposure. The 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 effect estimates were biased towards the null by one-third to one-half when using a single monitor. The results of Setton et al. (2011) and Van Roosbroeck et al. (2008) imply that failure to capture spatial variability in ambient NO_2 exposures can lead to biasing the effect estimate towards the null in time-series epidemiologic studies of short-term exposure. This is in agreement with the primary conclusion of the 2008 ISA for Oxides of Nitrogen U.S. EPA (2008c).

In the model formulation presented in Equation 2-12, α may be considered an effect modifier that interacts with $C_{a,csm}$ rather than contributing error to E_a. For example, Sarnat et al. (2013a) developed an effect modification model that used a_i, air exchange rate, as the effect modifier of exposure to NO_X, where a_i is defined in Section 2.6.1. The effect estimate was positive ($\beta = 1.9$) and statistically significant for NO_X ($p = 0.04$) for the model interaction term but not for the linear concentration and a_i terms, suggesting

effect modification by a_i. Further evidence of effect modification comes from an analysis in which the model (Equation 2-11) was stratified by low and high a_i; positive associations different from the linear estimate were observed for both low and high a_i, with a stronger and statistically significant association for the high a_i term. Given that most people spend upwards of 90% of their time indoors (Sarnat et al., 2013a), a_i is a major determinant of an individual's α. Exposure measurement errors affecting α, as described in Section 2.6.5.2, would add error to the effect modification term but not to the linear concentration term, as would be the case for Equation 2-11. The Sarnat et al. (2013a) paper was the first to apply the concept of effect modification to study health effects associated with NO_X exposure.

2.7 Summary and Conclusions

NO_X concentrations have generally decreased over the past 20 years and this trend continues. However, new diesel control technologies cause a greater proportion of NO_X to be present as NO_2 rather than NO. Moreover, diesel vehicles have become a relatively more important source as emissions from other major sources have decreased. Annual average NO_2 concentrations remain well below NAAQS levels, but 1-hour daily maximum levels appear to be exceeded in several near road studies. Background levels are much lower than ambient concentrations. In urban areas, NO_X and NO_2 concentrations exhibit a high degree of spatial variability, which presents difficulties for estimating exposure. Measurement of NO and NO_2 concentrations is also a challenge, and methods are under development for measuring true NO_2 concentration that improve interference problems associated with the current federal reference method. Concentrations are especially high near local sources, especially roadways with heavy traffic. Influence of distance from roadways is especially pronounced for NO, with a weaker influence of NO_2 concentrations.

Although total personal exposure to NO_2 includes ambient and nonambient components, this assessment is focused on the ambient component of personal NO_2 exposure, because it is relevant to review of the NAAQS. Personal exposure to ambient NO_2 can be estimated by a variety of techniques. These include models (e.g., dispersion models, land use regression models, and stochastic population exposure models), personal exposure measurements, and use of central site NO_2 concentration measurements as exposure surrogates. NO_2 exposure estimates are subject to error. These errors are influenced by spatial NO_2 concentration variability, time-activity data, air exchange characteristics of microenvironments, and accuracy and precision of instrumentation. Central site NO_2 concentration may be acceptable (i.e., minimally biased) for use as a surrogate for ambient NO_2 exposure in epidemiology studies of long-term exposure if the central site

monitor is located in close proximity to the entire study population (e.g., in a dense urban setting) or if there are either few or well-dispersed localized NO_2 sources such that the influence of spatial variability is minimized. Central site NO_2 measurements may be an acceptable NO_2 exposure surrogate for community time-series epidemiology studies if the central site monitor concentration captures the temporal variability of the true personal exposure to ambient NO_2. Recent time-series epidemiology studies of short-term exposure evaluating the effect of using a single monitor to represent exposure to ambient NO_2 demonstrate that use of a single monitor results in health effect estimates that are biased towards the null. This is in agreement with the findings of the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c). The effect of near-road exposures on adequacy of exposure estimates from central site monitors is not yet well characterized for epidemiologic studies.

References for Chapter 2

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CHAPTER 3 DOSIMETRY AND MODES OF ACTION FOR INHALED OXIDES OF NITROGEN

3.1 Introduction

This chapter has two main purposes. The first is to describe the principles that underlie the dosimetry of NO_2 and NO and to discuss factors that influence it. The second is to describe the modes of action that may lead to health effects that will be presented in Chapter 4 and Chapter 5. This chapter is not intended to be a comprehensive overview, but rather, it updates the basic concepts derived from NO_2 and NO literature presented in the 1993 AQCD and 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a, c, 1993) and introduces the recent relevant literature.

In Section 3.2, particular attention is given to chemical properties of inhaled NO_2 and NO that affect absorption, distribution, metabolism, and elimination. The net contribution of inhaled NO_2 and NO and subsequent reaction products are discussed in relation to those endogenously occurring. Because there have been few NO_2 dosimetry studies published since the 1993 AQCD (U.S. EPA, 1993), much of that information 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 extracellular lining fluid (ELF) and by reactions of NO with heme proteins, processes which play roles in both uptake and biological responses.

Section 3.3 highlights findings of studies published since the 2008 ISA (U.S. EPA, 2008a, c) that provide insight into the biological pathways affected by exposure to NO_2 and NO. Since common mechanisms lead to health effects from both short- and long-term exposure to NO_2 and NO, these pathways are discussed in this chapter rather than in later chapters. The related sections of health effects chapters are indicated. 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 are of greater interest, since mechanisms responsible for effects at low concentrations may not be identical to those occurring at high concentrations. Some studies at higher concentrations are included if they were early demonstrations of key mechanisms or if they are recent demonstrations of potentially important new mechanisms.

3.2 Dosimetry of Inhaled Oxides of Nitrogen

3.2.1 Introduction

This section provides a brief overview of NO_2 and NO dosimetry and updates information provided in the 2008 ISA (U.S. EPA, 2008c). 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 the net contribution of inhaled NO_2 and NO and subsequent reaction products in relation to those endogenously occurring.

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 Figure 2-14 and Figure 2-15). One-hour average, near-road (15 meters) NO_2 concentrations in Los Angeles, CA range from 3 ppb 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-hour average 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, due to its high reactivity, it is unlikely that these concentrations of inhaled NO_2 will diffuse through the ELF to reach the respiratory tract epithelium and less likely that NO_2 itself becomes systemically distributed. Therefore endogenous steady state levels of NO_2 in distant tissues are unlikely to be affected by inhaled NO_2 at ambient concentrations. The balance of reaction products from inhaled NO_2 relative to endogenous levels will also be considered.

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 Figure 2-14 and Figure 2-15). One-hour average, near-road (15 meters) NO concentrations in Los Angeles, CA range 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-hour average NO concentrations may still reach 250 ppb, but median NO concentrations are 5 ppb or less (Polidori and Fine, 2012). Comparison of NO_X data from Zhu et al. (2008) for the same roadway (Interstate 710), though on a different year, with that from Polidori and Fine (2012) suggests that on-road NO concentrations may exceed those near road. As will be

discussed these ambient NO concentrations are generally in the range of those occurring endogenously in the respiratory tract.

3.2.2 Dosimetry of NO_2

NO_2 is a highly reactive gas that occurs as a free 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 the entire respiratory tract surfaces (Bastacky et al., 1995). The ELF constituent composition shows appreciable heterogeneity with respect to anatomic site and species. Furthermore, both the alveolar surfaces and the conducting airway surfaces have a monomolecular layer of surface active lipids (Bernhard et al., 2004; Hohlfeld, 2002; Mercer et al., 1994), largely fully saturated, which reduce 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 non-electrolyte solute, and thus becomes subject to partitioning and reaction/diffusion characteristics like all small molecules. 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 for pulmonary 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 for Oxides of Nitrogen (U.S. EPA, 2008c, 1993) are briefly discussed below for thoroughness.

3.2.2.1 Mechanisms of Absorption of NO_2

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, 2008c), chemical reactions between NO_2 with ELF

constituents were more readily recognized as governing NO_2 absorption in the respiratory tract.

3.2.2.1.1 Reaction with ELF Water

Previous studies have demonstrated that it is not NO_2 but instead it is the NO_2 dimer, N_2O_4, that reacts with water to yield nitrite and nitrate (NO_3^-) (Schwartz and White, 1983; England and Corcoran, 1974) with more recent sophisticated analyses introducing greater detail (Finlayson-Pitts et al., 2003). However, in aqueous solutions, NO_2 undergoes rapid reaction with many solutes, in particular with solutes that are easily oxidized. Furthermore, at environmentally relevant concentrations of NO_2, 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 ppb 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. 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). Although during reactive uptake by pure water all reaction occurs via N_2O_4 regardless of the concentration of NO_2, this process becomes unlikely, and instead occurs via direct reactions of NO_2, in the presence of dissolved reactive substrates and at low, environmentally relevant concentrations of NO_2. The latter conditions resemble reactive uptake of NO_2 by the ELF that would entail direct reactions of NO_2, for example, with dissolved small molecular weight antioxidants like glutathione, 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 disconcerting 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). Enami et al. (2009) only measured nitrate and thereby these data do not strongly support their contention, except to suggest perhaps that some hydrolysis of NO_2 may be occurring

since nitrate was detected. Moreover, nitrite data are important because any excess nitrite formed (reaction with water generally yield a 1:1 ratio of nitrite and nitrate; thus, a yield of nitrite above 1 would be considered in excess) would be a main product formed in many one-electron oxidations by NO_2. Thus, by not measuring nitrite, an important index to assess oxidation by NO_2 was missed.

It should also be noted that Enami et al. (2009) conducted their experiments in the absence of oxygen which is important regarding the applicability of their model to the lung. At environmentally relevant concentrations and physiologic temperatures intrapulmonary gas phase NO_2 will exist in its monomeric form, plus 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.

3.2.2.1.2 Governing Determinants of NO_2 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 ELF reactive substrates (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) relative to its free radical reactions with biological substrates (further discussion below). However, since in the presence of aqueous phase reactants NO_2 absorption is robust, it is the rapid reactions with ELF substrates that maintain the net driving force for NO_2 mass transfer from the intrapulmonary 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 its 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, was aqueous substrate-dependent, and was 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 free 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 H^+ and nitrite. The concentration of the resulting nitrite is likely insufficient to alter physiological function since basal nitrite levels may not change appreciably due to an environmental exposure. 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 the proton load although the ELF buffering capacity is anticipated to compensate for environmentally-relevant exposure-related proton generation. Nitrite will diffuse into the underlying epithelial cells and vascular space wherein, in the presence of red blood cells, nitrite is oxidized to nitrate (Postlethwait and Bidani, 1989; Postlethwait and Mustafa, 1981).

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-fold 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, and 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 (rodent and some primate ELF contains significantly less urate than 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, dipalmitoyl phosphatidylcholine, was relatively unreactive towards NO_2 but subsequent studies documented that compressed monomolecular interfacial films of dipalmitoyl phosphatidylcholine inhibit NO_2 absorption in vitro (Connor et al., 2001). Similar to the bell-shaped dose/response related to NO_2 reaction with antioxidants, documenting whether surface active phospholipids (surfactant) inhibit NO_2 mass transfer in vivo is extremely challenging due to the fact that any in situ manipulations that disrupt the surface tension lowering actions of 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 intrapulmonary distribution of inhaled NO_2, local mass transfer rates, and thus dosimetry.

3.2.2.1.3 Reaction/Diffusion of ELF NO_2, Potential for Penetration to Underlying Cells

Since rapid ELF reactions constrain the diffusion of solute NO_2, exposure-related cellular perturbations within the lung are expected to be related to the ELF-derived products generated during reaction with NO_2, rather than by solute NO_2 per se. In support of this concept, one can estimate the distance (d) that NO_2 is able to diffuse before it chemically reacts with ELF constituent molecules in the ELF (e.g., antioxidants, proteins, lipids, etc.) using the Einstein-Smoluchowski equation:

$$d = \sqrt{2D\tau}$$

Equation 3-1

Where D is the molecular diffusion coefficient of NO_2 and tau (τ) is the time that NO_2 is allowed to diffuse into the ELF medium which is constrained by its rates of reaction and can be set to its half-life assuming pseudo first-order kinetics will apply.

The transit time (τ) then has the form:

$$\tau = \frac{\ln(2)}{\sum_i^n k_i c_i}$$

Equation 3-2

where the term $\sum_i^n k_i c_i$ represents the summation of the products of the rate constants (k_i) and concentrations (c_i) for all the reactive substances that are present in the ELF.

Replacing τ in Equation 3-1 yields:

$$d = \sqrt{\frac{2D \ln(2)}{\sum_i^n k_i c_i}}$$

Equation 3-3

This approach to estimate the penetration distance into the ELF was originally applied by Pryor (1992) to the lung surface penetration of ozone and later by Ford et al. (2002) for the diffusion distance of NO_2 in the cytoplasm and blood plasma. A diffusion coefficient D for NO_2 in water at 25 °C equal to 1.4×10^{-9} m^2/sec has been reported and will be used in calculations. 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.

In considering the classes of ELF biomolecules that react with NO_2, one may focus on the water-soluble small molecular weight antioxidants (SMWAOs; 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 for the SMWAOs 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 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 for the case

of glutathione, ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] was used to produce the intense chromophore $ABTS^{\bullet-}$ from its reaction with the glutathiyl radical.

Species and anatomical loci must be considered when selecting appropriate concentrations of reactive ELF biomolecules. Table 3-1 illustrates the SMWAO 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). Because the ELF only reaches dimensions that NO_2 is predicted to penetrate (i.e., 0.2 to 0.6 μm) in isolated regions of the alveolar spaces (Bastacky et al., 1995), it is reasonable to assume that NO_2 per se does not directly interact with most apical surfaces of the respiratory tract epithelial (Postlethwait et al., 1991b).

Table 3-1 Small molecular weight antioxidant concentrations in ELF and predicted penetration distances for NO_2.

Species - site	Ascorbate	Urate	Glutathione	$\sum_{i}^{n} k_i c_i$ (sec^{-1})	ELF penetration (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).

3.2.2.2 ELF Interactions with NO_2

3.2.2.2.1 In Vitro Studies

Small molecular weight antioxidants vary appreciably across anatomic sites and species. For example, due to the lack of urate oxidase, humans, primates, and select other species have increased levels of urate and 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 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 luminal 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 epithelial injury from NO_2 exposure. 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) since 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). Since the ELF varies throughout the respiratory tract, the heterogeneous distribution of epithelial injury observed from 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 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 gas phase NO_2 concentration, aqueous substrate concentrations, surface area, gas flow and related impacts on boundary layer diffusive resistance, pH, temperature, and others (Adgent et al., 2012; Bidani and Postlethwait, 1998).

3.2.2.2.2 Human Studies

In vivo studies

In vitro studies have clearly illustrated the role of antioxidants in mediating NO_2 uptake and membrane oxidation; however, the temporal dynamics of biological responses to NO_2 that occur in vivo are far more complex. Recognizing 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.

Antioxidant levels vary spatially between lung regions and temporally with NO_2 exposure. Kelly et al. (1996a) examined the effect of a four-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).

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, sample 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 the cascades leading to alterations in cell signaling, cell injury, inflammation, etc. Thus, placing ELF measures within the context of ELF turnover time, clearance of “stable” reaction products, and species generated/regenerated

as a consequence of secondary redox reactions should all be incorporated during data interpretation.

Ex vivo studies

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.

Nonetheless, such results must be placed in the context of secondary redox reactions as the reported measurements reflect net effects on individual antioxidants but may lend limited insights into the initial reactions of NO_2 within the ELF, and by extension, 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 glutathione (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 nitrite (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, resulting in an apparent, but potentially inaccurate, conclusion of direct loss of urate during subsequent analyses. In addition, some reports have suggested observations that include detecting 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 DNA spontaneous decomposes to its keto acid) and since high redox couples are maintained in the ELF and it 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, 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.

3.2.2.3 Regional and Total Respiratory Absorption of NO_2

There has been very limited work related to the quantification of NO_2 uptake since the 1993 AQCD for Oxides of Nitrogen (U.S. EPA, 1993) or the subsequent 2008 ISA (U.S. EPA, 2008c). Consequently, only an abbreviated discussion of this topical area is included.

3.2.2.3.1 Experimental Studies of NO_2 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 uptakes of 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), respectively. Uptake was not affected by a 4-hour prior exposure (naive versus previously exposed rats) to 40,400 ppb NO_2 and was constant over the 24-minute period during which uptake was determined.

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. However, the greater nasal than oral uptake on NO_2 is consistent with what is observed for O_3 as

described in Chapter 5 of the 2013 ISA for Ozone and Related Photochemical Oxidants (U.S. EPA, 2013b).

Overall, NO_2 fractional absorption (uptake efficiency) in the upper respiratory tract is greater in the nasal than oral passage and decreases with increasing ventilation rates. This causes a greater proportion of inhaled NO_2 to be delivered to the lower respiratory tract at higher ventilation rates associated with exercise. In humans, the breathing pattern shifts from nasal to oronasal during exercise relative to rest. Since 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. 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.

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 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 showed a linear relationship between NO_2 uptake and total inspired dose (25 to 330 μg NO_2). 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 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 how NO_2 uptake was measured (mass balance; wet chemical versus automated analyzer that 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 upper respiratory tract uptakes, 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 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 adult asthmatics 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 NO_2 uptake by 5-fold in these subjects. In an earlier study of seven healthy adults in which subjects were exposed to a NO_2/NO mixture containing 290 to 7,200 ppb NO_2 for brief (but unspecified) periods, Wagner (1970) reported that NO_2 uptake increased from 80% during normal respiration (V_T, 0.4 L) to 90% 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 female beagle dogs while 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. In large part, this increase in uptake may 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 uptake rate of NO_2 was 3-fold greater for the dogs during exercise than rest.

3.2.2.3.2 Dosimetry Models of NO_2 Uptake

There is a paucity of theoretical studies investigating NO_2 dosimetry. The original seminal dosimetry models 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 reactants to the tissues. They assumed reactions of NO_2 with constituents in the ELF as protective in that these reactions

reduced the flux of NO_2 to the tissues. Others have postulated that NO_2 reactants formed in the ELF, rather than NO_2 itself, could actually cause adverse responses (Velsor and Postlethwait, 1997; Postlethwait and Bidani, 1994; Overton, 1984). Two studies examined the influence of age on reactive gas dosimetry in humans (Ginsberg et al., 2005; Sarangapani et al., 2003). 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 between 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, due to the fact that children under age 5 have much a much smaller airway surface area in the extrathoracic (nasal) and alveolar regions (Sarangapani et al., 2003).

The predicted tissue dose and dose rate of NO_2 (NO_2 flux to liquid-tissue interface) is low in the trachea, increases to a maximum in the terminal bronchioles and the first generation of the pulmonary region, and then decreases rapidly with distal progression. The site of maximal NO_2 tissue dose is predicted to be fairly similar between 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 3-1 showed that NO_2 is not expected to go deeper than 0.2 to 0.6 μm into the ELF before reacting with substrates. The production of toxic NO_2 reactants in the ELF and the movement of the reactants 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 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.

3.2.2.4 Endogenous Generation, Metabolism, Distribution, and Elimination of NO_2

Along with CO, NO_2 is a criteria pollutant believed to be produced endogenously in the lung.[1] This endogenous production and function may have important implications for the interpretation of health effects studies. It is also interesting to note that organisms tend to be less sensitive to endogenously produced oxidants. For example, cells are quite resistant to hydrogen peroxide, an endogenous product of oxygen reduction in aerobic cells. NO_2 may be produced endogenously by various processes, including the acidification of nitrite ($2\ H^+ + 2\ NO_2^- \rightarrow 2HNO_2 \rightarrow H_2O + N_2O_3 \rightarrow NO + NO_2$) (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 occur where 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 forms nitrite and the antioxidant radicals. These reactions are so fast they only allow NO_2 to diffuse small distances in the submicrometer range before it reacts (see above, Ford et al., 2002). NO_2 is only slightly hydrophobic (Squadrito and Postlethwait, 2009) and faces no significant physical barriers to readily traverse biological membranes, but in view of its high reactivity, it is unlikely that NO_2 becomes 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,

[1] Evidence in support of a claim for endogenously produced ozone (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).

in combination with reactions of nitrite, especially with heme proteins, that yield nitrate, there is a constant cyclic flux of nitrite ↔ 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, 70% 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)], this would result in the net accumulation of approximately 0.3 µmoles 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, demonstrating that the ELF is a dynamic compartment and that small molecular weight reaction products (even though charged) move readily from the respiratory tract surface to the vascular space, further evidenced by nitrate which does not undergo many of the numerous reaction pathways possible for nitrite. Both nitrite and nitrate levels are very diet dependent and diet represents the primary source for both. Estimates of nitrite/nitrate stemming from NO production via nitric oxide synthase (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 non-dietary, endogenous biological activities. Thus, in compilation while it is clear that endogenous NO_2 is produced, how environmental exposures at current ambient NO_2 concentrations impact the overall balance of nitrite and nitrate, and more importantly, compares with endogenous production rates/amounts remains essentially unknown and introduces an appreciable degree of uncertainty when attempting to place low concentration ambient exposures into a biological context.

3.2.3 Dosimetry of NO

NO occurs within the respiratory tract gas phase due to: (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 intrapulmonary gas phase concentration (discussed below) relative to the inhaled ambient concentration.

While NO exists as a free radical, it is much less reactive than many other radical species except for select chemical interactions that are largely related to radical-radical reactions such as with the superoxide radical anion ($O_2^{\bullet -}$, that produces peroxynitrite; $ONOO^-$), thiyl radicals (e.g., cysteine, $Cys^{\bullet}$; glutathione, $GS^{\bullet}$; that produce S-nitrosothiols; RSNO), organic peroxyl radicals ($ROO^{\bullet}$) (Madej et al., 2008; Goldstein et al., 2004), and

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). 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 S-nitrosothiols, and by complex interactions within red blood cells that likely lead to the release of NO (Weitzberg et al., 2010). In combination, this results in the appearance of NO within the intrapulmonary 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 coming 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; 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, etc. 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 2007-2011 NHANES survey show a geometric mean eNO of 9.7 ppb in children (n = 1855; 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 (558M, 573F; 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 (23M, 28F, 1-5 years of age), a geometric mean eNO of 7 ppb (95% CI: 3, 12) has been reported (van der Heijden et al., In Press). 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 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 non-invasive 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 similar to oxygen and carbon monoxide. 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; 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 RBC 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/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 ppb concentrations of NO. The formation of RSNO within the ELF may contribute to the overall uptake (Torok et al., 2012) but it remains unclear the precise extent of contribution since formation of RSNO requires several steps due to the slow direct reactivity of NO with reduced thiols. 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. Consequently, one can reasonably predict that exposure to ambient NO may not significantly affect the

overall sequelae of NO absorption, metabolism, or downstream impacts on vascular homeostasis, or on lung and systemic biological processes. 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.

3.2.4 Metabolism, Distribution, and Elimination of Products Derived from Inhaled Oxides of Nitrogen

As stated earlier, NO_2 absorption may generate some nitrous acid (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 oxide of nitrogen stable product subsequently excreted in the urine. There has been concern that inhaled NO_2 may lead to N-nitrosamine production, many of which are carcinogenic, since 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 since 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 co-administered. 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 if mice were simultaneously given oral doses of amidopyrine and 4-methylpyrazole, an inhibitor of NDMA metabolism. Nevertheless, endogenous NO_2 production and the cyclic interconversion of nitrite and nitrate may provide the precursors that drive nitrosamine formation, especially since ELF nitrite is swallowed. However, because ambient NO_2 contributes only modest amounts of oxides of nitrogen 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 transform other chemicals that may be present in the body, in some cases 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. *N*-nitrosomorpholine is a nitrosamine that 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 to 1 g/kg morpholine simultaneously. *N*-nitrosomorpholine was found in the body of the exposed mice. Ninety-eight point four percent was labeled with ^{15}N that 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 co-exposed compounds, such as PAHs, via nitration reactions. Miyanishi et al. (1996) co-exposed 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 metabolite of pyrene (1-nitro-6/8-hydroxypyrene and 1-nitro-3hydroxypyrene) 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.

3.2.5 Summary

The uptake of inhaled NO_2 in the respiratory tract is governed by "reactive absorption" that 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 ELF substrates maintain a net driving force for NO_2 mass transfer from the intrapulmonary 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 respiratory tract epithelial. At high substrate concentrations, oxidative/cytotoxic products are at least partially quenched due to secondary antioxidant reactions. At low substrate concentration, ELF-derived oxidants/cytotoxic products have a lower probably of being intercepted by unreacted antioxidants and instead may reach underlying targets.

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, the breathing pattern shifts from nasal to oronasal during exercise relative to rest. Since 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. Experimental studies have shown 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. Since 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/cytotoxic products reaching target sites. 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.

The uptake of inhaled NO occurs against the background of endogenous NO production. 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 similar to oxygen and carbon monoxide. Blood acts as a near "infinite" sink for NO. Absorption of NO follows Henry's law for dissolution into the aqueous phase followed by diffusion into the vascular space where it interacts with RBC hemoglobin to ultimately form nitrate. 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. Given the high endogenous levels of NO in the respiratory tract, exposure to ambient NO may not generally affect the overall sequelae of its absorption, metabolism, or downstream impacts on vascular homeostasis or on lung and systemic biological processes.

3.3 Modes of Action for Inhaled Oxides of Nitrogen

3.3.1 Introduction

Mode of action refers to a sequence of key events and processes that result in a given toxic effect (U.S. EPA, 2005). Elucidation of mechanisms provides a more detailed understanding of these key events and processes (U.S. EPA, 2005). The purpose of this section of Chapter 3 is to describe the key events and pathways that may contribute to health effects resulting from short-term and long-term exposures to NO_2 and NO. Most of the emphasis will be placed on studies of NO_2 and NO, the two most prevalent forms

of NO_Y. The extensive research carried out over several decades in humans and in laboratory animals has yielded numerous studies on mechanisms by which NO_2 and NO exert their effects. This section will discuss some of the representative studies with particular emphasis on studies published since the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008a, c) and on studies in humans that inform biological mechanisms underlying responses to NO_2 and NO.

NO_2 is a free radical and a highly reactive oxidant gas (Table 3-2). It is well-appreciated that secondary oxidation 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 free radical gas that is more selective in its reactivity than NO_2 (Table 3-2). Once inhaled, NO rapidly passes through the alveolar capillary barrier into the circulation where it avidly binds to hemoglobin. Subsequent reactions with hemoglobin lead to the generation of circulating nitrate, nitrite, and methemoglobin.

Table 3-2 Chemical properties of NO_2 and NO that inform modes of action.

NO_2	NO
Free radical gas	Free radical gas
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 S-nitrosothiols 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 free radical reactions and lipid peroxidation	Quenches free radical reactions
Metabolites include nitrite and nitrate	Metabolites include nitrite and nitrate

Both NO_2 and NO are formed endogenously in cells and tissues (Sections 3.2.2.4 and 3.2.3). Formation of endogenous NO is catalyzed by nitric oxide synthases (NOS). In addition, three pathways contribute to the formation of endogenous NO_2: (1) acidification of nitrite usually occurring in the phagolysosomes, (2) reaction with carbonate to form nitrosoperoxylcarbonate anion which decomposes to carbonate anion and NO_2, and (3) the reaction of peroxidases using nitrite and hydrogen peroxide as substrates. These

enzymatic and non-enzymatic 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 circulating levels of NO_2 and NO species due to activity of the enterosalivary cycle (Weitzberg and Lundberg, 2013; Lundberg et al., 2011). Interconversion of reactive nitrogen species (i.e., nitrite, nitrate, and NO) has also been demonstrated in tissue and extracellular compartments. The contribution of environmentally-relevant levels of inhaled NO_2 and NO to levels of circulating nitrite and nitrate is thought to be minimal. 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). Since 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 potential pathways and modes of action responsible for the pulmonary and extrapulmonary effects of inhaled NO_2 and NO. For NO_2, this includes the formation of secondary oxidation products, activation of neural reflexes, initiation of inflammation, alteration of epithelial barrier function, enhancement of bronchial smooth muscle reactivity, modification of innate/adaptive immunity and remodeling of airways and alveoli. Mechanisms underlying the extrapulmonary effects of NO_2 are not well-understood however activation of neural reflexes and release of NO_2 metabolites or mediators from the lung to the bloodstream are possibilities. Inhaled NO may impact the pulmonary and systemic vasculature through interaction with heme proteins. Other effects of NO may be due to circulating nitrite, nitrate, and methemoglobin; due to interactions with redox active transition metals, and due to reactions with thiyl and superoxide radicals. Since endogenous NO is an important mediator of cell signaling, inhaled NO has the potential to disrupt cell signaling.

3.3.2 NO_2

3.3.2.1 Formation of secondary oxidation products

The 2008 ISA and 1993 AQCD (U.S. EPA, 2008a, c, 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 reaction products, such as nitrogen or oxygen radicals, generated via NO_2-mediated chemical reactions. Later effects may occur due to release of ROS/RNS by leukocytes responding to cell damage.

As summarized in the 2008 ISA and 1993 AQCD (U.S. EPA, 2008a, c, 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 employing in vitro systems point to the ability of antioxidants to both react with NO_2 to form reactive intermediates and to quench those reactive intermediates species (Velsor and Postlethwait, 1997). 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 2000 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 other antioxidants. 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, 2008c). For example, long term exposure to NO_2 resulted in decreased glutathione peroxidase activity in weanling mice which 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 since 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 changes phase II enzymes involved in antioxidant defense as well as in phase I and glycolytic enzymes which have been observed following NO_2 exposure.

Nitrite is a primary product of the chemical reactions of NO_2 in the respiratory tract. As discussed in Section 3.2.2.1.2, 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) suggesting that it may play a role in bronchodilation.

Besides nitrite, nitrated proteins, fatty acids and lipids may be formed in the respiratory tract following NO_2 exposure although experimental evidence is currently lacking (Sections 3.2.2 and 3.2.2.4). Nitration of proteins may cause inhibition of protein function and/or induce antigenicity. The presence of nitrated amino acids, such as 3-nitrotyrosine, in cells or tissues is viewed as an indicator of endogenous NO_2 and peroxynitrite formation. 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). Further discussion of the biological effects of these products of NO_2 metabolism is found in Section 3.3.4.

Toxicity resulting from NO_2 exposure is likely due to a product derived from the initial ELF substrate and/or secondary oxidants formed. These reaction products may not be long-lived due to short half-lives and/or continuous turnover of theELF. Studies in vitro have demonstrated quenching of NO_2-derived secondary oxidants that is dependent on concentrations of antioxidants. Thus quenching of reaction products by ELF antioxidants may limit damage to respiratory epithelium (Velsor and Postlethwait, 1997). The heterogeneous distribution of epithelial injury due to NO_2 may reflect ELF-dependent local effects, since the ELF is non-uniform in composition and quantity along the respiratory tract.

3.3.2.2 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 and decreased tidal volume and subsequent rapid shallow breathing. Sometimes pulmonary irritants also stimulate mild bronchoconstriction, bradycardia, and hypotension (Alarie, 1973). All of these pathways involve the vagal nerve.

In guinea pigs, concentration (5,200-13,000 ppb) and time (2-4 hours) dependent exposures to NO_2 by nose-cone resulted in statistically significant stimulated respiratory

rates and decreased tidal volumes that were reversible when animals were returned to clean air (Murphy et al., 1964). In contrast, no changes in these respiratory parameters were observed with 4-hour exposures to 16,000 and 50,000 ppb NO. Another study in 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 tidal volume 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), but not in humans (Bylin et al., 1985). In mice, statistically significant increases in respiratory rate and decreases in tidal volume 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 rats, continuous exposure to 800 ppb and higher concentrations of NO_2 resulted in elevated respiratory rates throughout life (Freeman et al., 1966). In human subjects, respiratory rates tended to decrease in humans exposed to 0-480 ppb for 20 minutes. The authors proposed that NO_2 did not act as a pulmonary irritant in humans at this exposure level (Bylin et al., 1985). In mice, the increase in respiratory rate observed in response to 100,000 ppb NO_2 for 8 minutes (McGrath and Smith, 1984) was lessened by continuous pre-exposure to 5,000 ppb NO_2 for 3 days, suggesting the development of a tolerance or attenuated response to NO_2 (U.S. EPA, 1993).

NO_2 has been shown to elicit a small increase in airway resistance consistent with mild bronchoconstriction in humans but not rabbits or guinea pigs [(Alarie, 1973) and below]. One study in human subjects at rest found a non-monotonic response to NO_2 in terms of airway resistance (Bylin et al., 1985). In this study, airway resistance was increased after 20 minutes of exposure to 250 ppb NO_2 and was decreased after 20 minutes 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. It should be noted that no increase in respiratory rate was observed in this study and that the authors proposed that NO_2 did not act as a pulmonary irritant at this exposure level. 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 acutely to 2,100 ppb NO_2 also exhibited increased airway resistance (von Nieding and Wagner, 1979). In addition, both FEV_1 and FVC were decreased in healthy human subjects exposed to 2,000 ppb NO_2 for 4 hours (Blomberg et al., 1999). These changes in pulmonary function are consistent with reflex bronchoconstriction. Since the response was lessened with each successive exposure on 4 consecutive days, the authors suggested the development of a tolerance or 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 (von Nieding and Wagner, 1979). This includes a study in rats whereby mast cell degranulation occurred after acute exposure to 500-1,000 ppb NO_2 (Thomas et al., 1967). In addition, a histamine-suppressive agent, but not atropine which inhibits vagal responses, 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). This study also demonstrated a decrease in arterial PO_2 and increase in the arterial to alveolar PO_2 gradient, reflecting impaired gas exchange, in humans with chronic lung disease immediately following 15 minutes of exposure to 4,000 and 5,000 ppb (but not 2,000 ppb) NO_2 (von Nieding and Wagner, 1979). 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-induced lipid peroxidation may promote mast cell-mediated changes in pulmonary function, albeit at high concentrations.

There is also 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. Furthermore, a decreased heart rate that 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). These results suggest that the decreased heart rate was due to a different mechanism than rapid stimulation of irritant receptors by NO_2. Subsequent studies by this same group found an increase in respiratory rate following a 24-hour exposure to 5,000 ppb NO_2, while exposure to 10,000 and 20,000 ppb NO_2 for 24 hours resulted in increased respiratory rates, impaired gas exchange, increased lung wet weight and increased lung water content (U.S. EPA, 2008c, 1993; Suzuki et al., 1982; Suzuki et al., 1981). These results suggest that some cardiovascular effects observed after exposure to high concentrations of NO_2 may be secondary to pulmonary edema which is known to stimulate pulmonary irritant receptors. Recently a controlled human exposure study reported an effect on heart rate variability, which is a measure of autonomic tone, at much lower concentrations of NO_2 (Huang et al., 2012b). While there was no indication of pulmonary edema in this study, a statistically significant increase in levels of the injury marker lactate dehydrogenase (LDH) was found in BAL fluid. Altered heart rate variability found in epidemiologic studies (Section 4.3.3.1) 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 tidal volume, stimulate reflex bronchoconstriction and induce bradycardia. Responses are rapid, concentration-dependent and variable between species. Evidence that reflex responses occur in humans is weak since no increases in respiratory rate have been reported as a result of NO_2 exposure. Some findings attributed to reflex bronchoconstriction in humans may be due to alternative pathways such as mast cell degranulation. However, the recent demonstration that NO_2 exposure results in altered heart rate variability suggests the possible activation of a neural reflex in humans. Attenuation of NO_2-mediated responses may occur with continuous or intermittent exposure. Lessening of the breathing pattern response occurred in rodents exposed acutely and continuously to NO_2 but not in rodents exposed chronically and continuously to NO_2. Attenuation of NO_2-mediated changes in pulmonary function occurred in human subjects exposed intermittently over several days.

3.3.2.3 Initiation of inflammation

As summarized in the 2008 ISA and 1993 AQCD (U.S. EPA, 2008a, c, 1993), NO_2 exposure-induced membrane perturbations resulted in the release of arachidonic acid and the formation of eicosanoid products (Section 4.2.4.2). Animal toxicological studies have found increases in concentrations of eicosanoids in BAL fluid immediately following exposure to NO_2 (Schlesinger et al., 1990). Controlled human exposure studies have also demonstrated increased levels of eicosanoids immediately following NO_2 exposure (Jörres et al., 1995). Eicosanoids play an important role in the recruitment of neutrophils. Interestingly higher concentrations and longer durations of exposure to NO_2 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 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. Increased levels of cytokines have also been documented following NO_2 exposure in controlled human studies (Section 4.2.4.1) (U.S. EPA, 2008c; Devlin et al., 1999). The cell signaling pathways responsible for upregulating cytokines at these lower levels of exposure to NO_2 are not clear.

Studies in rodents exposed acutely (1 hour to 3 days) to NO_2 (500-5,000 ppb) have demonstrated airways 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 airways inflammation in endobronchial biopsy tissue and in sputum, bronchial wash fluid and BAL fluid (Section 4.2.4.1). 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, 2008c; 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 exposures on airways inflammation. While neutrophilic inflammation was persistent over 4 consecutive days of exposure to 2000 ppb NO_2, other aspects of the lavage cell response were different compared to single exposure responses (Blomberg et al., 1999). Repeated exposures also led to the upregulation of cytokines characteristic of the Th2 inflammatory response and also to upregulation of ICAM-1 in respiratory epithelium (U.S. EPA, 2008c; Pathmanathan et al., 2003). Upregulation of ICAM-1 suggests a potential mechanism for the persistent leukocyte influx that was observed (Blomberg et al., 1999). In a study of repeated exposure to 4000 ppb over 6 consecutive days, numbers of mast cells, macrophages and total lymphocytes were decreased compared with responses to a single exposure (Sandström et al., 1992; Rubinstein et al., 1991). Furthermore, repeated exposure to 1,500 ppb NO_2 resulted in reduction in lymphocyte subpopulations (Sandström et al., 1992). Allergic inflammatory responses to NO_2 are discussed in Sections 3.3.2.6.2, 3.3.2.6.3, and 4.2.4.3.

3.3.2.4 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. This could potentially lead to the loss of ELF solutes or proteins that could diffuse down their concentration gradient from the lung to the blood. Increases in vascular permeability may also occur, leading to the influx of plasma proteins such as albumin into the airway lumen.

As summarized in the 2008 ISA and 1993 AQCD (U.S. EPA, 2008a, c, 1993), numerous studies have demonstrated increases in the injury biomarkers, protein, albumin, LDH and shed epithelial cells in BAL fluid following exposure to NO_2 (Sections 4.2.4.1 and

4.2.4.2). Since 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 concentrations that are higher than ambient-relevant levels. 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., 1982a). 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). Both BAL fluid total protein and serum Clara Cell secretory protein (CC16) were increased in selenium-deficient rats exposed to NO_2. Selenium supplementation diminished this response providing evidence that the selenium-containing enzyme glutathione peroxidase played an important mitigating role.

Increases in lung permeability due to high concentrations of NO_2 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 ppb and 10,000 ppb NO_2 for 3 or 25 days resulted in epithelial degeneration and necrosis and proteinaceous edema (Barth et al., 1995), while exposure to 1,000-10,000 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 blood stream 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 circulation (U.S. EPA, 2008c; Gilmour et al., 1996) and/or to the immunocompetent and inflammatory cells underlying the epithelium which 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). In addition, increased epithelial permeability may contribute to the activation of neural reflexes and the stimulation of smooth muscle receptors (Dimeo et al., 1981) by allowing greater access of agonist.

Susceptibility to NO_2 exposure-induced cytotoxicity was investigated in several mice strains with differing genetic backgrounds (Kleeberger et al., 1997). This study found a strong genetic component of NO_2 susceptibility that differed from the genetic component involved in susceptibility to O_3. In addition, the genetic component contributed to the attenuation of responses that was seen following repeated exposures.

3.3.2.5 Enhancement of bronchial smooth muscle reactivity

Exposure to NO_2 enhances the inherent reactivity of airway smooth muscle in healthy and asthmatic human subjects (Folinsbee, 1992) (Section 4.2.2) 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 2008 ISA (U.S. EPA, 2008c) and in Section 4.2.2, 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. Asthmatics exhibited greater sensitivity than nonasthmatics when similarly exposed. In addition, several studies found that NO_2 exposure enhanced airways responsiveness to specific stimuli such as allergens in mild allergic asthmatics.

Exercise during exposure to NO_2 appeared to modify airway responsiveness in asthmatics (Folinsbee, 1992) (Section 4.2.2.2). Mechanisms by which this occurs are not understood but two hypotheses have been postulated. First, exercise-induced refractoriness, which has been demonstrated in some asthmatics, may alter responsiveness to NO_2 (Magnussen et al., 1986). A second hypothesis is that nitrites formed by NO_2-mediated reactions in the ELF mediate 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 reactive nitrogen species have bronchodilatory effects. For example, endogenous S-nitrosothiols are an important modulator of airway responsiveness in asthmatics and in eosinophilic inflammation (Lee et al., 2011b; Que et al., 2009).

Animal toxicological studies have also demonstrated NO_2-enhanced responsiveness of airways to nonspecific and specific challenges, as summarized in the 2008 ISA and 1993 AQCD (U.S. EPA, 2008a, c, 1993) and in Sections 4.2.2 and 5.2.8. 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 since no enhanced responsiveness was seen at 2 and 19 hours post-exposure to NO_2. The rapidity of the response suggests reflex bronchoconstriction (Section 3.3.2.2) as a possible underlying mechanism. A 7-day exposure to 4,000 ppb NO_2 also increased airway responsiveness to histamine in guinea pigs (Kobayashi and Shinozaki, 1990). Eicosanoids were proposed to play a role in this transient response. In addition, a recent 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 airways disease. A subchronic study demonstrated dose-dependent increases in airway responsiveness to histamine in NO_2-exposed guinea pigs (1,000-4,000 ppb, 6-12 weeks) (U.S. EPA, 2008c; Kobayashi and Miura, 1995). Specific airways resistance in the absence of a challenge agent was also increased, which indicates the development of airways obstruction. This finding suggests airway remodeling as a possible underlying mechanism for AHR. Another subchronic exposure study found a delayed bronchial response, measured as increased respiration rate and 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 since 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 asthmatic subjects exposed to NO_2 (Mohsenin, 1987b).

Furthermore, different inflammatory pathways may underly NO_2-mediated AHR (Krishna and Holgate, 1999). First, there is some evidence that mast cell activation may contribute to NO_2 exposure-induced AHR. As discussed in Section 3.3.2.2, acute exposure to NO_2 led to mast cell activation in rats and humans. 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, neutrophils and other inflammatory cell types can release mediators such as IL-13, IL-17 and TNF-α that can alter the calcium sensitivity of the smooth muscle and enhance a contractile response to a stimulus (Kudo et al., 2013). Thirdly, chronic inflammation can lead to structural changes in the airway walls that enhance the contractile response of the smooth muscle to a given stimuli (Cockcroft and Davis, 2006c). Whether or not NO_2 exposure enhances intrinsic contractility of airway smooth muscle by these mechanisms is unknown. Fourthly, increased peroxynitrite formation occurring during inflammatory states may play a role. Generally, peroxynitrite is produced by reaction of NO and superoxide, subsequently reacts with CO_2 to form the nitrosoperoxylcarbonate anion ($ONOOCO_2^-$), which decomposes to carbonate radical and NO_2 (Section 3.2.2.4). Recent studies have provided evidence that endogenous peroxynitrite contributes to AHR in animal models of allergic airways disease (Section 3.3.2.6.2). These studies demonstrate that NO metabolism is dysfunctional in inflamed lungs and enhances peroxynitrite formation. Amelioration of the dysfunction resulted in

less nitrative stress and reduced AHR (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 is found in and contributes to AHR and allergic airway disease in animal models (Section 3.3.2.6.2).

It has been hypothesized that NO_2-mediated impairment of epithelial barrier function may play a role in antigen accessibility to immune tissue, resulting in activation of immune responses that trigger AHR (Section 3.3.2.4). In addition, NO_2-mediated impairment of epithelial barrier function could allow greater access of mediators to sensory receptors on nerve fibers. This could lead to enhanced activation of neural pathways and airway smooth muscle contraction (Hesterberg et al., 2009; Cockcroft and Davis, 2006c). Conditions where epithelial barrier function is impaired may increase responses to allergens, mediators and/or NO_2. Allergic inflammation, especially eosinophil activation and release of eosinophil cationic protein (ECP), may cause damage to the airway epithelium in allergic airways disease (Ohashi et al., 1994). This damage may result in epithelial shedding and mucociliary dysfunction. Epithelial shedding could lead to greater exposure of sensory nerve endings and enhanced activation of neural reflexes by mediators. In addition, epithelial shedding and mucociliary dysfunction may allow greater access of allergens to the airway epithelium and submucosa. This may explain the close relationship which has been observed between epithelial shedding and AHR. While NO_2 exposure has been shown to enhance the immune response to allergens (Section 3.3.2.6.2), it is not know whether this mechanism is responsible for NO_2-mediated AHR in allergic airways disease.

3.3.2.6 Modification of innate/adaptive immunity

Host defense depends on effective barrier function and on innate immunity and adaptive immunity (Al-Hegelan et al., 2011). The effects of NO_2 on barrier function in the airways were discussed above (Section 3.3.2.4). This section focuses on the mechanisms by which NO_2 impacts innate and adaptive immunity. Both tissue damage and foreign pathogens are triggers for the activation of the innate immune system. This results in the influx of inflammatory cells such as neutrophils, mast cells, basophils, eosinophils, monocytes and dendritic cells and the generation of cytokines such as TNF-α, IL-1, IL-6, KC and IL-17. Further, innate immunity encompasses the actions of complement and collections, and the phagocytic functions of macrophages, neutrophils and dendritic cells. In addition to immune cells, airway epithelium contributes to innate immune responses. Innate immunity is highly dependent on cell signaling networks involving TLR4 in airway epithelium and other cell types. Adaptive immunity provides immunologic

memory through the actions of B and T-cells. Important links between the two systems are provided by dendritic cells and antigen presentation.

3.3.2.6.1 Impairment of host defenses

As summarized in the 2008 ISA (U.S. EPA, 2008c), 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 of the lung of infectious and non-infectious particles. Altered alveolar macrophage function and other potential mechanisms such as increases in pro-inflammatory mediators and cytokines, increased IgE concentrations, interactions with allergens and altered lymphocyte subsets, reflect modification of innate and/or adaptive immunity. These changes may underly susceptibility to infection, which have been observed in animals exposed to NO_2.

Controlled human studies have demonstrated reduced mucociliary clearance due to depressed ciliary function, depressed alveolar macrophage phagocytic activity and superoxide production 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 4.2.5). 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 since ICAM-1 is a major receptor for rhino and respiratory syncytial viruses. Finally, enhanced susceptibility of airways 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 in children is consistent with these results (Section 4.2.5.1).

As summarized in the 2008 ISA (U.S. EPA, 2008a, c) 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. This includes 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., 1989a; Schlesinger et al., 1987; Schlesinger and Gearhart, 1987; Suzuki et al., 1986; Greene and Schneider, 1978; Dowell et al., 1971). Infectivity models have shown increased mortality and decreased bactericidal activity (U.S. EPA, 2008c; Jakab, 1987; Miller et al., 1987; Ehrlich, 1980; Ehrlich et al., 1977), as a result of NO_2 exposure. Further discussion is found in Section 4.2.5 and 5.2.9.

3.3.2.6.2 Exacerbation of allergic airways 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 T helper cell (Th2) cytokines that can cause airway smooth muscle contraction and recruit mast cells. They 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 or cytolysis resulting in release of ECP and other mediators (Erjefält et al., 1999). Th2 cytokines also activate B lymphocyte 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, 2006c) and to other responses occurring greater than 3-8 hours following an antigen challenge.

Exogenous NO_2

As summarized in the 2008 ISA (U.S. EPA, 2008c) and in Section 4.2.4.3, exposure to NO_2 affects several steps in the acute immune response to inhaled allergens. Several controlled human studies found that NO_2 exposure enhanced airways responsiveness to specific stimuli such as house dust mite (HDM) allergen (Jenkins et al., 1999; Tunnicliffe et al., 1994) in mild allergic asthmatics. 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 airway smooth muscle cell contraction.

Controlled human exposure studies also demonstrated that NO_2 exposure exacerbated the late phase asthmatic response to allergen challenge in mild allergic asthmatics. 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 asthmatic subjects 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 polymorphonuclear leukocytes and increased levels of ECP in BAL 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., 2005a; 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 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 HDM-sensitive asthmatics exposed to 400 ppb NO_2 for 3 hours (Witten et al., 2005).

Late phase responses were also investigated in animal models of allergic airways disease. 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 hours/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 challenged mice found decreased eosinophilic inflammation in response to 5,000 ppb NO_2 (Hubbard et al., 2002; Proust et al., 2002). These results in animal models provide some evidence of NO_2-mediated enhancement of late phase responses, however results were somewhat inconsistent. 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 challenged mouse model may not mimic the eosinophil degranulation or cytolysis that is 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 airways disease. These results provide evidence for NO_2-induced exacerbation of allergic airways disease both in the presence and absence of an allergen challenge.

Endogenous NO_2

Several recent animal toxicological studies have explored the role of endogenous NO and peroxynitrite on allergic airways disease in animal models. In one study, upregulating the enzyme eNOS (and presumably NO production) decreased airway inflammation, AHR and remodeling 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 iNOS expression and 3-nitrotyrosine immunostaining in airway epithelium were reported, as were diminished epithelial injury and apoptosis. Since 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 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 AHR in a guinea pig model of asthma (Maarsingh et al., 2009). Similarly, increased L-arginine levels reduced peroxynitrite formation and AHR 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. Increased L-arginine levels also 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 AHR 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 metaplasia, NFκB activation and AHR 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 the enhanced AHR 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. Upregulation of iNOS was observed and it was greater in distal airways compared with more proximal airways of asthmatics. In addition, levels of DHE+ cells capable of producing reactive oxygen species (such as superoxide) were higher in both the bronchial wash and BAL fluid of asthmatics compared with healthy subjects. Levels of arginase were also higher in BAL fluid of asthmatics 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. 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 asthma 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 reactive nitrogen species are found in and contribute to allergic airway disease in animal models. Inhaled NO_2 may exacerbate allergic airways disease by adding to the lung burden of reactive nitrogen species in inflammatory states.

3.3.2.6.3 Th2 skewing and allergic sensitization

A controlled human exposure study demonstrated that repeated daily exposures 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 4.2.4.3). 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 persistent leukocyte influx. A separate study by these same investigators found that neutrophilic inflammation was persistent over 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 4.2.4.3 and 5.2.6.2). In one study, exposure of guinea pigs to 3000 or 9000 ppb NO_2 increased the numbers of eosinophils in nasal epithelium and mucosa after two weeks (Ohashi et al., 1994). In the other, exposure to 4000 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.

A recent study in mice provides evidence that NO_2 may act as an adjuvant promoting the development of allergic airways 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 and $CD4^+$ T-cells biased towards to Th2 and Th17 phenotypes in the blood. These results are consistent with an allergic asthma phenotype in humans. Furthermore, eosinophilic inflammation, mucus gene upregulation and ovalbumin-specific IgE production were found to be dependent on Toll receptor 2 (TLR2) and MyD88 pathways. TLR2 is known to promote dendritic cell maturation, 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 naïve $CD4^+$ 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 co-stimulatory molecules and produce cytokines which 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 which 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 NO_2 exerts its effects. It should additionally be noted that airway inflammation is seen in mice exposed to 15,000 ppb, but not 10,000 ppb, NO_2 for 1 hour and that pulmonary damage is minimal in this model (Martin et al., 2013).

It should be noted that another study failed to find that NO_2 acted as an adjuvant in a mouse model of allergic airway disease (Alberg et al., 2011). In this study 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 involving the timing between 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 reactive nitrogen and oxygen species can alter the balance between tolerance and allergic sensitization due to an inhaled agent (Ckless et al., 2011). Some activities of dendritic and T-cells, such as maturation of antigen presenting capacity of dendritic cells, dendritic cell stimulation of $CD4^+$ T-cells and polarization of T-cells are redox-sensitive. Endogenous reactive nitrogen and oxygen species are produced by a variety of respiratory tract cells including epithelial, dendritic, T lymphocytes, macrophages, neutrophils and eosinophils, especially during inflammation. Peroxynitrite formation, myeloperoxidase activity and/or nitrite acidification may also be enhanced during inflammation and contribute to endogenous NO_2 levels. Reactive oxygen and nitrogen species are thought to promote the allergic phenotype. Air pollution-derived exogenous reactive nitrogen and oxygen species can potentially contribute to oxidative/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 via Th2 skewing and allergic sensitization.

3.3.2.7 Remodeling of airways and alveoli

As summarized in the 2008 ISA (U.S. EPA, 2008a, c) and 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 exhibited the greatest sensitivity to NO_2 exposure, while the nasal cavity was not much affected. Cells most injured in the centriacinar region were the ciliated cells of the bronchiolar epithelium and type 1 cells of the alveolar epithelium. These were replaced with nonciliated bronchiolar 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. Since collagen is an important structural protein in the lung and since 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 was also found to enhance pre-existing emphysema in animal models (U.S. EPA, 2008c). 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 noted. 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 hours/day, 5 days/week for 24 months found an additional response (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 function growth seen in epidemiological studies is not clear. Effects of NO_2 exposure on lung morphology in rats has been shown to be age-dependent (U.S. EPA, 2008a, c, 1993). Six-week old rats exposed to NO_2 for 6 weeks were more sensitive to the effects of NO_2 exposure than one day-old rats exposed for six 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.

3.3.2.8 Transduction of extrapulmonary responses

While the respiratory tract has been viewed as the primary target of the effects of inhaled NO_2, effects outside the respiratory tract have been demonstrated in controlled human exposure and toxicological studies (U.S. EPA, 2008a, 1993). These include hematological effects and effects on the heart, central nervous system, liver, kidneys and on reproduction and development. Some studies have explored the potential carcinogenicity of NO_2. Many, but not all, of these extrapulmonary effects have been observed in animal models at concentrations that are higher than ambient-relevant levels. Epidemiologic evidence of associations between NO_2 exposure and extrapulmonary effects has also been described (Section 4.3).

Given the reactivity of NO_2, extrapulmonary effects would likely be due to NO_2 reaction products rather than to NO_2 itself. One mechanism by which a NO_2-derived reaction product could mediate extrapulmonary effects would involve the activation of pulmonary irritant receptors (Section 3.3.2.2). As summarized in the 2008 ISA and 1993 AQCD (U.S. EPA, 2008a, c, 1993), effects of chronic NO_2 exposure in animal models include a reduction in PaO_2 and a reduction in heart rate. This reduction in heart rate was not accompanied by an increase in respiratory rate, suggesting that pulmonary irritant receptors were not involved (Section 3.3.2.2 and 5.3.3). However, altered vagal responses were seen in animals exposed acutely to a high concentration of NO_2, but not to a lower concentration over several weeks. Much weaker evidence exists for activation of pulmonary irritant receptors in humans (Section 3.3.2.2). Controlled human exposure studies have examined the effects of NO_2 on heart rate and heart rate variability (Section 4.3.3). Older studies and one newer study failed to find statistically significant changes in heart rate at ambient-relevant concentrations of NO_2. However, changes in heart rate variability found in one recent study suggest the possibility that NO_2 exposure may lead to effects on autonomic nervous system that are mediated via pulmonary irritant receptors (Huang et al., 2012a).

Alternatively, NO_2-derived reaction products in the lung may “spillover” into the circulation or lead to the “spillover” of other mediators 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. 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).

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 1993 AQCD (U.S. EPA, 2008a, c, 1993), one controlled human exposure study demonstrated that NO_2 exposure for a few hours resulted in a reduction in blood pressure (Linn et al., 1985b), 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. 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.

Although unknown, NO_2-derived reaction products or mediators may transduce an oxidative or other stress signal from the lung to the circulation. As summarized in the 2008 ISA and 1993 AQCD (U.S. EPA, 2008a, c, 1993), 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 4.3.6.2). Studies in 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 4.3.6.3). However, changes in hematocrit and hemoglobin did not occur following longer-term exposure to NO_2. Additionally, blood lipids were altered by exposure to NO_2 for several weeks in obese rats. Studies in animal models have demonstrated increases in blood glutathione levels resulting from NO_2 exposure (U.S. EPA, 2008c). Recent controlled human exposure studies (Section 4.3.6.2) also found NO_2 exposure-induced changes in blood lipids and increased levels of plasma soluble lectin-like receptor for oxidized low-density lipoprotein (Channell et al., 2012; Huang et al., 2012a). Changes in peripheral blood inflammatory cells and tissue markers of inflammation have also been observed following exposure to NO_2. As summarized in the 2008 ISA (U.S. EPA, 2008c), controlled human exposure studies demonstrated changes in lymphocyte numbers and subsets in the peripheral blood following exposure to NO_2 (Frampton et al., 2002; Sandström et al., 1992). More recently, markers of

inflammation were observed in myocardial tissue of NO_2-exposed rats (Li et al., 2011a) (Section 4.3.6.3).

As summarized in the 2008 ISA and 1993 AQCD (U.S. EPA, 2008a, c, 1993), NO_2 exposure in animals results in effects on the brain, the liver and xenobiotic metabolism. A recent study in rats also demonstrated neurological effects of exposure to 2,500-10,000 ppb NO_2 for seven days (Li et al., 2012a) (Section 5.4.4.1). In addition, animal studies demonstrated reproductive and developmental effects resulting from exposure to NO_2 during gestation (U.S. EPA, 2008c). This included decreased litter size and neonatal weight, lipid peroxidation of maternal lungs and placenta, retarded intrauterine development, and disturbances in neuromotor development (Section 5.4). Epidemiologic evidence of associations between exposure to NO_2 and reproductive and developmental effects has also been described (Section 5.4). The involvement in these effects of NO_2-mediated activation of pulmonary irritant receptors or spillover of NO_2 metabolites or inflammatory mediators from the lung to the circulation is not known.

There is no clear evidence that NO_2 acts as a carcinogen (U.S. EPA, 2008a, c, 1993) (Section 5.6). 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 where urban level concentrations were used (Koehler et al., 2011, 2010). Some studies demonstrated that inhaled NO_2 at high concentrations can contribute to the formation of mutagens and carcinogens if other precursor chemicals are found in body; e.g., N-nitrosomorpholine from morpholine and nitro-pyrene from pyrene (U.S. EPA, 2008c) (Section 3.2.4). However inhaled ambient NO_2 may not contribute significantly to the body burden of nitrite that can be derived from other NO_2 sources.

3.3.3 NO

As summarized in the 2008 ISA, 1993 AQCD (U.S. EPA, 2008a, c, 1993), and a recent review (Hill et al., 2010), the synthesis of endogenous NO in cells is catalyzed by three different isoforms of NO synthases (eNOS, iNOS, nNOS). 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 free radical. 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 it there may be overlap between endogenous and exogenous NO in terms of biological targets and pathways. The following discussion focuses on mechanisms underlying the effects of both endogenous and exogenous inhaled NO.

Since 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 the smooth muscle relaxation and vasodilation of pulmonary and systemic vessels, and possibly for bronchodilator effects. Inhaled NO rapidly diffuses across the alveolar capillary barrier and 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 non-protein 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 free radical, to produce the potent oxidant peroxynitrite (Hill et al., 2010). Peroxynitrite subsequently reacts with CO_2 to form the nitrosoperoxylcarbonate anion ($ONOOCO_2^-$), followed by decomposition to carbonate radical and NO_2 (Section 3.2.2.4).

Endogenous NO is formed in the respiratory tract at high levels (Section 3.2.3) 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 derived from nasal airways is thought to play a role in sinus host defense through bacteriostatic activity. In addition, NO derived from 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, 2008c). 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 asthmatics 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 and thus its role in inflammatory lung disease is not clear. While it is known that 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 since it has been correlated with inflammation, epithelial injury and clinical exacerbations of asthma (Anderson et al., 2011) (Section 3.3.2.6.2). Furthermore, preferential iNOS upregulation was found in the distal airways compared with more proximal airways in asthmatics. This is of interest since 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 airways disease (Section 3.3.2.6.2).

NO exposure has been shown to alter pulmonary function, morphology and vascular function (U.S. EPA, 2008a, c, 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 5000 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 which are environmentally relevant. Although it can cause both pulmonary and systemic vasodilation, effects on pulmonary vasculature occur at relatively lower concentrations

than 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, 2008c).

Endogenous NO is an important mediator of cardiovascular homeostasis. It has anti-inflammatory and anti-thrombotic 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 and aortic S-nitrosothiols in rats exposed acutely to 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 the exposure to very high concentrations of inhaled NO over the course of a week (17,000 ppb NO for 6 hours/day for 7 days) led to increases in mRNA for aortic endothelin-1 and 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 enough 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 methemogobin formation in the blood. Further discussion of the biological functions of NO metabolites is found below.

3.3.4 Metabolites of NO and NO_2

3.3.4.1 Nitrites/Nitrates

Recently it has been proposed that nitrite is a storage form of NO since 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 cGMP levels and HSP20 expression, decrease CYP450 activity and alter HO-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 S-nitrosothiols. 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.

3.3.4.2 S-Nitrosothiols

Exogenous and endogenous NO can increase S-nitrosothiols, protein S-glutathionylation and thiol oxidation by cysteinyl thiol-dependent pathways (Hill et al., 2010). All of these post-translational protein modifications can act as redox switches to initiate cell signaling events or alter enzyme activity. While NO does not react directly with thiol groups, it can form S-nitrosothiols via reactions with thiyl groups and through intermediate formation of N_2O_3 or metal nitrosyls. S-nitrosothiols are thought to serve as a storage or delivery form of NO and play a role in cell signaling.

High concentrations of S-nitrosothiols are found in the lung where they act as endogenous bronchodilators (Que et al., 2009). In addition, S-nitrosothiols suppress

inflammation by decreasing activation of the transcription factor NFκB (Marshall and Stamler, 2001). Furthermore, augmentation of airway S-nitrosothiols by ethyl nitrite inhalation protected against LPS-induced lung injury in an animal model (Marshall et al., 2009). Several findings suggest an inverse relationship between endogenous airway S-nitrosothiol levels and AHR. 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 asthmatics than in nonasthmatics (Que et al., 2009). GSNOR expression was inversely correlated with S-nitrosoglutathione content. In addition, GSNOR activity in BAL fluid was increased and inversely correlated with AHR in human asthma (Que et al., 2009). Third, levels of airway S-nitrosothiols were inversely correlated with AHR in human subjects with eosinophilic inflammation (Lee et al., 2011b).

3.3.4.3 Nitrated fatty acids and lipids

Nitration of fatty acids and lipids can occur in vivo under conditions of inflammation, infection or ischemia/reperfusion; following exposure to exogenous NO_2 and NO; and possibly by reaction with nitrite (Higdon et al., 2012; Khoo et al., 2010). 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 is likely due to posttranslational 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 PPARγ and the ARE pathway and suppression of NFκB and STAT-1 pathways (Bonacci et al., 2011). In a mouse model, nitro-oleic acid upregulated vascular eNOS and HO-1 and inhibited angiotensisn II-induced hypertension (Khoo et al., 2010; Zhang et al., 2010). Nitro-oleic acid protected against ischemia/reperfusion injury in a mouse model (Rudolph et al., 2010). Nitro-oleic acid also activated matrix metalloproteinases (a pro-inflammatory effect) through thiol alkylation in vitro and inhibited matrix metalloproteinase expression in macrophages through activation of PPARγ (Bonacci et al., 2011). Matrix metalloproteinase was also suppressed in a mouse model of atherosclerosis.

3.3.4.4 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. The presence of nitrated amino acids, such as 3-nitrotyrosine, in cells or tissues is an indicator of NO_2 and/or peroxynitrite formation.

3.3.5 Summary

This section summarizes the key events and pathways that contribute to health effects resulting from short-term and long-term exposures to NO_2 and NO (Table 3-3). Both older studies and studies published since the 2008 ISA (U.S. EPA, 2008c) and both studies conducted in humans and studies conducted in animals provide insight into the biological mechanisms which are affected by exposure to NO_2 and NO. While studies conducted at more environmentally-relevant concentrations are of greater interest, some studies at higher concentrations are recent demonstrations of potentially important new mechanisms.

NO_2: Formation of secondary oxidation products

Studies in in vitro systems have shown that antioxidants react with NO_2 to form reactive intermediates and subsequently quench those reactive intermediates species. Consistent with these findings, 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 that modulating antioxidant status alters levels of injury biomarkers. Health effects resulting from NO_2 exposure are likely due to reactive intermediates or secondary oxidation products formed following initial reaction with ELF substrates. These reaction products likely activate the following pathways.

NO_2: Activation of neural reflexes

NO_2 is classified as a pulmonary irritant. Irritant responses such as altered breathing patterns and bradycardia have been demonstrated in animal models using high concentrations of NO_2. Increased airway resistance has been observed in humans but not in animals exposed to high concentrations of NO_2. This has been attributed to reflex bronchoconstriction, however non-neural mechanisms may underly this response since the changes in airway resistance were not accompanied by altered breathing patterns. A

recent controlled human exposure study reported an effect of NO_2 exposure on heart rate variability, which is a measure of autonomic tone, at much lower concentrations of NO_2. This suggests the activation of a neural reflex in humans.

NO_2: Initiation of inflammation

Increased levels of respiratory tract eicosanoids have been found in NO_2-exposed humans and animals. These products may arise from lipid peroxidation and/or membrane perturbation and play a role in neutrophil recruitment. A recent study demonstrated NFκB activation in airway epithelium of animals exposed to high concentrations of NO_2. NFκB activation generally results in the synthesis and/or release of proinflammatory cytokines. The influx of inflammatory cells in NO_2-exposed animals and humans consists of neutrophils and other cells types. Repeated exposure of humans to NO_2 led to upregulation of the adhesion cell molecule ICAM-1, persistent neutrophil inflammation, and the upregulation of Th2 cytokines, the latter of which is characteristic of a pro-allergic response. Studies involving repeated exposure of humans to NO_2 also found a reduction in lymphocyte subpopulations and other changes which may be characteristic of impaired host defense mechanisms. NO_2 exposure-induced inflammation generally occurs at higher concentrations in humans however some effects of NO_2 on allergic inflammation may occur at lower concentrations (see below).

NO_2: Alteration of epithelial barrier function

Epithelial barrier function is an important component of host defense. Increases in injury biomarkers characteristic of impaired barrier function have been measured in the respiratory tract of NO_2-exposed humans and animals. This impairment may result from lipid peroxidation and/or membrane perturbation and generally occurs only at higher concentrations in humans. Under extreme conditions, increases in lung permeability may cause death due to pulmonary edema. More subtle increases in lung permeability may enhance the translocation of an antigen to the immune cells underlying the epithelium which are involved in allergic responses or may contribute to activation of neural reflexes and/or the stimulation of smooth muscle receptors by allowing greater access of agonist.

NO_2: Enhancement of bronchial smooth muscle reactivity

Exposure to NO_2 enhances the inherent reactivity of airway smooth muscle in healthy and asthmatic human subjects and in animals. Several mechanisms may play a role in this response, including impaired epithelial barrier function (as mentioned above), enhancement of neural pathways leading to airway smooth muscle contraction,

oxidative/nitrative stress, mast cell activation and enhanced smooth muscle cell contractility. Inflammation, especially allergic inflammation (see below), and airway remodeling may contribute to these processes. Experiments in animals demonstrated that NO_2 exposure resulted in mast cell degranulation, production of eicosanoids (which may sensitize receptors on nerve fibers) and eosinophilic inflammation. Studies in humans also suggested a role for mast cell degranulation in NO_2-exposure induced AHR.

NO_2: Modification of innate/adaptive immunity

Innate and adaptive immunity encompass many different cell types and processes which provide defense against foreign pathogens, repair tissue damage, and/or promote an allergic phenotype. Studies in animals and humans exposed to NO_2 demonstrated impairment of mucociliary clearance and alveolar macrophage phagocytic activity. In addition, NO_2 exposure resulted in increased mortality in animal infectivity models and altered lymphocyte subpopulations in humans (repeated exposures). These changes are characteristic of impaired host defense. In addition, studies in humans and animals found that NO_2 exposure resulted in exacerbation of asthma and allergic airways disease. In humans, both early and late phase asthmatic responses (e.g., AHR and eosinophil activation following a specific allergen challenge) were enhanced by NO_2 exposure. In allergic animals, late phase responses (IgE, eosinophilia) were enhanced by NO_2 exposure. Furthermore, recent studies in animal models of allergic airways disease and in human subjects with asthma provide evidence that increased levels of endogenous peroxynitrite/NO_2 are linked to AHR and other features of allergic airways disease. These findings raise the possibility that exogenous and endogenous NO_2 act through similar pathways to exacerbate allergic airways disease. In addition, NO_2 exposure resulted in Th2 skewing/allergic sensitization in animals and human subjects. This included increased expression of Th2 cytokines in human respiratory epithelium and the development of nasal eosinophilia and enhanced mast cell responses in animals. Furthermore recent studies provide evidence that NO_2 may act as an adjuvant promoting the development of allergic airways disease in response to an inhaled allergen. These findings suggest that exposure to NO_2 may lead to the development of allergic airways disease.

NO_2: Remodeling of airways and alveoli

Morphologic changes in response to NO_2 exposure generally consist of epithelial damage in the centriacinar region, followed by hyperplastic repair. Emphysematous-like lesions have been reported at high concentrations of NO_2. The relationship between morphologic changes observed in animal models and decrements in pulmonary function is unclear.

NO_2: Transduction of extrapulmonary responses

NO_2 exposure results in effects outside the respiratory tract in both humans and animals, especially at higher concentrations. Given the reactivity of NO_2, these extrapulmonary effects are likely secondary to the formation of an NO_2 reaction product. Two possible mechanisms underlying extrapulmonary responses are activation of neural reflexes via pulmonary irritant receptors and spillover of NO_2 reaction products or inflammatory mediators from the respiratory tract into the circulation. Experimental evidence in animals demonstrated that exposure to high concentrations of NO_2 activates vagal pathways and results in bradycardia. Evidence for pulmonary irritant responses in humans is weak although a recent study demonstrating NO_2 exposure-induced changes in heart rate variability is suggestive of this possibility. Another recent study found that NO_2 exposure resulted in the presence of a vasoactive substance in the plasma of human volunteers, providing evidence that spillover of a reaction product or inflammatory mediator into the circulation may transduce the signal from the respiratory tract.

NO: Initiating events

Due to its hydrophobic nature, selective reactivity and high affinity for heme proteins, inhaled NO rapidly diffuses across the alveolar capillary barrier and reacts with soluble guanylate cyclase in the pulmonary arterial smooth muscle and with hemoglobin in red blood cells. Inhaled NO at high concentrations is used clinically and results in selective pulmonary vasodilation.

Metabolites of NO_2 and NO

Major metabolites of NO_2 and NO include nitrites and nitrates, S-nitrosothiols, and nitrated fatty acids, lipids, amino acids and proteins. With the exception of nitrate, all have been shown to be biologically active. However, there is little available evidence to support a role for these metabolites in mediating the effects of NO_2 and NO considered in this ISA.

Table 3-3 Biological pathways, key events and endpoints.

Biological Pathways	Key Events	Downstream Endpoints
NO_2: Initiating Event-formation of secondary oxidation products	Lipid Peroxidation	
	Antioxidant depletion	
	↑ROS/RNS	
	Thiol oxidation	
NO_2: Activation of neural reflexes	Altered breathing patterns	
	Possible reflex bronchoconstriction	↑ Airway resistance
	Bradycardia	
NO_2: Initiation of inflammation	↑Eicosanoid production	Neutrophil influx
	NFκB Activation	↑Pro-inflammatory cytokines
	ICAM-1 upregulation	Persistent neutrophil influx
NO_2: Alteration of epithelial barrier function	↑ BAL fluid protein or albumin	Impaired host defense
	↑ Access of antigen to immune cells	Allergic inflammation
	↑ Access of agonist to airway smooth muscle	AHR
	↑ Access of mediators to irritant receptors	AHR
NO_2: Enhancement of bronchial smooth muscle reactivity	↑ROS/RNS	AHR
	Mast cell degranulation	
	Allergic inflammation	
	Enhanced airway smooth muscle cell contractility: inflammatory mediators or airway remodeling	
	Sensitization of irritant receptors	
NO_2: Modification of innate/adaptive immunity	Altered mucociliary clearance	Impaired host defense: ↑Animal infectivity
	Altered macrophage phagocytosis	
	Altered lymphocyte subsets	
	↑Early phase and late phase responses to allergen (AHR, allergic inflammation)	Exacerbation of asthma and allergic airways disease
	↑Late phase responses in allergic model (allergic inflammation)	
	↑Endogenous NO_2 and peroxynitrite	
	Upregulation of Th2 cytokines	Th2 skewing/Allergic sensitization
	Nasal eosinophilia	
	Enhanced mast cell responses	
	Adjuvant activity: dendritic cell activation	
NO_2: Remodeling of airways and alveoli	Morphologic changes to centriacinar region	Unknown

Table 3-3 (Continued): Biological pathways, key events and endpoints.

Biological Pathways	Key Events	Downstream Endpoints
NO_2: Transduction of extrapulmonary responses	Activation of neural reflex	Possibly altered HRV
	Spillover into circulation	Presence of vasoactive substance in plasma
NO: Initiating events	Rapid diffusion across alveolar capillary barrier	Lack of respiratory tract effects
	Reaction with heme proteins	Selective pulmonary vasodilation
Metabolites of NO_2 and NO	Various biological activities	Unknown

3.4 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 airways and biological responses that result from activation of intra- and inter-cellular signaling pathways in a variety of tissues. Key to this process is the reaction of NO_2 with components of the ELF of the respiratory tract and the reaction of NO with heme proteins in the circulation. These chemical interactions are responsible for targeting of oxides of nitrogen species to different tissues. Inhaled NO_2 may contribute to the endogenous body burden of NO_2 species, especially in the respiratory tract.

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CHAPTER 4 INTEGRATED HEALTH EFFECTS OF SHORT-TERM EXPOSURE TO OXIDES OF NITROGEN

4.1 Introduction

This chapter summarizes, integrates, and evaluates the evidence for various health effects associated with short-term (i.e., 1 month or less, see Section 1.4) 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 4.2), cardiovascular effects (Section 4.3), as well as total mortality (Section 4.4). Reproductive and developmental effects have also been examined in relation to short-term exposure to oxides of nitrogen. However, this evidence is evaluated with long-term exposure studies in Chapter 5, because associations are often compared among various short- and long-term exposure periods that are difficult to distinguish.

Individual sections for major outcome 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 groups of related endpoints (e.g., airway hyperresponsiveness, pulmonary inflammation, lung function) and then specific scientific discipline (i.e., epidemiology, toxicology).

Sections for each of the major outcome categories (i.e., respiratory, cardiovascular, total mortality) conclude with an integrated summary of the assessment of evidence and conclusions regarding causality. A determination of causality was made for each major outcome category by evaluating the evidence for each category independently with the causal framework (described in the Preamble to the ISA). Findings for cause-specific mortality (i.e., respiratory, cardiovascular) are used to assess the continuum of effects and inform the causality determinations for respiratory and cardiovascular effects. The causality determination for total mortality (Section 4.4) is based primarily on the evidence for non-accidental causes of mortality combined, but is 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 exposure or ambient concentrations in animal toxicological, controlled human

exposure, and epidemiologic studies considered relevant to this ISA (i.e., up to 5,000 ppb NO_2 or NO) as described in Section 1.1. Studies that examined higher concentrations of oxides of nitrogen were evaluated particularly to inform mode of action. Causality determinations 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 exposures or ambient concentrations of oxides of nitrogen and relationship with the outcome), and other biases could be ruled out with reasonable confidence. This evaluation involved consideration of the strength of study design and analytical methods as well as the potential for selection bias, publication bias, and confounding. Aspects used in the evaluation and integration of evidence to form a causal classification are described in more detail in the Preamble.

Epidemiologic studies of short-term exposure to oxides of nitrogen rely primarily on temporal variation in exposure (e.g., day-to-day variations in ambient NO_2 concentrations) and health effects. For the assessment of potential confounding, epidemiologic studies of short-term exposure were evaluated for the extent to which they considered other factors associated with health outcomes and exhibited similar temporal variation as exposures to oxides of nitrogen. These potential confounding factors can include meteorological factors, season, long-term time trends, medication use, and copollutant exposures. 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 also may result from poorly measured factors. However, potential confounding was assessed as the extent to which the collective literature base examined factors well documented in the literature to be associated with exposure to oxides of nitrogen and health outcomes (e.g., meteorological factors, others specified above). Epidemiologic studies examine various averaging times of NO_2, NO and NO_X concentrations (e.g., 24-h avg, daily 1-h max) and present effect estimates for associations with health outcomes scaled to various changes in concentrations, e.g., interquartile range for the study period or an arbitrary unit such as 10 ppb. To increase comparability among studies, the ISA presents effect estimates for a given averaging time scaled to the same increment. For short-term exposure, effect estimates are scaled to a 20-ppb increase for 24-h avg NO_2 or NO, a 40-ppb increase for 24-h avg NO_X, a 30-ppb increase for 1-h max NO_2 or NO, and a 60-ppb increase for 1-h max NO_X. These increments were derived by calculating the U.S. nationwide percentile distributions for various averaging times, and they represent the estimated difference between the median (a typical pollution day) and the 95th percentile (a more polluted day) for a given averaging time.

Controlled human exposure and animal toxicological studies can provide direct evidence for health effects related to pollutant exposures. They also can be used to address uncertainties in the epidemiologic evidence, for example, potential confounding. Experimental studies additionally can provide biological plausibility for observed effects by describing key events to inform modes of action. Thus, the integration of evidence across a spectrum of related endpoints, including cause-specific mortality, and across disciplines was used to inform uncertainties for any particular endpoint or discipline due to factors such as publication bias, selection bias, and confounding by copollutant exposures.

4.2 Respiratory Effects

4.2.1 Introduction

The 2008 ISA for Oxides of Nitrogen concluded that there was sufficient evidence to infer that a causal relationship is likely to exist between short-term exposure to NO_2 and respiratory effects (U.S. EPA, 2008c). This conclusion was based primarily on a large body of epidemiologic evidence consistently indicating associations between increases in ambient NO_2 concentrations and increases in respiratory-related hospital admissions and emergency department (ED) visits with supporting evidence for increases in respiratory symptoms in children with asthma. Copollutant modeling results generally indicated robust associations with adjustment for copollutants, such as ozone (O_3), carbon monoxide (CO), or particulate matter (i.e., $PM_{2.5}$, PM_{10}), supporting an independent effect of ambient NO_2 exposure. Biological plausibility was provided, in particular, by observations of NO_2-induced airway hyperresponsiveness (AHR) in adults with asthma following <1 to 6-hour exposures to NO_2 at concentrations in the range of 100 to 300 ppb, on the order of peak 1-h maximum ambient concentrations examined in epidemiologic studies. The 2008 ISA for Oxides of Nitrogen also noted some support for pulmonary inflammation and impaired host defenses in controlled human exposure and animal toxicological studies, albeit at higher concentrations of 1,500 to 5,000 ppb NO_2. There was less consistent evidence for NO_2-associated lung function decrements, as examined in controlled human exposure and epidemiologic studies. However, epidemiologic studies in adults and children found associations with lung function measured by supervised spirometry (U.S. EPA, 2008c).

Substantial evidence in support of a relationship between short-term NO_2 exposure and respiratory effects was provided by the coherence of findings across disciplines for related outcomes. Specifically, evidence for increases in AHR and pulmonary

inflammation in controlled human exposure, animal toxicological, and to a limited extent, epidemiologic studies provided biological plausibility for respiratory symptoms in children with asthma which in turn, provided biological plausibility for increases in asthma hospital admissions and ED visits. Although there was coherence of evidence across related outcomes and disciplines, a major uncertainty that remained regarding the respiratory effects of short-term ambient NO_2 exposure was the high correlations of NO_2 with other traffic-related pollutants and the potential for NO_2 to serve primarily as an indicator for another or mixture of combustion-related 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 the evidence for ambient NO_2-associated increases in respiratory hospital admissions and ED visits (including those for asthma), increases in pulmonary inflammation, oxidative stress, and respiratory symptoms in children with asthma, as well as increases in respiratory mortality. The discussion of the evidence is organized by outcome with results from recent studies, where available, evaluated in the context of those from previous studies.

4.2.2 Airway Hyperresponsiveness

Inhaled pollutants such as NO_2 may have direct effects on lung function or they may enhance the inherent responsiveness of the airways to challenge by bronchoconstricting agents. Challenge agents can be classified as nonspecific (e.g., histamine, 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 primarily focuses on changes in airway responsiveness to bronchial challenge attributable to NO_2 in individuals with asthma. Discussed in Section 3.3.2.5, toxicological studies have demonstrated increased airway responsiveness to nonspecific challenges following short-term exposures to 4,000 ppb NO_2 (Kobayashi and Shinozaki, 1990). Described in Section 4.2.4.3 (Allergic Inflammation), altered responses to specific allergens following NO_2 exposure have also been demonstrated in human and animal studies. There is a wide range of airways responsiveness that is influenced by many factors, including medications, cigarette smoke, air pollutants, respiratory infections, occupational exposures, disease status, and respiratory irritants.

There are several notable changes and additions to the discussion of airway responsiveness in this ISA as compared to Section 3.1.3 of the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c). Only one new experimental study (Riedl et al., 2012) and a new meta-analysis (Goodman et al., 2009) of NO_2 associated increases in airway responsiveness have been published since the 2008 ISA. Accordingly, this ISA focuses primarily on meta-analyses of airway responsiveness data rather than specifics of individual studies. Relative to Table 3.1-2 in the 2008 ISA, Table 4-1 and Table 4-2 include new or previously not included data (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). Based on data in Table 4-1 and Table 4-2, an updated meta-analysis (Table 4-3) and detailed methodology are provided. Consistent with conclusions reached in the 2008 ISA, the updated meta-analysis (Table 4-3) shows that in individuals exposed to NO_2 at rest, 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 in individuals with asthma. Finally, a section has been added evaluating the potential of various factors to affect airways hyperresponsiveness independently or in conjunction with NO_2 exposure (Section 4.2.2.3).

Responses to bronchial challenge are typically quantified in terms of the provocative dose (PD) or concentration (PC) of an agent required to produce a 20% reduction in forced expiratory volume in 1 second (FEV_1) (PD_{20}, PC_{20}, respectively) or a 100% increase in specific airway resistance (PD_{100}, PC_{100}, respectively). In the general population, airway responsiveness is log-normally distributed with individuals having AHR tending to be those with asthma (Postma and Boezen, 2004); although, the airway responsiveness of individuals with asthma extends into the normal range (Cockcroft, 2010). 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). 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).

Due to their predisposition for AHR, individuals with asthma generally require 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 two of fifteen 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 nonspecific 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 AHR 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 AHR 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 exacerbations (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 AHR to one agent in subjects with asthma may also enhance airway responsiveness to other challenge agents. Transient increases in AHR 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.

4.2.2.1 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 (3-h) exposures in healthy adults (U.S. EPA, 2008c). Studies of airway responsiveness in healthy individuals were generally conducted using volunteers of 18 to 35 + years of age. Mohsenin (1988) found that a 1-h resting exposure to 2,000 ppb NO_2 increased responsiveness to methacholine. A mild increase in responsiveness to carbachol was observed following a 3-h 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-h 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 of

healthy subjects (Ahmed et al., 1983b; Hazucha et al., 1983). Two meta-analyses of the available literature confirm 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 airways responsiveness in healthy individuals following NO_2 exposure are not available.

4.2.2.2 Individuals with Asthma

The 2008 ISA for Oxides of Nitrogen reported that increases in nonspecific airway responsiveness were observed 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 (U.S. EPA, 2008c). Enhanced airway responsiveness to allergens was found in individuals with asthma at exposures as low as 260 ppb for 30 minutes (U.S. EPA, 2008c). 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, 2008c).

As an update to Table 3.1-2 in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), Table 4-1 and Table 4-2 present studies for which individual data were available to evaluate the fraction of subjects whose airway responsiveness increased or decreased following exposure to NO_2. 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. For studies that assessed AHR 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 Table 4-1 and Table 4-2 in an attempt to reduce the heterogeneity between studies. Selection of the earliest time point assessing AHR 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 Table 4-1 and Table 4-2 are sorted by NO_2 exposure concentration and, as such, studies that evaluated multiple NO_2 exposure concentrations appear in multiple rows. The statistical significance reported in studies for changes in AHR 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 AHR following NO_2 exposure with some studies reaching statistical significance. Fewer studies showed no effect or a tendency for decreased AHR following NO_2. Published since the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), the one recent study reported a statistically significant decrease in AHR following NO_2, but the authors attributed the protective effect of NO_2 to chance (Riedl et al., 2012).

Table 4-1 Resting exposures to NO_2 and airway responsiveness in subjects with asthma.

Reference	N	NO_2 (ppb)	Exp. (min)	Challenge Type	End Point	Time Post-exp (min)	Change in AHR[a]		Average PD ± SE[b]		p-value[c]
							+	–	Air	NO_2	
Ahmed et al. (1983b)	20	100	60	CARB	sGaw	NA	13	7	6.0 ± 2.4	2.7 ± 0.8	NA
Ahmed et al. (1983a)	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	p <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
Tunnicliffe et al. (1994)	8	400	60	HDM	FEV_1	IM	8	0	-14.62 ΔFEV_1	-18.64 ΔFEV_1	0.009

Table 4-1 (Continued): Resting exposures to NO_2 and airway responsiveness in subjects with asthma.

Reference	N	NO_2 (ppb)	Exp. (min)	Challenge Type	End Point	Time Post-exp (min)	Change in AHR[a]		Average PD ± SE[b]		p-value[c]
							+	–	Air	NO_2	
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.

Abbreviations: BIR, birch; CARB, carbachol; COLD, cold-dry air; FEV_1, forced expiratory volume in 1 s; 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 AHR: number of individuals showing increased (+) or decreased (−) AHR after NO_2 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]Statistically significance of increase in AHR to bronchial challenge following NO_2 exposure compared to filtered air. Statistical tests varied between studies, e.g., sign test, t-test, analysis of variance.

[d]Statistically significance for all asthmatics from analysis by Dawson and Schenker (1979). Orehek et al. (1976) only tested for differences in subsets of individuals classified as "responders" and "nonresponders."

Table 4-2 Exercising exposures to NO_2 and airway responsiveness in subjects with asthma.

Reference	N	NO_2 (ppb)	Exp. (min)	Challenge Type	End Point	Time Post-exp (min)	Change in AHR[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.
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	CAT	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.

Table 4-2 (Continued: Exercising exposures to NO_2 and airway responsiveness in subjects with asthma.

Reference	N	NO_2 (ppb)	Exp. (min)	Challenge Type	End Point	Time Post-exp (min)	Change in AHR[a]		Average PD ± SE[b]		p-value[c]
							+	–	Air	NO_2	
Avol et al. (1988)	37	600	120	COLD	FEV_1	60	13 [D]	16 [D]	-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.

Abbreviations: AHR, airway hyperresponsiveness; CARB, carbachol; CAT, cat allergen; COLD, cold-dry air; 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 AHR: number of individuals showing increased (+) or decreased (−) AHR after NO_2 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]Statistically significance of increase in AHR to bronchial challenge following NO_2 exposure compared to filtered air. Statistical tests varied between studies, e.g., sign test, t-test, analyses of variance.

[d]Number of individuals having an increase or decrease in AHR 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 filter air than NO_2, i.e., a protective effect of NO_2 exposure.

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 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 statistically increased responsiveness, whereas only 52% of subjects exposed with exercise tended to have increased responsiveness. The analyses of Folinsbee (1992) and Kjaergaard and Rasmussen (1996) effectively assessed nonspecific responsiveness since few studies of allergen responsiveness were available.

The analyses conducted by Folinsbee (1992) were detailed in the 1993 AQCD for Oxides of Nitrogen (U.S. EPA, 1993). Results of these analyses appeared in Table 15-10 of that 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 nonspecific responsiveness appeared in Table 3.1-3 on the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c). The overall conclusion of that modified analysis was that NO_2 exposures conducted during rest, but not exercise, in the range of 200 and 300 ppb NO_2 for 30-minute exposures and at 100 ppb NO_2 for 60-minute exposures increased nonspecific responsiveness in individuals with asthma. Due to differences in study protocols (e.g., rest versus exercise) in the NO_2-AHR literature, the original (Folinsbee, 1992) and updated meta-analyses in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) assessed only the fraction of individuals experiencing increased or decreased airway responsiveness following NO_2 exposure.

A recent study by 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, 2008c), which was limited to nonspecific responsiveness in subjects with asthma in an attempt to reduce the heterogeneity between 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, 2008c), Goodman et al. (2009) found 64% (95% CI: 58%, 71%) of subjects with asthma exposed at rest to NO_2 experience 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 a reduction in PD and increased 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 further concluded that the effects of NO_2 exposure on airway responsiveness were sufficiently small so as not to be considered adverse. By this assessment, the authors concluded that a −50% change in the PD would be considered adverse, whereas the effect of NO_2 exposure was a −27% (95% CI: −37%, −18%) reduction 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. 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. The nature of the relationship between NO_2 exposure and airway responsiveness, as well as factors potentially affecting within- and between-study variability in observed responses, is discussed later in this section.

Based on the summary data in Table 4-1 and Table 4-2, the fraction of individuals experiencing a NO_2-induced increase in airway responsiveness can be assessed in a manner consistent with the analysis conducted by Folinsbee (1992). The magnitude of NO_2-induced changes in PD were not evaluated due to considerable variability in exposure protocols and the potential for this variability in protocols to affect estimates of PD (see Section 4.2.2.3). Specifically, a two-tailed sign test was used to assess the statistical significance of directional changes in AHR 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 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 may be calculated in Microsoft® Office Excel® 2007 as:

For $AHR^+ > (AHR^+ + AHR^-)/2$

p-value = 2 * (1−BINOMDIST(AHR^+ -1, (AHR^+ + AHR^-), 0.5,TRUE))

For $AHR^+ < (AHR^+ + AHR^-)/2$

p-value = 2 * BINOMDIST(AHR^+, (AHR^+ + AHR^-), 0.5,TRUE)

For $AHR^+ = AHR^-$

p-value = 1.00

where: AHR^+ and AHR^- are the number of individuals in Table 4-1 and Table 4-2 having an increase or decrease in airway responsiveness, respectively, under a specified set of conditions (i.e., NO_2 concentration, exercise versus rest during exposure, nonspecific versus allergen challenge); the BINOMDIST function in Excel® returns the binomial distribution probability given the total number of increases (AHR^+), number experiencing a change (AHR^+ + AHR^-), probability of a change (0.5), for the cumulative distribution (indicated by the logical, TRUE); and the multiplication by two provides the probability for a two-tailed test. Table 4-3, Table 4-4, and Table 4-5 present the fraction of individuals experiencing a NO_2-induced increase in airway responsiveness to nonspecific agents, specific allergens, and all challenge types, respectively.

Table 4-3 updates Table 3.1-3 of the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) and is consistent with the prior conclusion that 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 airways responsiveness were not observed following the exercising exposures to NO_2. As discussed in Section 4.2.2.3, the literature on airway responsiveness supports the development of a refractory period following bouts of exercise. An effect of exercise refractoriness is consistent with NO_2-induced increases in airway responsiveness following resting but not excercising exposures.

Table 4-3 Fraction of subjects with asthma having NO_2-induced increase in airway hyperresponsiveness to a nonspecific 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)
$100 \leq [NO_2] < 200$	0.66 (87; p = 0.005)	0.59 (17; n.s.)	0.67 (70; p = 0.006)
$200 \leq [NO_2] \leq 300$	0.59 (199; p = 0.011)	0.55 (163; n.s.)	0.78 (36; p = 0.001)
$[NO_2] > 300$	0.57 (94; n.s.)	0.49 (61; n.s.)	0.73 (33; p = 0.014)
All $[NO_2]$	0.60 (380; p <0.001)	0.54 (241; n.s.)	0.71 (139; p <0.001)

Abbreviations: 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 versus 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 Table 4-1 and Table 4-2 having a change (+/-) in nonspecific AHR.

Table 4-4 Fraction of subjects with asthma having NO_2-induced increase in specific airway hyperresponsiveness 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.)
$100 \leq [NO_2] < 200$	0.50 (26; n.s.)	—	0.50 (26; n.s.)
$200 \leq [NO_2] \leq 300$	0.55 (56; n.s.)	0.55 (11; n.s.)	0.56 (45; n.s.)
$[NO_2] > 300$	0.56 (48; n.s.)	0.48 (40; n.s.)	1.00 (8; p = 0.008)
All $[NO_2]$	0.55 (130; n.s.)	0.49 (51; n.s.)	0.58 (79; n.s.)

Abbreviations: n.s., less than marginal statistical significance (p >0.10)

[a]See Footnote "a" of Table 4-3.

[b]Analysis is for the 130 subjects with asthma in Table 4-1 and Table 4-2 having a change (+/-) in specific allergen AHR.

Table 4-5 Fraction of subjects with asthma having NO_2-induced increase in airway hyperresponsiveness regardless of challenge types.

NO_2 Concentration, ppb	All Exposures[a,b]	Exposure during Exercise[a,b]	Exposure at Rest[a,b]
$[NO_2]$ = 100	0.61 (76; p = 0.08)	—	0.61 (76; p = 0.08)
100 ≤ $[NO_2]$ <200	0.62 (113; p = 0.014)	0.59 (17; n.s.)	0.63 (96; p = 0.018)
200 ≤ $[NO_2]$ ≤ 300	0.58 (255; p = 0.008)	0.55 (174; n.s.)	0.65 (81; p = 0.007)
$[NO_2]$ >300	0.57 (142; n.s.)	0.49 (101; n.s.)	0.78 (41; p <0.001)
All $[NO_2]$	0.59 (510; p <0.001)	0.53 (292; n.s.)	0.67 (218; p <0.001)

Abbreviations: n.s., less than marginal statistical significance (p >0.10)

[a]See Footnote "a" of Table 4-3.

[b]Analysis is for the 510 subjects with asthma in Table 4-1 and Table 4-2 having a change (+/-) in AHR.

In general, Table 4-4 does not show significant effects of NO_2 exposure on airway responsiveness to allergen challenge. This may be, in part, due to the small number of individuals in the analysis. The lack of statistical significance in Table 4-4 does not necessarily diminish the potential importance of allergen exposures. Eighty percent of children with asthma are thought to be sensitized to common household allergens (O'Byrne et al., 2009). Individuals with asthma may experience an early phase response to allergen challenge with declines in lung function within 30 minutes; and, approximately half of those having an early phase response also have a late phase response with a decline in lung function 3-8 hours after the challenge (O'Byrne et al., 2009; Cockcroft and Davis, 2006c). The early response, which may be reversed with bronchodilators, primarily reflects release of histamine and other mediators by airway mast cells; whereas, the late response reflects enhanced airways inflammation and mucous production and 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. The degree of airway responsiveness 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 effects, the responsiveness to allergens in animals and humans is also addressed in Sections 3.3.2.6 (Modification of Innate/Adaptive Immunity) and 4.2.4.3 (Allergen Inflammation).

With all challenge types considered, Table 4-5 shows statistically significant increases in airway responsiveness across all exposure concentration in subjects with asthma exposed at rest. However, given differing mechanisms of effect (see discussion of Bronchial Challenge Agent in Section 4.2.2.3), preference should be given to the analysis of nonspecific responsiveness (Table 4-3) over the combined analysis of specific and nonspecific agents (Table 4-5).

4.2.2.3 Factors Affecting Airway Hyperresponsiveness and Dose-response

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 been found following periods of exercise than rest (Freedman et al., 1988). The refractory period from exercise had also 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 3.3.2.3 and 4.2.4.1). Refractory periods following exercise of 40 minutes to 3 hours has been reported (Dryden et al., 2010).

Controlled NO_2 exposure studies may also provide some insight into the effect of exercise on airways responsiveness. Jörres and Magnussen (1991) and Strand et al. (1996) provide individual subject PD_{100} for methacholine and histamine, respectively, on both a control day (no exposure, no exercise) and following a filtered air exposure with exercise. There was a slight tendency for the PD_{100} to be lower following the filtered air exposures relative to control (no exposure, no exercise) with roughly 53% of the

individuals having a lower PD_{100} following filtered air (with exercise). Thus, these two studies do not support an effect of exercise on AHR in studies evaluating effects of NO_2 exposure. However, 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 does support the conclusion that exercise diminishes the subsequent responsiveness to bronchial challenge. Jörres and Magnussen (1990) found a statistically significant increase in airway responsiveness to SO_2 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 SO_2 inhalation 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 was shown in Table 4-3.

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 significant ($p < 0.00001$), two-fold 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 also cause a partial reversal of bronchospasm versus the use of other measures such as specific airway resistance (sRaw). Variations in the delivery of bronchial challenge agents and methods of assessing airway response may affect comparisons of provocative doses between NO_2 studies.

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. The asthmatic response to specific allergens may include an early response

(within 30 minutes of challenge), which primarily reflects release of histamine and other mediators by airways mast cells as well as a response (typically 3-8 hours post challenge), which reflects enhanced airways inflammation and mucous production (O'Byrne et al., 2009; Cockcroft and Davis, 2006c). The degree of early airway responsiveness to allergen challenge 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). Even similarly delivered nonspecific, direct acting agents may differently affect the lung. 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 between bronchial challenge agents is complicated by the differing mechanisms by which they initiate bronchoconstriction.

Subject Selection

Exercise is a major trigger of asthma symptoms in between 60 and 90 percent 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 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 (see Table 4-2). 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 utilizing resting versus exercising exposures.

Medication Usage

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 two-fold reduction in airway responsiveness relative to an unmedicated baseline (Reddel et al., 2009). There was a wide range in restrictions on asthma

medication usage between NO_2 studies. For example, Hazucha et al. (1983) required that subjects not receive steroid therapy or daily bronchodilator therapy for a month prior to bronchial challenge testing. Other studies 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 from analysis since 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). The use of asthma medications or dietary supplements may have affected the ability of studies to identify effects 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, statistically significant increases in sRaw following a 20-minute resting exposure to 240 ppb NO_2 and significant decreases in sRaw following exposure to 480 ppb NO_2 has been reported in healthy individuals (Bylin et al., 1985), and individuals with asthma exhibited similar trends in sRaw responses to NO_2 exposure. 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 airway 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 airways responsiveness has been investigated by numerous investigators, some of the more conclusive findings are described here. Moss and Oldham (2006) reported a PC of methacholine producing a 200% increase in airway resistance in Balb/c mice of 12× lower than in B6C3F1 mice. However, the B6C3F1 mice airways were 1.6× larger and their ventilation was smaller by 0.9× than the Balb/c mice. Given these differences in airway size and breathing rate, the estimated dose of methacholine delivered to the airways was equivalent between the species. Wanner et al. (1985) found a strong correlation between the decrease in FEV_1 following histamine challenge and the estimated 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 increased with decreasing inhaled particle size from 10 μm to 1 μm (mass median aerodynamic diameter). These studies demonstrate lower airway responsiveness for distal versus proximal deposition of challenge agents. Thus, these studies are supportive of the supposition proposed by Bylin (1993).

The recent review by Brannan and Lougheed (2012) has also specifically identified reduced airway caliber as a predictor of airway responsiveness. However, other studies not described here have concluded that airway caliber was not a predictor of airway responsiveness. Although this may seem counter to the dosimetric discussion above, 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 more strongly suggests an effect of the surface dose of challenge agents to the conducting airways on airways responsiveness than as a function of airway caliber, per se. The dose of bronchial challenge agents to the conducting airways may have been affected by numerous factors within and between studies evaluating the effect of NO_2 on airway responsiveness. Although it is clear that such factors could contribute to variability within and between 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 Time of Challenge Post-exposure

With respect to the data in Table 4-1 and Table 4-2, bronchial challenges were delivered an average of 60 minutes post-exposure. For nonspecific 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

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 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 two hours post exposure.

Although there is strong evidence for a refractory period following 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 NO_2 Exposures

To mimic a daily commute, Strand et al. (1998) exposed adults with asthma on four sequential days to either filtered air or 260 ppb NO_2 for 30 minutes during rest. The early phase response to allergen challenge was significantly increased by NO_2 exposure. The allergen-induced fall in FEV_1 for the 4 days was, on average, -2.5 versus -0.4% after air ($p = 0.018$). The late phase response to allergen challenge was also significantly greater after NO_2 with an average decrement in FEV_1 of -4.4 versus -1.9% after air ($p = 0.009$) for the 4 days. 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.

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 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 AHR data at the time of enrollment in their study and

AHR data following resting exposures to filtered air. Little to no discernible change was observed between AHR at inclusion and following the resting exposure which suggests that experimental procedures (other than exposure to NO_2) did not affect AHR. In the study by Jenkins et al. (1999), although the average PD_{20} to HDM were similar following a 3- and 6-hour filter air exposure with exercise, 5 of 10 subjects had greater than a 2× difference in their PD_{20} between the two air exposures. Unfortunately, this study does not allow the contribution of daily variability in airway responsiveness versus an effect of exposure duration to be discerned.

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 lack of a clear dose-response relationship may suggest that some factors cause a diminution of responses at higher versus lower intake doses.

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 than exercise, despite a lower intake dose of NO_2 during the resting exposures, is consistent with an effect of exercise refractoriness. 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. The choice of bronchial challenge agent and method of delivery each also would have likely contributed to variability between studies. Limited evidence also suggests airway dilation at higher intake doses could reduce airway responsiveness. Overall, the effects of exercise refractoriness 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, between studies such as the choice of challenge agents, challenge delivery method, physiological endpoint used to quantify airway responses, severity of disease, 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. Bylin et

al. (1988) found statistically significant effects of NO_2 on airway responsiveness at 270 ppb, but not 140 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 versus 0.94 mg at 100 ppb). Tunnicliffe et al. (1994) found a significant and larger increase in airway responsiveness at 400 ppb as compared to tendency for increased responsiveness at 100 ppb. These three studies (Tunnicliffe et al., 1994; Bylin et al., 1988; Orehek et al., 1976), for resting exposure to NO_2 are supportive of increasing airways responsiveness with increasing NO_2 concentration in individuals with asthma. 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 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) should allow for a valid comparison across studies since the air control would, theoretically, adjust for many methodological differences between studies. However, even after this adjustment, clear differences between resting and exercising exposures exist, presumably because exercise itself causes real effects on airway responsiveness. It may not be possible to adequately remove the influence of some methodological factors such as exercise 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, especially those using resting exposure, are supportive of a dose-response relationship showing increasing airway responsiveness with increasing NO_2 exposure concentration.

4.2.2.4 Summary of Airway Hyperresponsiveness

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. The airway responsiveness of individuals with asthma is generally greater than in healthy age-matched controls; although, the airway

responsiveness of those with asthma extends into the normal range. 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 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 between NO_2 studies including subject activity level (rest versus exercise) during NO_2 exposure, asthma medication usage, choice of airway challenge agent (e.g., direct and indirect nonspecific stimuli), method of administering the bronchoconstricting agents, and physiological endpoint used to assess airway responsiveness. These intra-study differences likely contribute to considerable variability and uncertainty in comparison of factors such as the provocative dose and lung function response to bronchial challenge agents. Studies that evaluated effects of NO_2 in subjects with asthma at multiple NO_2 concentrations under resting conditions generally show increasing airway responsiveness with an increase in exposure concentration.

Controlled human exposure studies have shown significant effects of NO_2 exposure on airway responsiveness in both healthy individuals and those with asthma. In healthy individuals, increases in nonspecific airway responsiveness were observed in the range of 1,500 to 2,000 ppb NO_2 for 3-hour exposures. In those with asthma, statistically significant effects on responsiveness to nonspecific challenge were reported following exposures as low as 100 ppb NO_2, although most studies showing significant effects were in the range of 300 ppb NO_2 or greater. Enhanced airway responsiveness to allergens in asthmatics was found at exposures of as low as 260 ppb for 30 minutes. Evidence to describe key events to inform modes of action for NO_2-induced AHR include the effects of NO_2 on bronchial smooth muscle reactivity (Section 3.3.2.5) and innate/adaptive immunity (Section 3.3.2.6). Given the methodological differences between studies, several meta-analyses including those in Table 4-3, Table 4-4, and Table 4-5 have assessed the fraction of individuals experiencing a change in airway responsiveness. In individuals exposed to NO_2 at rest, increases in nonspecific airway responsiveness (Table 4-3) 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.

4.2.3 Lung Function

Compared with evidence for AHR, the 2008 ISA for Oxides of Nitrogen reported weak evidence for the direct effects of NO_2 exposure on changes in lung function, particularly in controlled human exposure studies and epidemiologic studies of adults (U.S. EPA,

2008c). The evidence was weak in healthy adults and those with asthma or chronic obstructive pulmonary disease (COPD) alike. In previous epidemiologic studies, the most robust evidence comprised associations in children in the general population between increases in ambient NO_2 concentration and decrements in lung function as measured by supervised spirometry. Evidence in children with asthma was based on unsupervised lung function measurements and was inconsistent. Most recent studies were epidemiologic and supported associations between ambient NO_2 concentrations and lung function decrements in children with asthma and children in the general population.

4.2.3.1 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 and children in the general population. Across the various populations examined, results are less consistent for lung function measured under unsupervised conditions, primarily peak expiratory flow (PEF) at home. Most results indicate lung function decrements in association with NO_2; associations were inconsistent for NO and NO_X. Ambient concentrations of oxides of nitrogen, locations, and time periods for epidemiologic studies of lung function are presented in Table 4-6.

Table 4-6 Mean and upper percentile oxides of nitrogen concentrations in epidemiologic studies of lung function.

Study[a]	Location	Study Period	Exposure Metric Analyzed	Mean/Median Concentration (ppb)	Upper Percentile Concentrations (ppb)
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	-
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]
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

Table 4-6 (Continued): Mean and upper percentile oxides of nitrogen concentrations in epidemiologic studies of lung function.

Study[a]	Location	Study Period	Exposure Metric Analyzed	Mean/Median Concentration (ppb)	Upper Percentile Concentrations (ppb)
Liu et al. (2009b) Dales et al. (2009a)	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
Hernández-Cadena et al. (2009)	Mexico City, Mexico	May-Sept 2005	1-h max NO_2	57	75th: 69 Max: 116
Delfino et al. (2008a)	Riverside, CA	July-Dec 2003	24-h avg personal NO_2	28.6	Max: 105.7
	Whittier, CA	July-Dec 2004	24-h avg central site NO_2	25.0	Max: 29.2
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 NO_2 (6 a.m.-10 a.m.)	NR	NR
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
Odajima et al. (2008)	Fukuoka, Japan	Apr-Sept 2002	3-h avg (7 p.m.-10 p.m.) NO_2	20.0	Max: 51.3
		Oct 2002 - Mar 2003		11.0	Max: 49.0
Gillespie-Bennett et al. (2011)	Bluff, Dunedin, Christchurch, Porirua, Hutt Valley, New Zealand	Sept 2006	4-week avg NO_2	3.9	NR
Wiwatanadate and Trakultivakorn (2010)	Chiang Mai, Thailand	Aug 2005-June 2006	24-h avg NO_2	17.2	90th: 26.5 Max: 37.4
Just et al. (2002)	Paris, France	Apr-June 1996	24-h avg NO_2	28.6[c]	Max: 59.0[c]
Yamazaki et al. (2011)	Yotsukaido, Japan	Oct-Dec 2000	1-h avg (6 p.m.-7 p.m.) NO_2	32.6	-
McCreanor et al. (2007)	London, U.K.	Nov-March 2003-2005	2-h avg (10:30 a.m. - 12:30 p.m.) NO_2	Oxford St: 75.5[c] Hyde Park: 11.5[c]	Max: 154[c] Max: 77.7[c]

Table 4-6 (Continued): Mean and upper percentile oxides of nitrogen concentrations in epidemiologic studies of lung function.

Study[a]	Location	Study Period	Exposure Metric Analyzed	Mean/Median Concentration (ppb)	Upper Percentile Concentrations (ppb)
Qian et al. (2009a)	Boston, MA Denver, CO Madison, WI New York City, NY Philadelphia, PA San Francisco, CA	Feb 1997-Jan 1999	24-h avg NO_2	20.8	75th: 25.5 Max: 60.7
Silkoff et al. (2005)	Denver, CO	Winters	24-h avg NO_2		
		1999-2000		16	75th: 30, Max: 54
		2000-2001		29	75th: 36, Max: 54
Harre et al. (1997)	Christchurch, New Zealand	June-Aug 1994	24-h avg NO_2	NR	NR
Peacock et al. (2011)	London, U.K.	Oct 1995-Oct 1997	1-h max NO_2	51.4	75th: 56
Canova et al. (2010)	Padua, Italy	Summer/Fall 2004, Winter/Summer /Fall 2005	24-h avg NO_2	27.2[c]	48.1[c]
Wiwatanadate and Liwsrisakun (2011)	Chiang Mai, Thailand	Aug 2005-June 2006	24-h avg NO_2	17.2	90th: 26.5 Max: 37.4
Lagorio et al. (2006)	Rome, Italy	May-June, Nov-Dec 1999	24-h avg NO_2	37.6[c]	Max: 54.3[c]
Maestrelli et al. (2011)	Padua, Italy	1999-2003	24-h avg NO_2	Across seasons and years: 20.9-37.0[c]	Range of 75th: 23.0-42.5[c]
Hiltermann et al. (1998)	Bilthoven, the Netherlands	July-Oct 1995	24-h avg NO_2	11.2[c]	22.5[c]
Higgins et al. (2000); Higgins et al. (1995)	Widnes, Runcorn, U.K.	Aug, year NR	24-h avg NO_2	NR	Max: 44.7[c]
Park et al. (2005)	Incheon, Korea	Mar-June 2002	24-h avg NO_2	Control days: 31.6 Dust days: 20.7	
Steerenberg et al. (2001)	Utrecht, the Netherlands	Feb-Mar 1998	24-h avg NO_2 24-h avg NO	28.2[c] 30.2[c]	Max: 44.7[c] Max: 168[c]
	Bilthoven, the Netherlands		24-h avg NO_2 24-h avg NO	25.5[c] 7.4[c]	Max: 49.5[c] Max: 85.6[c]
Linn et al. (1996)	Upland, Rubidoux, Torrance, CA	School yr, 1992-1994	24-h avg NO_2	33	Max: 96
Moshammer et al. (2006)	Linz, Austria	School yr, 2000-2001	8-h avg NO_2 (12-8 a.m.)	9.3[c]	75th: 11.4[c]
Oftedal et al. (2008)	Oslo, Norway	Nov 2001-Dec 2002	24-h avg NO_2	14.4[c]	Max: 59.2[c]
Chang et al. (2012)	Taipei, Taiwan	Dec 1996-May 1997	6-day avg NO_2	31.8	75th: 41.7

Table 4-6 (Continued): Mean and upper percentile oxides of nitrogen concentrations in epidemiologic studies of lung function.

Study[a]	Location	Study Period	Exposure Metric Analyzed	Mean/Median Concentration (ppb)	Upper Percentile Concentrations (ppb)
Castro et al. (2009)	Rio de Janeiro, Brazil	May, June, Sept, Oct 2004	24-h avg NO_2	49.2[c]	Max: 115[c]
Bagheri Lankarani et al. (2010)	Tehran, Iran	NR	24-h avg NO_2 24-h NO 24-avg NO_X	75.5, 17.6[c] 51.6, 40.4[c] 72.9, 38.8[c]	Max: 119, 25.5[c] Max: 85.1, 110$_c$ Max: 122, 94.7[c]
Eenhuizen et al. (2013)	3 study areas, the Netherlands	Oct 2000-Nov 2001	24-h avg NO_2	16.0[c]	75th: 23.2[c] Max: 47.9[c]
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
Scarlett et al. (1996)	Surrey, U.K.	June-July 1994	1-h max NO_2	34.9	Max: 82
Timonen and Pekkanen (1997)	Kuopio, Finland	Feb-Apr 1994	24-h avg NO_2	Urban: 14.9[c] Suburban: 7.4[c]	Max: 41.5[c] Max: 27.1[c]
Ranzi et al. (2004)	Emiglia-Romagna, Italy	Feb-May 1999	24-h avg NO_2	Urban: 37.0[c] Rural: 18.51[c]	NR NR
Ward et al. (2000)	West Midlands, U.K.	Jan-Mar 1997 May-July 1997	24-h avg NO_2	NR	NR
van der Zee et al. (2000); van der Zee et al. (1999)	Rotterdam, Bodegraven/Reeuwij, Amsterdam, Meppel, Nunspeet, the Netherlands	Winter 1992-1993 Winter 1993-1994 Winter 1994-1995	24-h avg NO_2	27.1, 17.6[c] 25.5, 13.3[c] 25.0, 11.7[c]	Max: 50, 44.2[c] Max: 40.4, 28.7[c] Max: 43.6, 30.3[c]
Roemer et al. (1998)	Multiple locations: Sweden, Finland, Norway, the Netherlands, Germany, Czech Republic, Hungary, Italy, Greece	Winter 1993-1994	24-h avg NO_2	Across locations: 6.7-39.8[c]	-
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
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
Thaller et al. (2008)	Galveston, TX	Summers 2002, 2003, 2004	24-h avg NO_2 1-h max NO_2 24-h avg NO_X 1-h max NO_X	1.2 3.2 1.3 3.6	Max: 7.1 Max: 27.7 Max: 7.4 Max: 36.6
Schindler et al. (2001)	Aarau, Basel, Davos, Geneva, Lugano, Montana, Payerne, Wald, Switzerland	NR	24-h avg NO_2	19.5[c]	69.3[c]
Cakmak et al. (2011a)	14 Canadian cities	Mar 2006-Mar 2007	24-h avg NO_2	12.6	95th: 29.4

Table 4-6 (Continued): Mean and upper percentile oxides of nitrogen concentrations in epidemiologic studies of lung function.

Study[a]	Location	Study Period	Exposure Metric Analyzed	Mean/Median Concentration (ppb)	Upper Percentile Concentrations (ppb)
Steinvil et al. (2009)	Tel Aviv, Israel	Sept 2002-Nov 2007	24-h avg NO_2	19.3	75th: 25.3 Max: 59.9
Son et al. (2010)	Ulsan, Korea	2003-2007	24-h avg NO_2	21.4	75th: 26.1 Max: 44.8

[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).

NR = not reported.

Children with Asthma

In contrast with studies reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), several recent studies of children with asthma conducted spirometry under supervised conditions, and most indicated a relationship with short-term NO_2 exposure (Figure 4-1 and Table 4-7). Studies of supervised spirometry measured lung function weekly, biweekly, or seasonally. Evidence for lung function measured daily by subjects at home was 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 Liwsrisakun, 2011; Odajima et al., 2008; Just et al., 2002; Mortimer et al., 2002). Results were inconsistent between U.S. multicity studies (NCICAS, ICAS) (O'Connor et al., 2008; Mortimer et al., 2002). However, several studies that reported no association with home lung function measurements did not provide quantitative results, including NCICAS (Odajima et al., 2008; Just et al., 2002; Mortimer et al., 2002). Thus, assessing the relative magnitude and precision of their results was not possible. A relationship between ambient NO_2 and PEF was indicated in children diagnosed 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.

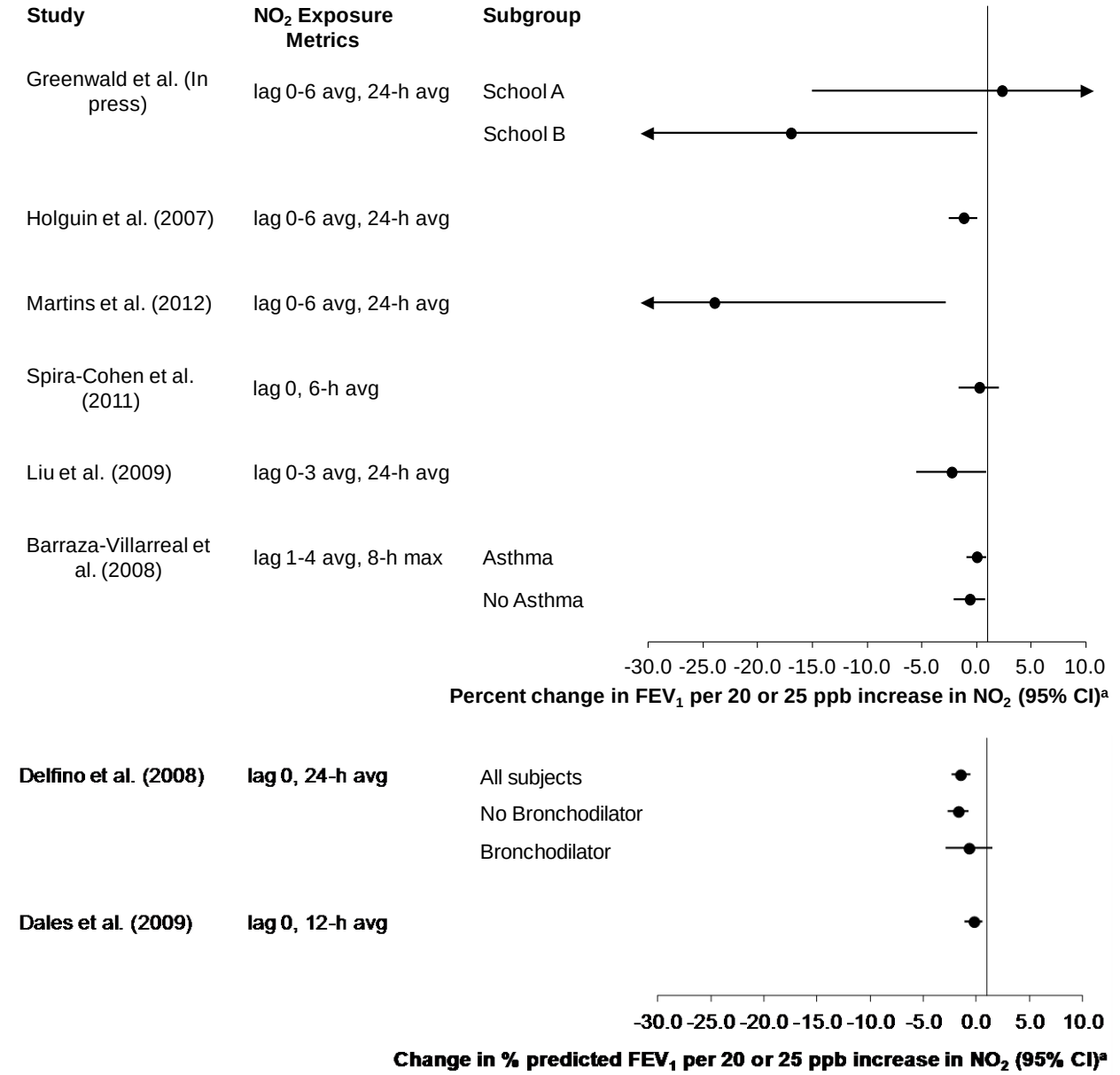
With respect to the populations examined, most studies assessed asthma as parental report of physician-diagnosed asthma. Children were recruited mostly from schools, supporting the likelihood that study populations were representative of the general population of children with asthma. Study populations 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 ICS (Hernández-Cadena et al., 2009; Liu et al., 2009b) or not taking controller bronchodilators (Delfino et al., 2008a). The limited results for larger associations in ICS nonusers together with observations for NO_2-associated lung function decrements in populations with high prevalence of atopy (53-100%) (Martins et al., 2012; O'Connor et al., 2008; Holguin et al., 2007) are supported by findings for NO_2-induced increases in allergic inflammation (Section 4.2.4.3) and findings for mast cell degranulation (which leads to histamine release) in mediating NO_2-induced lung function decrements (Section 3.3.2.2). Bronchodilator use has been shown to reduce AHR in response to a challenge agent (Section 4.2.2.3).

For children with asthma, key evidence for NO_2-associated lung function decrements was provided by studies with strong exposures assessment characterized by personal monitoring (Delfino et al., 2008a), modeling outdoor measurements at school and other locations with time-activity data (Martins et al., 2012) or outdoor school monitoring (Greenwald et al., 2013; Spira-Cohen et al., 2011; Holguin et al., 2007). These studies examined limited lags of NO_2 exposure and 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 modeled personal exposures provided evidence of an effect of outdoor NO_2 on lung function. Among children with asthma in the Los Angeles, CA area, slightly larger decrements in % predicted FEV_1 were found with 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). But, a Spearman correlation of 0.43 between personal and central site NO_2 indicated that ambient NO_2 had some influence on personal exposures. 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 2.6.5.2).

Among studies of outdoor school NO_2, associations with FEV_1 were found with lag 0-6 day avg NO_2 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 4-1 and Table 4-7). Between two El Paso schools, associations were limited to the school characterized by a larger percentage of Mexican-American children, higher BMI, and higher outdoor pollutant concentrations (Greenwald et al., 2013). No association with FEV_1 was found in children with asthma in Bronx, NY with school NO_2 averaged over the 6-h school day (Spira-Cohen et al., 2011). An effect of outdoor NO_2 was indicated 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 lung function measures were related to a single 4-week average of NO_2.



Note: All results are from recent studies and are organized by population examined and then generally in order of decreasing study strength (e.g., exposure assessment method, potential confounding considered).

[a]Effect estimates are standardized to a 20-ppb increase for 24-h avg NO_2, and a 25-ppb increase for 6-h to 12-h avg or 8-h max NO_2. Study details and quantitative results are reported in Table 4-7.

Figure 4-1 Associations between ambient or personal NO_2 concentrations and FEV_1 in children with asthma.

Table 4-7 Epidemiologic studies of lung function in children and adults with respiratory disease.

Study	Study Population and Methodological Details	Exposure Metrics Analyzed	Lag day Analyzed	Subgroup Analyzed (if applicable)	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Children with Asthma						
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 potential confounding by school, temperature, relative humidity, indoor NO.	NO_2–School outdoor NO_2–School indoor All 24-h avg	0-4 avg	School A School B School A School B	FEV_1: 2.3% (-15, 24%) -17% (-32, 0.12%) 38% (-12, 116%) -14% (-32, 7.2%)	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 (r = 0.77).
Holguin et al. (2007)‡	Ciudad Juarez, Mexico N = 194, ages 6-12 yr, 78% mild, intermittent asthma, 58% with atopy. Repeated measures. Supervised spirometry. Examined biweekly for 4 mo. 87% participation. Self-report of physician-diagnosed asthma. Linear and nonlinear mixed effects model with random effect for subject and school adjusted for sex, BMI, day of week, season, maternal and paternal education, passive smoking exposure.	NO_2–School outdoor 24-h avg Homes 397 meters from schools	0-6 avg	Asthma, n = 31	FEV_1: -1.2% (-2.5, 0.06%)	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 4-7 (Continued): Epidemiologic studies of lung function in children and adults with respiratory disease.

Study	Study Population and Methodological Details	Exposure Metrics Analyzed	Lag day Analyzed	Subgroup Analyzed (if applicable)	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. 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 or dampness in home, fireplace in home, pets in home because changed at least 1 pollutant effect estimate >10%.	NO_2-Subject-level 24-h avg Estimated from school outdoor NO_2, 20 city monitors, MM5/CHIMERE modeling, and daily activity patterns	0-6 avg8		FEV_1: -24% (-45, -2.8%) FEV_1/FVC: -11% (-21, 0.49%) $FEF_{25-75\%}$: -39% (-71, -6.0%) FEV_1 after bronchodilator: 18% (3.4, 32%)	For FEV_1: w/PM_{10}: -31% (-96, 35%) w/benzene: -3.7% (-33, 25%) w/ethylbenzene: -18% (-50, 14%) Benzene robust to NO_2 adjustment, PM_{10} and ethylbenzene attenuated. Correlations negative or weakly positive. Spearman r = -0.72 to -0.55 for PM_{10}, -0.43 to 0.14 for VOCs.
Spira-Cohen et al. (2011)‡	Bronx, NY N = 40, ages 10-12 yr, 100% nonwhite, 44% with asthma ED visit or hospital admission in previous 12 mo. Repeated measures. Supervised spirometry. Examined daily for 1 mo. 454 observations. 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. 89% time indoors.	NO_2–school 6-h avg (9 a.m.-3 p.m.) Most children walk to school.	0		FEV_1: 0.23% (-1.6, 2.1%) PEF: 0.92% (-1.0, 2.8%)	Personal EC associated with lung function and was robust to NO_2 adjustment.

Table 4-7 (Continued): Epidemiologic studies of lung function in children and adults with respiratory disease.

Study	Study Population and Methodological Details	Exposure Metrics Analyzed	Lag day Analyzed	Subgroup Analyzed (if applicable)	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Liu et al. (2009b)‡	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. Most subjects live within 10 km of sites.	0		FEV_1: -1.2% (-3.2, 0.84%) $FEF_{25-75\%}$: -4.8% (-8.6, -0.94%)	FEV_1 w/$PM_{2.5}$: 1.2% (-3.8, 6.4%) FEV_1 w/SO_2: -2.0% (-6.9, 3.1%) $PM_{2.5}$ association robust to NO_2 adjustment, SO_2 attenuated. Spearman r = 0.71 for $PM_{2.5}$, 0.18 for SO_2.
			0-3		FEV_1: -2.3% (-5.5, 0.92%) $FEF_{25-75\%}$: -8.0 (-14, -1.6%)	
Dales et al. (2009a)‡	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. Most subjects live within 10 km of sites Mean 1.6 and 2.2 h/day spent outdoors.	0		Evening % predicted FEV_1: -0.23 (-1.1, 0.59) Diurnal change FEV_1: -0.69% (-1.3, 0.07%)	Evening FEV_1: NO_2 becomes positive with $PM_{2.5}$ adjustment. Diurnal change FEV_1: NO_2 robust to adjustment for $PM_{2.5}$ or SO_2. SO_2 and $PM_{2.5}$ attenuated slightly. Moderate correlation between NO_2 and $PM_{2.5}$. Pearson r = 0.68.

Table 4-7 (Continued): Epidemiologic studies of lung function in children and adults with respiratory disease.

Study	Study Population and Methodological Details	Exposure Metrics Analyzed	Lag day Analyzed	Subgroup Analyzed (if applicable)	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Barraza-Villarreal et al. (2008)‡	Mexico City, Mexico N = 163-179, ages 6-14 yr, 54% persistent asthma, 89% with atopy. Repeated measures. Supervised spirometry. Examined every 15 days for mean 22 weeks. 1,503 observations. Children with asthma recruited from pediatric clinic. Children without asthma were friends or schoolmates. Asthma severity assessed by pediatric allergist. Linear mixed effects model with random effect for subject and adjusted for sex, BMI, lag 1 minimum temperature, ICS use, time. Adjustment for outdoor activities, smoking exposure, anti-allergy medication use, and season did not alter results.	NO_2-Central site 8-h max Monitors within 5 km of school or home. Spearman correlation coeffiecient for school vs. central site: r = 0.21	1-4 avg		FEV_1	No copollutant model. $PM_{2.5}$ associated with FEV_1 and FVC. Moderate correlation with NO_2. Pearson r = 61.
				Asthma, n = 126	0% (-0.87, 0.87%)	
				No asthma, n = 50	-0.64% (-2.1, 0.82%)	
					FVC	
				Asthma, n = 129	-0.11% (-1.2, 0.97%)	
				No asthma, n = 45	-0.91% (-2.6, 0.76%)	
Hernández-Cadena et al. (2009)‡	Mexico City, Mexico N = 85, ages 7-12 yr, 62% mild, intermittent asthma, 90% with 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		% change FEV_1 after bronchodilator use -39% (-64, 5.4%)	No copollutant model. O_3, not $PM_{2.5}$ associated with FEV_1.

Table 4-7 (Continued): Epidemiologic studies of lung function in children and adults with respiratory disease.

Study	Study Population and Methodological Details	Exposure Metrics Analyzed	Lag day Analyzed	Subgroup Analyzed (if applicable)	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Yamazaki et al. (2011)‡	Yotsukaido, Japan N = 17, ages 8-15 yr. Repeated measures. Supervised peak flow collected before medication use. Examined daily during long-term stay in hospital. No air conditioning in hospital. Permitted to go outside if asthma stable. Lack of generalizability. 1,198 morning and evening 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.	0		No quantitative data. PEF decreases with increasing NO_2 0 to 23 hours before measurement. Stronger associations at 0 h and 12 h.	Only 3-pollutant model analyzed. $PM_{2.5}$ also associated with evening PEF. Moderate correlation with $PM_{2.5}$. r = 0.62,
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 1 to 16 10-day periods. 416 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-Personal 24-h avg Monitoring checked daily.	0-1 avg 0	All subjects All subjects	% predicted FEV_1 -1.7 (-3.2, -0.19) -1.5 (-2.3, -0.57)	w/ 1-h max $PM_{2.5}$: -1.3 (-2.8, 0.22) Weak correlation with NO_2. Spearman r = 0.38 for personal $PM_{2.5}$, 0.36 for central site. $PM_{2.5}$ robust to NO_2 adjustment. Lack of associations with EC, OC. Central site NO_2 w/personal $PM_{2.5}$: -0.86 (-2.6, 0.89)
				No bronchodilator, n = 37	-1.7 (-2.7, -0.75)	
				Bronchodilator use, n = 16	-0.70 (-2.9, 1.5)	
		NO_2-Central site 24-h avg Central site with 5 or 10 km of homes Central site and personal r = 0.43	0	All subjects	-1.3 (-2.4, -0.15)	

Table 4-7 (Continued): Epidemiologic studies of lung function in children and adults with respiratory disease.

Study	Study Population and Methodological Details	Exposure Metrics Analyzed	Lag day Analyzed	Subgroup Analyzed (if applicable)	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 and symptoms with exercise or cold exposure or family history of asthma. 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.	O_3 associated with PEF.
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. Recruitment from intervention of physician feedback. 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 industrial source. Median distance to site = 2.3 km	1-5 avg		% predicted FEV_1: -1.3 (-1.9, -0.78) % predicted PEF: -1.6 (-2.2, 1.1)	Only 3-pollutant model analyzed. $PM_{2.5}$, SO_2, CO, O_3 also associated. Moderate correlations with NO_2. r = 0.59 for $PM_{2.5}$ and SO_2, 0.54 for CO. Weak correlation with O_3. r = -0.31
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. Recruitment from hospital outpatients. GEE adjusted for time trend, day of week, pollen, temperature, humidity.	NO_2–Central site 24-h avg Average of 11 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 4-7 (Continued): Epidemiologic studies of lung function in children and adults with respiratory disease.

Study	Study Population and Methodological Details	Exposure Metrics Analyzed	Lag day Analyzed	Subgroup Analyzed (if applicable)	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Wiwatanadate and Trakultivakorn (2010)‡	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. 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 in L/min -1.8 (-5.4, 1.8) 2.6 (-1.2, 6.4)	
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. 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 3-pollutant model analyzed. SPM associated with PEF in warm season. Weak correlation with NO_2. r = 0.30 for 24-h avg.
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, 1 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–outdoor home NO_2–indoor home	4-week avg		Per log increase NO_2: Evening FEV_1 (mL) -88 (-191, 15) Evening PEF (L/min) -10 (-21, 0.98) Evening FEV_1 (mL) -13 (-26, -0.38) Evening PEF (L/min) -0.97 (-2.3, 0.36)	No copollutant model. No other pollutants examined.

Table 4-7 (Continued): Epidemiologic studies of lung function in children and adults with respiratory disease.

Study	Study Population and Methodological Details	Exposure Metrics Analyzed	Lag day Analyzed	Subgroup Analyzed (if applicable)	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Adults with Respiratory Disease						
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. Recruitment from advertisements and volunteer databases. Mixed effects model with random effect for subject and adjusted for temperature, relative humidity.	NO_2–on site of outdoor exposure 2-h avg	2-h		FEV_1: -1.2% (-2.2,-0.22%) $FEF_{25-75\%}$: -4.3% (-7.9, -0.65%)	$FEF_{25-75\%}$ w/UFP: -0.45% (-0.73,-0.17%) w/EC: -1.0% (-2.1, 0.06%) w/$PM_{2.5}$: -0.84% (-2.0, 0.34%) Moderate correlation with NO_2. Spearman r = 0.58 for UFP, EC, 0.60 for $PM_{2.5}$
			22-h Post-exposure		FEV_1: -0.73% (-2.0,-0.51%) $FEF_{25-75\%}$: -4.2% (-8.7, -0.61%)	
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 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.	NO_2–Central site 24-h avg Average of all monitors within 20 miles of subject ZIP code centroid			PEF	w/SO_2: -0.52% (-3.9, 2.9%) w/PM_{10}: -3.6% (-7.7, 0.46%) w/O_3: -3.1% (-5.9, -0.24%) SO_2 slightly attenuated with NO_2 adjustment. PM_{10}, O_3 not associated with PEF. Correlations NR.
			0	All subjects	-0.69% (-1.3, -0.06%)	
				Placebo	-0.28% (-1.4, 0.85%)	
				Beta-agonist	-1.1% (-2.1, -0.05%)	
				ICS	-0.62% (-1.6, 0.38%)	
			0-2 avg	All subjects	-0.62% (-1.6, 0.38%)	

Table 4-7 (Continued): Epidemiologic studies of lung function in children and adults with respiratory disease.

Study	Study Population and Methodological Details	Exposure Metrics Analyzed	Lag day Analyzed	Subgroup Analyzed (if applicable)	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Silkoff et al. (2005)	Denver, CO N = 34 with COPD, mean age 66, 67 yr, panel 1 and 2, 75% with severe COPD Repeated measures. Home PEF. Recruitment from outpatient clinics, research registries, advertisements. Mixed effects model with random effect for subjects and adjusted for temperature, relative humidity, barometric pressure.	NO_2–Central site 24-h avg 1 city site	0 1 2		No quantitative data. Negative, positive, and null associations across NO_2 lags.	No copollutant model. Mixed positive, negative, null associations for $PM_{2.5}$, PM_{10}, O_3
Harre et al. (1997)	Christchurch, New Zealand N = 40 with COPD, ages 55-83 yr, nonsmokers Repeated measures. Home PEF. Recruitment from doctors' offices, COPD support group, advertisements. 66% participation. Log linear model adjusted for temperature, wind speed, day of study, CO, PM_{10}, SO_2.	NO_2–central site 24-h avg # sites NR	1		PEF -0.72% (-1.5, 0.07%)	Only 4-pollutant model analyzed. PM_{10}, CO, SO_2 not associated with PEF.
Peacock et al. (2011)‡	London, U.K. N = 28-94 with COPD, 40-83 yr Repeated measures. Home PEF. Examined daily for 21-709 days. Recruitment from outpatient clinic. GEE adjusted for temperature, season. Lung function measures adjusted for indoor temperature and time spent outdoors.	NO_2–central site 1-h max 1 city site	1		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 OR w/PM_{10}: 0.97 (0.81, 1.2) OR w/BC: 1.1 (0.84, 1.3)

Table 4-7 (Continued): Epidemiologic studies of lung function in children and adults with respiratory disease.

Study	Study Population and Methodological Details	Exposure Metrics Analyzed	Lag day Analyzed	Subgroup Analyzed (if applicable)	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
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		No quantitative data. NO_2 shows null associations with PEF and FEV_1.	CO associated with PEF. Moderate correlation with NO_2. Spearman r = 0.48.
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: 1.8 (0.60, 3.0) Average PEF: -0.40 (-0.80, 0) Units of PEF not reported.	Only multipollutant models analyzed. No associations with PM_{10}, SO_2, O_3.
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	0 0-6 avg		Diurnal change PEF -0.75 (-8.1, 6.6) L/min -3.0 (-16, 10) L/min	No association found with O_3, PM_{10}, or BS.

Table 4-7 (Continued): Epidemiologic studies of lung function in children and adults with respiratory disease.

Study	Study Population and Methodological Details	Exposure Metrics Analyzed	Lag day Analyzed	Subgroup Analyzed (if applicable)	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Higgins et al. (1995)	Widnes, Runcorn, U.K. N = 47 with asthma, 10 with COPD, 10 with asthma and COPD, 8 with wheeze, ages 18-81 yr, 70% with atopy.	NO_2–central site 24-h avg 1 site per city	0, 1, 0-1 avg		No quantitative data. NO_2 reported to have some effect on PEF.	O_3 and SO_2 associated with PEF. NO_2 effect estimate attenuated with adjustment for SO_2.
Higgins et al. (2000)	N = 31 with asthma, 3 with COPD, 63% with atopy. Repeated measures. Home PEF. Examined daily for 28 days. Recruitment from doctors' offices. Model specifics including covariates NR.		0		<33th percentile: ref 33-67th percentile -3.3 (-7.9, 1.4) >67th percentile -4.7 (-9.6, 0.21)	O_3 and spore count associated with PEF.
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 in L/min 0.45 (-1.0, 1.9)	PM_{10} and CO associated with PEF.
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 as beta-agonist users (>6/yr for 3 yr), diagnosis clinically confirmed. 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 city sites	0		% predicted FEV_1: 1.1 (-6.6, 8.7)	No copollutant model. O_3, SO_2, personal or central site $PM_{2.5}$, PM_{10} not associated with FEV_1. Correlations NR.

Table 4-7 (Continued): Epidemiologic studies of lung function in children and adults with respiratory disease.

Study	Study Population and Methodological Details	Exposure Metrics Analyzed	Lag day Analyzed	Subgroup Analyzed (if applicable)	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Lagorio et al. (2006)	Rome, Italy N = 11, ages 18-64 yr, 100% mild, intermittent asthma, N = 11, ages 40-64 yr, COPD Repeated measures. Supervised spirometry. Examined 2/week for two 1-mo periods. Mean 9, 15 observations/subject. 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	Asthma COPD	% predicted FEV_1: -2.0 (-3.2, -0.75) -2.3 (-3.6, -1.0)	No copollutant model. Lung function associated with $PM_{2.5}$, PM_{10} in adults with COPD not asthma. Moderate correlations with NO_2. Spearman r = 0.43 for $PM_{2.5}$, 0.45 for PM_{10}.
Children in the General Population						
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 (8 a.m.-11 p.m.) 24-h avg	15-h avg 0-2 avg	Urban Suburban Urban Suburban	PEF mL/min -17 (-35,0) 7, p >0.05 0, p >0.05 6, p >0.05	No copollutant model. PM_{10} and BS also associated with PEF. Correlations NR.
		NO – central site 15-h (8 a.m.-11 p.m.) 24-h avg Site within 2 km of schools	15-h avg 0-2 avg	Urban Suburban Urban Suburban	1, p >0.05 0, p >0.05 -6 (-12, 0) 6, p >0.05	

(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. 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 = 0.61 correlation with personal NO_2
			0		p.m. FEV_1 mL
					-5.2 (-13, 2.3)
					p.m. FVC
					-3.6 (-12, 4.6)
					Diurnal change FEV_1
					-7.8 (-14, -1.5)
					Diurnal change FVC
					-2.2 (-9.6, 4.9)
						Associations found with $PM_{2.5}$, weak for O_3.
						NO_2 association reported to lose statistical significance with $PM_{2.5}$ adjustment.
						Weak correlation with $PM_{2.5}$. r = 0.25

Table 4-7 (Continued): Epidemiologic studies of lung function in children and adults with respiratory disease.

Study	Study Population and Methodological Details	Exposure Metrics Analyzed	Lag day Analyzed	Subgroup Analyzed (if applicable)	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. 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%)	w/$PM_{2.5}$: -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}.
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		Results in figure Lag 1-3 avg similar to 1-7, slightly smaller than 1-30 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
Chang et al. (2012)‡	Taipei, Taiwan N = 2,919, ages 12-16 yr Cross-sectional. Supervised spirometry. Recruitment from schools. 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}

Table 4-7 (Continued): Epidemiologic studies of lung function in children and adults with respiratory disease.

Study	Study Population and Methodological Details	Exposure Metrics Analyzed	Lag day Analyzed	Subgroup Analyzed (if applicable)	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
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. 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 24-h avg School was within 2 km of homes.	1 1-2 avg 1-3 avg		PEF, Liters/minute 0.04 (-0.58, 0.65) -0.60 (-1.3, 0.14) -1.7 (0.02)	No copollutant model. Associations also found with PM_{10}, weaker associations with CO, SO_2.
Bagheri Lankarani et al. (2010)‡	Tehran, Iran N = 562, elementary school. Repeated measures. Examined daily for 6 weeks. 158 case-days. Case crossover with control dates as two weeks before and after case date. Conditional logistic regression adjusted for daily temperature, lag 0-6 avg PM_{10}.	NO – central site 24-h avg 2 city sites	0-6 avg		PEF <50% predicted OR: 18 (1, 326)	PM_{10} associated with decreased odds of large PEF decrement.
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 kPA*s/L (+ = worse) 0 (-0.04, 0.04) -0.02 (-0.06, 0.03)	No associations with PM_{10} or BS. Moderate correlations with NO_2. Pearson r = 0.47 for PM_{10}, 0.60 for BS.

Table 4-7 (Continued): Epidemiologic studies of lung function in children and adults with respiratory disease.

Study	Study Population and Methodological Details	Exposure Metrics Analyzed	Lag day Analyzed	Subgroup Analyzed (if applicable)	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Peacock et al. (2003)	Rochester upon Medway, U.K. N = 177, ages 7-13 yr, 25% with wheeze Repeated measures. Home PEF. Examined daily for 13 weeks. 14-63 observations/subject. Recruitment from rural and urban schools. Individual subject regressions adjusted for day of week, date, and temperature. Pooled estimates obtained using weighting method.	NO_2 – schools 24-h avg 1-h max	0-4 avg		PEF: -0.20 (-3.0, 2.6) PEF>20%:2.3 (1.0,5.4) PEF: 1.2 (-1.5, 3.9) PEF>20%:1.3 (0.5,3.4)	No copollutant model. $PM_{2.5}$ also associated with PEF decrement >20%. Correlation NR.
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. 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 1-h max	1		$FEV_{0.75}$ 0.30 (-0.29, 0.89%) FVC 5.5% (-5.1, 17%)	Association found with PM_{10} Weak to moderate correlations with NO_2. r = 0.07.
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 1-4 avg	Urban Suburban Urban Suburban	FEV_1: 11 (-14, 35) -6.5 (-40, 27) PEF: 13 (-24, 50) -22 (-87, 43)	Associations found for SO_2 in urban group. Weak correlations with NO_2. r = 0.22.
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. Recruitment from schools. GLM adjusted for sex, medication use, symptom status, temperature, humidity	NO_2 – central site 24-h avg # sites NR	0		No quantitative data. Figure shows null association in group with and without atopy.	$PM_{2.5}$ associated with PEF in urban group.

Table 4-7 (Continued): Epidemiologic studies of lung function in children and adults with respiratory disease.

Study	Study Population and Methodological Details	Exposure Metrics Analyzed	Lag day Analyzed	Subgroup Analyzed (if applicable)	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
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 2 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 0 in symptomatic group	No spool Associations with $PM_{2.5}$ equally inconsistent.
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 general population. 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	Urban Suburban Urban Suburban	OR: 0.96 (0.79, 1.2) OR: 0.77 (0.54, 1.1) OR: 1.1 (0.93, 1.3) OR: 0.99 (0.72, 1.4)	Associations found for PM_{10}, BS, SO_4, 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. 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, Liters/minute 0.15 (-0.19, 0.49) 0.23 (-1.2, 1.6)	Association found with PM_{10} and BS, but not consistently across lags

Table 4-7 (Continued): Epidemiologic studies of lung function in children and adults with respiratory disease.

Study	Study Population and Methodological Details	Exposure Metrics Analyzed	Lag day Analyzed	Subgroup Analyzed (if applicable)	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Adults in the General Population						
Strak et al. (2012)‡	Utrecht area, the Netherlands N = 31, adults ages 19-26 yr, all healthy, nonsmoking Repeated measures. Supervised spirometry. Examined 3-7 times. 107 observations. Recruitment from university. Well defined outdoor exposures at various traffic/non-traffic sites. Outcomes measured before and after outdoor exposures. 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 – on site of outdoor activity 5-h avg	0-h 2-h 18-h		FVC: -4.3% (-7.4, -1.0) -3.5% (-6.5, -0.43%) -4.5% (-7.4, -1.4)	FVC w/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 attenuated with NO_2 and NO_X
		NO_X – on site of outdoor activity 5-h avg	0-h 2-h 18-h Post-exposure		-1.6% (-2.6, -0.51%) -2.0% (-4.9, -0.16%) -2.5% (-5.4, -0.69%)	
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. Supervised spirometry. Most examined 3 times. 118 observations. 1-h outdoor exposures during cycling in low and high traffic areas. Recruitment from public advertisements. 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 liters 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, $PM_{2.5}$

Table 4-7 (Continued): Epidemiologic studies of lung function in children and adults with respiratory disease.

Study	Study Population and Methodological Details	Exposure Metrics Analyzed	Lag day Analyzed	Subgroup Analyzed (if applicable)	Effect Estimate (95% CI) Single-Pollutant Model[a]	Copollutant Examination
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. GLM, covariates not specified.	NO_2 and NO_X – central site 24-h avg, 1-h max 1 site 2.5-7.6 miles from beaches	0		No quantitative data. NO_2 and NO_X reported not to be significantly associated with lung function.	
Schindler et al. (2001)	Aarau, Basel, Davos, Geneva, Lugano, Montana, Payerne, Wald, Switzerland N = 3,912, ages 18-60 yr, nonsmokers 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%)	w/TSP: -1.2% (-3.8, 1.6%)
Van Der Zee et al. (2000)	Rotterdam, Bodegraven/Reeuwijk, Amsterdam, Meppel, Nunspeet, the Netherlands N = 274, ages 50-70 yr, no symptoms in previous 12 mo Repeated measures. Home PEF. Examined daily for 3 mo. Recruitment from mailings. Logistic regression adjusted for minimum temperature, day of week, time trend, influenza,	NO_2 – central site 24-h avg 1 site per community	0	Urban Suburban	PEF decrement >10% OR: 0.85 (0.59, 1.2) OR: 0.72 (0.50, 1.05)	No copollutant model. PEF associated with PM_{10} and SO_4 in urban group.
			0-4 avg	Urban Suburban	OR: 0.46 (0.20, 1.08) OR: 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 4-7 (Continued): Epidemiologic studies of lung function in children and adults with respiratory disease.

Study	Study Population and Methodological Details	Exposure Metrics Analyzed	Lag day Analyzed	Subgroup Analyzed (if applicable)	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. 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.
Steinvil et al. (2009)‡	Tel Aviv, Israel N = 2,380, mean age 43 (SD:11) yr, healthy nonsmokers Cross-sectional. Supervised spirometry. Recruitment from ongoing survey of individuals attending health center. 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 mL: -16 (-64, 33) -55 (-103, -6.3) -97 (-181, -13)	For lag 5 w/SO_2: -7.8 (-72, 56) w/CO: -19 (-88, 50) SO_2 and CO results robust with adjustment for NO_2. High correlations with NO_2. Pearson r = 0.70 for SO_2, 0.75 for CO.

Table 4-7 (Continued): Epidemiologic studies of lung function in children and adults with respiratory disease.

Study	Study Population and Methodological Details	Exposure Metrics Analyzed	Lag day Analyzed	Subgroup Analyzed (if applicable)	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. 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–avg 13 central site	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.
		NO_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)	

Note: Studies are organized by population examined and then generally in order of study strength (e.g., exposure assessment method, potential confounding considered). GLM = Generalized linear model, BC = black carbon, SO_2 = sulfur dioxide, BTEX = benzene, toluene, Ethylbenzene, xylene, BMI = body mass index, $PM_{2.5}$ = particulate matter less than 2.5 µm in aerodynamic diameter, EC = elemental carbon, VOCs = volatile organic compounds, ICS = inhaled corticosteroid, SES = socioeconomic status, O_3 = ozone, GEE = generalized estimating equations, OC = organic carbon, NCICAS = National Cooperative Inner-city Asthma Study, ICAS = Inner City Asthma Study, CO = carbon monoxide, SPM = suspended particulate matter, UFP = ultrafine particles, PM_{10} = particulate matter less than 10 µm in aerodynamic diameter, NR = not reported, PNC = Particle number concentration, TSP = Total suspended particles.

[a]Effect estimates were standardized to a 20-ppb increase in 24-h avg NO_2, a 25-ppb increase in 5-h to 12-h avg or 8-h max NO_2, a 30-ppb increase 1-h or 2-h avg NO_2, and a 50-ppb increase in 5-h avg NO_X.

‡Recent study published since the 2008 ISA for Oxides of Nitrogen.

Compared with more spatially resolved estimates of NO_2 exposure, evidence for associations with lung function decrements was less robust 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., 2009a; Hernández-Cadena et al., 2009; Liu et al., 2009b; O'Connor et al., 2008). In the U.S. multicity ICAS cohort, a 20-ppb increase in 24-h avg NO_2 at lag 1-5 day avg was associated with a -1.3-point (95% CI: -1.9, -0.78) change in percent predicted FEV_1 (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). Several studies did not report quantitative results, but 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 4-7). 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 1 site. The central site NO_2 assessment method did not appear to influence results.

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 examined day of the week, smoking exposure, or asthma medication use. Few studies analyzed copollutant models, and while Holguin et al. (2007) found that neither $PM_{2.5}$ nor elemental carbon (EC) was associated with FEV_1 among children with asthma in Ciudad Juarez, Mexico, most studies found associations with $PM_{2.5}$ as well as with PM_{10}, black carbon (BC), EC, sulfur dioxide (SO_2), ozone (O_3) or volatile organic compounds (VOCs). 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 robust in others. Copollutant effect estimates adjusted for NO_2 generally were robust. 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., 2009a; Liu et al., 2009b) (Table 4-7). NO_2 associations with FEV_1 diurnal change were robust to $PM_{2.5}$ and SO_2 adjustment (Dales et al., 2009a). 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 but did not eliminate 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). Results from copollutant analyses with personal and central site NO_2 indicate that ambient NO_2 may partly serve as an indicator of personal $PM_{2.5}$ but also provide evidence for independent effects on FEV_1 of personal and ambient NO_2.

Adults with Respiratory Disease

Most previous and recent studies of lung function in adults with asthma or COPD were based on PEF measured at home and produced inconsistent associations with ambient NO_2 concentrations (U.S. EPA, 2008c). 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 previous studies with supervised spirometry found associations with ambient NO_2 concentrations (McCreanor et al., 2007; Lagorio et al., 2006) whereas the recent study did not (Maestrelli et al., 2011) (Table 4-7). The majority of studies found no NO_2-associated lung function decrements in adults with asthma or COPD (Maestrelli et al., 2011; Canova et al., 2010; Hiltermann et al., 1998) or mixed associations (Peacock et al., 2011; Wiwatanadate and Liwsrisakun, 2011; Park et al., 2005; Silkoff et al., 2005; Higgins et al., 1995) among the various lung function parameters or NO_2 exposure lags examined. Most 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. There were more studies of adults with asthma than adults with COPD, but evidence was equally inconsistent for the two conditions. Similar associations between increases in ambient NO_2 concentrations and FEV_1 decrements were found in adults with COPD and asthma in Rome, Italy (Lagorio et al., 2006).

With respect to exposure assessment, most studies examined 24-h NO_2. In a study of adults with COPD in London, U.K., 1-h max NO_2 showed mixed associations among the various lung function measures examined (Peacock et al., 2011). Among all studies, no clear pattern of association was found for a particular lags of exposure (0, 1, 2, or 2- to 7-day avg). NO_2 exposures were assessed primary from central site measurements, and results were equally inconsistent for NO_2 exposures assigned from 1 site or averaged from multiple city sites. However, in a study in London, U.K., with stronger exposure assessment, NO_2 measured on site of outdoor exposures on a high-traffic road (allowing only diesel buses and taxis) and in a park was associated with decrements FEV_1 and $FEF_{25-75\%}$ in adults with mild to moderate asthma (McCreanor et al., 2007). Results indicated associations 2- to 22-hours after exposure. A 30-ppb increase in 2-h avg NO_2

with a lag of 2 hours was associated with a -1.24 L (95% CI: -2.26, -0.23) change in FEV_1 and -4.4 L/min (95% CI: -8.1, -0.68) change in $FEF_{25-75\%}$.

In addition to the inconsistent evidence in adults with asthma or COPD, there is uncertainty regarding an independent association of NO_2 from that of copollutants. Lagorio et al. (2006) found lung function decrements in association with NO_2 but not $PM_{2.5}$ or PM_{10}. But, in most cases, associations were also found for copollutants (Peacock et al., 2011; Qian et al., 2009b; McCreanor et al., 2007; Higgins et al., 2000). Studies reporting copollutant correlations found moderate correlations (Spearman r = 0.43-0.60 for UFP, EC, $PM_{2.5}$, PM_{10}) (McCreanor et al., 2007; Lagorio et al., 2006). In the few studies with copollutant modeling, NO_2-PEF effect estimates were attenuated with adjustment for SO_2 in the U.S. multicity study of adults with asthma (Qian et al., 2009b) and for PM_{10} and black smoke (BS) in the study of adults with COPD in London, U.K. (Peacock et al., 2011). Copollutant effect estimates were robust 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.

Children in the General Population

As in other populations, the 2008 ISA for Oxides of Nitrogen indicated associations between ambient NO_2 concentrations and lung function decrements in children in the general population as measured using supervised spirometry but not home PEF (U.S. EPA, 2008c). All recent studies conducted supervised spirometry, and most found associations with ambient NO_2 concentrations. Studies recruited children from schools, and reflecting the general population, examined groups of children with prevalence of respiratory conditions such as asthma and allergy of 5 to 72%. Several recent studies were cross-sectional. Most 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 (Chang et al., 2012; Oftedal et al., 2008). In children in the Netherlands, no association was found between 24-h avg NO_2 and interrupter resistance (Eenhuizen et al., 2013), a measure of airway resistance. In controlled human exposure studies, examination of airway resistance has been limited to adults. But, some evidence shows NO_2-induced increases in airway resistance (Section 3.3.2.2).

A relatively large body of previous studies, which were conducted in various European countries, did not provide evidence of NO_2-associated decrements in PEF. These studies were similar to studies of supervised lung function in examining study populations that included children with symptoms, asthma, or atopy, 24-h avg NO_2 measured at central sites, and exposures lagged 0 to 3 days or averaged over 5 to 7 days. Collectively, results indicated null associations (Ranzi et al., 2004; Ward et al., 2000; van der Zee et al., 1999) or NO_2-associated increases in PEF (Peacock et al., 2003; Roemer et al., 1998; Timonen and Pekkanen, 1997), including a population with 77% asthma and 70% atopy prevalence (Ranzi et al., 2004).

With respect to exposure assessment, a majority of evidence was for NO_2. NO was associated with lung function among children in Iran (Bagheri Lankarani et al., 2010) but not consistently among children in the Netherlands (Steerenberg et al., 2001). With the exception of Scarlett et al. (1996), studies found associations with NO_2 measured at or next to schools (Castro et al., 2009; Moshammer et al., 2006). Lung function decrements were also associated with home outdoor NO_2 estimated with dispersion modeling but not measured at central sites (Oftedal et al., 2008). These model estimates corresponded well with measured outdoor concentrations. Among children in three southern California communities, Linn et al. (1996) indicated that central site NO_2 measurements may represent temporal variation in personal exposures by finding a correlation of 0.61 between the two metrics. Most studies examined 24-h avg NO_2. Associations also were found with NO_2 averaged over 4 to 10 hours (Chang et al., 2012; Moshammer et al., 2006) and 8-h max NO_2 (Barraza-Villarreal et al., 2008) but not 1-h max NO_2 (Chang et al., 2012; Scarlett et al., 1996). Lung function decrements were found with NO_2 lagged 0 or 1 day and averaged over 3, 7, or 30 days, without a clear difference among lags in the magnitude of association. Among children in Oslo, Norway, associations with lung function were larger for lag 1-30 avg NO_2 than lag 1-2 avg or 1-7 avg (Oftedal et al., 2008). With adjustment for age 1-year or lifetime NO_2, the association for short-term NO_2 was attenuated, but exposure metrics were highly correlated (r = 0.70–0.77).

In most studies, an association of ambient NO_2 with lung function in children independent of copollutants was not clearly distinguished. Among children in Oslo, Norway, decrements in lung function were associated with increases in ambient NO_2 not PM_{10} or $PM_{2.5}$ (no quantitative results reported) (Oftedal et al., 2008). But, other studies found associations with PM_{10}, $PM_{2.5}$, and BS, which showed a range of correlations with NO_2 (r = 0.25-0.62). Few studies conducted copollutant modeling. In a study of children in three southern California communities, Linn et al. (1996) did not provide quantitative results for copollutant analyses and only indicated that NO_2 effect estimates lost statistical significance with adjustment for $PM_{2.5}$. Robust $PM_{2.5}$-adjusted effects were estimated among children in Austria, whose exposures were assessed from a monitoring

site adjacent to the school (Moshammer et al., 2006). A 25-ppb increase in lag 1 of 8-h avg NO_2 (12 a.m.-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 $PM_{2.5}$ (r = 0.54). $PM_{2.5}$ 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.

Adults in the General Population

In studies reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), 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 by peak flow meter (Van Der Zee et al., 2000). Recent studies examined lung function with supervised spirometry but also provided inconsistent evidence. 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 results. Van Der Zee et al. (2000) found no association in adults with or without respiratory symptoms.

Studies noteworthy for examining lung function before and after repeated outdoor exposures produced mixed evidence for associations with NO_X averaged over 1 to 5 hours (Strak et al., 2012; Weichenthal et al., 2011; Thaller et al., 2008). Associations were not found in adults cycling in various traffic and non-traffic locations or lifeguards working outdoors, whose NO_2 exposures were assessed from a central site (Weichenthal et al., 2011; Thaller et al., 2008). However, decreases in FVC and FEV_1 were found in healthy adults after 5-hour NO_2 and NO_X exposures measured on site of outdoor activity (Strak et al., 2012). A 25-ppb increase in NO_2 and 50-ppb increase in NO_X was associated with a 4.3% (95% CI: 1.0, 7.4%) and 1.6% (0.51, 2.6%) decrease in FVC, respectively, immediately after exposures. Decrements persisted 18 hours after exposure.

Other studies of adults in the general population also were mixed in finding ambient NO_2-associated lung function decrements. Contributing to the supporting evidence were recent cross-sectional studies, including a study of 15 Canadian communities that adjusted for age, sex, income, and smoking and considered potential confounding by temperature and humidity (Cakmak et al., 2011a). Studies similarly assessed 24-h avg NO_2 from central sites, primarily 1 per city. Son et al. (2010) was unique in comparing various methods of exposure assessment. NO_2-associated decreases in FVC in subjects ages 7-97 years in Ulsan, Korea were similar among NO_2 averaged across 13 city monitors, measured at the nearest monitor, and estimated by spatial interpolation methods

(i.e., inverse distance weighting, kriging). Overall, results from this study may have weaker implications because potential confounding by meteorological factors or other time-varying factors was not considered. With respect to lags of exposure, lung function decrements in adults were found with lag 0 day NO_2 and lags 0-2 day and 0-3 day avg NO_2 (Cakmak et al., 2011a; Son et al., 2010; Schindler et al., 2001). Among healthy adults in Tel Aviv, Israel, Steinvil et al. (2009) found decreases in FEV_1 in association with increases in lag 0-6 day avg of 24-h avg NO_2, but results generally were mixed among the various lags of exposure examined.

In adults, an NO_2-associated decrement in lung function independent of copollutants was not clearly demonstrated. Studies also found associations with various PM components, SO_2, CO, and O_3; and copollutant correlations were often high (r = 0.56 to 0.75). Some studies found NO_2 effect estimates to be attenuated with adjustment for TSP (Schindler et al., 2001) or SO_2 or CO (Steinvil et al., 2009) but found copollutant effect estimates to remain robust to NO_2 adjustment. Son et al. (2010) found robust NO_2 associations with FVC with adjustment for O_3 but did not analyze copollutant models with PM_{10}, SO_2, or CO because they covaried with NO_2. In contrast, Strak et al. (2012), who measured pollutants on the locations of outdoor exposures, found that FVC remained associated with NO_2 with adjustment for particle number concentration (PNC), O_3, $PM_{2.5}$, PM_{10}, $PM_{2.5-10}$, $PM_{2.5}$ absorbance, EC, and metal components of $PM_{2.5}$ (e.g., -3.0% [95% CI: -7.2, 1.4%] per 25-ppb increase in 5-h avg NO_2 with adjustment for PNC). The association between NO_X and FVC was attenuated with adjustment for PNC. Copollutants were weakly to moderately correlated with NO_2 and NO_X, except for EC and absorbance (r = 0.67-0.87). Effect estimates for EC, absorbance, and PNC were attenuated with adjustment for NO_2 or NO_X, indicating that NO_2 or NO_X may have confounded the associations of copollutants.

4.2.3.2 Controlled Human Exposure Studies

Most controlled human exposure studies examined adults, and consistent with epidemiologic findings, numerous controlled human exposure studies, most of which were reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), generally reported minimal effects on lung mechanics in healthy adults or those with asthma or COPD. Study details are presented in Table 4-8, but 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.

Among healthy adults, Huang et al. (2012b) conducted a study to examine the health effects of NO_2 exposure alone and in combination with exposure to concentrated ambient

particles (CAPs). Healthy adults did not experience any pulmonary function changes 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 hours/day for 2 consecutive days did not induce pulmonary function changes with the exception of a 1.5% drop in forced vital capacity (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 even had slight improvements, though not statistically significant, in FVC and FEV_1 during and after NO_2 exposure compared to air.

Since ambient NO_2 exposures occur with copollutants, other studies have examined co-exposures to NO_2 and O_3. Hazucha et al. (1994) found no effect on pulmonary function after exposure to 600 ppb NO_2 for 2 hours. However, significantly greater 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, though the increase in resistance with co-exposure was significantly less than O_3 alone and NO_2 exposure along did affect lung function (Adams et al., 1987).

Controlled human exposure studies examining potentially at-risk lifestages or populations, including older adults and those diagnosed with COPD and asthma, also have not consistently found decrements in lung function with NO_2 exposure. Healthy, older adults exposed to 300-400 ppb NO_2 for 2-3 hours did not experience decrements in lung function compared to air controls, though there were slight differences between smokers and nonsmokers in FEV_1 (Gong et al., 2005; Morrow et al., 1992). Exposure of older adults diagnosed with COPD to 300 ppb NO_2 for 3 hours showed consistent reductions in FVC that reached significance at the end of exposure (Morrow et al., 1992), while Vagaggini et al. (1996) reported decreases in FEV_1 in subjects with COPD exposed to 300 ppb NO_2 for 1 h. In contrast, Linn et al. (1985a) and Gong et al. (2005) reported that exposure to 400-2,000 ppb for 1-2 hours has no effect on lung function in adults with COPD.

Whereas NO_2 consistently induced increases in AHR in adults with asthma (Section 4.2.2.2), 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 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 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).

Subjects with asthma have also been screened for pulmonary function changes in response to pollutant co-exposures. Koenig et al. (1987) exposed adolescents with asthma to 300 ppb NO_2 in combination with 120 ppb O_3, with or without 70 $\mu g/m^3$ H_2SO_4 or 50 ppb HNO_3, and reported no changes in pulmonary function. Jenkins et al. (1999) investigated the effects of exposure to 200 ppb NO_2 for 6 hours (with or without 200 ppb O_3) or 400 ppb NO_2 for 3 hours (with or without 400 ppb O_3) and found no change in lung function in adults with asthma following NO_2 exposures. Significant decreases in FEV_1 were found following the 3-hour exposure to O_3 and $O_3 + NO_2$.

Overall, ambient concentrations of NO_2 do not consistently contribute to decrements in lung function in controlled human exposure studies in adults with chronic respiratory disease, particularly COPD. Studies more frequently report NO_2-induced decrements in lung function, but these also vary across studies. This evidence suggests that NO_2 exposure has minimal effects on lung function in the range of 200-4,000 ppb for 30 minutes to 6 hours.

Table 4-8 Controlled human exposure studies of NO_2 and lung function.

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)	Before and after exposure
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	Before, during, and after 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 W	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 3h, (2) 1,500 ppb for 3h; (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 3h + 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
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
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

Table 4-8 (Continued): Controlled human exposure studies of NO_2 and lung function.

Study	Disease status[a]; n; Sex; Age (mean ± SD)	Exposure Details (Concentration; Duration)	Endpoints Examined
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
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
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 $\dot{V}_E$= 32L/min)	Pulmonary function tests before 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
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
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
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

Table 4-8 (Continued): Controlled human exposure studies of NO_2 and lung function.

Study	Disease status[a]; n; Sex; Age (mean ± SD)	Exposure Details (Concentration; Duration)	Endpoints Examined
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
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

[a]Subjects were healthy individuals unless described otherwise.

4.2.3.3 Summary of Studies of Lung Function

Recent epidemiologic evidence consistently indicates associations between short-term increase in ambient NO_2 concentration and decrements in lung function in children with asthma (ages 6-18 years), as measured by supervised spirometry. The evidence in children with asthma that was reviewed in the 2008 ISA for Oxides of Nitrogen was based primarily on unsupervised PEF measurement and was inconsistent (U.S. EPA, 2008c). The results for FEV_1 are not supported by findings from a controlled human exposure study of adolescents with asthma, which showed no effect. Previous and recent epidemiologic evidence indicates ambient NO_2-associated decrements in lung function in children in the general population. Most studies recruited children from schools, increasing the likelihood that study populations were representative of the general population. Consistent with overall results, studies did not find more marked effects of ambient NO_2 exposure on lung function in children with asthma than in children without asthma (Barraza-Villarreal et al., 2008; Holguin et al., 2007). Bronchodilator use was associated with smaller ambient NO_2-associated lung function in children with asthma (Delfino et al., 2008a) but not adults with asthma (Qian et al., 2009b). Recent

epidemiologic studies of adults with asthma or COPD or adults in the general population also measured lung function with spirometry, but collectively studies were mixed in finding associations with NO_2. Similarly, most controlled human exposure studies did not find ambient-relevant NO_2 exposures (200-4,000 ppb) of 30 minutes to 6 hours to consistently induce decrements in lung function healthy adults, adults with asthma, or adults with COPD.

In addition to the lack of experimental evidence to directly support epidemiologic observations in children, the mechanisms underlying NO_2-related decrements in lung function are not well delineated. There is not strong evidence in humans for the direct effects of inhaled NO_2 on activating neural reflexes; however, there is some evidence for mast cell degranulation mediating changes in lung function (Section 3.3.2.2). Mast cell degranulation leads to histamine release, describing a role for allergic inflammation in mediating NO_2-induced lung function decrements. These findings provide biological plausibility for the ambient NO_2-associated decreases in lung function found in populations of children with asthma that have high prevalence of atopy (53-100%) and groups of children with asthma not using anti-inflammatory ICS (Hernández-Cadena et al., 2009; Liu et al., 2009b). Controlled human exposure studies of adults with atopic asthma and the single study in adolescents with asthma did not find lung function decrements with NO_2 exposures of 120-400 ppb (for 30 minutes to 6 hours) but did find a decrease with 1,000 ppb NO_2 (3 hours).

Key epidemiologic evidence was provided by studies with relatively strong exposure assessment characterized by measuring or modeling personal exposures (Martins et al., 2012; Delfino et al., 2008a; Oftedal et al., 2008) and measuring NO_2 at schools (Greenwald et al., 2013; Holguin et al., 2007) or on site of outdoor exposures (Strak et al., 2012; McCreanor et al., 2007). Lung function was also associated with NO_2 measured at one available city monitoring site, the closest site, or averaged among city-wide monitors. Comparisons among central site exposure assessment methods did not clearly indicate differences in association, but stronger associations with lung function were found for individual-level NO_2 than central site NO_2 (Delfino et al., 2008a; Oftedal et al., 2008) and for school outdoor NO_2 than indoor NO_2 (Greenwald et al., 2013).

A majority of the supporting evidence was for 24-h avg NO_2, with more variable results for NO or NO_X. For shorter averaging times (1-h max, 3- to 10-h avg), a few studies found associations with lung function (Chang et al., 2012; Moshammer et al., 2006); most did not (Peacock et al., 2011; Spira-Cohen et al., 2011; Barraza-Villarreal et al., 2008; Odajima et al., 2008; Mortimer et al., 2002; Scarlett et al., 1996). In adults with outdoor exposures in traffic and non-traffic locations, lung function decrements were associated with NO_2 averaged over 2 to 5 hours and measured on site of outdoor

exposures (Strak et al., 2012; McCreanor et al., 2007). Decrements in lung function were found with lags of 2-22 hours after outdoor exposures (Strak et al., 2012; McCreanor et al., 2007), ambient NO_2 concentrations lagged 0 or 1 day, and multiday averages of 2 to 30 days. Evidence was not more robust for a particular lag of NO_2 exposure. Some studies found stronger associations for multiday average than single-day concentrations (Castro et al., 2009; Liu et al., 2009b; Delfino et al., 2008a); others found similar associations (Qian et al., 2009b; Lagorio et al., 2006; Schindler et al., 2001). The range of mean ambient NO_2 concentrations was 4.5-49.2 ppb for 24-h avg NO_2 and 11.5-75 ppb for 1- to 5-h avg NO_2.

In most studies, an association of ambient NO_2 with lung function independent of copollutants was not clearly demonstrated. However, some studies provided supporting evidence. Some studies found associations with NO_2 but not copollutants such as $PM_{2.5}$, PM_{10}, EC, CO, or SO_2 (Oftedal et al., 2008; Holguin et al., 2007; Lagorio et al., 2006). A wide range of correlations were reported between NO_2 and copollutants (r = 0.18-0.75). In studies with copollutant modeling, NO_2-lung function associations were attenuated (Liu et al., 2009b; Qian et al., 2009b; Schindler et al., 2001) or reported to lose statistical significance (Linn et al., 1996) with adjustment for copollutants such as $PM_{2.5}$, PM_{10}, EC, BC, SO_2, and UFP where pollutants were measured at central sites. Copollutant effect estimates were robust to adjustment for NO_2. Among studies that measured or modeled personal NO_2 or measured NO_2 at schools or on site of outdoor activity, most found NO_2 associations with adjustment for pollutants such as $PM_{2.5}$, PM_{10}, EC, $PM_{2.5}$ metal components, PNC, and O_3 (Strak et al., 2012; Delfino et al., 2008a; McCreanor et al., 2007; Moshammer et al., 2006). The attenuation of copollutant effect estimates with adjustment for NO_2 in some of these studies indicated that NO_2 may have confounded copollutant associations (Strak et al., 2012; Delfino et al., 2008a; Moshammer et al., 2006).

4.2.4 Pulmonary Inflammation, Injury, and Oxidative Stress

The evidence for NO_2-related increases in AHR (Section 4.2.2) shows coherence with evidence indicating effects on pulmonary inflammation, as pulmonary inflammation can mediate AHR (Section 3.3.2.5). Both are characteristic features of respiratory conditions such as asthma and COPD, and both lines of evidence describe key events informing the modes of action by which ambient NO_2 exposure may lead to increases in respiratory symptoms (Section 4.2.6) as well as respiratory hospital admissions and ED visits (Section 4.2.7). 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 3.3.2.3). Further, NO_2-induced increases in ROS and RNS may

initiate inflammation (Section 3.3.2.1), as many transcription factors regulating expression of pro-inflammatory cytokines are redox sensitive. Both pulmonary inflammation and oxidative stress can cause tissue injury. However, there is not strong evidence for the effects of NO_2 on pulmonary oxidative stress or injury.

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, 2008c). There was coherence with findings from the few available epidemiologic studies in children with asthma and children in the general population, 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, pulmonary injury, and oxidative stress. Biological indicators of pulmonary inflammation, injury, and oxidative stress included those measured in exhaled breath or bronchoalveolar or nasal lavage fluid. Indicators of systemic inflammation in blood are evaluated in the context of cardiovascular effects in Section 4.3.

4.2.4.1 Controlled Human Exposure Studies

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) cited several studies addressing the effects of NO_2 exposure on markers of airway inflammation (i.e., differential cell counts, cytokines, prostaglandins), injury (i.e., lactate dehydrogenase (LDH) and protein concentrations), and oxidative stress (i.e., antioxidant molecules and enzymes). Study details are presented in Table 4-9, but overall, 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 bronchoalveolar lavage fluid (BALF). The coherence 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 polymorphonuclear cells (PMNs).

Several studies reported increases in PMNs and other inflammatory markers following NO_2 exposure. In a study by Frampton et al. (2002), adults exposed to 1,500 ppb NO_2 had increased PMNs in BALF 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 BALF protein concentration was reported, but lymphocytes were decreased in peripheral blood and increased in BALF after 600 ppb NO_2. Consistent with

Frampton et al. (2002), several studies reported an increase in PMNs in BALF 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 were not altered following NO_2 exposure in these studies. 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 BALF from adults exposed to 2,000 ppb, whereas 2,000 ppb exposure repeated over 4 days did not increase expression of IL-6 and IL-8 in biopsies of the bronchial epithelium (Pathmanathan et al., 2003); however this study did find significant increases in IL-5 and IL-13, both of which contribute to allergic inflammation (Section 4.2.4.3). Based on this group of studies, NO_2 exposure can induce pulmonary inflammation in healthy human adults, though evidence does not demonstrate NO_2-induced pulmonary injury.

The few available studies did not consistently demonstrate NO_2-induced pulmonary inflammation in adults with asthma. Jörres et al. (1995) exposed healthy adults and those with asthma to 1,000 ppb NO_2 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, this was not accompanied by any changes in cell counts in the BALF. 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. 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.

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 following exposure to 2,000 ppb NO_2. Kelly et al. (1996a) examined the kinetics of antioxidant response in the respiratory tract after exposure to 2,000 ppb NO_2 and found reduced levels of uric acid in bronchial wash and BALF 1.5 hours post-exposure, elevated levels at 6 hours, and control levels by 24 hours. Ascorbic acid decreased in bronchial wash and BALF at 1.5 hours but returned to baseline levels by 6 hours. Glutathione was increased at 1.5 and 6 hours in the bronchial wash, but no changes in glutathione were found in the BALF or for reduced glutathione and malondialdehyde at any time after exposure. These observations suggest that pulmonary antioxidants are modulated by NO_2 exposure.

Table 4-9 Controlled human exposure studies of NO_2 and pulmonary inflammation, injury, and oxidative stress.

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	BALF 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 W	Cell counts from bronchial biopsies, BW, and BALF 1.5-h post-exposure; protein concentration, IL-8, MPO, hyaluronic acid, glutathione, ascorbic acid, and uric acid in BALF 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 16h post-exposure. LDH activity, t-PA 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	BALF cell viability and differential counts 3.5-h post-exposure, IL-1 activity in BALF 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), 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 BALF 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 BALF 18-h post-exposure

Table 4-9 (Continued): Controlled human exposure studies of NO_2 and pulmonary inflammation, injury, and oxidative stress.

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	BALF analysis 1 h after exposure (cell counts, histamine, prostaglandins
Kelly et al. (1996a)	n = 44; Median: 24 yr (Range: 19-45 yr)	2,000 ppb for 4 h; Exercise 15 min on/15 min off at 75 W	Antioxidant concentrations and malondialdehyde in BALF and bronchial wash at 1.5, 6, or 24-h post-exposure
Pathmanathan et al. (2003)	n = 12; 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 W	Quantification of cytokines in airway biopsies by immunohistochemistry
Riedl et al. (2012)	n = 31; Range: 18-50 yr	350 ppb for 2 h; Exercise 15 min on/15 min off at $\dot{V}_E$= 15-20 L/min	Sputum sample cell count (alveolar macrophages, lymphocytes, PMNs, and eosinophils).
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 BALF 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

[a]Subjects were healthy individuals unless described otherwise.

4.2.4.2 Toxicological Studies

Animal toxicological studies reported limited evidence of pulmonary injury with ambient-relevant short-term exposures to NO_2 but more consistently indicate effects with long-term exposure (Section 5.2.7.2). Few studies have been published since the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) and the results reported are consistent with those from past studies. Study details are presented in Table 4-10.

Pulmonary Inflammation

Animal studies have examined similar endpoints to those in controlled human exposure studies (Section 4.2.4.1) to assess pulmonary inflammation after NO_2 exposure, but effects of NO_2 are inconsistent across disciplines. While studies in humans demonstrated

increases in BALF PMNs after NO_2 exposure, several studies in animals found no significant changes in BALF 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 BALF from rabbits exposed to 1,000 ppb NO_2 for 3, 7, and 14 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 alveolar macrophages (AMs) ex vivo to NO_2 at concentrations as low as 100 ppb and found that in vivo exposure led to significant decreases in eicosanoid levels in as little as 4 hours, while ex vivo exposure of AMs led to significant increases in cyclooxygenase and lipoxygenase activity and slight, but not significant, increases in eicosanoids. 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 BALF at 1,000 ppb, but not at 3,000 ppb. This study also investigated effects of O_3 and co-exposures of NO_2 and O_3 and suggested that eicosanoid response is more sensitive to O_3 exposure than NO_2.

Pulmonary Injury

In addition to NO_2-induced changes in inflammatory cells and mediators, 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 BALF 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. A study published by 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, though 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 Clara cells increased in the bronchioles and bronchi relative to air controls following a 3-day exposure to 5,000 ppb, but the number of Clara 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, though long-term studies (Section 5.2.10) report more profound impacts of exposure.

In addition to pulmonary injury observed at the morphologic level, molecular markers of injury have also been described in some studies. Continuous exposure to 400 ppb NO_2 for one week resulted in increased BALF 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 BALF 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 in LDH in BALF. Rose et al. (1989b) did not find any changes in LDH in BALF 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 BALF protein and LDH levels in a variety of models (Robison et al., 1993; Robison and Forman, 1993; Schlesinger et al., 1990; Last and Warren, 1987).

Oxidative Stress and Antioxidant Status

Oxidant gases are known to impair antioxidant defenses and contribute to oxidant stress in the upper and lower airways. The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) did not discuss the toxicological evidence relating to effects of NO_2 on antioxidants or oxidative stress, but a limited number of previously published studies have evaluated oxidative stress at ambient-relevant 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 (non-protein sulfhydryls, Vitamin C, and Vitamin E) were not affected in lung homogenates. Furthermore, there was no change in activity levels of antioxidant enzymes including glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glutathione S-transferase (GSH), 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 have also investigated the effects of NO_2 on glutathione and oxidized glutathione levels in the BALF 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 significant. Conversely, significant increases in oxidized glutathione were reported in the BALF, 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 BALF. GSH and SOD also increased in BALF after exposure, though 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 BALF. SOD also increased in BALF, but only 48 hours post-exposure. Oxidized lipids were transiently increased immediately after exposure in BALF 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 BALF 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., 1982b). Additional support for an influence of Vitamin E is provided by observations that NO_2-induced increases in BALF 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 in animals.

Table 4-10 Animal toxicological studies of NO_2 and pulmonary inflammation, injury, and oxidative stress.

Study	Species (Strain); Lifestage; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Barth et al. (1995)	Rat (Sprague Dawley); Male, 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); Male, n = 5/group	5,000, 10,000, and 20,000 ppb NO_2 for 3 or 25 days	Clara cell morphology, cellular proliferation, epithelial proliferation

Table 4-10 (Continued): Animal toxicological studies of NO_2 and pulmonary inflammation, injury, and oxidative stress.

Study	Species (Strain); Lifestage; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
de Burbure et al. (2007)	Rat (Wistar); 8 weeks; Male, 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 6h/day for 5 days; (1-3) Animals had selenium-deficient or selenium-supplemented diets.	BALF lipid peroxidation, antioxidative enzyme levels, protein concentration, cell counts, oxidant production, and selenium levels, 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, BALF and lung homogenate biochemical analysis (protein concentration, LDH, glucose-6-phosphate dehydrogenase, alkaline phosphatase, glutathione reductase, and glutathione peroxidase)
Hatch et al. (1986)	Guinea Pig (Hartley); Young adult; n =5 -16/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	BALF protein and antioxidant concentrations
Ichinose et al. (1988)	Mice (ICR), Hamster (Golden), Rat (Wistar), Guinea Pig (Hartley); 10 weeks; Male	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, antioxidative protective enzymes, total proteins, TBA reactants, non-protein sulfhydryls in lung homogenates
Last and Warren (1987)	Rat (Sprague Dawley); Male	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, BALF protein content and lavagable enzyme activities
Müller et al. (1994)	Rat (Sprague Dawley); Male, n = 4	800, 5,000, and 10,000 ppb NO_2 for 1 and 3 days	BALF cell counts and protein concentration, phospholipid component, SP-A, morphological changes,
Mustafa et al. (1984)	Mice (Swiss Webster); 8 weeks; Male, 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)
Ohashi et al. (1994)	Guinea Pig (Hartley); Female, n = 10/group	3,000 and 9,000 ppb NO_2 for 6 h/day, 6 times/week for 2 weeks	Ciliary activity and morphological observations
Pagani et al. (1994)	Rat (CD Cobs); Male	5,000 and 10,000 ppb NO_2 for 24 h and 7 days	Analysis of BALF 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 BALF and histopathological evaluation

Table 4-10 (Continued): Animal toxicological studies of NO_2 and pulmonary inflammation, injury, and oxidative stress.

Study	Species (Strain); Lifestage; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Robison and Forman (1993)	Rat (Sprague Dawley); Male	100, 1,000, 5,000, and 20,000 ppb NO_2 for 1, 2, and 4 h	Enzymatic production of arachidonate metabolites in AMs, cyclooxygenase products
Robison et al. (1993)	Rat (Sprague Dawley); n >4/group	500 ppb NO_2 for 8h/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
Rose et al. (1989b)	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 BALF (LDH, albumin, macrophages)
Sherwin et al. (1972)	Guinea Pig; Male, 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; Male, n = 9/group	400 ppb NO_2 continuously for 1 week	Protein concentration in BALF
Schlesinger (1987a)	Rabbit (New Zealand White); Male, 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 2h/day for 2, 6, or 13 days	Cell counts in BALF, AM function
Schlesinger et al. (1990)	Rabbit (New Zealand White); Male, 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 BALF

4.2.4.3 Allergic Inflammation

As described in Section 4.2.2.2, controlled human exposure studies in adults with asthma and allergy demonstrated increases in AHR in response to NO_2 exposure or to NO_2 followed by an allergen challenge. These observations are supported by several findings in controlled human exposure and animal toxicological studies that NO_2 exposure or NO_2 exposure followed by an allergen challenge resulted in increased indicators of allergic inflammation. This includes increases in Th2 cytokines and IgE and the influx and/or activation of eosinophils and neutrophils. Results provide evidence that NO_2 exposure may lead to exacerbation of allergic airways disease (discussed below and in Section 3.3.2.6) and the development of allergic airways disease (discussed below and in Section 3.3.2.6). These results provide support for epidemiologic evidence of

NO_2-associated increases in inflammation in children with asthma and allergy (Section 4.2.4.4).

Exacerbation of allergic airways disease

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) 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 4-11). 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. BALF and bronchial wash fluid were collected 19 hours after allergen challenge. NO_2 exposure for 30 minutes increased PMN in BALF and bronchial wash fluid and increased eosinophil cationic protein (ECP) in bronchial wash fluid compared with air exposure (Barck et al., 2002). Reduced cell viability of BALF cells and reduced volume of BALF were also reported. ECP is released by activated eosinophils, is toxic to respiratory epithelial cells, and is 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, 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, 2008c), these studies were well-designed and indicate that brief exposures to 260 ppb NO_2 can enhance allergen responsiveness in individuals with asthma.

Additional studies have been performed using longer NO_2 exposures (Table 4-11). 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 house dust mite (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.

A recent study of adults with mild allergic asthma also did not provide evidence of NO_2-induced increases in allergic asthma (Table 4-11) (Riedl et al., 2012). Exposure to 350 ppb NO_2 for 2 hours with intermittent exercise followed by methacholine challenge 1.5 hours later resulted in increased levels of blood IgM, and decreased levels of sputum IgG4, IL-4, eotaxin, RANTES and fibrinogen measured 22 hours after exposure. Subjects that were 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 allergen-induced airway inflammatory responses, it should be noted that markers of eosinophil activation were not measured.

As noted in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), 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 BALF 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.

Allergic inflammatory responses were also investigated in animal models of allergic airways disease (Table 4-12). 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 BALF 7 days post-exposure to NO_2. In addition, an increase in the ratio of inflammatory cells (lymphocytes, neutrophils and eosinophils) to alveolar macrophages was observed 7 days post-exposure to NO_2 although the total number of lavagable cells was not changed.

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 BALF 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 significant eosinophil degranulation or cytolysis, which are characteristic features 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 high quality 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. 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 3.3.2.6).

Development of allergic airways disease

While the majority of studies of allergic inflammation were conducted in individuals with asthma and allergy or in animal models of allergic airways disease, two studies were conducted in naïve individuals or animals. As reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), one study examined the effects of repeated NO_2 exposures in bronchial biopsy tissue obtained from healthy human subjects who were exercising at a light rate (Table 4-11). Exposure to 2,000 ppb NO_2 for 6 hours on 4 consecutive days increased expression of the cytokines IL-5, IL-10, and IL-13 and the intercellular adhesion molecule ICAM-1 in bronchial epithelium (Pathmanathan et al., 2003). IL-5 and IL-13 are characteristic of a Th2 inflammatory response, with IL-5 known to promote eosinophilia and IL-13 known to promote AHR and mucus production. A study in guinea pigs also 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 4-12). The observed increase in numbers of airway eosinophils and expression of Th2 cytokines in these two studies

suggest that inhaled NO_2 may promote Th2 skewing and allergic sensitization (Section 3.3.2.6).

Table 4-11 Controlled human exposure studies of NO_2 and allergic inflammation.

Study	Disease status[a]; 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, percentage 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 4h 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
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 airways 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	NAR 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

Table 4-11 (Continued): Controlled human exposure studies of NO_2 and allergic inflammation.

Study	Disease status[a]; Age; N; Sex	Exposure Details	Endpoints Examined
Witten et al. (2005)	Adults with asthma and HDM 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 four 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)
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 $\mu g/m^3$ 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
Pathmanathan et al. (2003)	Healthy adults Mean age: 26 yr N = 8 M, 4 F	2,000 ppb NO_2 for 4 h/day for 4 days	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)

[a]Subjects were healthy individuals unless described otherwise.

Table 4-12 Animal toxicological studies of NO_2 and allergic inflammation.

Study	Species (Strain); Lifestage; Sex; N	Exposure Details	Endpoints Examined
Gilmour et al. (1996)	Rats (Brown Norway); six 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: BALF total and differential cell counts EPO 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 day 0 and 7 Challenge with OVA aerosol (1% in PBS) 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: BALF- total and differential cell counts; LDH Histopathology analysis mRNA levels of Gob5, Muc5AC, Th2, dendritic cell chemokine CCL20 and eotaxin-1

Table 4-12 (Continued): Animal toxicological studies of NO_2 and allergic inflammation.

Study	Species (Strain); Lifestage; Sex; N	Exposure Details	Endpoints Examined
Ohashi et al. (1994)	Guinea pigs (Hartley); F; n = 10/group	(1) 3,000 ppb NO_2 for 6 h/day, 6 day/week, for 2 weeks (2) 9,000 ppb NO_2 for 6 h/day, 6 day/week, for 2 weeks	Pathology of mucosal samples: accumulation of eosinophils, epithelial injury, mucociliary dysfunction (taken 24 h after end of exposure period)

4.2.4.4 Epidemiologic Studies

The observations described in the preceding sections for NO_2-induced increases in inflammation, particularly increases in allergic inflammation, provide support for the epidemiologic evidence for associations of ambient or personal NO_2 with increases in inflammation in children with asthma and allergy. Evidence also supports associations in children in the general population but is inconsistent in adult populations. 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 additional examination of potential copollutant confounding and potential at-risk populations. Ambient NO_2 concentrations, locations, and time periods for epidemiologic studies of pulmonary inflammation and oxidative stress are presented in Table 4-13.

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 American Thoracic Society (ATS, 2000a). eNO assessment methods also accounted for NO in the collection room, although eNO has not been shown to be a reliable indicator of exposure (Section 3.2.3). Although not examined in controlled human exposure or animal toxicological studies of NO_2 exposure, several observations support epidemiologic findings. NO_2 exposure has been shown to increase some pro-inflammatory cytokines and increase neutrophils and eosinophils (Sections 3.3.2.6, 4.2.4.1, 4.2.4.2), which can activate inducible nitric oxide synthase or produce NO in the lung during an inflammatory response (Barnes and Liew, 1995). Further, eNO commonly is higher in children and adults with asthma and increases during acute exacerbations (Carraro et al., 2007; Jones et al., 2001; Kharitonov and Barnes, 2000).

Table 4-13 Mean and upper percentile concentrations of oxides of nitrogen in epidemiologic studies of pulmonary inflammation and oxidative stress.

Study[a]	Location	Study Period	Oxide of Nitrogen Metric Analyzed	Mean/Median Concentration (ppb)	Upper Percentile Concentrations (ppb)
Liu et al. (2009b)	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
	Riverside, CA		8-h max NO_2	Central Site: 39.3	Max: 72.4
	Whittier, CA			Central Site: 35.1	Max: 96
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
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
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]
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[c] Max: 19.7[c]
Lin et al. (2011)	Beijing, China	June 2007	24-h avg NO_2	24.3	NR
		Sept 2007		30.4	NR
		Dec 2007		45.3	NR
		June 2008		26.6	NR
		Sept 2008		25.9	NR
Berhane et al. (2011)	13 Southern CA 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
Patel et al. (2013)	New York City, NY	May-June 2005	24-h avg NO_2	Median: 23.3	NR

Table 4-13 (Continued): Mean and upper percentile concentrations of oxides of nitrogen in epidemiologic studies of pulmonary inflammation and oxidative stress.

Study[a]	Location	Study Period	Oxide of Nitrogen Metric Analyzed	Mean/Median Concentration (ppb)	Upper Percentile Concentrations (ppb)
Jalaludin et al. (2004)	Western and Southwestern Sydney, Australia	Feb-Dec 1994	15-h avg (6 a.m.-9 p.m.) NO_2	15.0	Max: 47.0
Qian et al. (2009a)	Boston, MA; Denver, CO; Madison, WI; New York City, NY; Philadelphia, PA; 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 of Means across seasons and years: 20.9-37.0[c]	Range of 75th: 23.0-42.5[c]
Steerenberg et al. (2001)	Utrecht	Feb-Mar 1998	24-h avg NO_2	28.2[c]	Max: 44.7[c]
			24-h avg NO	30.2[c]	Max: 168[c]
	Bilthoven the Netherlands		24-h avg NO_2	25.5[c]	Max: 49.5[c]
			24-h avg NO	7.4[c]	Max: 85.6[c]
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)		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 NR	24-h avg NO_2	17.3[c]	Max: 28.3[c]
			24-h avg NO	6.3[c]	Max: 34.5[c]
Steenhof et al. (2013) Strak et al. (2012)	the Netherlands, city NR	Mar-Oct 2009	5-h avg NO_2	36	Max: 96
			5-h avg NO_X	20	Max: 34
Adamkiewicz et al. (2004)	Steubenville, OH	Sept-Dec 2000	1-h avg NO_2	9.2	75th: 12.8, Max: 32.9
			24-h avg NO_2	10.9	75th: 14.6, Max: 23.8
			1-h avg NO	15	75th: 16.1, Max: 215
			24-h avg NO	11.2	75th: 14.2, Max: 70.7
Weichenthal et al. (2011)	Ottawa, ON, Canada	NR	1-h avg NO_2	High traffic: 4.8	Max: 11
				Low traffic: 4.6	Max: 10
Chimenti et al. (2009)	Palermo, Sicily, Italy	Nov	7-day avg NO_2	31.7[c]	NR
		Feb		27.1[c]	NR
		July, year NR		33.9[c]	NR
Madsen et al. (2008)	Oslo, Norway	Jan-June 2000	24-h avg NO_2	NR	NR
			7-day avg NO_2	NR	NR

Table 4-13 (Continued): Mean and upper percentile concentrations of oxides of nitrogen in epidemiologic studies of pulmonary inflammation and oxidative stress.

Study[a]	Location	Study Period	Oxide of Nitrogen Metric Analyzed	Mean/Median Concentration (ppb)	Upper Percentile Concentrations (ppb)
Timonen et al. (2004)	Amsterdam, the Netherlands	Nov 1998-June 1999	24-h avg NO_2	22.7[c]	75th: 28.7, Max:49.7[c]
	Erfurt, Germany	Oct 1998-Apr 1999		15.4[c]	75th: 19.6, Max:43.5[c]
	Helsinki, Finland	Nov 1998-Apr 1999		16.5[c]	75th: 18.9, Max:35.9[c]

[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 and 0.815 for NO assuming standard temperature (25 °C) and pressure (1 atm).

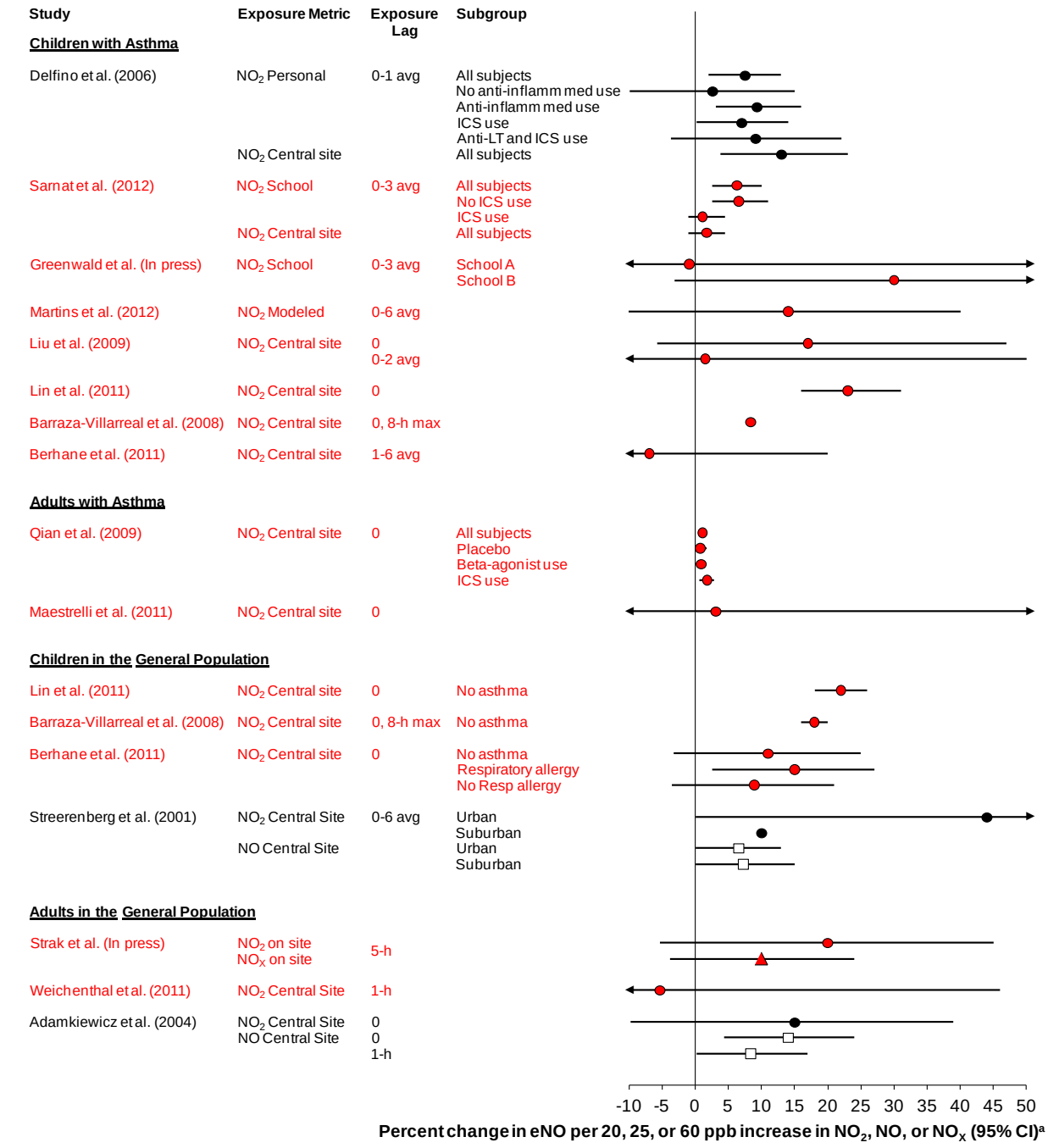
NR = not reported.

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 studies varied in whether they assessed asthma severity or required current symptoms in subjects. Across studies, associations varied in strength and precision; however, most results indicated a pattern of increasing eNO with increasing short-term NO_2 exposure (Figure 4-2 and Table 4-14). Most studies analyzed multiple endpoints, pollutants, lags of exposure, or subgroups; however, with a few exceptions (Liu et al., 2009b; Barraza-Villarreal et al., 2008), there was a pattern of association found across the multiple comparisons reducing the likelihood of associations found by chance alone or publication bias.

Key evidence was provided from studies with strong NO_2 exposure assessment, comparison of various exposure metrics, and examination of copollutant confounding. 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 4-2 and Table 4-14), 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 subject-level outdoor exposures based on monitoring, modeling, and daily activity patterns (Martins et al., 2012), or monitoring at schools (Greenwald et al., 2013; Sarnat et

al., 2012; Holguin et al., 2007). 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, although personal NO_2 was examined as a 24-h avg, and central site NO_2 (1 per community) was analyzed as an 8-h max (Delfino et al., 2006). Based on interquartile range, a 17-ppb increase in 24-h avg personal NO_2 was associated with a 1.6 (95% CI: 0.43, 2.8)-ppb increase in eNO, and a 12-ppb increase in 8-h max central site NO_2 was associated with a 1.4 (95% CI: 0.39, 2.3)-ppb increase in eNO. Personal and central site NO_2 were moderately correlated (Spearman r = 0.46), indicating that despite the potential for greater exposure measurement error due to spatial variability in ambient NO_2 concentrations and variation in time-activity patterns (Sections 2.5.1, 2.5.2, 2.5.3, 2.6.5.2), daily variation in ambient NO_2 represents some daily variation in personal NO_2 exposures that is associated with eNO. Among children with wheeze in Portugal who spent more than 22 hours per day indoors, a 20-ppb increase in 1-week avg subject-level NO_2 was associated with a 13.9 ppb (95% CI: -12.4, 40.2) increase in eNO and a -2.6 unit (95% CI: -4.8, -1.4) decrease in 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 2.6.5.2).



Note: Studies are organized by study population and then generally in order of decreasing study strength (e.g., exposure assessment method, potential confounding considered). Red=recent studies, Black=previous studies, Circles=NO_2, Squares=NO, Triangles=NO_X.

[a]Effect estimates are standardized to a 20 ppb, 25 ppb, 30 ppb, and 60-ppb increase for 24-h avg NO_2 or NO, 8-h max NO_2 or NO, 1-h avg NO_2 or NO, and 5-h avg NO_X, respectively. Study details and quantitative results reported in Table 4-14.

Figure 4-2 Associations of personal or ambient NO_2, NO, or NO_X with exhaled nitric oxide (eNO) in various populations.

Table 4-14 Epidemiologic studies of pulmonary inflammation, injury, and oxidative stress.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	Effect Estimate[a] (95% CI) Single-Pollutant Model[b]	Copollutant Examination
Children with Asthma						
Delfino et al. (2006)	Riverside, Whittier, CA N = 45, ages 9-18 yr, persistent asthma and exacerbation in previous 12 mo. Repeated measures. Examined for 4-8 10-day follow-up periods, 372 observations. Recruitment in schools of nonsmokers from nonsmoking 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 relative humidity, personal temperature, and follow-up period. Adjustment for city, daily beta agonist use, weekend did not alter results.	NO_2-Personal 24-h avg Compliance assessed with motion detectors. Monitoring checked daily.	0	All subjects	1.1% (-2.0, 4.3%)	No quantitative results for copollutant model. w/ $PM_{2.5}$, EC, or OC: NO_2 results robust. Decrease in precision. Copollutant results robust to NO_2 adjustment. Weak correlations for personal exposures. Spearman r = 0.20-0.31. Stronger correlations for central site pollutants. r = 0.25-0.70).
			0-1 avg	All subjects	7.5% (2.0, 13%)	
				No anti-inflammatory medication use, n = 14	2.6% (-9.9, 15%)	
				Anti-inflammatory medication use, n = 31	9.3% (3.1, 16%)	
				ICS use, n = 19	7.0% (0.23, 14%)	
				Anti-LT + ICS use, n = 12	9.1% (-3.7, 22%)	
		NO_2–central site 8-h max	0 0-1 avg		0.98% (-5.4, 7.3%) 13% (3.8, 23%)	

Table 4-14 (Continued): Epidemiologic studies of pulmonary inflammation, injury, and oxidative stress.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	Effect Estimate[a] (95% CI) Single-Pollutant Model[b]	Copollutant Examination
Sarnat et al. (2012)‡	El Paso, TX and Ciudad Suarez, Mexico N = 29 per city, ages 6-12 yr, asthma and current symptoms. Repeated measures. Examined weekly for 16 weeks, 697 observations. Recruitment from schools representing a gradient of traffic, subjects from nonsmoking 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	0-4 avg	All subjects	6.3% (2.5, 10%)	w/O_3: 8.8% (4.6, 13%) No copollutant model with $PM_{2.5}$ or $PM_{10-2.5}$. $PM_{2.5}$ and $PM_{10-2.5}$ associated with eNO, weakly correlated with NO_2. Spearman r = -0.39 to 0.32 for $PM_{2.5}$; -0.24 to 0.04 for $PM_{10-2.5}$.
				No ICS use, n = 10	6.6% (2.6, 11%)	
				ICS use, n = 19	1.1% (-8.9, 12%)	
		NO_2–School indoor			0.53% (0.11, 1.0%)	
		NO_2–Central site 1 site in El Paso, TX			1.7% (-1.0, 4.5%)	
		All 24-h avg				
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	0-4 avg	School A	-0.86% (-38, 58)	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).
				School B	30% (-3.1, 73%)	
		NO_2–School indoor		School A	-16% (-53, 47%)	
				School B	14% (-19, 60%)	
		All 24-h avg				
Holguin et al. (2007)‡	Ciudad Juarez, Mexico N = 194, ages 6-12 yr, 78% mild, intermittent asthma, 58% with atopy. Repeated measures. Examined biweekly for 4 mo. 87% participation. Self-report of physician-diagnosed asthma. Linear and nonlinear mixed effects model with random effect for subject and school adjusted for sex, BMI, day of week, season, maternal and paternal education, passive smoking exposure	NO_2–School outdoor 24-h avg Homes 397 meters from schools	0-6 avg	Asthma, n = 31 No asthma, n = 41	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.

Table 4-14 (Continued): Epidemiologic studies of pulmonary inflammation, injury, and oxidative stress.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	Effect Estimate[a] (95% CI) Single-Pollutant Model[b]	Copollutant Examination
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 changed at least 1 pollutant effect estimate >10%.	NO_2-Subject-level 24-h avg Estimated from school outdoor NO_2, 20 city monitors, MM5/CHIMERE modeling, and daily activity patterns	0-6 avg		14% (-12, 40%) EBC pH: -2.6% (-3.8, -1.4%)	For EBC pH only: w/PM_{10}: 0.08 (-3.0, 3.6) w/benzene: -1.7 (-3.6, 0.26) w/ ethylbenzene: -1.6 (-3.7, 0.49) PM_{10} robust to NO_2 adjustment. VOCs attenuated to null. Correlations negative or weakly positive. Spearman r = -0.72 to -0.55 for PM_{10}, -0.43 to 0.14 for various VOCs.
Flamant-Hulin et al. (2010)‡	Clermont-Ferrand, France N = 104, mean age: 10.7 (SD: 0.7) yr 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 All 24-h avg	0-4 avg		≥ 14.3 vs. <14.3 ppb NO_2 log eNO	No copollutant model. $PM_{2.5}$, acetylaldehyde associated with eNO.
				Asthma	0 (-0.14, 0.14)	
				No asthma	-0.09 (-0.22, -0.04)	
		NO_2-School indoor All 24-h avg		Asthma	0 (-0.13, 0.14)	
				No asthma	-0.16 (-0.11, -0.20)	

Table 4-14 (Continued): Epidemiologic studies of pulmonary inflammation, injury, and oxidative stress.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	Effect Estimate[a] (95% CI) Single-Pollutant Model[b]	Copollutant Examination
Liu et al. (2009b)‡	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. Most subjects live within 10 km of sites.	0 1 0-2 0 1 0-2		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 w/$PM_{2.5}$: 31% (-30, 145%) w/SO_2: 43% (-10, 126%) $PM_{2.5}$ estimate less attenuated with NO_2 adjustment. High correlation with $PM_{2.5}$, weak with SO_2. Spearman r = 0.71 for $PM_{2.5}$, 0.18 for SO_2.
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. Subjects selected from 437 initial respondents. GEE adjusted for temperature, relative humidity, BMI, asthma.	NO_2-Central site 24-h avg Site 650 meters from schools.	0 1 	All subjects Asthma No asthma Asthma No Asthma	22% (18, 26%) 23% (16, 31%) 22% (18, 26%) 12% (4.0, 20%) 9.5% (5.8, 13%)	w/BC: 5.6% (0.38, 11%) w/$PM_{2.5}$: 14% (9.5, 19%) BC robust to NO_2 adjustment, $PM_{2.5}$ reduced but positive. Weak to moderate correlations with NO_2. Spearman r = 0.30 for $PM_{2.5}$, 0.68 for BC.

Table 4-14 (Continued): Epidemiologic studies of pulmonary inflammation, injury, and oxidative stress.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	Effect Estimate[a] (95% CI) Single-Pollutant Model[b]	Copollutant Examination
Barraza-Villarreal et al. (2008)‡	Mexico City, Mexico N = 163-179, ages 6-14 yr, 54% persistent asthma, 89% with atopy. Repeated measures. Examined every 15 days for mean 22 weeks. 1,004 observations. Children with asthma recruited from pediatric clinic. Children without asthma were friends or schoolmates, 72% atopy. Asthma severity assessed by pediatric allergist. Linear mixed effects model with random effect for subject and adjusted for sex, BMI, lag 1 minimum temperature, ICS use, time. Adjustment for outdoor activities, smoking exposure, anti-allergy medication use, and season did not alter results.	NO_2-Central site 8-h max Monitors within 5 km of school or home. Spearman correlation coeffiecient for school vs. central site: r = 0.21	0	Asthma, n = 126	8.4% (7.9, 9.0%)	No copollutant model. $PM_{2.5}$ and O_3 also associated with eNO and IL-8. Weak to moderate correlations with NO_2. Pearson r = 0.61 for $PM_{2.5}$, 0.21 for O_3.
				No asthma, n = 50	18% (16, 20%)	
					IL-8:	
				Asthma, n = 129	1.2% (1.1, 1.3%)	
				No asthma, n = 45	1.1 (0.93, 1.2%)	
					pH:	
				Asthma, n = 119	-0.5% (-1.5, 0.5%)	
				No Asthma, n = 44	0.25% (-1.7, 2.2%)	
Romieu et al. (2008)‡	Mexico City, Mexico N = 107, mean age 9.5 yr. 48% persistent asthma, 90% with 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. MDA 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 MDA 0.13 (-0.10, 0.35)	No copollutant model. O_3, $PM_{2.5}$, distant to closest avenue, and 4.5-h traffic count also associated with MDA. Moderate correlation with NO_2. Pearson r = 0.44 for O_3 and 0.54 for $PM_{2.5}$.

Table 4-14 (Continued): Epidemiologic studies of pulmonary inflammation, injury, and oxidative stress.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	Effect Estimate[a] (95% CI) Single-Pollutant Model[b]	Copollutant Examination
Berhane et al. (2011)‡	13 Southern CA towns N = 2,240, 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, age, sex, race/ethnicity, asthma, asthma medication use, history of respiratory allergy, eNO collection time, BMI 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.		Asthma, n = 169 No asthma, n = 2,071	-6.9% (-33, 20%) 11% (-3.2, 25%)	No copollutant model. $PM_{2.5}$, PM_{10}, O_3 associated with eNO in all groups.
				Respiratory allergy, n = 1,167	15% (2.6, 27%)	Moderate correlations with NO_2. Pearson r = 0.47 for $PM_{2.5}$, 0.49 for PM_{10}, 0.15 for O_3.
				No respiratory allergy, n = 1,073	8.9% (-3.6, 21%)	
Adults with Asthma						
Qian et al. (2009a)‡	Boston, MA; New York, NY; Denver, CO; Philadelphia, PA; San Francisco, CA; Madison, WI. N = 119, ages 12-65 yr, 100% persistent asthma Repeated measures. Examined every 2-4 weeks for 16 weeks. 480 person-days. No information on participation rate. Study population representative of full cohort. 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.	NO_2-Central site 24-h avg Average of all monitors within 20 miles of subject ZIP code centroid	0	All subjects	1.1% (0.52, 1.7)	w/PM_{10}: 0.69% (-0.09, 1.8) w/O_3: 0.94% (0.43, 1.5) w/SO_2: 1.2% (0.52, 1.9) Copollutant effect estimates attenuated. Correlations NR.
				Placebo	0.79% (-0.08, 1.7)	
				Beta-agonist use	0.86% (0.08, 1.6)	
				ICS use	1.8% (0.62, 2.9)	
			0-3	All subjects	0.94% (0.09, 1.8%)	

Table 4-14 (Continued): Epidemiologic studies of pulmonary inflammation, injury, and oxidative stress.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	Effect Estimate[a] (95% CI) Single-Pollutant Model[b]	Copollutant Examination
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 as beta-agonist users (>6/yr for 3 yr), diagnosis clinically confirmed. 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	All subjects, n = 32	3.1 (-89, 95)	No copollutant model. O_3 and SO_2 but not personal or central site $PM_{2.5}$ or PM_{10} associated with eNO. Correlations NR.
				Nonsmokers, n = 22	2.9 (-120, 126)	
					EBC pH	
				All subjects, n = 32	(-1.1, 1.1)	
				Nonsmokers, n = 22	0.01 (-1.4, 1.4)	
Children in the General Population						
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 3.1 (1.3, 4.9) log	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.
					EBC pH	
			0 0-3 avg		-0.05 (-0.79, 0.68) -0.11 (-1.2, 1.0)	

Table 4-14 (Continued): Epidemiologic studies of pulmonary inflammation, injury, and oxidative stress.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	Effect Estimate[a] (95% CI) Single-Pollutant Model[b]	Copollutant Examination
Chen et al. (2012a)‡	New Taipei City, Taiwan N = 100, mean age 10.6 (SD: 2.5 yr), 33% asthma, 33% allergic rhinitis Repeated measures. Examined 3-4 times/mo for 10 mo. 824 observations. Recruitment from schools. A priori recruitment of children with and without asthma or atopy. Participants similar to nonparticipants. Atopy confirmed by study physician. Mixed effects model adjusted for age, BMI, upper respiratory infection, asthma/allergic rhinitis attack, asthma medication use, temperature, humidity, day of week, sampling time, sex, school, parental education, parental atopy, 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. Weak to moderate to correlations with NO_2. Pearson r = 0.61 for $PM_{2.5}$, -0.01 for O_3.
Steerenberg et al. (2001)	Utrecht and Bilthoven, 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. Nonstandardized eNO collection. 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	0-6 avg	Urban Suburban Urban Suburban	44% (0, 88%)[c] 10%, p >0.05 IL-8 (units NR) OR: 1.1, p >0.05 OR: 1.0, 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 2 km of schools	0-6 avg	Urban Suburban Urban Suburban	6.6% (0, 13%)[c] 7.3% (0, 15%)[c] IL-8 (units NR) OR: 1.1, p >0.05 OR: 0.95, p >0.05	

Table 4-14 (Continued): Epidemiologic studies of pulmonary inflammation, injury, and oxidative stress.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	Effect Estimate[a] (95% CI) Single-Pollutant Model[b]	Copollutant Examination
Steerenberg et al. (2003)	the Netherlands N = 115, ages 7-12 yr, 75% with pollen and/or HDM atopy, 59% with asthma. Cross-sectional. Recruitment from schools. 72% participation rate. Allergic sensitization confirmed by skin prick test. Nonstandardized eNO collection. Linear regression model adjusted for age, sex, gas cooking, unvented water heater, smoking exposure, presence of a cold. No consideration for potential confounding by meteorological factors.	NO_2 and NO-central site 24-h avg Site within 2 km of schools	1 0-6 avg		No quantitative data. Lag 1 NO and NO_2 associated with eNO in group with HDM and pollen atopy. No consistent association for Lag 0-6 avg NO or NO_2.	No copollutant model. Lag 1 CO, $PM_{2.5}$, pollen associated with eNO.
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 0 0-3 avg		EBC 8-isoprostane 1.7 (0.63, 2.7) log 3.1 (1.3, 4.9) log EBC pH -0.05 (-0.79, 0.68) -0.11 (-1.2, 1.0)	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.

Table 4-14 (Continued): Epidemiologic studies of pulmonary inflammation, injury, and oxidative stress.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	Effect Estimate[a] (95% CI) Single-Pollutant Model[b]	Copollutant Examination
Salam et al. (2012)‡	Anaheim, Glendora, Long Beach, Mira Loma, Riverside, San Dimas, Santa Barbara, Upland, CA, Children's Health Study N = 940, ages 6-11 yr, 14% with asthma, 56% with respiratory allergy. Cross-sectional. Recruitment from schools. Subjects representative of full cohort. Two different methods used for eNO measurement. Linear regression model adjusted for age, sex, ethnicity, asthma, respiratory allergy, parental education, smoking exposure, community, month of eNO collection. No consideration for potential confounding by meteorological factors.	NO_2-Central site 24-h avg 1 site per community	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. Weak correlation with NO_2. Spearman r = 0.36.
Adults in the General Population						
Strak et al. (2012)‡	Utrecht area, the Netherlands N = 31, adults ages 19-26 yr, all healthy, nonsmoking	NO_2 and NO_X–on site of outdoor activity 5-h avg	0-h post-exposure	NO_2 NO_X	20% (-5.4, 45%) 10% (-3.8, 24%)	w/PNC: -21% (-53, 11%) for NO_2, -6.2% (-15, 2.6%) for NO_X.

Table 4-14 (Continued): Epidemiologic studies of pulmonary inflammation, injury, and oxidative stress.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	Effect Estimate[a] (95% CI) Single-Pollutant Model[b]	Copollutant Examination
Steenhof et al. (2013)‡	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. Outcomes measured before and after outdoor exposures. Heart rate maintained during intermittent exercise. Multiple comparisons could results in higher probability of associations found by chance alone. Mixed effects model adjusted for temperature, relative humidity, season, high/low pollen, respiratory infection.		2-h	NO_2	IL-6 66% (-10, 142) NAL protein 60% (0, 121)[c]	w/EC: 12% (-17, 41%) for NO_2, 1.6% (-8.0, 11%) for NO_X. PNC results robust to NO_2/NO_X adjustment. EC and Abs attenuated. For IL-6: w/PNC: 95% (0, 190%) w/OC: 67% (-10, 144) Copollutant results robust. Robust NO_2 results found for NAL protein. 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.
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. 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 Potential differential exposure measurement error for personal PM species and VOCs and central site NO_2.	1-h 4-h Post-exposure		-5.3% (-57, 46%) -32% (-70, 6.7%)	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.

Table 4-14 (Continued): Epidemiologic studies of pulmonary inflammation, injury, and oxidative stress.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	Effect Estimate[a] (95% CI) Single-Pollutant Model[b]	Copollutant Examination
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. 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 $PM_{2.5}$.
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. GLM adjusted for age, alcohol consumption, smoking status, hour of examination, respiratory disease, BMI, # cigarettes/day, smoking exposure, education, temperature.	NO_2-Central site NO_2-residential estimated with dispersion model No information on model validation.	0-7 avg		CC16 30% (7.8, 57%) 3.8% (-7.3, 16%)	No copollutant model. Associations found for $PM_{2.5}$ (central site and home). Moderate correlation with NO_2. Spearman r for home = 0.59.
Adamkiewicz et al. (2004)	Steubenville, OH N = 29, adults median age 71 yr, nonsmoking, 28% with asthma, 24% with COPD. Repeated measures. Examined weekly for 12 weeks. 138-244 total observations. 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 1-h avg 24-h avg 1 site	0 0		15% (-9.7, 39%) 8.4% (0.21, 17%) 14% (4.4, 24%)	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.

Table 4-14 (Continued): Epidemiologic studies of pulmonary inflammation, injury, and oxidative stress.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	Effect Estimate[a] (95% CI) Single-Pollutant Model[b]	Copollutant Examination
Timonen et al. (2004)	Amsterdam, the Netherlands; Erfurt, Germany; Helsinki, Finland N = 121, adults mean ages 71, 65, 68 yr, 100% with coronary heart disease, 18% with asthma, 19% with COPD. Repeated measures. Examined every 2 weeks for 6 mo. 1,249 total observations. GLM adjusted for different covariates depending on city but included time trend, temperature, relative humidity, barometric pressure, weekday.	NO_2–central site 24-h avg 1 site per city			CC16	No quantitative results for copollutant model. PNC and $PM_{2.5}$ associated with CC16. $PM_{2.5}$ result robust to NO_2 adjustment. Weak to high correlation with NO_2. Spearman r = 0.82 Erfurt, 0.35 and 0.49 other cities.
			0	Amsterdam	14% (-8.4, 41%)	
				Erfurt	15% (-13, 52%)	
				Helsinki	13% (-18, 54%)	
			0-4 avg	Amsterdam	26% (-12, 80%)	
				Erfurt	0.38% (-34, 52%)	
				Helsinki	59% (-15, 195%)	

Note: Studies are organized by population examined and then generally in order of study strength (e.g., exposure assessment method, potential confounding considered). ICS = inhaled corticosteroid, LT = leukotrienes, GLM = generalized linear mixed effects model, GEE = generalized estimating equation, EBC = exhaled breath condensate, eNO = exhaled nitric oxide, TBARS = thiobarbituric acid reactive substances, BMI = body mass index, IL-8 = interleukin-8, MDA = malondialdehyde, NR = not reported, PMNs = polymorphonuclear leukocytes, HDM = house dust mite, iNOS = inducible nitric oxide synthase, NAL = nasal lavage, CC16 = clara cell protein.

[a]Results are presented for exhaled nitric oxide unless otherwise specified.

[b]Effect estimates are standardized to a 20 ppb for 24-h avg NO_2 or NO, 25 ppb for 8-h max NO_2 or NO, 30-ppb increase for 1-h avg or 5-h avg NO_2 or NO, and a 60-ppb increase for 5-h avg NO_X.

[c]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.

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Evidence also pointed 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) and school indoor NO_2 (Greenwald et al., 2013; Sarnat et al., 2012) (Figure 4-2 and Table 4-14). 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. There was evidence of association with central site NO_2, which was moderately to strongly correlated (Spearman r = 0.63-0.91) with school NO_2 (Sarnat et al., 2012). The results suggest that 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 of eNO with school NO_2 may be attributable to school measurements better representing spatial variability in NO_2. Spatial variability has been characterized to influence exposure measurement error (Section 2.6.5.2). 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 that had weaker methodology characterized by cross-sectional design and comparison of eNO between low and high NO_2 (means 10.1 and 17.4, respectively for lag 1-4 day avg) (Flamant-Hulin et al., 2010).

Several studies found associations of pulmonary inflammation or oxidative stress with ambient NO_2 measured at central sites. Included among these studies were those using central sites located within 5 km of subjects' homes or schools (Lin et al., 2011; Barraza-Villarreal et al., 2008; Romieu et al., 2008). In particular, among children in Beijing, China examined before and after the 2008 summer Olympics, NO_2 measured within 650 meters of subjects' schools (lag 0 day of 24-h avg) was associated with eNO. NO_2 was not associated with eNO in a cross-sectional study of children with asthma in 13 southern California communities (Berhane et al., 2011). Central site NO_2 was associated with indicators of inflammation such as IL-8 and exhaled breath condensate pH and indicators of oxidative stress. Among children in Windsor, ON, Canada, an increase in 24-h avg NO_2 (measured at one of two sites within 10 km of subjects' homes) was associated with increases in exhaled breath condensate thiobarbituric acid reactive substances (TBARS), an indicator of lipid peroxidation, weakly associated with eNO, and not associated with another lipid peroxidation indicator, 8-isoprostane (Liu et al., 2009b).

With regard to confounding, most studies adjusted for temperature and humidity, with a few additionally evaluating asthma medication use (Sarnat et al., 2012; Liu et al., 2009b; Delfino et al., 2006). Most studies found associations with copollutants such as $PM_{2.5}$,

PM_{10}, $PM_{10-2.5}$, EC, organic carbon (OC), VOCs, O_3, and SO_2. These copollutants showed a wide range of correlations with NO_2 (Pearson or Spearman r = 0.18 to 0.80).

Among studies with personal or school NO_2 monitoring, some provided evidence for the effects of NO_2 independent from other pollutants. Among children with asthma in southern California, robust eNO-NO_2 associations were found with adjustment for personal $PM_{2.5}$, EC, and OC, which were weakly correlated with personal NO_2 (Spearman r = 0.20-0.33) (Delfino et al., 2006). Effect estimates for copollutants were robust to adjustment for NO_2. Reporting copollutant-adjusted results only for EBC pH, Martins et al. (2012) found that associations for subject-level estimates of outdoor NO_2 exposure were relatively robust to adjustment for VOCs, which showed no or negative correlations with NO_2 (range of Spearman correlation coeffiecient across four visits: r = -0.42 to 0.03). VOC estimates were attenuated to the null with adjustment for NO_2. NO_2 effect estimates were attenuated to null with PM_{10}, but PM_{10} and NO_2 were negatively correlated (r = -0.55 to -0.82). The studies conducted in El Paso, TX and Ciudad Juarez, Mexico did not analyze copollutant models with the copollutants associated with eNO (Greenwald et al., 2013; Sarnat et al., 2012). In the El Paso schools, increases in eNO were found with increases in both school NO_2 and BTEX (benzene, toluene, ethylbenzene, xylene, traffic-related VOCs) (Greenwald et al., 2013). Because of the high correlation (Pearson r = 0.77) between NO_2 and BTEX, an independent association for NO_2 is not discernible. However, analyses of the combined El Paso/Ciudad Juarez population indicated an independent association of ambient school NO_2 with eNO. BTEX was not analyzed, but PM_{10}, $PM_{10-2.5}$, and $PM_{2.5}$ were weakly correlated with NO_2 in most schools (r = -0.28 to 0.34) (Sarnat et al., 2012). Further, NO_2 associations were less variable across schools than were copollutant associations, and in a school in Ciudad Juarez, NO_2 but not copollutants, was associated with eNO. An independent association for NO_2 was not clearly demonstrated for children in Windsor, ON, Canada because of a high NO_2-$PM_{2.5}$ correlation (r = 0.71) (Liu et al., 2009b). In a copollutant model, the effect estimates for NO_2 were attenuated somewhat with adjustment for SO_2 and largely with adjustment for $PM_{2.5}$ (Figure 4-2 and Table 4-14). Smaller changes were found in the SO_2 and $PM_{2.5}$ estimates with adjustment for NO_2.

Studies of children with asthma did not clearly identify potential factors that may modify ambient NO_2-associated increases in pulmonary inflammation, primarily based on post-hoc analyses. Associations were not found to differ by sex (Sarnat et al., 2012; Liu et al., 2009b; Delfino et al., 2006). Results did not clearly indicate whether use of anti-inflammatory ICS influences ambient NO_2-associated increases in pulmonary inflammation. Larger associations were found in children with daily ICS use (Delfino et al., 2006), but in children not using ICS in other studies (Sarnat et al., 2012; Liu et al., 2009b). The latter studies did not report frequency of ICS use. Because of the

heterogeneity in the definition of ICS use and lack of assessment of ICS compliance, ICS use could represent well-controlled or more severe asthma across populations, which could contribute to the inconsistent evidence for effect modification by ICS use. 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

In the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), there were no epidemiologic studies of pulmonary inflammation specifically in adults with asthma. Recent studies, both of which examined predominately adults with persistent asthma and central site NO_2 and adjusted for temperature and humidity, had contrasting results. A U.S. multicity (Boston, MA; New York, NY; Philadelphia, PA; San Francisco, CA; Madison, WI) study nested within an asthma medication trial found an association with eNO that was robust to copollutant adjustment (Qian et al., 2009a). Among all subjects, a 20-ppb increase in lag 0 day of 24-h avg NO_2 (averaged from monitors located within 20 miles of subjects' homes) was associated with a 0.26 ppb (95% CI: 0.12, 0.40) increase in eNO. 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 provided evidence for the independent effects of ambient NO_2-assoiciated exposure. eNO was associated with 24-h avg SO_2, weakly associated with 24-h avg PM_{10}, but not with 24-h avg O_3. The NO_2-eNO association was slightly attenuated (0.16 ppb [95% CI: -0.02, 0.34] increase per 20-ppb increase in lag 0 day NO_2) with adjustment for 24-h avg PM_{10} and increased with adjustment for O_3 and SO_2 (Qian et al., 2009a). In turn, adjustment for NO_2 attenuated effect estimates for the examined copollutants, indicating that the copollutant associations were confounded by NO_2.

Children in the General Population

Together with the few studies described in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), most recent studies found associations between increases in ambient NO or NO_2 and increases in pulmonary inflammation or oxidative stress in populations of

children not restricted to those with asthma. Many of these studies also examined children with asthma and similarly analyzed multiple endpoints, pollutants, lags of exposure, or subgroups. With a few exceptions (Patel et al., 2013; Steerenberg et al., 2001), there was a pattern of association found across the multiple comparisons reducing the likelihood of associations found due to chance alone or publication bias. Results were consistent for eNO (Figure 4-2 and Table 4-14). In the Southern California Children's Health Study, cross-sectional analyses indicated that NO_2 was associated with eNO (Berhane et al., 2011) but not methylation of inducible nitric oxide synthase (Salam et al., 2012), which is the enzyme thought to induce increases in eNO. However, these results do not appear to be discordant since methylation was not associated with eNO. Based on more limited examination, associations were found with IL-8 (Barraza-Villarreal et al., 2008; Steerenberg et al., 2001) and exhaled breath condensate 8-isoprostane (Patel et al., 2013). Associations were not found with exhaled breath condensate pH or inflammatory cells such as PMNs or eosinophils (Patel et al., 2013; Chen et al., 2012a; Barraza-Villarreal et al., 2008; Steerenberg et al., 2001). Ambient NO_2 was associated with pulmonary inflammation and oxidative stress in populations of children with prevalence of asthma ranging from 7.5 to 59% and allergy ranging from 20 to 89% (Patel et al., 2013; Berhane et al., 2011; Lin et al., 2011; Steerenberg et al., 2003; Steerenberg et al., 2001). With the exception of Flamant-Hulin et al. (2010), studies demonstrated associations in groups without asthma or allergy (Berhane et al., 2011; Lin et al., 2011; Steerenberg et al., 2003), suggesting that increases in ambient NO_2 exposure may increase pulmonary inflammation in healthy children.

NO- and NO_2-(24-h avg) associated increases in pulmonary inflammation were reported in some (Lin et al., 2011; Steerenberg et al., 2003; Steerenberg et al., 2001) but not all (Chen et al., 2012a; Flamant-Hulin et al., 2010; Holguin et al., 2007) studies with exposure ascertained from schools or central sites located 0.65 to 2 km from subjects' schools. However, Chen et al. (2012a) did not provide quantitative results, and results from Flamant-Hulin et al. (2010) were based on cross-sectional comparisons of high and low school-based NO_2.

With the exception of Steerenberg et al. (2001), repeated measures studies adjusted for temperature (Patel et al., 2013; Lin et al., 2011; Barraza-Villarreal et al., 2008). Lin et al. (2011) additionally adjusted for relative humidity. Many cross-sectional studies adjusted for age, sex, parental education, and smoking exposure (Salam et al., 2012; Berhane et al., 2011; Flamant-Hulin et al., 2010; Steerenberg et al., 2003). A limitation of the evidence in children overall was the uncertainty in discerning an independent association of NO_2 exposure among multiple pollutants examined. Studies found increases in pulmonary inflammation and oxidative stress in association with copollutants such as $PM_{2.5}$, PM_{10}, BC, O_3, SO_2, carbon monoxide (CO), and pollen. In the few studies that

reported copollutant correlations, weak correlations were reported for O_3 (r = 0.15, 0.21) (Berhane et al., 2011; Barraza-Villarreal et al., 2008), and moderate to strong correlations were reported for PM and BC (r = 0.47-0.80) (Patel et al., 2013; Berhane et al., 2011; Barraza-Villarreal et al., 2008). Evidence for an independent association with NO_2 was found among children in Beijing, China examined before and after the 2008 Olympics in a study that was noteworthy also for the large number of measurements per child and measurement of pollutants at a site 650 meters from schools (Lin et al., 2011). NO_2 effect estimates adjusted for BC or $PM_{2.5}$ were attenuated 2 to 4 fold but remained positive (e.g., 22% [95% CI: 18, 26%] increase in eNO per 20-ppb increase in lag 0 day of 24-h avg NO_2 to 5.6% [95% CI: 0.38, 11%] with adjustment for BC). Adjustment for NO_2 attenuated the association with $PM_{2.5}$ but not BC. These results indicated that some of the NO_2 association was confounded, by BC in particular, but NO_2 was also independently associated with eNO in this population of children in Beijing.

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, 2008c) and recent studies conducted in older adults and adults performing outdoor exercise, results point to increases in pulmonary inflammation more clearly in association with increases in 24-h avg ambient NO or NO_2 than NO_X or NO_2 averaged up to 5 hours. Copollutant-adjusted associations were found with 24-h avg NO (Adamkiewicz et al., 2004) and with 5-h avg NO_X or NO_2 for some outcomes (Steenhof et al., 2013; Strak et al., 2012). Pulmonary inflammation was indicated as increases in eNO, nasal lavage IL-6, and indicators of pulmonary injury and lung permeability such as Clara cell protein (CC16) and nasal lavage protein levels. The findings for pulmonary injury and lung permeability have weak support from controlled human exposure and animal toxicological studies as that evidence was inconsistent (Sections 4.2.4.1, 4.2.4.2). With respect to species of oxides of nitrogen, associations were found with ambient NO, NO_2, and NO_X.

Ambient NO_2 was not consistently associated with increases in pulmonary inflammation in populations of mostly healthy adults performing outdoor exercise for <1 to 5 hours. A strength of these studies is the examination of effects in subjects while outdoors, whose exposures are likely to be more correlated with ambient NO_2 measured at central sites than they are for individuals in indoor locations. In these studies, subjects had 3-5 separate outdoor exposure periods, in some cases, in locations representing a gradient of traffic volume. Among adults running or cycling outdoors for 35-90 minutes, eNO and inflammatory cell counts were not associated with NO_2 measured at central sites (Weichenthal et al., 2011; Chimenti et al., 2009) (Figure 4-2 and Table 4-14). 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 spatial variability 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. Further, 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). eNO also was associated with EC, Absorbance coefficient (Abs), and PNC (Strak et al., 2012), whereas IL-6 also was associated with $PM_{2.5}$ and OC (Steenhof et al., 2013). Independent associations for NO_X and NO_2 were not clearly demonstrated because of high copollutant correlations (e.g., r = 0.75 for NO_X and PNC and 0.71 for NO_2 and EC). 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. However, the associations of nasal lavage IL-6 and protein with NO_2 remained after adjustment for PNC or other copollutants (e.g., 66% [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.

Increases in pulmonary inflammation were associated with 24-h avg NO or NO_2 measured at central monitoring sites among older adults (mean ages: 65-71 years) (Madsen et al., 2008; Adamkiewicz et al., 2004), including those with coronary heart disease (Timonen 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; Timonen et al., 2004). However, there was 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.

4.2.4.5 Summary of Studies of Pulmonary Inflammation, Injury, and Oxidative Stress

Overall, results from recent epidemiologic studies augmented the body of evidence reviewed in the 2008 ISA for Oxides of Nitrogen indicating associations between short-term increases in ambient NO_2 exposure and increases in pulmonary inflammation in children with asthma. There was heterogeneity in the strength and precision of associations, but there was a pattern of association found across the various lags of exposure and endpoints examined. A majority of the evidence was for NO_2-associated increases in eNO (Figure 4-2 and Table 4-14). The studies of adults with asthma produced contrasting results. The epidemiologic observations are supported by demonstrations of allergic inflammation in several (but not all) controlled human exposure studies of adults with asthma and allergy following exposures to allergen plus 260 ppb NO_2 for 15-30 minutes or 400 ppb NO_2 for 6 hours. Although these findings are in adults with asthma and allergy, the allergic inflammation was characterized by an increase in eosinophil number and activation of eosinophils and/or neutrophils, both of which have been linked with NO production during an inflammatory response. Allergic inflammation was also enhanced by a 3-hour exposure to 5,000 ppb NO_2 in a rat model of allergic airways disease. These results provide evidence for NO_2-induced exacerbation of allergic airways disease both in the presence and absence of an allergen challenge (Section 3.3.2.6). Observations of increased numbers of airway eosinophils and expression of Th2 cytokines in healthy individuals or guinea pigs following exposure to 2,000-3,000 ppb NO_2 provide evidence that repeated or prolonged exposure to NO_2 may lead to the development of a pro-allergic inflammatory response and promote Th2 skewing and allergic sensitization (Section 3.3.2.6). Epidemiologic studies generally did not find NO_2-associated changes in inflammatory cell counts in children with asthma.

Results from controlled human exposure studies demonstrated NO_2-induced pulmonary inflammation in healthy adults, most consistently as increases in PMNs in healthy adults. Whereas epidemiologic studies did not consistently find NO_2-associated increases in pulmonary inflammation in adults (examined during outdoor exercise), results were consistent in children in the general population and older adults. In contrast, pulmonary inflammation was not consistently affected in experimental animals with a wide range of NO_2 exposures (800-5,000 ppb for 6 hours to 2 weeks).

Rather than increases in oxidative stress, in the limited available controlled human exposure studies, results indicated NO_2-induced changes in antioxidant concentrations in BALF. Such observations also were made in experimental animals, but changes in antioxidant capacity appeared to be transient. Further, there was heterogeneity in animal toxicological studies that made it difficult to draw conclusions across studies. A few

epidemiologic studies reported associations between ambient NO_2 concentrations and indicators of lipid peroxidation in exhaled breath condensate of children with asthma and children in the general population. Across disciplines, there were a few observations of NO_2-induced pulmonary injury as demonstrated by alterations in epithelial barrier function. However, in experimental studies, results were inconsistent at ambient-relevant NO_2 exposures. Effects are concentration dependent and observed at concentrations of NO_2 above 5,000 ppb as discussed in Section 3.3.2.4. However, this evidence combined with that from morphologic studies suggest the slight injury to the airway can result from short-term NO_2 exposure.

With respect to potential at-risk populations, several epidemiologic studies did not find larger NO_2-associated increases in pulmonary inflammation or oxidative stress among children with asthma than children without asthma (Patel et al., 2013; Berhane et al., 2011; Lin et al., 2011; Flamant-Hulin et al., 2010; Barraza-Villarreal et al., 2008). Evidence did not consistently indicate that NO_2-associated pulmonary inflammation differed by sex (Sarnat et al., 2012; Liu et al., 2009b; Delfino et al., 2006), ICS use (Sarnat et al., 2012; Liu et al., 2009b; Qian et al., 2009a; Delfino et al., 2006), or respiratory allergy (Sarnat et al., 2012; Berhane et al., 2011; Steerenberg et al., 2003).

A majority of the epidemiologic evidence for pulmonary inflammation and oxidative stress was for NO_2; however, associations also were found with NO and NO_X. With respect to averaging times, most evidence was for 24-h avg, with a few results indicating associations with 8-h max NO_2. Associations with shorter averaging times (i.e., <1 to 5 hours), examined primarily in adults performing outdoor exercise, were inconsistent. The ranges of mean concentrations were 6.5-45 ppb for outdoor 24-h avg NO_2, 4.5-39 ppb for 24-h avg personal NO_2, and 6.3-30 ppb for 24-h avg NO. Increases in pulmonary inflammation and oxidative stress were found with single-day NO_2 lags of 0 or 1 day and multiday averages of 2- to 7-days. Increases in pulmonary inflammation were found 0 to 2 hours after outdoor exposures (Steenhof et al., 2013; Strak et al., 2012). Among studies that compared various lags, several found larger associations for multiday average (e.g., 0-1 avg to 0-7 avg) than single-day NO_2 concentrations (e.g., 0 or 1) (Patel et al., 2013; Liu et al., 2009b; Madsen et al., 2008; Delfino et al., 2006; Timonen et al., 2004; Steerenberg et al., 2001).

The evidence in children with asthma is substantiated by several studies with strong exposure assessment characterized by personal monitoring, modeling individual outdoor exposures, or school-based monitoring. Among studies that compared various exposure assessment methods, Delfino et al. (2006) found similar associations with 24-h avg personal and 8-h max central site NO_2, and Sarnat et al. (2012) found stronger associations for school than central site NO_2. Observations that indoor school NO_2 was

not associated with pulmonary inflammation (Greenwald et al., 2013; Sarnat et al., 2012) or that home or school indoor NO_2 concentrations were negligible (Martins et al., 2012) provide additional support for associations of pulmonary inflammation with ambient NO_2. Increases in pulmonary inflammation in adults also were associated with higher NO_X and NO_2 measured at the locations of outdoor exposure (Strak et al., 2012) and in children with NO_2 or NO measured 0.65 to 2 km of schools (Lin et al., 2011; Steerenberg et al., 2003; Steerenberg et al., 2001).

Most epidemiologic studies found NO_2-associated increases in pulmonary inflammation and oxidative stress with adjustment for temperature and relative humidity. Because pulmonary inflammation and oxidative stress were associated with other pollutants (e.g., $PM_{2.5}$, EC, other PM constituents) that were correlated with NO_2 in many studies, an association of pulmonary inflammation specifically with NO_2 exposure was not identified in all studies. However, among the small group of studies that examined copollutant confounding, most demonstrated an independent association with NO_2. Particularly among children with asthma, associations of pulmonary inflammation with personal or modeled NO_2 exposure were relatively robust in copollutant models with $PM_{2.5}$, PM_{10}, EC or VOCs (Martins et al., 2012; Delfino et al., 2006). With few exceptions (Strak et al., 2012; Liu et al., 2009b), studies found increases in pulmonary inflammation in association with NO_2 measured at central sites or at the locations of outdoor exposures, after adjusting for BC, PM_{10}, $PM_{2.5}$, PNC, SO_2, or O_3 (Steenhof et al., 2013; Lin et al., 2011; Qian et al., 2009a; Adamkiewicz et al., 2004). Copollutant associations adjusted for NO_2 were robust in some cases (Lin et al., 2011; Delfino et al., 2006; Adamkiewicz et al., 2004) and attenuated in other cases (Steenhof et al., 2013; Martins et al., 2012; Qian et al., 2009a). Thus, in some studies, NO_2 appeared to confound copollutant associations. Overall, results from copollutant modeling provided evidence for the effects of NO_2 on pulmonary inflammation independent of the effects of other ambient pollutants. Sarnat et al. (2012) found increases in eNO in association with outdoor and indoor school NO_2. The 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 a different pollutant mixture in the indoor and outdoor environments. Thus, the coherence of evidence for eNO related to indoor and outdoor NO_2 exposure further supports the independent effects of NO_2 exposure on pulmonary inflammation.

4.2.5 Host Defense

The respiratory tract is protected from exogenous pathogens and particles through a variety of lung host defense mechanisms that include mucociliary clearance, particle transport and detoxification by alveolar macrophages, and innate and adaptive immunity.

Animal toxicological studies provide clear evidence for NO_2-induced susceptibility to bacterial or viral infection with some coherence with results from controlled human exposure and epidemiologic studies. Providing mechanistic support for these observations, some toxicological studies show NO_2-induced impairments in alveolar macrophage function as characterized by decreased superoxide anion release and diminished phagocytic activity. Effects on mucociliary clearance are not in a consistent direction, but the exact mechanism by which it may impair host defense is not well characterized. Study details for animal toxicological and controlled human exposure studies are presented in Table 4-15 and Table 4-16, respectively. Study details for epidemiologic studies are presented in Table 4-18, Table 4-21, and Table 4-22.

4.2.5.1 Susceptibility to Bacterial or Viral Infection

Toxicological Studies

A large body of evidence, provided by studies reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), demonstrates increased susceptibility of rodents to viral or bacterial infection following short-term NO_2 exposure. These studies used a variety of experimental approaches that generally includes 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. 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.

In a series of studies, Goldstein et al. (1974); 1973) examined bactericidal activity and clearance in mice challenged with radiolabeled *Staphylococcus aureus* either 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, dose-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 significant differences compared to air controls at NO_2 concentrations less than 7,000 ppb, the 17-hour exposure preceding challenge was significant for concentrations greater than 2,300 ppb. Parker et al. (1989) also used radiolabeled bacteria to determine 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 published 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 1 hour. 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 increases mortality from bacterial infection. Illing et al. (1980) exposed mice to 2,000 ppb NO_2 with continuous exercise for 3 hours while Ehrlich et al. (1977) exposed mice to 3,000 ppb NO_2 for 3 hours; both studies subsequently exposed mice to an aerosol of *Streptococcus pyogenes* and measured 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, exposure to 500 ppb for more than 3 months was required to result in NO_2-related increases in infection mortality. 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 *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 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. Although Pathmanathan et al. (2003) found increased expression of 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 LDH release. Consistent with Frampton et al. (2002); Goings et al. (1989) reported no increase 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. Although not significant, 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.

Epidemiologic Studies

Epidemiologic studies examined respiratory infections as hospital admissions (Section 4.2.7.3), ED visits (Section 4.2.7.4), and parental reports of incidence and duration of respiratory infections. Several studies found associations with ambient NO_2 concentrations in children (Mehta et al., 2013; Stern et al., 2013; HEI Collaborative Working Group, 2012; Zemek et al., 2010; Ségala et al., 2008; Just et al., 2002). Studies varied in the specific respiratory infection examined (e.g., bronchiolitis, ear infection, any respiratory infection), and some associations were estimated imprecisely, with wide 95% CIs (Figure 4-4 and Figure 4-5). Studies indicated associations in groups with respiratory disease, i.e., children with asthma (Just et al., 2002) and adults with COPD (Faustini et

al., 2013). A multicity study in Canada did not find an association between ambient NO_2 concentrations and ED visits for respiratory infections among subjects of all ages (Stieb et al., 2009).

Ambient NO_2-associated increases in respiratory infection were found across a wide range of ages in children, from infants ages 0-1 years (Stern et al., 2013) to schoolchildren ages 7-15 years (Just et al., 2002). Among infants ages 0-1 years, higher 1-week average NO_2 was associated with longer duration of respiratory infections but not incidence of infections (Stern et al., 2013). Several studies found respiratory infections in children ages 0-5 years in associations with multiday (5- to 7-day) averages of NO_2 concentration (Mehta et al., 2013; Stern et al., 2013; HEI Collaborative Working Group, 2012; Zemek et al., 2010). In addition to NO_2, respiratory infections were associated with ambient concentrations of copollutants such as BS, CO, and PM_{10}. Among adults with COPD in Italy, the association between NO_2 and LTRI was robust to adjustment for PM_{10} (Faustini et al., 2013). Other studies did not examine copollutant models, and studies conducted in Paris, France reported high correlations for NO_2 with BS, PM_{10}, and SO_2 (r = 0.74-0.92) (Ségala et al., 2008; Just et al., 2002), adding some uncertainty regarding an independent association with NO_2.

4.2.5.2 Mucociliary and Alveolar Clearance

Airborne substances that are small enough to be respired may be trapped in the epithelial lining fluid (ELF) in the conducting airways and physically removed or cleared from the airway by ciliated epithelial cells. Recent and previous animal toxicological studies have demonstrated that exposure to high concentrations of NO_2, generally above 5,000 ppb, functionally impair pulmonary clearance and damage the ciliated epithelium of the airway; however, exposure to NO_2 at 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 has been limited to previous studies, which were reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c). No recent studies have been conducted that investigate the effects of NO_2 on mucociliary clearance.

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, 7, or 14-day exposures; however, patterns in clearance, measured as the fraction of retained radioactive tracer microspheres, were

reported be significantly different than controls at both 310 or 1,030 ppb NO_2 over 14 days of exposure. Similarly, 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 exposures; both studies reported accelerated clearance of particles. A study published by Ohashi et al. (1994) found different results as guinea pigs exposed to 3,000 or 9,000 ppb NO_2 for 6 hours per day, 6 days per week for 2 weeks had concentration-dependent reductions in ciliary activity. This study, however, used excised nasal tissues from exposed animals and reported ciliary beat measured by light refraction which is less representative of ambient human exposure.

In a controlled human exposure study of healthy adults, Helleday et al. (1995) used fiberoptic bronchoscopy to measure ciliary activity and found decreased ciliary activity 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 proper controls and subject blinding.

4.2.5.3 Alveolar Macrophages

Resident AMs have an integral role in detoxifying and/or clearing the lung of infectious and noninfectious particles. The ability of AMs to perform this duty is dependent upon several factors including the number and type of AMs, viability, mobility, phagocytic activity, efficient enzyme activity, and secretion of inflammatory mediators. In animal toxicological studies and controlled human exposure studies, examination of the effect of NO_2 on AM function has been limited to those reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) as there are no recent studies.

Toxicological Studies

Previous studies reported NO_2 exposure to induce slight morphological differences and increases in AM numbers in BALF (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 4-15 for study details). Robison et al. (1990) and Robison et al. (1993) exposed rat AMs to 100-20,000 ppb for 1 hour in vitro and found a dose-dependent decrease in superoxide production, ranging from 81% -55% of control levels after 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 3-10 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, though inconsistencies are present across studies that could be the results 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. (1989b) 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 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.

Controlled Human Exposure Studies

Similar to animal toxicological studies, controlled human exposure studies did not consistently demonstrate that NO_2 concentrations relevant to ambient concentrations can alter AM characteristics (see Table 4-16 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 BALF 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).

4.2.5.4 Summary of Studies of Host Defense

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 provided 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. 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 averages) 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 some associations were estimated imprecisely, with wide 95% CIs (Figure 4-4 and Figure 4-5). Epidemiologic evidence indicated associations in children and in study populations with respiratory disease (i.e., children with asthma, adults with COPD). Whereas an 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 associations were found with highly correlated copollutants such as BS, PM_{10}, and SO_2 (r = 0.74 to 0.92).

Also providing biological plausibility for NO_2-induced impaired host defense, studies have characterized potential mechanisms underlying susceptibility to infection. Although results vary across studies, some animal toxicological and controlled human exposure 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. There was heterogeneity across studies in animal species, strain, and sex that could 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 relevant NO_2 exposure.

Table 4-15 Animal toxicological studies of NO_2 and lung host defense.

Study	Species (Strain); Lifestage; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Amoruso et al. (1981)	Rat (Sprague-Dawley); Female, n = 4/group	1,300, 1,900, and 3,000 ppb NO_2 for 3 h	Analysis of BALF 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; Female; 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); Female; 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); Male; 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); Male; 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 4-15 (Continued): Animal toxicological studies of NO_2 and lung host defense.

Study	Species (Strain); Lifestage; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Goldstein et al. (1977)	Rat (Sprague-Dawley); Female	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); Male; n = 10/group	3,000 ppb NO_2 for 6 h/day, 5 days/week up to 21 days	Histopathological evaluation, analysis of BALF, and AM function and morphology
Illing et al. (1980)	Mice (CD-1); 5-6 weeks; Female; 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); Male; 19-23 weeks; n = 6/group	4,000 ppb NO_2 continuously up to 10 days	BALF 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, BALF cell counts
Purvis and Ehrlich (1963)	Mice (Swiss Webster and albino); n >25/group	1,500, 2,500, 3,500, and 5,000 ppb NO_2 for 2h; *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, 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); Male, 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. (1989b)	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 BALF (LDH, albumin, macrophages)
Schlesinger (1987b)	Rabbits (New Zealand white); Male, 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)

Table 4-15 (Continued): Animal toxicological studies of NO_2 and lung host defense.

Study	Species (Strain); Lifestage; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Sherwood et al. (1981)	Mice (Swiss albino); Male; 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); Male, 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

Table 4-16 Controlled human exposure studies of lung host defense.

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 4h; Exercise for 15 min on/15 min off at $\dot{V}_E$= 50 L/min	BALF macrophage superoxide production and phagocytosis
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 3h, (2) 1,500 ppb for 3h; (1,2) Exercise 10 min on/20 min off at $\dot{V}_E$= ~4 times resting	BALF cell viability and differential counts 3.5h post-exposure, inactivation of influenza virus by BALF cells, IL-1 activity in BALF 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 3h, (2) 1,500 ppb for 3h; (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 3.5h post-exposure, influenza and RSV challenge in BALF 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 2h (2) 3,000 ppb for 2h (3) 1,000 or 2,000 ppb for 2h	Nasal wash virus isolation and count 4d after virus administration. Serum and nasal wash antibody response 4w after virus administration.

Table 4-16 (Continued): Controlled human exposure studies of lung host defense.

Study	n, Sex; Age (mean ± SD)	Exposure Details (Concentration; Duration)	Endpoints Examined
Helleday et al. (1995)	n = 8 nonsmokers; Median: 26 yr (Range: 24-35), 8 smokers, Median: 29 yr (Range: 28-32)	3,500 ppb for 20 min; Exercise last 15 min at 75 W	Bronchial wash and BALF analysis (protein concentration, differential cell counts, AM function)
(Pinkston et al., 1988)	Human alveolar macrophages 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

4.2.6 Respiratory Symptoms and Asthma Medication Use

The evidence described in preceding sections for NO_2-induced AHR (Section 4.2.2) and increases in pulmonary inflammation (Section 4.2.4) characterizes key events to inform the mode of action by which NO_2 exposure may increase respiratory symptoms. Epidemiologic studies reviewed in the 2008 ISA for Oxides of Nitrogen consistently found increased respiratory symptoms in children with asthma and children in the general population in association with higher indoor NO_2, personal NO_2, and ambient NO_2 concentrations (U.S. EPA, 2008c). There was weak support from a controlled human exposure study of adolescents. NO_2-associated increases in respiratory symptoms in adults with asthma were found in previous epidemiologic studies but inconsistently found in controlled human exposure studies. Controlled human exposure studies in healthy adults generally did not observe respiratory symptoms with NO_2 exposure. Recent studies, most of which were epidemiologic, continued to demonstrate associations between short-term increases in ambient NO_2 concentration and increases in respiratory symptoms in children with asthma and children in the general population.

4.2.6.1 Epidemiologic Studies

The most robust evidence for associations between ambient oxides of nitrogen and respiratory symptoms is demonstrated for NO_2 for children with asthma and children in the general population. Across the various populations examined, symptom data were collected by having subjects or their parents complete daily diaries for periods of two weeks to several months. Heterogeneity in the number of consecutive days of follow-up and the frequency of diary collection from study subjects did not appear to influence

results. Ambient NO_2 and NO_X concentrations, locations, and time periods for epidemiologic studies of respiratory symptoms are presented in Table 4-17.

Table 4-17 Mean and upper percentile concentrations of oxides of nitrogen in epidemiologic studies of respiratory symptoms.

Study[a]	Location	Study Period	Exposure Metric Analyzed	Mean/Median Concentration (ppb)	Upper Percentile Concentrations (ppb)
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 NO_2 (6 a.m.-10 a.m.)	NR	NR
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
Schildcrout et al. (2006)	Albuquerque, NM; Baltimore, MD; Boston, MA; Denver, CO; San Diego, CA; St. Louis, MO; Toronto, ON, Canada	Nov 1993-Sept 1995	24-h avg NO_2	Across cities: 17.8-26.0	90th: Across cities 26.7-36.9 ppb
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
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
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
Gent et al. (2003)	New Haven county, CT	Aug 200-Feb 2004	NO_2 – avg time NR	NR	NR
Holguin et al. (2007)	Ciudad Juarez, Mexico	2001-2002	1-week avg NO_2	18.2	NR

Table 4-17 (Continued): Mean and upper percentile concentrations of oxides of nitrogen in epidemiologic studies of respiratory symptoms.

Study[a]	Location	Study Period	Exposure Metric Analyzed	Mean/Median Concentration (ppb)	Upper Percentile Concentrations (ppb)
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
Patel et al. (2010)	New York City and nearby suburb, NY	2003-2005, months NR	24-h avg NO_2	NR	NR
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
Romieu et al. (2006)	Mexico City, Mexico	Oct 1998-Apr 2000	1-h max NO_2	66	Max: 298
Gillespie-Bennett et al. (2011)	Bluff, Dunedin, Christchurch, Porirua, Hutt Valley, New Zealand	Sept 2006	4-week avg NO_2	3.9	NR
Just et al. (2002)	Paris, France	Apr-June 1996	24-h avg NO_2	28.6[b]	Max: 59.0[b]
Schwartz et al. (1994)	Watertown, MA; Kingston-Harriman, TN; St. Louis, MO; Steubenville, OH; Portage, WI; Topeka, KS	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
Andersen et al. (2008a)	Copenhagen, Denmark	Dec 1998-Dec 2004	24-h avg NO_2 24-h avg NO_X	11.8 15.2	75th: 14.6 75th: 18.4
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
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
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
Peel et al. (2011)	Atlanta, GA	Aug 1998-Dec 2002	1-h max NO_2	41.7	90th: 65.6 Max: 109.2
Wiwatanadate and Liwsrisakun (2011)	Chiang Mai, Thailand	Aug 2005-June 2006	24-h avg NO_2	17.2	90th: 26.5 Max: 37.4
Maestrelli et al. (2011)	Padua, Italy	1999-2003	24-h avg NO_2	Across seasons and years: 20.9-37.0[b]	75th: 23.0-42.5[b]
Hiltermann et al. (1998)	Bilthoven, the Netherlands	July-Oct 1995	24-h avg NO_2	11.2[b]	22.5[b]

Table 4-17 (Continued): Mean and upper percentile concentrations of oxides of nitrogen in epidemiologic studies of respiratory symptoms.

Study[a]	Location	Study Period	Exposure Metric Analyzed	Mean/Median Concentration (ppb)	Upper Percentile Concentrations (ppb)
Boezen et al. (1998)	Amsterdam	Winter 1993-1994	24-h avg NO_2	24.5[b]	Max: 40.4[b]
	Meppel, the Netherlands			14.2[b]	Max: 28.9[b]
Forsberg et al. (1998)	Landskrona, Sweden	Jan-Mar, yr NR	24-h avg NO_2	16.2[b]	38.1[b]
Feo Brito et al. (2007)	Ciudad Real	May-June 2000-2001	24-h avg NO_2	17.4[b]	Max: 35.6[b]
	Puertollano, Spain			29.5[b]	Max: 100.5[b]
Annesi-Maesano et al. (2012b)	Multiple metropolitan locations, France	May-Aug 2004	24-h avg NO_2	9.9[b]	Max: 38.9[b]
Karakatsani et al. (2012)	Amsterdam, the Netherlands	Oct 2002-Mar 2004	24-h avg NO_2	20.4[b]	51.8[b]
	Athens, Greece			21.2[b]	59.0[b]
	Birmingham, U.K.			18.3[b]	44.2[b]
	Helsinki, Finland			12.1[b]	41.4[b]
von Klot et al. (2002)	Erfurt, Germany	Sept 1996-Nov 1997	24-h avg NO_2	24.5[b]	Max: 63.3[b]
Laurent et al. (2009)	Strasbourg, France	2004, all yr	24-h avg NO_2	18.6[b]	NR
			Dispersion model		
Carlsen et al. (2012)	Reykjavik, Iceland	Mar 2006-Dec 2009	24-h avg NO_2	11.7[b]	52.9[b]
			1-h max	27.4[b]	Max: 64.4[b]
Silkoff et al. (2005)	Denver, CO	Winters	24-h avg NO_2		
		1999-2000		16	75th: 30, Max: 54
		2000-2001		29	75th: 36, Max: 54
Harre et al. (1997)	Christchurch, New Zealand	June-Aug 1994	24-h avg NO_2	NR	NR
Peacock et al. (2011)	London, U.K.	Oct 1995-Oct 1997	1-h max NO_2	51.4	75th: 56
Higgins et al. (1995)	Widnes, Runcorn, U.K.	Aug, year NR	24-h avg NO_2	NR	Max: 44.7[b]
Desqueyroux et al. (2002)	Paris, France	Oct 1995-Mar 1996	1-h max NO_2	31.4[b]	Max: 68.1[b]
		Apr-Sept 1996		26.1[b]	Max: 56.4[b]

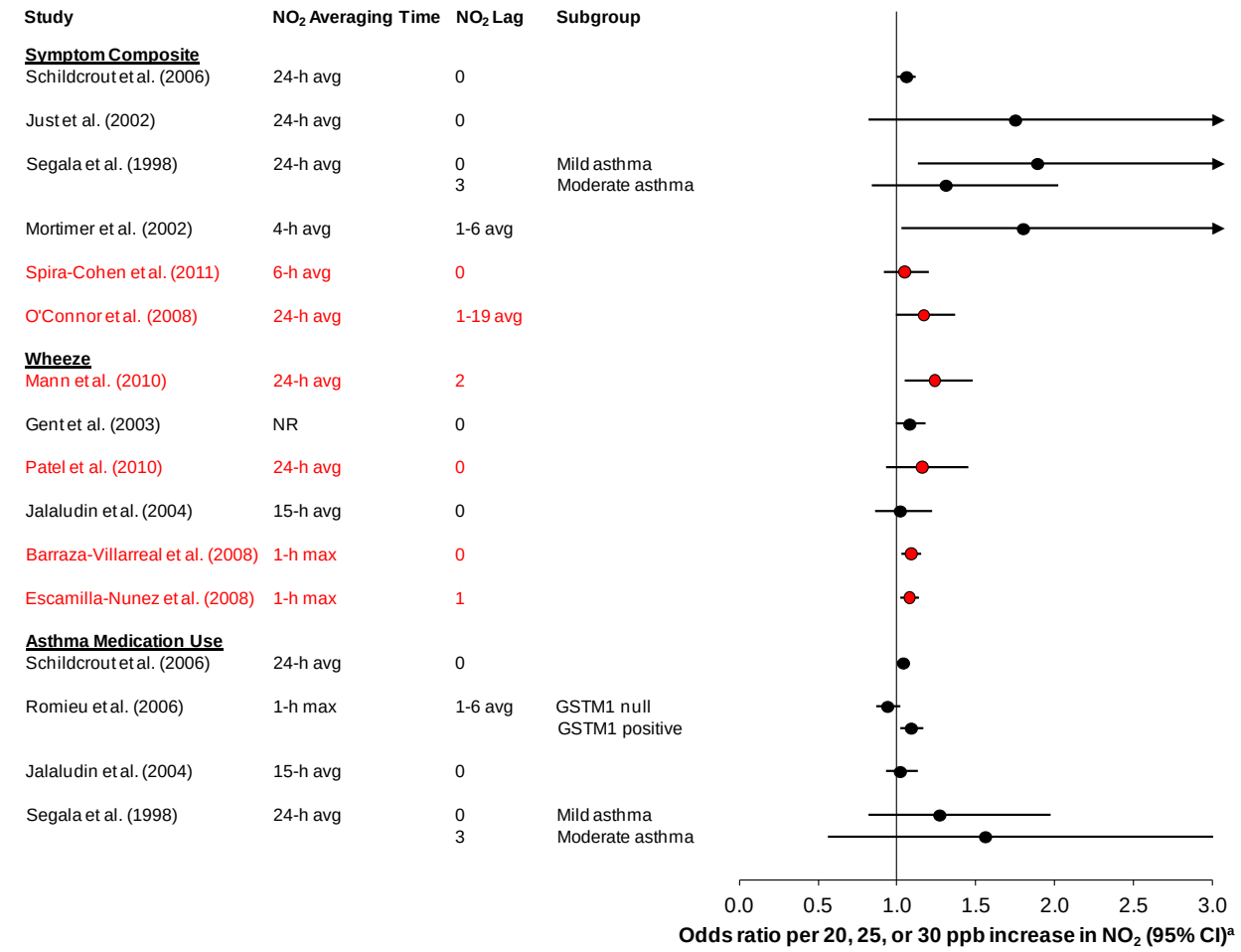
[a]Studies presented in order of first appearance in the text of this section.

[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).

NR = not reported.

Children with Asthma

Several recent studies add to the evidence for increases in respiratory symptoms in children with asthma associated with increases in ambient NO_2. Across previous and recent studies, there was heterogeneity in the strength and precision of association; however, most results indicated a pattern of elevated risk of respiratory symptoms across the various symptoms and lags of NO_2 exposure examined (Figure 4-3 and Table 4-18). The robustness of association was demonstrated in a meta-analysis of 24 mostly European studies and some U.S. studies, including several reviewed in the 2008 ISA for Oxides of Nitrogen. 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 all studies, 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).



Note: Studies are presented in order of decreasing study strength (e.g., exposure assessment method, potential confounding considered). Red=recent studies, Black=previous studies. Study details and quantitative results are reported in Table 4-18.

[a]Effect estimates are standardized to a 20-ppb increase for 24-h avg or 15-h avg NO_2, 25 ppb for 4-h avg, 6-h avg or 8-h max NO_2, and 30 ppb for 1-h max NO_2.

Figure 4-3 Associations of ambient NO_2 concentrations with respiratory symptoms and asthma medication use in children with asthma.

Table 4-18 Epidemiologic studies of respiratory symptoms and asthma medication use in children.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	Odds Ratio (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Children with Asthma						
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 and symptoms with exercise or cold exposure or family history of asthma. 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 Largest OR		Morning symptoms: 1.80 (1.03, 3.15)	w/O_3 (summer): 1.66 (0.90, 3.06) 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 NR.
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. 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 industrial source. 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. $PM_{2.5}$, SO_2, CO and O_3 also associated Moderate correlations with NO_2. r = 0.59 for $PM_{2.5}$ and SO_2, 0.54 for CO. Negative correlation for O_3. r = -0.31

Table 4-18 (Continued): Epidemiologic studies of respiratory symptoms and asthma medication use in children.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	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. 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 Average of multiple sites within 50 miles 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 0.68 for SO_2, 0.26 to 0.64 for PM_{10}, 0.63 to 0.92 for CO.
Zora et al. (2013)	El Paso, TX N = 36, mean age 9.3 (SD: 1.5) yr, 47% with atopy. Repeated measures. Daily asthma control questionnaire given by parents for 13 weeks, weekly. Questionnaire ascertains symptoms, activity limitations, asthma medication use. Parent report of physician-diagnosed asthma. Linear mixed effects model adjusted for random subject effect and humidity, temperature, school.	NO_2-school outdoor 24-h avg	0-4 avg		Asthma control score	No copollutant models analyzed for subgroups. BC, benzene, toluene, also associated with poorer asthma controls. 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
				No allergy, n = 19	-0.29 (-1.07, 0.49)	
				Allergy, n = 17	0.56 (-0.17, 1.28)	
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 nonsmoking 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. Excluded potential confounding by medication use, cold symptoms.	NO_2-school outdoor 24-h avg	0-4 avg		No quantitative results reported. Associations reported to be consistent with the null.	

Table 4-18 (Continued): Epidemiologic studies of respiratory symptoms and asthma medication use in children.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	Odds Ratio (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Holguin et al. (2007)	Ciudad Juarez, Mexico N = 194, 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, BMI, day of week, season, maternal and paternal education, passive smoking exposure	NO_2–School outdoor 24-h avg Homes 397 meters from schools.	0-6 avg	Asthma, n = 31 No asthma, n = 41	No quantitative results reported. Air pollutant exposures reported not to be associated with respiratory outcomes.	Road density at home and school reported not to be associated with respiratory symptoms.
Spira-Cohen et al. (2011)	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. Mixed effects model with subject as random effect adjusted for height, sex, temperature. Adjustment for school (indicator of season) did not alter results. 89% time indoors.	NO_2–School outdoor 6-h avg (9 a.m.-3 p.m.) Most children walk to school.	0, 1, 0-1 avg		Total symptoms: 1.05 (0.92, 1.20) Wheeze: 1.10 (0.87, 1.39)	Personal EC associated with symptoms with NO_2 adjustment. No quantitative data reported.

Table 4-18 (Continued): Epidemiologic studies of respiratory symptoms and asthma medication use in children.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	Odds Ratio (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Patel et al. (2010)	New York City and nearby suburb, NY N = 249, ages 14-20 yr, 57 with asthma, 192 without asthma. Repeated measures. Daily symptom diaries for 4-6 weeks, collected weekly. Recruitment from schools. Self-report of physician-diagnosed asthma. GLMM with random effect for subject and school and adjusted for weekend, daily maximum 8-h avg 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	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.
				All subjects	1.04 (0.92, 1.17)	
				Asthma, n = 57	1.16 (0.93, 1.45)	
				No asthma, n = 192	0.88 (0.75, 1.03)	
					Chest tightness	
				All subjects	1.10 (0.96,1.25)	
				Asthma, n = 57	1.25 (1.00, 1.55)	
				No asthma, n = 192	0.96 (0.75, 1.23)	
Barraza-Villarreal et al. (2008)	Mexico City, Mexico N = 163-179, ages 6-14 yr, 54% persistent asthma, 89% with atopy. Repeated measures. Symptom data collected every 15 days for mean 22 weeks. Children with asthma recruited from pediatric clinic. Children without asthma were friends/schoolmates. Asthma severity assessed by pediatric allergist. Linear mixed effects model with random effect for subject and adjusted for sex, BMI, lag 1 minimum temperature, ICS use, time. Adjustment for outdoor activities, smoking exposure, anti-allergy medication use, and season did not alter results.	NO_2–Central site 1-h max Site within 5 km of school or home. Spearman correlation coeffiecient for school vs. central site: r = 0.21	0	Asthma, n = 126	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 $PM_{2.5}$, 0.21 for O_3.
			0-1 sum	No asthma, n = 45	Cough 1.28 (1.04, 1.57)	

Table 4-18 (Continued): Epidemiologic studies of respiratory symptoms and asthma medication use in children.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	Odds Ratio (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Escamilla-Nuñez et al. (2008)	Mexico City, Mexico N = 197, ages 6-14 yr, 147 with asthma, 43% with persistent asthma, 89% atopy; 50 without asthma, 79% with atopy. Part of same cohort as above. 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	 Asthma, n = 147 No Asthma, n = 50	Cough 1.07 (1.02, 1.12) 1.23 (1.03, 1.47)	Only multipollutant model with O_3 and $PM_{2.5}$. NO_2 associations persist.
				 Asthma, n = 147	Wheeze: 1.08 (1.02, 1.14)	
Romieu et al. (2006)	Mexico City, Mexico N = 151, mean age 9 yr, mild or moderate asthma. 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. GEE adjusted for supplementation group, minimum temperature, smoking exposure, asthma severity, time.	NO_2–Central site 1-h max Site within 5 km of home	1-6 avg	Genotype GSTM1 null GSTM1 positive	Cough 1.09 (1.00, 1.19) 1.19 (1.11, 1.27)	No copollutant model. Associations with O_3 found with different variants. Moderate correlation with NO_2. Pearson r = 0.57 for O_3 and PM_{10}
				GSTP1 Ile/Ile or Ile/Val GSTP1 Val/Val	1.19 (1.11, 1.27) 1.08 (0.99, 1.18)	
				 GSTM1 null GSTM1 positive	BD use 0.94 (0.87, 1.02) 1.09 (1.02, 1.17)	
				GSTP1 Ile/Ile or Ile/Val GSTP1 Val/Val	1.08 (1.02, 1.14) 0.94 (0.85, 1.04)	

Table 4-18 (Continued): Epidemiologic studies of respiratory symptoms and asthma medication use in children.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	Odds Ratio (95% CI) Single-Pollutant Model[a]	Copollutant Examination
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. Daily symptom diaries for 3 mo, collected weekly. Recruitment from hospital outpatients. GEE adjusted for time trend, day of week, pollen, temperature, humidity.	NO_2–Central site 24-h avg Average of 11 sites	0		Asthma 1.75 (0.82, 3.70) Night cough 2.11 (1.20, 3.71) Respiratory infection 7.19 (2.53, 20.4)	No copollutant model. BS associated with cough and infection. High correlation with NO_2. Pearson r = 0.92.
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–outdoor home 24-h avg 1 measure per subject	4-week avg		Per log increase NO_2 Lower respiratory symptoms: 1.09 (0.78, 1.51) Reliever Inhaler: 1.47 (0.96, 2.26)	No copollutant model. No other pollutants examined.
		NO_2–indoor home 24-h avg Multiple measures per subject			Lower respiratory symptoms: 1.14 (1.12, 1.16) Reliever Inhaler: 1.14 (1.11, 1.17)	
Gent et al. (2003)	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. GEE adjusted for season, day of week, date, and motor vehicle factor obtained by source apportionment.	NO_2-central site Avg time NR # sites NR	0		Not reported	w/source apportionment factor of EC, Zn, Pb, Cu, Se: 1.08 (0.99, 1.18). Factor results robust to NO_2 adjustment. Moderate correlation with NO_2. Pearson r = 0.49.

Table 4-18 (Continued): Epidemiologic studies of respiratory symptoms and asthma medication use in children.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	Odds Ratio (95% CI) Single-Pollutant Model[a]	Copollutant Examination
Mann et al. (2010)	Fresno, Clovis, CA N = 315, 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. ARIMA with imputed wheeze values for 7.6% days and GEE adjusted for fitted daily mean wheeze, home ownership, race, sex, asthma severity, panel group, 6-month 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	w/$PM_{10-2.5}$ all subjects 1.14 (0.95, 1.37). $PM_{10-2.5}$ association robust to NO_2 adjustment. Weak correlation with NO_2. r = 0.12.
				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)	
Jalaludin et al. (2004)	Sydney, Australia N = 125, mean age 9.6 yr, 45 with wheeze, asthma, and AHR, 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. 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: 1.02 (0.86, 1.22) Wet cough: 1.13 (1.00, 1.27) Beta agonist use: 1.02 (0.93, 1.13)	NO_2 associations found in children with asthma and AHR but examined only in multipollutant model with O_3 and PM_{10}. Negative or weak correlation with NO_2. r= -0.31 for O_3, 0.26 for PM_{10}.

Table 4-18 (Continued): Epidemiologic studies of respiratory symptoms and asthma medication use in children.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	Odds Ratio (95% CI) Single-Pollutant Model[a]	Copollutant Examination
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. GEE adjusted for day of week, time trend, temperature, humidity, age, sex.	NO_2-central site 24-h avg # sites NR			Incident asthma	No copollutant model. Associations also found with PM_{13} and SO_2. Moderate correlations with NO_2. Pearson r = 0.55 for PM_{13}, 0.61 for BS, 0.54 for SO_2.
			0	Mild asthma, n = 43	1.89 (1.13, 3.15)	
			3	Moderate asthma, n = 41	1.31 (0.84, 2.02)	
					Beta agonist use	
			3	Mild asthma, n = 43	1.27 (0.82, 1.97)	
			0	Moderate asthma, n = 41	1.56 (0.51, 4.7)	
Children in the General Population						
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. Logistic regression adjusted for lag 1 temperature, day of week, city.	NO_2-central site 24-h avg 1 site per community			Cough	Cough w/PM_{10}: 1.46 (0.98, 2.19) w/O_3: 1.61 (1.08, 2.41) w/SO_2: 1.42 (0.90, 2.22) PM_{10} less attenuated with adjustment for NO_2. O_3 robust, SO_2 reduced. Moderate correlations with NO_2. r = 0.35 for PM_{10}, 0.35 for $PM_{2.5}$, 0.28 for sulfate
			0		1.21 (0.92, 1.59)	
			0-3 avg		1.61 (1.08, 2.40)	
					LRS:	
			1		1.44 (0.96, 2.16)	
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		LRS	No copollutant model. Association also found with CO. Correlation NR.
				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)	

Table 4-18 (Continued): Epidemiologic studies of respiratory symptoms and asthma medication use in children.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	Odds Ratio (95% CI) Single-Pollutant Model[a]	Copollutant Examination
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	24-h avg		Wheeze	For age 0-1 yr
				Age 0-1 yr	3.13 (1.27, 7.77)	NO_2 w/PM_{10}:
				Age 2-3 yr	1.71 (0.94, 3.10	2.46 (0.72, 8.4)
		NO_X-central site 24-h avg 1 site with 15 km		Age 0-1 yr	3.26 (1.14, 9.26)	NO_X with PM_{10}:
				Age 2-3 yr	1.80 (0.87, 3.72)	2.36 (0.59, 9.37)
						NO_2 and NO_X results also attenuated with UFP adjustment. PM_{10} associations also attenuated.
						Moderate correlations for PM_{10} with NO_2 and NO_X. Spearman r = 0.40, 0.43. High correlations for CO with NO_2 and NO_X. r = 0.74, 0.75
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 2 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	No copollutant model.
				Winter	0.78 (0.57, 1.09)	$PM_{2.5}$ associated with cough.
				Summer	1.14 (1.01, 1.27)	
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 yrs 4and5. GEE adjusted for temperature, humidity.	NO_2-central site 1-h max	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 Average of 10 sites			Wheeze 1.01 (0.98, 1.04) Cough 1.03 (1.00, 1.06)	

Table 4-18 (Continued): Epidemiologic studies of respiratory symptoms and asthma medication use in children.

Study	Study Population and Methodological Details	Oxide of Nitrogen Metrics Analyzed	Oxide of Nitrogen Lag Day	Subgroup Analyzed (if applicable)	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. High follow-up participation. GAM adjusted for study week, sex, siblings, nursery care, prenatal maternal smoking, postnatal maternal smoking, birth weight, maternal atopy, 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) Respiratory tract infection duration NO_2<26 ppb: ref NO_2>26 ppb: 1.18 (1.00, 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 42 days. 111,000 person-days. Recruitment from referral center for home cardiorespiratory monitoring of infants. Limited generalizability. Apnea events collected electronically. 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)	w/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 then generally in order of study strength (e.g., exposure assessment method, potential confounding considered). NCICAS = National Cooperative Inner-city Asthma Study, ICAS = Inner City Asthma Study, CAMP = Childhood Asthma Management Program, GEE = generalized estimating equations, BMI = body mass index, GLMM = Generalized linear mixed model, ICS = inhaled corticosteroid, LRS = lower respiratory symptoms, GLM = generalized linear model, NR = not reported, GAM = generalized additive model, BD = Bronchodilator

[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

Study populations were recruited from schools, asthma or allergy clinics, or doctors' offices. Asthma assessment included parental report of physician-diagnosed asthma or clinical examination. Neither of these methodological issues appeared to affect whether an association was found. In a-priori-determined comparisons of children with and without asthma, one found stronger associations in children with asthma (Patel et al., 2010); another found stronger associations in children without asthma but with 72% prevalence of atopy (Barraza-Villarreal et al., 2008; Escamilla-Nuñez et al., 2008).

Many asthma study populations had high prevalence of atopy (47-100%), 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. However, they are supported by findings from experimental studies demonstrating increases in NO_2-induced allergic responses in adults with asthma and animal models of allergic disease (Section 4.2.4.3). 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. Jalaludin et al. (2004) found elevated risk in children with asthma and AHR, not children without AHR, but in a 3-pollutant model that is difficult to interpret because of potential multicollinearity.

The evidence in children with asthma is substantiated by results from several U.S. multicity studies indicating associations between increases in ambient NO_2 concentration and increases in a composite index of respiratory symptoms. These studies each examined 6-8 cities across the U.S., and only four cities were common to multiple studies (Table 4-18). In each study, NO_2 exposures were assigned as the average of measurements from multiple city monitors, and concentrations lagged up to 6 days were associated with symptoms. In NCICAS and CAMP, respectively, ORs for asthma symptoms were 1.48 (95% CI: 1.03, 3.15) for a 30-ppb increase in lag 1-6 day avg of 4-h avg (6 a.m.-10 a.m.) NO_2 (Mortimer et al., 2002) and 1.05 (95% CI: 1.01, 1.09) for a 20-ppb increase in lag 0-2 day sum of 24-h avg NO_2 (Schildcrout et al., 2006). 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).

While a strength of the study was the proximity of most subjects to a monitor (median 2.3 km), a limitation is the examination of 19-day avg concentrations of NO_2. Most other evidence, whether from multi- or single-city, indicates associations of respiratory symptoms with shorter lags of NO_2 up to a few days. There is lack of biological plausibility for symptoms occurring with NO_2 exposure on the order of weeks, and there

is greater potential for residual temporal confounding. ICAS could not examine shorter lag periods because symptom data were collected with a time resolution of two weeks.

Among the studies covering smaller geographic regions, primarily one or two cities, many examined outdoor NO_2 exposures at home or schools. School-based NO_2 exposures were imprecisely associated with symptoms among children in Bronx, NY (6-h avg school-day (9 a.m.-3 p.m.) (Spira-Cohen et al., 2011), and not consistently associated with respiratory symptoms among children with asthma in El Paso, TX and Ciudad Juarez, Mexico (4-day avg)(Zora et al., 2013; Sarnat et al., 2012; Holguin et al., 2007). Zora et al. (2013) found a larger association between higher outdoor school 4-day avg NO_2 and poorer asthma control (composite of symptoms, activity limitation and asthma medication use) among children with asthma who also had allergies. Outdoor home NO_2 was weakly associated with respiratory symptoms among children with asthma in multiple New Zealand towns (Gillespie-Bennett et al., 2011). However, daily symptoms 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 respiratory symptoms and reliever inhaler use.

Among studies examining NO_2 exposures assigned from central sites, most found associations with respiratory symptoms in children with asthma. In many cases, including multiple Mexico City studies, the monitor was within 2-5 km of children's homes or schools (Barraza-Villarreal et al., 2008; Escamilla-Nuñez et al., 2008; Romieu et al., 2006). Results were equally robust for NO_2 measured at 1 central site per location or averaged over multiple sites (Mann et al., 2010; Patel et al., 2010; Gent et al., 2003; Just et al., 2002).

Collectively, the multicity and single-city studies demonstrated increases in respiratory symptoms among children with asthma in association with 24-h avg NO_2. Studies conducted in Mexico City found associations with 1-h max NO_2 (Barraza-Villarreal et al., 2008; Escamilla-Nuñez et al., 2008; Romieu et al., 2006). NO_2 averaged over 4 hours in the morning was associated with asthma symptoms in the multicity NCICAS cohort (Mortimer et al., 2002), but school-day 6-h avg NO_2 showed weak associations with symptoms in children in South Bronx, NY (Spira-Cohen et al., 2011). Collectively, respiratory symptoms were associated with NO_2 lagged 0 to 2 days and averaged over 2 to 7 days. In comparison of various lags, several found stronger associations with multiday averages of NO_2 than single-day lags (Mann et al., 2010; Patel et al., 2010; Escamilla-Nuñez et al., 2008; Romieu et al., 2006; Mortimer et al., 2002). But some found no difference (Schildcrout et al., 2006; Just et al., 2002) between single- and multi-day lags of NO_2 exposure.

In addition to NO_2, most studies found associations with copollutants such as PM_{10}, $PM_{2.5}$, $PM_{10-2.5}$, EC, BC, BS, SO_2, and O_3. These copollutants showed a wide range of correlations with NO_2 (r = 0.23-0.64), with higher correlations reported for CO and BS/BC (r = 0.54-0.92). Multicity studies analyzed multipollutant models (3 pollutants) which have limited implications because of potential multicollinearity (O'Connor et al., 2008; Mortimer et al., 2002) or joint effect models (Schildcrout et al., 2006). In CAMP, joint effects of NO_2 with CO, SO_2, or PM_{10} were similar to NO_2 single-pollutant effects (Schildcrout et al., 2006). In studies covering smaller regions, NO_2-wheeze associations were robust to adjustment for $PM_{10-2.5}$ among children in California (OR: 1.14 [95% CI: 0.95, 1.37] per 20-ppb increase in lag day 2 of 24-h avg NO_2) (Mann et al., 2010) and to adjustment for source apportionment factor comprising EC, zinc, lead, copper, and selenium in New Haven County, CT (OR: 1.08 [95% CI: 0.99, 1.18] per unit increase in lag 0 NO_2, unit not reported) (Gent et al., 2003). Thus, the few copollutant-adjusted results provide evidence for an independent association of NO_2 with respiratory symptoms in children. In the El Paso, TX study, outdoor school NO_2, BC, and VOCs were associated with poorer asthma control in children who also had allergies that were not in the whole study population (Zora et al., 2013). Copollutant models only were analyzed for the whole study population, and it is not clear whether NO_2 associations confounded these copollutants that showed a wide range of correlations with NO_2 (Spearman r = 0.19-0.71). 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 average (Belanger et al., 2013; 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 Respiratory Disease

Previous and recent evidence indicates associations of ambient NO_2 concentrations with respiratory symptoms and asthma medication use among adults with asthma or bronchial hyperresponsiveness. However, among adults with COPD, examined primarily in studies reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), associations of ambient oxides of nitrogen with respiratory symptoms were inconsistent. Most studies were conducted in Europe.

Asthma

Adults with asthma or bronchial hyperresponsiveness were recruited primarily from clinics, doctors' offices, and administrative databases and represented a mix of asthma severity and prevalence of ICS use and atopy. NO_2-associated increases in respiratory symptoms (e.g., total respiratory symptoms, cough, wheeze) were found in most studies (Maestrelli et al., 2011; Wiwatanadate and Liwsrisakun, 2011; von Klot et al., 2002; Boezen et al., 1998; Forsberg et al., 1998). However, a study of adults in four European countries found no association (Karakatsani et al., 2012). Although NO_2 induced allergic inflammatory responses in subjects with asthma and animal models of allergic disease (Section 4.2.4.3), ambient NO_2 was not associated with respiratory symptoms with adults with asthma and allergy (Feo Brito et al., 2007) or with severe allergic rhinitis in affected adults (Annesi-Maesano et al., 2012b). In addition to respiratory symptoms, evidence pointed to ambient NO_2-associated increases in asthma medication use in adults, primarily bronchodilators but also ICS. These associations were found in studies of individual subjects (von Klot et al., 2002; Forsberg et al., 1998; Hiltermann et al., 1998) and time-series or case-crossover studies of asthma medication sales (Carlsen et al., 2012; Laurent et al., 2009).

Most studies assigned NO_2 exposure from a single central site located in the community. Beta-agonist sales were associated with NO_2 estimated at the census block level using a dispersion model which showed high correlation with ambient concentrations (r = 0.87) (Laurent et al., 2009). Symptoms and asthma medication sales were associated with increases in 24-h avg ambient NO_2, with Carlsen et al. (2012) finding stronger associations of beta-agonist sales with 1-h max than 24-h avg NO_2. Across studies, respiratory symptoms were associated with lag 0 NO_2. Increases in medication use or sales, in particular, were associated more strongly with increases in 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). Medication use or sales also were associated with 2-week average NO_2, for which it is more difficult to control for confounding by weather (Carlsen et al., 2012; von Klot et al., 2002).

For both respiratory symptoms and medication, most studies found associations with copollutants such as SO_2, CO, BS, PM_{10}, $PM_{2.5}$, and UFP. Copollutants were not associated with most respiratory symptoms among adults with asthma in the Netherlands (Boezen et al., 1998). Few studies conducted copollutant analyses. Wiwatanadate and Liwsrisakun (2011) did not provide quantitative data but only reported that lag 5 NO_2 was not associated with nighttime symptoms with adjustment for lag 5 SO_2. Among adults with asthma in Germany, the association between lag 0-4 day avg NO_2 and medication use was robust to adjustment for UFP or $PM_{2.5}$ (e.g., OR: 1.31 [95% CI: 1.06, 1.61] per 20-pbb increase in lag 0-14 day avg of 24-h avg NO_2, with adjustment for UFP,

Pearson r = 0.66), but the NO_2-wheeze association was attenuated with adjustment for UFP (OR: 1.03 [95% CI: 0.82, 1.29]) (von Klot et al., 2002). Copollutant effect estimates were attenuated with NO_2 adjustment. Thus, an independent association was found for medication use, but confounding by UFP was indicated for wheeze.

COPD

Studies of adults with COPD were conducted mostly in Europe and found no association (Desqueyroux et al., 2002; Higgins et al., 1995) between ambient NO_2 and respiratory symptoms or inconsistent associations across the lags of exposure or range of outcomes examined (Peacock et al., 2011; Silkoff et al., 2005; Harre et al., 1997). These studies recruited subjects from clinics and advertisements. Results were equally inconsistent for symptoms such as cough, wheeze, dyspnea, total symptoms and medication use. There was no pattern of association 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 $PM_{2.5}$, PM_{10}, 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 PM_{10} or BS (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 was uncertainty regarding independent associations with NO_2.

Children in the General Population

Together, most previous and recent studies found associations between short-term increases in ambient NO_2 and respiratory symptoms in children in the general population, with the strongest evidence provided by the U.S. multicity Six Cities study (Schwartz et al., 1994). Overall, evidence was more robust for cough than symptoms such as wheeze and shortness of breath, which are associated more with asthma. Associations were demonstrated in school-aged children recruited primarily from schools and also from an allergy clinic or birth cohort, suggesting study populations were representative of the general populations. NO_2-associated increases in respiratory symptoms also were found in infants (Stern et al., 2013; Andersen et al., 2008a; Peel et al., 2007). These results may

have weaker implications because symptoms such as wheeze are common in infancy and may not necessarily be correlated with respiratory morbidity later in life. Further, Peel et al. (2007) examined apnea in infants on home cardiorespiratory monitors, a group that may not be representative of the general population. The health status of study populations was not always specified. Some studies demonstrated NO_2-associated increases in respiratory symptoms in children with high (72, 79%) prevalence of atopy (Barraza-Villarreal et al., 2008; Escamilla-Nuñez et al., 2008) or parental history of asthma (Rodriguez et al., 2007). Other studies found no association among children without asthma (Patel et al., 2010) or children with 27% asthma prevalence (Ward et al., 2002).

A majority of the supporting evidence was for 24-h avg NO_2, which was assigned from central sites, one site per city or average of multiple sites per city. Studies in Mexico City demonstrated associations with 1-h max NO_2 measured at sites within 5 km of children's schools or homes (Barraza-Villarreal et al., 2008; Escamilla-Nuñez et al., 2008). Among children in Australia followed from birth to age 5 years, Rodriguez et al. (2007) found slightly larger increases in cough for increases in 24-h avg than 1-h max NO_2. Most studies examined multiple lags of NO_2, and associations were found with lag day 0 and 2- to 5-day averages of NO_2. The U.S. multicity study found larger associations with multiday average NO_2 than lag day 0 NO_2 (Schwartz et al., 1994), whereas an Australian study found larger associations with lag 0 than lag 0-4 day avg NO_2 (Rodriguez et al., 2007). In the U.S. Six Cities study, a 20-ppb increase in lag 0-3 day avg of 24-h avg NO_2 was associated with increased cough with an OR of 1.61 (95% CI: 1.08, 2.43) (Schwartz et al., 1994). A nonlinear association was found, in which cough was found to increase with increasing NO_2 up to the median (among all study cities) of 13 ppb but not with higher NO_2 concentrations.

Copollutant models were not analyzed in most studies, and respiratory symptoms also were associated with copollutants such as PM_{10}, $PM_{2.5}$, UFP, BS, CO, SO_2, or O_3. Studies that reported data indicated a wide range of correlations between NO_2 and copollutants, with higher correlations found for UFP, BS, and CO (r = 0.61-0.75). Copollutant models were not analyzed with CO (Moon et al., 2009; Andersen et al., 2008a). In the Copenhagen, Denmark study of infants, NO_2 and NO_X associations were attenuated and became imprecise with adjustment for PM_{10} or UFP (Andersen et al., 2008a). Odds ratios for PM_{10} and UFP also were attenuated; thus, an independent effect was not demonstrated for either NO_2 or copollutants. In the U.S. Six Cities study, although NO_2 effect estimates were reduced with adjustment for PM_{10} or SO_2 (r = 0.36 and 0.51, respectively), they remained positive (Schwartz et al., 1994). The ORs for a 20-ppb increase in lag 0-3 day avg of NO_2 were 1.37 (95% CI: 0.88, 2.13) with PM_{10} adjustment and 1.42 (95% CI: 0.90, 2.28) with SO_2 adjustment. The PM_{10} odds ratio was

robust to NO_2 adjustment, whereas the SO_2 odds ratio was reduced. These results from the Six Cities study indicate some confounding of NO_2 associations with symptoms but also support an independent association with NO_2.

4.2.6.2 Controlled Human Exposure Studies

Similar to epidemiologic studies, controlled human exposure studies did not provide strong evidence for NO_2-induced increases in respiratory symptoms in adults with COPD. However, unlike epidemiologic studies, they also did not provide strong evidence in adults with asthma. In fact, the majority of controlled human exposure studies that assessed respiratory symptoms before, during, or after exposure to NO_2 did not find changes, regardless of the subjects' age or disease status. Most of these studies were reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), and the recent study published since the last review does not materially change the previous conclusion. Study details are presented in Table 4-19, but 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 reported no change in symptoms, as measured by symptom score, in healthy subjects or in adults with asthma or COPD (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; Kleinman et al., 1983), though a few studies reported statistically nonsignificant increases in symptom score following NO_2 exposure (Frampton et al., 2002; Hackney et al., 1978).

Other studies in adults with asthma or COPD reported small, 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), though Koenig et al. (1987) found a statistically nonsignificant increase in symptom score in adolescents with COPD following NO_2 exposure. 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.

Other studies investigated the effects of co-exposure to NO_2 and O_3 on respiratory symptoms, although Adams et al. (1987) and Hazucha et al. (1994) did not find evidence of effects of NO_2 (600 ppb for 1-2 hours) on symptoms related to O_3 exposure (200-300 ppb for 1-2 hours) when exposed simultaneously or sequentially.

Table 4-19 Controlled human exposure studies of respiratory symptoms.

Study	Disease status[a]; n, Sex; Age (mean ± SD)	Exposure Details (Concentration; Duration)	Time of Symptom Assessment
Adams et al. (1987)	(1-3) n = 20 M, 20 F F= 21.4 ± 1.5 yr M= 22.7 ± 3.3 yr	(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)	Following 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 3h + 2,000 ppb peak for 15 min/h; (1-3) Exercise 10 min on/20 min off at $\dot{V}_E$= ~4 times resting	Following 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	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	After each exposure
Hazucha et al. (1994)	(1,2) n = 21F; 22.9 ± 3.6 yr	(1) 600 ppb NO_2 for 2h, air for 3h, 300 ppb O_3 for 2h, (2) air for 5h, 300 ppb O_3 for 2 h; (1,2) Exercise for 15 min on/15 min off at $\dot{V}_E$= 35 L/min	Not reported
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	Immediately and 6 and 24 h 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	Before, immediately after, and day 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)	(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

Table 4-19 (Continued): Controlled human exposure studies of respiratory symptoms.

Study	Disease status[a]; n, Sex; Age (mean ± SD)	Exposure Details (Concentration; Duration)	Time of Symptom Assessment
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	Before, during, immediately after, 1 day after and 1 week 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	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	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)	2,300 ppb for 5 h	Before, during, and 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 2h; 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)	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	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 $\dot{V}_E$= 25 L/min; Inhalation challenge with house dust mite antigen after NO_2 exposure	Before and after exposure and 6 h after allergen challenge

[a]Subjects were healthy individuals unless described otherwise.

4.2.6.3 Summary of Studies of Respiratory Symptoms

Consistent with studies reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), recent epidemiologic studies generally found associations between increases in ambient NO_2 concentrations and increases in respiratory symptoms among children with asthma and children in the general population. The evidence in children with asthma is weakly supported by findings in a controlled human exposure study of adolescents with asthma. The robustness of epidemiologic evidence was demonstrated by results from U.S. multicity studies (Schildcrout et al., 2006; Mortimer et al., 2002; Schwartz et al., 1994) and a meta-analysis of children with asthma (Weinmayr et al., 2010). Results did not clearly indicate larger effects in children with asthma than children without asthma (Patel et al., 2010; Barraza-Villarreal et al., 2008).

Epidemiologic results also indicated associations of ambient NO_2 concentrations with respiratory symptoms in adults with asthma. Associations with asthma medication use or sales were found more consistently in adults with asthma than children with asthma. These findings are only weakly supported by results from controlled human exposure studies, as only a few found increases in respiratory symptoms in adults with asthma following exposure to 120-350 ppb NO_2 for 30 minutes to 3 hours. Among adults with COPD, both controlled human exposure and epidemiologic studies were inconsistent in showing NO_2-related increases in respiratory symptoms. Respiratory symptoms were not examined in healthy adults in epidemiologic studies, but most controlled human exposure studies did not find increases in respiratory symptoms in healthy adults following exposures of 300-2,300 ppb NO_2 for 1 to 5 hours.

Respiratory symptoms were associated more consistently with NO_2 measured at central monitoring sites than schools (Zora et al., 2013; Sarnat et al., 2012; Spira-Cohen et al., 2011; Holguin et al., 2007). Several of the school studies were conducted in the same or neighboring communities. A majority of evidence was for 24-h avg NO_2; however, associations also were found with shorter averaging times, 4-h avg (6 a.m.-10 a.m.) in a U.S. multicity study (Mortimer et al., 2002) and 1-h max (Carlsen et al., 2012; Barraza-Villarreal et al., 2008; Rodriguez et al., 2007). Comparisons of averaging times did not clearly indicate larger ORs for 24-h avg NO_2 or 1-h max NO_2 (Carlsen et al., 2012; Rodriguez et al., 2007). Increases in respiratory symptoms were found with single-day lags of ambient NO_2 concentrations of 0 to 7 days and multiday averages of 2 to 7 days. In some studies, associations were inconsistent among the lags examined. Several studies found stronger associations for multiday averages of NO_2, including multicity U.S. studies (Mortimer et al., 2002; Schwartz et al., 1994) and most studies of asthma medication use or sales (Carlsen et al., 2012; von Klot et al., 2002; Hiltermann et al.,

1998). The range of mean ambient NO_2 concentrations in studies was 7-40.4 ppb for 24-h avg NO_2 and 18-37.4 ppb for shorter averaging times. With respect to the concentration-response relationship, the Six Cities Study of children found a nonlinear relationship with cough that was evident in the lower range of the 24-h avg NO_2 distribution, i.e., days with <13 ppb NO_2 (Schwartz et al., 1994).

Several studies provide evidence for associations between indoor home NO_2 exposures with respiratory symptoms among children with asthma (Belanger et al., 2013; Lu et al., 2013; Gillespie-Bennett et al., 2011; Hansel et al., 2008) and between reductions in indoor NO_2 and reduction in symptoms (Pilotto et al., 2004). Evidence is inconsistent in adults with COPD (Hansel et al., 2013). Indoor NO_2 exposures at ice arenas also were associated with respiratory symptoms in hockey players (Salonen et al., 2008). These findings for indoor NO_2 support an independent effect of NO_2 exposure. Among studies of outdoor NO_2, a small percentage examined copollutant models. While some results indicated confounding by copollutants, several observations supported an independent association for ambient NO_2 exposure. In studies of children with asthma, associations of NO_2 with respiratory symptoms were robust to adjustment for $PM_{10-2.5}$, O_3, or a source factor comprising EC and $PM_{2.5}$ metal components (Mann et al., 2010; Gent et al., 2003; Mortimer et al., 2002). In the Six Cities study, the NO_2-cough association was reduced in magnitude and precision with adjustment for PM_{10} or SO_2 but remained positive (Schwartz et al., 1994), indicating only partial confounding of the NO_2 association. In adults, adjustment for UFP, which was highly correlated with NO_2 (r = 0.63-0.92), did not affect the NO_2 association with medication use but attenuated the association with symptoms (Andersen et al., 2008a; von Klot et al., 2002). Copollutant associations were reduced with adjustment for NO_2, indicating that NO_2 may have confounded copollutant associations.

Biological plausibility for effects in children and adults with asthma is provided by evidence for NO_2-induced AHR in adults in asthma (Section 4.2.2) and evidence for NO_2-induced increases in allergic inflammation in adults with asthma and animal models of allergic disease (Section 4.2.4.3). Consistent with the latter, ambient NO_2-associated increases in respiratory symptoms were found in children with atopy as determined by skin prick test (Barraza-Villarreal et al., 2008; Escamilla-Nuñez et al., 2008). And, larger NO_2-associated increases in respiratory symptoms were found in children with asthma who also had allergy, although based on analyses of small groups (Zora et al., 2013; Mann et al., 2010). In contrast, such associations were not demonstrated in adults with asthma and pollen allergy (Feo Brito et al., 2007) or adults with severe allergic rhinitis (Annesi-Maesano et al., 2012b). Similarly, a recent controlled human exposure study of adults with allergic asthma did not find NO_2-induced increases in allergic inflammation, and increases in symptoms were found only during, not after, exposure. Nonetheless,

there is robust evidence for NO_2-associated increases in respiratory symptoms in children with asthma and evidence describing underlying mechanisms. Because increases in respiratory symptoms can lead to the seeking of medical treatment, this evidence provides biological plausibility for epidemiologic associations observed between short-term increases in ambient NO_2 concentration and increases in respiratory-related hospital admissions, ED visits, and physician visits as described in Section 4.2.7.

4.2.7 Respiratory Hospital Admissions and Emergency Department Visits

4.2.7.1 Summary of Findings from the 2008 ISA for Oxides of Nitrogen

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, 2008c), consisted of the first thorough evaluation of respiratory morbidity in the form of respiratory-related hospital admissions and ED visits. Of the studies evaluated, the majority consisted of single-city, time-series studies that examined all respiratory hospital admissions or ED visits with additional cause-specific studies of asthma and COPD. Studies of all respiratory and asthma hospital admissions and ED visits consistently reported positive associations with short-term NO_2 exposures. These associations were generally found to be robust and independent of the effects of ambient particles or gaseous copollutants (U.S. EPA, 2008c). The strongest evidence was from respiratory studies that focused on children and older adults (65+ years of age) and asthma studies that focused on all ages and children. The 2008 ISA for Oxides of Nitrogen found limited evidence for associations between short-term NO_2 exposure and other respiratory hospital admission and ED visit outcomes, such as COPD. This evidence supporting NO_2-associated increases in respiratory-related 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, 2008c).

4.2.7.2 Evaluation of Studies Published Since 2008 ISA for Oxides of Nitrogen

Since the completion of the 2008 ISA for Oxides of Nitrogen, relatively fewer studies have examined the association between short-term exposure to ambient NO_X or NO_2 and

respiratory-related hospital admissions and ED visits. The following sections characterize recent studies in the context of the collective body of evidence evaluated in the 2008 ISA for Oxides of Nitrogen. Where possible, the emphasis within this section is on multicity studies, which allow for the examination of the relationship of short-term exposures to NO_X or NO_2 with respiratory-related hospital admissions or ED visits over a large geographic area using a common statistical methodology. The remaining evaluated studies consist of single-city studies conducted over long durations or in areas with large populations with a specific emphasis on studies that provide insight into the relationship between short-term exposure to NO_X or NO_2 and respiratory-related hospital admissions and ED visits with respect to potential copollutant confounding, seasonal differences in risk estimates, or factors that may modify risk of NO_2-related hospital admissions and ED visits.

In this ISA, respiratory-related hospital admissions and ED visits are evaluated separately because it is likely that a small percentage of respiratory ED visits will be admitted to the hospital. Therefore, ED visits may represent potentially less serious, but more common, outcomes. Additionally, results are presented as either a collection of respiratory diagnoses or as an individual diagnosis (e.g., asthma). Table 4-20 presents characteristics of studies discussed within this section along with the air quality characteristics of the city, or across all cities, evaluated in each study.

Table 4-20 Mean and upper percentile concentrations of respiratory-related hospital admission and emergency department visit studies published since the 2008 ISA for Oxides of Nitrogen.

Study	Location	Type of Visit (ICD9/10)	Years	Metric	Mean Concentration (ppb)	Upper Percentile of Concentrations (ppb)
Hospital Admissions						
Cakmak et al. (2006)	10 Canadian cities	Hospital Admissions: All Respiratory (466, 480-486, 490-494, 496)	1993-2000	24-h avg	21.4	Max: 44 – 134
Wong et al. (2009)	Hong Kong	Hospital Admissions: All Respiratory (460-519) COPD (490-496) Acute Respiratory Disease (460-466, 480-487)	1996-2002	24-h avg	31.2	75th: 37.0 Max: 89.4
Dales et al. (2006)	11 Canadian cities	Hospital Admissions: All Respiratory (799.0, 799.1, 786.0, 769, 768.9, 770.8, 486)	1986-2000	24-h avg	21.8	95th: 21 – 43
Faustini et al. (2013)	6 Italian cities	Hospital Admissions: All Respiratory (460-519) COPD (490-492,494,496) LRTI (466, 480-487)	2001-2005	24-h avg	24.1-34.6	NR
Son et al. (2013)	8 South Korean cities	Hospital Admissions: Respiratory diseases (J05, J18, J20, J21, J40-42, J44-46, J67) Asthma (J45, J46) Allergic disease (J30, J45, L20)	2003-2008	24-h avg	11.5-36.9	NR
Samoli et al. (2011)	Athens, Greece	Hospital Admissions: Asthma (493, 493.9)	2001-2004	1-h max	44.4	75th: 53.1
Ko et al. (2007b)	Hong Kong	Hospital Admissions: Asthma (493)	2000-2005	24-h avg	28.3	75th: 33.8 Max: 79.5

Table 4-20 (Continued): Mean and upper percentile concentrations of respiratory-related hospital admission and emergency department visit studies published since the 2008 ISA for Oxides of Nitrogen.

Study	Location	Type of Visit (ICD9/10)	Years	Metric	Mean Concentration (ppb)	Upper Percentile of Concentrations (ppb)
Ko et al. (2007a)	Hong Kong	Hospital Admissions: COPD (491, 492, 496)	2000-2004	24-h avg	27.2	75th: 34.0 Max: 83.8
HEI Collaborative Working Group (2012) Mehta et al. (2013)	Ho Chi Minh City, Vietnam	Hospital Admissions: Acute Lower Respiratory Infection (J13-16, 18, 21)	2003-2005	24-h avg	11.7	Max: 29.2
Ségala et al. (2008)	Paris, France	Hospital Admissions: Bronchiolitis	1997-2001	24-h avg	27.0	Max: 90.4
Grineski et al. (2010)	Phoenix, AZ	Hospital Admissions: Asthma (493)	2001-2003	1-h max (Evening hours: 4 p.m.-11 pm)	46.0	75th: 57.0 Max: 79.0
ED Visits						
Darrow et al. (2011a)	Atlanta, GA	ED Visits: All Respiratory (460-466, 477, 480-486, 491-493, 496, 786.09)	1993-2004	1-h max 24-h avg Commute (7 a.m.-10 a.m., 4 p.m.-7 p.m.) Day-time (8 a.m.-7 p.m.) Night-time (12 a.m.-6 a.m.)	1-h max: 43 24-h avg: 22 Commute: 21 Day-time: 17 Night-time: 25	75th: 1-h max: 53 24-h avg: 28 Commute: 27 Day-time: 22 Night-time: 35 Max: 1-h max: 181 24-h avg: 74 Commute: 97 Day-time: 82 Night-time: 97
Stieb et al. (2009)	7 Canadian cities	ED Visits: Respiratory Infection (464, 466, 480-487) Asthma (493) COPD (490-492, 494-496)	1992-2003	24-h avg	9.3 – 22.7	75th: 12.3 – 27.6
Strickland et al. (2010)	Atlanta, GA	ED Visits: Asthma (493.0-493.9, 786.09)	1993-2004	1-h max	23.3	NR

Table 4-20 (Continued): Mean and upper percentile concentrations of respiratory-related hospital admission and emergency department visit studies published since the 2008 ISA for Oxides of Nitrogen.

Study	Location	Type of Visit (ICD9/10)	Years	Metric	Mean Concentration (ppb)	Upper Percentile of Concentrations (ppb)
Strickland et al. (2011)	Atlanta, GA	ED Visits: Asthma (493.0-493.9, 786.09)	1993-2004	1-h max	Central monitor: 42.0 Unweighted average: 27.7 Population-weighted average: 22.0	NR
Li et al. (2011b)	Detroit, MI	ED Visits: Asthma (493)	2004-2006	24-h avg	15.7	75th: 21.2 Max: 55.2
Villeneuve et al. (2007)	Edmonton, Canada	ED Visits: Asthma (493) Influenza (487)	1992-2002	24-h avg	50th: 17.5 Summer 50th: 28.5 Winter	75th: 22.0 Summer 75th: 35.5 Winter
Jalaludin et al. (2008)	Sydney, Australia	ED Visits: Asthma (493)	1997-2001	1-h max	23.2	Max: 59.4
Arbex et al. (2009)	Sao Paulo, Brazil	ED Visits: Bronchitis (J40, J41, J42) Emphysema (J43) COPD (J44)	2001-2003	1-h max	63.0	75th: 78.6 Max: 204.6
Ségala et al. (2008)	Paris, France	ED Visits: Bronchiolitis	1997-2001	24-h avg	27.0	Max: 90.4
Zemek et al. (2010)	Edmonton, Canada	ED Visits: Otitis Media (382.9)	1992-2002	24-h avg	21.9	75th: 27.6
Orazzo et al. (2009)	6 Italian cities	ED Visits: Wheeze	1996-2002	24-h avg	21.4 – 41.2	NR

Table 4-20 (Continued): Mean and upper percentile concentrations of respiratory-related hospital admission and emergency department visit studies published since the 2008 ISA for Oxides of Nitrogen.

Study	Location	Type of Visit (ICD9/10)	Years	Metric	Mean Concentration (ppb)	Upper Percentile of Concentrations (ppb)
Physician Visits						
Burra et al. (2009)	Toronto, Canada	Physician Visits: Asthma (493)	1992-2001	1-h max	39.2	95th: 60 Max: 105
Sinclair et al. (2010)	Atlanta, GA	Physician Visits: Asthma Upper Respiratory Infection Lower Respiratory Infection	1998-2002	1-h max	1998-2000: 49.8 2000-2002: 35.5 1998-2002: 41.7	NR
Villeneuve et al. (2006b)	Toronto, Canada	Physician Visits: Allergic rhinitis	1995-2000	24-h avg	25.4	Max: 71.7

4.2.7.3 Hospital Admissions Studies

Of the recent studies evaluated that examined the association between short-term exposure to concentrations of oxides of nitrogen and respiratory-related hospital admissions, the majority focus on short-term exposures to NO_2 in single-city studies. Most of these studies are limited in that they encompass short durations (<5 years) or a small number of daily hospital admissions (i.e., <2 admissions per day). A comprehensive list of these studies can be found in Supplemental Table S4-1. (U.S. EPA, 2013d). The following section evaluates the multi- and single-city studies detailed in Table 4-20 that examined the association between short-term NO_2 concentrations and respiratory-related hospital admissions.

Respiratory Disease

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. The results from these studies are consistent with those studies evaluated in the 2008 ISA for Oxides of Nitrogen (Figure 4-4). Several of these studies also examined potential effect modification by SES, influenza, age, and sex; the results of which are discussed later in the section.

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 detail later in the section). 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 Wong et al. (2009) in a study conducted in Hong Kong aimed to examine whether influenza modifies the relationship between air pollution exposure and hospital admissions. Wong et al. (2009) observed a 3.2% (95% CI: 1.9, 4.5) increase in all respiratory hospital admissions for all ages at lag 0-1 days for a 20-ppb increase in 24-h avg NO_2 concentrations.

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 potential 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 admissions association through the use of multipollutant models, which are not informative due to potential multicollinearity issues.

The results of Cakmak et al. (2006) and Wong et al. (2009), are further supported by Son et al. (2013) in a study that examined the association between short-term exposures to air pollution and respiratory-related hospital admissions in 8 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, percent 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. The authors did not conduct copollutants 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.

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 6 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 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) also indicated that the NO_2 association was independent of that with PM_{10}. In a copollutant model with PM_{10}, 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]).

Cause-specific Respiratory Outcomes

Asthma

The 2008 ISA for Oxides of Nitrogen generally found consistent evidence of a positive association between short-term NO_2 exposures and asthma hospital admissions. Generally, studies that examined the effect of short-term NO_2 exposures on asthma hospital admissions have been 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 Panel (2007)], but it is unlikely a systematic positive bias would be introduced. In contrast, to account for this difficulty, the majority of studies on asthma ED visits (Section 4.2.7.4) have excluded ages <2 years in analyses.

Samoli et al. (2011), in a time-series study conducted in Athens, Greece, 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); however, the magnitude of the association was small compared to that observed for SO_2 and PM_{10}. An examination of additional lags (quantitative results not presented) revealed similar associations at lag 2 and a 0-2 day 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]). However, there was evidence of confounding of the NO_2 association when SO_2 was included in the model, which was most highly correlated with NO_2 ($r = 0.66$). In the copollutant model with SO_2 the NO_2 effect estimate was no longer positive (-4.3% [95% CI: -16.9, 10.2]).

Ko et al. (2007b) conducted a more extensive analysis than Samoli et al. (2011) to examine associations between short-term air pollution exposures and asthma hospital admissions at both single- and multi-day lags. In a time-series analysis conducted in Hong Kong, which included all ages, 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). These results are consistent with those of Son et al. (2013) in 8 South Korean cities, which found the strongest association between short-term NO_2 exposures and asthma as well as allergic disease hospital admissions, which encompasses asthma, at lag 0 (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 multi-day 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 copollutants analysis in Samoli et al. (2011). This attenuation occurred even though NO_2 and O_3 were not well correlated (r = 0.34) in Hong Kong.

COPD

Of the studies evaluated in the 2008 ISA for Oxides of Nitrogen, relatively few examined the association between short-term NO_2 exposure and COPD hospital admissions; however, these studies provided initial evidence of a positive association. Consistent with the 2008 ISA for Oxides of Nitrogen, a few recent studies have focused on the outcome of COPD hospital admissions, and these studies further support the initial evidence observed in the 2008 ISA for Oxides of Nitrogen. Faustini et al. (2013), in the 6 Italian city analysis discussed above, also examined the association between short-term NO_2 concentrations and COPD hospital admissions. Unlike the pattern of associations observed for total respiratory 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 with positive, albiet smaller associations 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}, the association between NO_2 and COPD hospital admissions remained robust (3.9% [95% CI: -1.7, 9.8]; lag 0).

In a study conducted in Hong Kong, 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). 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 3 and 4 pollutant models, which are difficult to interpret. However, when comparing single-pollutant results for NO_2 with the other pollutants examined (O_3, $PM_{2.5}$, and PM_{10}), similar patterns of associations were observed across pollutants.

Respiratory Infection

To date, very few studies have examined the association between short-term NO_2 exposures and respiratory infection hospital admissions. Overall, these studies have generally not provided consistent evidence of an association (U.S. EPA, 2008c). 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 Collaborative Working Group, 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 (see Section 4.2.7.4) 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 conduct copollutants analyses. 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 6 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 effects 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 results remained robust (7.8% [95% CI: -6.5, 24.2]).

Sensitivity Analyses and Effect Modification of Relationships between NO_2 and Respiratory-Related Hospital Admissions

Model Specification

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 8 South Korean cities, conducted a sensitivity analyses to identify if risk estimates changed depending on the degrees of freedom (df) used to control for temporal trends and meteorology covariates (i.e., temperature, humidity, and barometric pressure). The authors reported that this association between short-term NO_2 exposures and all of the respiratory hospital admission outcomes examined was sensitive to using less than 6 degrees of freedom (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 for all respiratory outcomes.

Examination of Seasonal Differences

In addition to examining the association between short-term NO_2 concentrations and respiratory-related hospital admissions in all-year analyses, some studies also conducted seasonal analyses. When evaluating these studies it is important to note that the differences in the locations examined across studies complicate the ability to draw overall conclusions regarding the seasonal patterns of associations.

Son et al. (2013), in the study of 8 South Korean cities, examined potential seasonal differences across respiratory hospital admission outcomes. For all outcomes examined, the association with NO_2 was largest in magnitude during the summer (Respiratory Diseases: 8.3% [95% CI: 2.8, 14.3], lag 0; 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). Across the 8 cities, NO_2 concentrations were lowest during the summer season (<20 ppb compared to >24 ppb in the other seasons).

The asthma hospital admission results of Son et al. (2013) are supported by Samoli et al. (2011) in a study conducted in Athens, Greece. 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 percent increase was observed for the summer (28.7% [95% CI: -3.4, 71.3]).

Additional studies conducted in Hong Kong and Ho Chi Minh, Vietnam highlight the vast differences observed in seasonal analyses based on geographic location. In studies conducted in Hong Kong that examined asthma hospital admissions (Ko et al., 2007b) and COPD hospital admissions (Ko et al., 2007a), larger associations were observed in the cold season (i.e., December to March) than the warm season.

Mehta et al. (2013) in the examination of acute lower respiratory infection (ALRI) hospital admissions in Vietnam examined potential seasonal differences in associations by dividing the year into the dry (November-April) and rainy seasons (May-October). Unlike the other pollutants examined in the study for which concentrations differed drastically between these seasons, mean NO_2, concentrations were similar across seasons: 23.1 ppb in the dry season and 21.2 ppb in the rainy season. However, NO_2 was strongly correlated with PM_{10} (r = 0.78) only during the dry season; the correlation dropped to r = 0.18 in the rainy season. In seasonal analyses, Mehta et al. (2013) reported that NO_2 was consistently associated with ALRI hospital admissions in the dry season (35.9% [95% CI: 3.0, 79.3] per 20-ppb increase in 24-h avg NO_2, lag 1-6 day avg), with no evidence of an association in the rainy season. No pollutants were associated with ALRI hospital admissions during the rainy season. In copollutant analyses for the dry season NO_2 was robust to the inclusion of other pollutants (SO_2, O_3, or PM_{10}) with the magnitude of the effect remaining constant or increasing slightly. In the dry season analyses, the PM_{10} results were robust to all pollutants in copollutant models except NO_2, where PM_{10} risk estimates were dramatically attenuated. These results, along with the high correlation between PM_{10} and NO_2, complicate the interpretation of the NO_2 results.

Lifestage

The 2008 ISA for Oxides of Nitrogen found evidence of consistent associations between NO_2 and respiratory-related hospital admissions for both children and older adults (65+ years of age). Recent studies that conducted age stratified analyses add to this body of evidence.

In the study of asthma hospital admissions in Hong Kong, Ko et al. (2007b) found evidence of positive associations across each age range (i.e., 0-14, 14-65, and 65+) with the strongest evidence for ages 0-14 (15.5% [95% CI: 10.9, 20.6]) and 65+ (8.9% [95% CI: 5.4, 13.0]) at lag 0-4 days for a 20-ppb increase in 24-h avg NO_2 concentrations. Son et al. (2013) also reported children 0-14 years of age to be at increased risk of respiratory-related hospital admissions (i.e., allergic disease, asthma, and respiratory) compared to ages 15-64, 65-74, and ≥ 75 years. However, the authors observed limited evidence for increased risk in older adults (≥ 75 years), with older adults exhibiting a greater risk compared to the other age ranges >15 years, but only for allergic diseases (Son et al., 2013). Samoli et al. (2011) in the study of pediatric asthma hospital admissions in Athens, Greece reported evidence of increased risk of NO_2-associated pediatric asthma ED visits in children 0-4 years of age compared to those 5-14 years of age. However, the interpretation of these results requires caution due to the examination of a separate age category of children less than age 5 years in whom asthma diagnoses are less reliable.

Wong et al. (2009) examined potential differences by lifestage for respiratory-related hospital admissions in Hong Kong without taking into consideration influenza intensity. In models that examined the baseline effect of lag 0-1 days NO_2 exposure on all ages and those 65 years of age and older, the authors found evidence for positive associations across age ranges for all respiratory, acute respiratory disease, and COPD hospital admissions. Only for all respiratory hospital admissions was the association larger in ages 65 years and older (4.0 [95% CI: 2.4, 5.7] for a 20-ppb increase in 24-h avg NO_2 concentrations) compared to all ages (3.2 [95% CI: 1.9, 4.5]).

Sex

Of the studies evaluated a limited number examined whether there were differences in the risk of NO_2-associated respiratory-related hospital admissions by sex. Cakmak et al. (2006), in the 10 Canadian city study, found evidence of increased risk of NO_2-associated respiratory hospital admissions for males (2.6% [95% CI: -0.1, 5.3]) compared to females (0.7% [95% CI: -2.1, 3.3]) at lag 1.4 days for a 20-ppb increase in 24-h avg NO_2 concentrations. Additionally, in a study of pediatric asthma hospital admissions, Samoli et al. (2011) observed evidence of larger effects in males (13.6% [95% CI: 0.7, 28.2]; lag 0 for a 30-ppb increase in 1-h max NO_2 concentrations) compared to females (-3.4% [95% CI: -12.4, 6.6]; lag 0) for NO_2-associated pediatric asthma ED visits.

Race/Ethnicity

Of the studies evaluated, only Grineski et al. (2010), in a study conducted in Phoenix, Arizona, examined whether race/ethnicity modified the association between NO_2 exposure and asthma hospital admissions focusing on children <14 years of age. It is important to note that in this study the authors used evening (4 – 11 pm) NO_2 concentrations to represent the time of day when children would not be in school and more likely to be outside (Grineski et al., 2010). In these analyses, differences in race/ethnicity were examined by using a specific race or ethnicity as a referent category. In this analysis, black children were found to be at increased risk of NO_2-related asthma ED visits compared to Hispanic children, but no difference was observed between black and white children. However, among children with the same health insurance status (i.e., private insurance), black children were found to be at increased risk of asthma hospital admissions compared to Hispanic and white children.

Pre-existing disease

The potential for pre-existing diseases to modify risk of respiratory-related hospital admissions was examined by Faustini et al. (2013) in 6 Italian cities and Wong et al. (2009) in Hong Kong. Faustini et al. (2013) examined whether individuals with COPD were at increased risk of hospital admissions compared to individuals that had both a LRTI and COPD. The authors found evidence of increased risk of hospital admissions for people with LRTI and COPD (6.9% increase [95% CI: -4.3, 19.4] at lag 0-5 days for a 20-ppb increase in 24-h avg NO_2 concentrations) compared to individuals with only COPD (3.1% increase [95% CI: -2.6, 9.2]; lag 0-5 days for a 20-ppb increase in 24-h avg NO_2 concentrations).

Wong et al. (2009) examined the potential modification of the relationship between ambient air pollution, including NO_2, and respiratory hospital admissions by influenza intensity in Hong Kong. Influenza intensity was defined as a continuous variable using the proportion of weekly specimens positive for influenza A or B instead of defining influenza epidemics. This approach was used to avoid any potential bias associated with the unpredictable seasonality of influenza in Hong Kong where there are traditionally two seasonal peaks, which is in contrast to the single peaking influenza season in the U.S. (Wong et al., 2009). Across respiratory-related hospital admission endpoints, when examining influenza intensity, Wong et al. (2009) only observed increased risk with higher levels of influenza intensity for COPD hospital admissions in the population 65 years of age and older with an additional increase in NO_2-associated hospital admissions above baseline of 1.6% (95% CI: 0.2, 3.1).

Socioeconomic Status (SES)

Potential differences in associations betwen $NO_2$and respiratory-related hospital admissions by SES were examined using measures of insurance status, education level, income, and socioeconomic position. In a study examining insurance status, Grineski et al. (2010) reported evidence that children ages <14 years who had no insurance were at greater risk of NO_2-associated asthma hospital admissions compared to children who had private insurance or Medicaid.

Cakmak et al. (2006) conducted a more extensive analysis of SES indicators in 10 Canadian cities. Focusing on education level, the authors reported consistent risk of respiratory hospital admissions across individuals with different levels of educational attainment (~2% increase for <Grade 9, Grades 9-13, and some university or trade school), but a marked reduction in NO_2-related risk for individuals with a university diploma. When examining income quartiles, Cakmak et al. (2006) reported evidence of increased risk of NO_2-associated respiratory hospital admissions for those in the lowest quartile of income, with much lower risk found for individuals in the remaining quartiles.

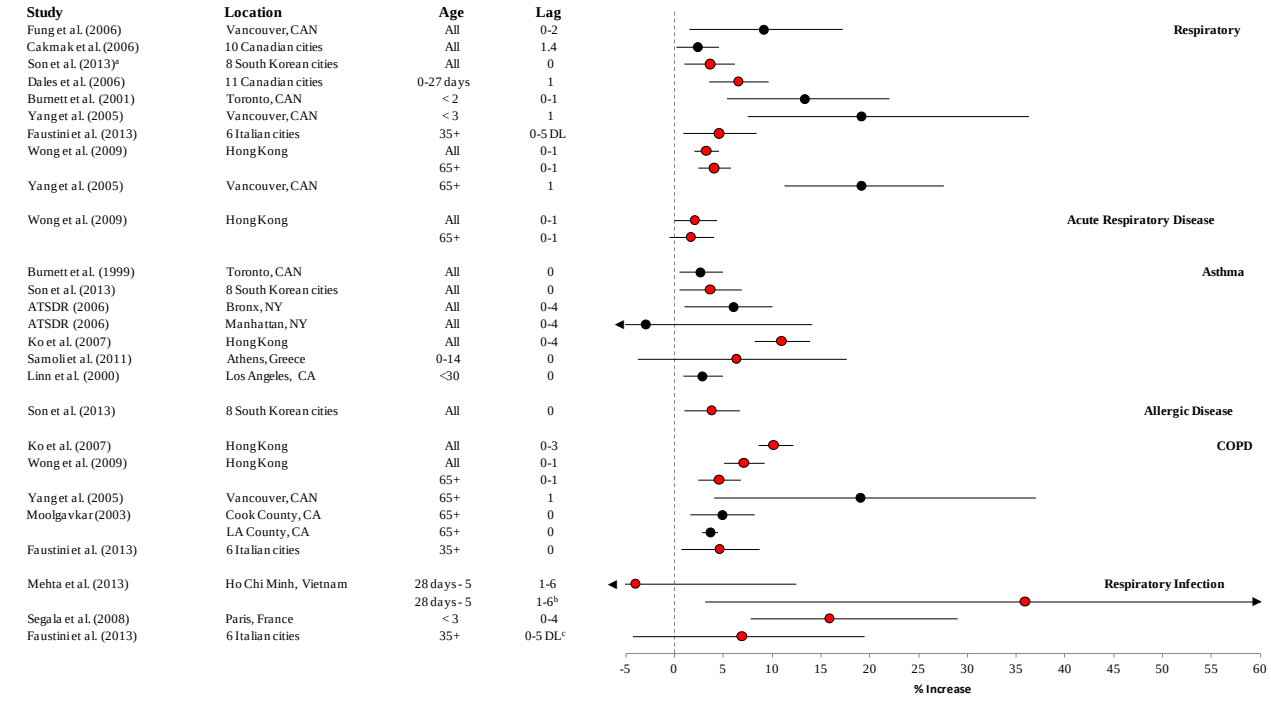
HEI Collaborative Working Group (2012) did not observe evidence to support the conclusions of Grineski et al. (2010) and Cakmak et al. (2006). HEI Collaborative Working Group (2012) examined SES and reported evidence that risk associated with NO_2 was greater in those individuals that resided in the higher quartiles (i.e., have the lowest district-level poverty prevalence). The authors speculate that these results could be due to the fact the districts that comprise the higher quartiles are located in the urban center of Ho Chi Minh City, Vietnam, and results may reflect the effects of increased exposures of residents living in the city center (HEI Collaborative Working Group, 2012).

Summary of Hospital Admission Studies

Recent studies that examined the association between short-term NO_2 exposure and respiratory-related hospital admissions found consistent positive associations for both all respiratory and cause-specific outcomes. The evidence from recent studies supports the results from the U.S. and Canadian studies evaluated in the 2008 ISA for Oxides of Nitrogen (Figure 4-4). Similar to the 2008 ISA for Oxides of Nitrogen, the majority of recent studies focused on all respiratory hospital admissions and provided more limited evidence from studies that examined cause-specific outcomes. However, the overall body of evidence indicates an association with both asthma and COPD hospital admissions with more limited evidence for respiratory infection. Across outcomes, the strongest associations were primarily observed within the first few days after exposure (i.e., lags ranging from 0-3 days). Most studies also demonstrated that associations between short-

term NO_2 exposures and respiratory-related hospital admissions remained relatively robust in copollutant models (i.e., similar in magnitude or attenuated slightly, but remaining positive). Overall, in the majority of studies NO_2 was not found to be highly correlated with other combustion-related pollutants (i.e., $r<0.60$ for CO and $PM_{2.5}$).

An examination of model specification indicates the NO_2-respiratory hospital admissions relationship is senstive to using less than 6 df per year to account for temporal trends, but robust to alternative lags and df for weather covariates (Son et al., 2013). Studies that examined potential seasonal differences in associations across outcomes provide some evidence of seasonal differences with NO_2 effects being greater in the summer in studies conducted in the U.S., Canada, and Europe, but the season with the largest effect was found to vary depending on the study location (i.e., cold season in some Asian cities). A number of studies examined potential effect modifiers of the association between NO_2 exposure and respiratory-related hospital admissions. These studies continue to provide evidence of larger effects for children and older adults with preliminary evidence that black children, individuals with pre-existing diseases, and individuals of low SES (i.e., no health insurance, low educational attainment, or low income) may be at increased risk of an NO_2-related respiratory hospital admission. Additionally, there was inconsistent evidence for differences in the risk of NO_2-related respiratory hospital admissions by sex.



Note: Effect estimates were standardized to a 20-ppb increase in 24-h avg or 30-ppb increase in 1-h max NO_2 concentrations. All effect estimates presented [except Ségala et al. (2008), which focused on winter time air pollution], are for all-year analyses.
Black symbols = U.S. and Canadian studies from the 2008 ISA for Oxides of Nitrogen,
Red symbols = recent studies.
DL = distributed lag.
a = Respiratory diseases for this study was defined as croup, pneumonia, bronchiolitis, respiratory infection inlcuding bronchitis, chronic obstructive pulmonary disease, asthma, and pneumonitis;
b = dry season results; and
c = lower respiratory tract infections in individuals with COPD.

Figure 4-4 Percent increase in respiratory-related hospital admissions for a 20 ppb increse in 24-h avg or 30-ppb increase in 1-h max NO_2 concentrations from U.S. and Canadian studies evaluated in the 2008 ISA for Oxides of Nitrogen and recent studies.

Table 4-21 Corresponding percent increase (95% CI) for studies presented in Figure 4-4.

Study	Location	Age	Lag	Avg Time	% Increase (95% CI)
Respiratory Diseases					
Fung et al. (2006)	Vancouver, CAN	All	0-2	24-h avg	9.1 (1.5, 17.2)
Cakmak et al. (2006)	10 Canadian cities	All	1.4	24-h avg	2.3 (0.2, 4.5)
Son et al. (2013)[b]	8 South Korean cities	All	0	24-h avg	3.6 (1.0, 6.1)
Dales et al. (2006)	11 Canadian cities	0-27 days	1	24-h avg	6.5 (3.5, 9.6)
Burnett et al. (2001)[a]	Toronto, CAN	<2	0-1	1-h max	13.3 (5.3, 22.0)
Yang et al. (2003)[a]	Vancouver, CAN	<3	1	24-h avg	19.1 (7.4, 36.3)
Faustini et al. (2013)	6 Italian cities	35+	0-5	24-h avg	4.6 (0.9, 8.3)
Wong et al. (2009)	Hong Kong	All	0-1	24-h avg	3.2 (1.9, 4.5)
		65+	0-1	24-h avg	4.0 (2.4, 5.7)
Yang et al. (2003)[a]	Vancouver, CAN	65+	1	24-h avg	19.1 (11.2, 27.5)
Asthma					
Burnett et al. (1999)[a]	Toronto, CAN	All	0	24-h avg	2.6 (0.5, 4.9)
Son et al. (2013)	8 South Korean cities	All	0	24-h avg	3.6 (0.5, 6.8)
ATSDR (2006)	Bronx, NY	All	0-4	24-h avg	6.0 (1.0, 10.0)
	Manhattan, NY	All	0-4	24-h avg	-3.0 (-18.0, 14.0)
Ko et al. (2007b)	Hong Kong	All	0-4	24-h avg	10.9 (8.1, 13.8)
Samoli et al. (2011)	Athens, Greece	0-14	0	1-h max	28.7 (-3.4, 71.3)
Linn et al. (2000)	Los Angeles, CA	<30	0	24-h avg	2.8 (0.8, 4.9)
Allergic Disease					
Son et al. (2013)	8 South Korean cities	All	0	24-h avg	3.8 (1.0, 6.6)
COPD					
Ko et al. (2007a)	Hong Kong	All	0-3	24-h avg	10.1 (8.5, 12.2)
Wong et al. (2009)	Hong Kong	All	0-1	24-h avg	7.1 (5.1, 9.1)
		65+	0-1	24-h avg	4.6 (2.4, 6.8)

Table 4-21 (Continued): Corresponding percent increase (95% CI) for studies presented in Figure 4-4.

Study	Location	Age	Lag	Avg Time	% Increase (95% CI)
Yang et al. (2005)	Vancouver, CAN	65+	1	24-h avg	19.0 (4.0, 37.0)
Moolgavkar (2003)	Cook County, IL	65+	0	24-h avg	4.9 (1.6, 8.2)
	LA County, CA	65+	0	24-h avg	3.6 (2.8, 4.5)
Faustini et al. (2013)	6 Italian cities	35+	0	24-h avg	4.6 (0.6, 8.6)
Acute Respiratory Disease					
Wong et al. (2009)	Hong Kong	All	0-1	24-h avg	2.1 (-0.1, 4.3)
		65+	0-1	24-h avg	1.7 (-0.6, 4.0)
Respiratory Infection					
Mehta et al. (2013)	Ho Chi Minh, Vietnam	28 days-5 yr	1-6	24-h avg	-4.0 (-18.0, 12.5)
		28 days-5 yr	1-6[c]	24-h avg	35.9 (3.0, 79.3)
Ségala et al. (2008)	Paris, France	<3	0-4	24-h avg	15.9 (7.7, 29.0)
Faustini et al. (2013)	6 Italian cities	35+	0-5[d]	24-h avg	6.9 (-4.3, 19.4)

Note: Studies correspond to studies presented in Figure 4-4.

[a]U.S. and Canadian studies from the 2008 ISA for Oxides of Nitrogen.

[b]Respiratory diseases for this study was defined as croup, pneumonia, bronchiolitis, respiratory infection inlcuding bronchitis, chronic obstructive pulmonary disease, asthma, and pneumonitis.

[c]Dry season results.

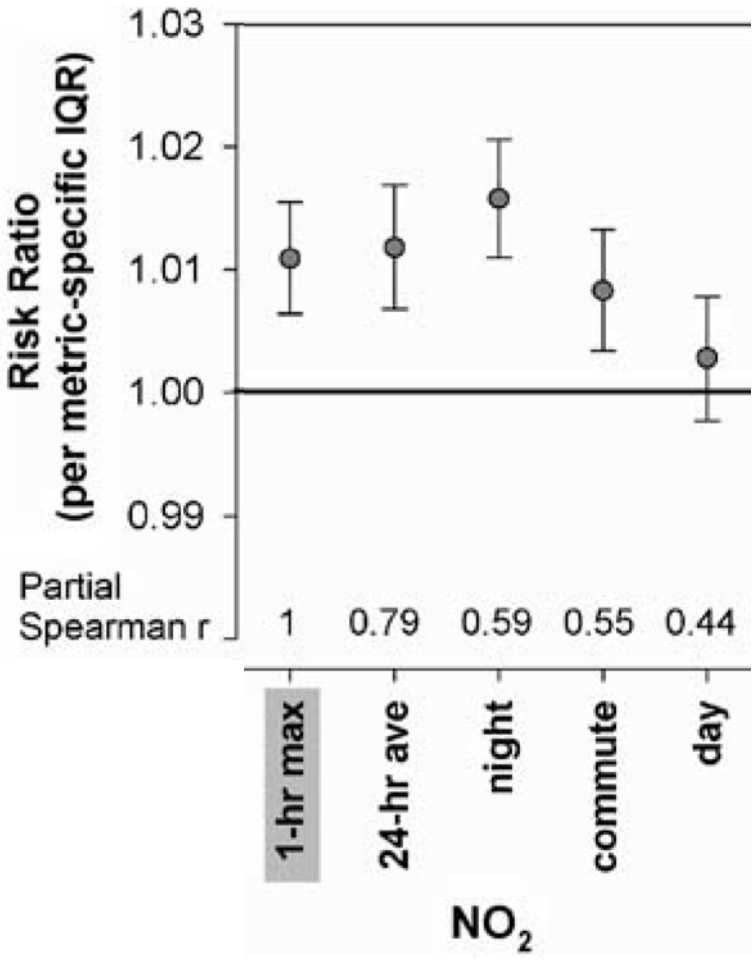
[d]Lower respiratory tract infections in individuals with COPD.

4.2.7.4 Emergency Department Visits

As mentioned previously, ED visit studies are evaluated separately because they often represent less serious and more common respiratory-related outcomes. Similar to the hospital admission studies evaluated above, the majority of ED visit studies that evaluated respiratory-related outcomes have been conducted in individual cities. However, compared to the hospital admission studies, a larger number of these studies have been conducted over longer study durations (i.e., >5 years). The following section evaluates the multi- and single-city studies detailed in Table 4-20 that examined the association between short-term NO_2 concentrations and respiratory-related ED visits. Several studies of ED visit are not included in this section because they do not fit the criteria outlined in Section 4.2.7.2. A comprehensive list of these studies can be found in Supplemental Table S4-1 (U.S. EPA, 2013d)

Respiratory Disease

ED visit studies evaluated in the 2008 ISA for Oxides of Nitrogen that focused on all respiratory outcomes were limited in number and focused almost exclusively on study populations consisting of all ages. These studies reported evidence of consistent positive associations between short-term NO_2 exposures and all respiratory ED visits. Building on the studies conducted in Atlanta, GA, and evaluated in the 2008 ISA for Oxides of Nitrogen, Peel et al. (2005), Tolbert et al. (2007), and Darrow et al. (2011a) conducted an analysis to examine whether the association between short-term air pollution exposures and respiratory ED visits differed depending on the exposure metric used (i.e., 1-h max, 24-h avg, commuting period [7:00 a.m. to 10:00 a.m.; 4:00 p.m. to 7:00 p.m.], daytime avg [8:00 a.m. to 7:00 p.m.] and night-time avg [12:00 a.m. to 6:00 a.m.]). 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. (2011a) reported relatively consistent results (using an a priori lag of 1 day) across exposure metrics with the largest estimate found for the night-time average and the smallest for the daytime metrics (Figure 4-5). 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, which is the other metric for NO_2 often used in epidemiologic studies.



Note: Partial Spearman correlation coeffiecient 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. (2011a)

Figure 4-5 Risk ratio and 95% CIs for associations between various lag 1 NO_2 metrics and respiratory ED visits.

Cause-specific Respiratory Outcomes

Asthma

In the 2008 ISA for Oxides of Nitrogen there was consistent evidence of a positive association between short-term NO_2 exposures and asthma ED visits with 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 Darrow et al. (2011a) and Tolbert et al. (2007). However, unlike Darrow et al. (2011a) and Tolbert et al. (2007), which used a single-site centrally located monitor, 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) observed a 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 robust to the inclusion of O_3 (quantitative results not presented).

Additional evidence for an association between short-term increases in NO_2 concentrations and asthma ED visits comes from studies conducted in Canada (Villeneuve et al., 2007) and Australia (Jalaludin et al., 2008). Villeneuve et al. (2007) in a study conducted in Edmonton, Alberta, Canada, in the population aged 2 years and older, 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). 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), but in copollutant models with CO, NO_2 associations with asthma ED visits remained robust (quantitative results not provided).

In a study conducted in Sydney, Australia 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. In addition to conducting the analysis focusing on ages 1-14, the authors also examined whether risks varied among age ranges within this study population. In the 1-14 years of age analysis, 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. The NO_2-asthma ED visit association was found to remain robust in copollutants models with the magnitude of the effect ranging from 4.2-6.1% increase in asthma ED visits. Additionally, NO_2 was moderately to weakly correlated with the other pollutants examined.

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 7 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. 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 versus 3-h avg pollutant concentrations).

Wheeze

Additional evidence for an association between NO_2 and respiratory-related ED visits comes from a study focusing on children, (ages 0-2 years) and conducted in 6 Italian cities (Orazzo et al., 2009). In this study, Orazzo et al. (2009) used data on wheeze extracted from medical records as an indicator of lower respiratory disease. This study examined daily counts of wheeze in relation to air pollution using a time-stratified case-crossover approach in which control days were matched on day of week in the same month and year as the case day. PM_{10}, SO_2, CO, and O_3 were also evaluated, but no correlations with NO_2 were reported or copollutants analyses conducted. The authors reported positive associations between short-term 24-h avg NO_2 exposures and wheeze ED visits when examining various lag averages (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.

COPD

To date, the majority of studies that have examined the association between short-term NO_2 exposures and COPD have focused on hospital admissions, with very limited evidence for ED visits. In the 7 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 avg 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 versus 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 Sao Paulo, Brazil, for

individuals over the age of 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, i.e., 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 conduct copollutant analyses, but NO_2 was moderately correlated with PM_{10} (r = 0.60), SO_2 (r = 0.63), and CO (r = 0.56).

Respiratory Infection

Recently some studies have examined the effect of air pollution on ED visits attributed to respiratory-related infections. Stieb et al. (2009), in their study of 7 Canadian cities, also examined the association between short-term NO_2 concentrations and respiratory infection ED visits. The authors reported postive 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), discussed in Section 4.2.7.3, in a study of winter (October-January) air pollution in Paris, France, 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 for a 20-ppb increase in 24-h avg NO_2 concentrations). 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 a new outcome for NO_2, 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 (7.9% [95% CI: 1.6, 12.8] for a 20-ppb increase in 24-h avg NO_2 concentrations). Interestingly the pattern of associations for CO was similar to that observed for NO_2, but the authors did not report correlations between pollutants or conduct copollutant analyses.

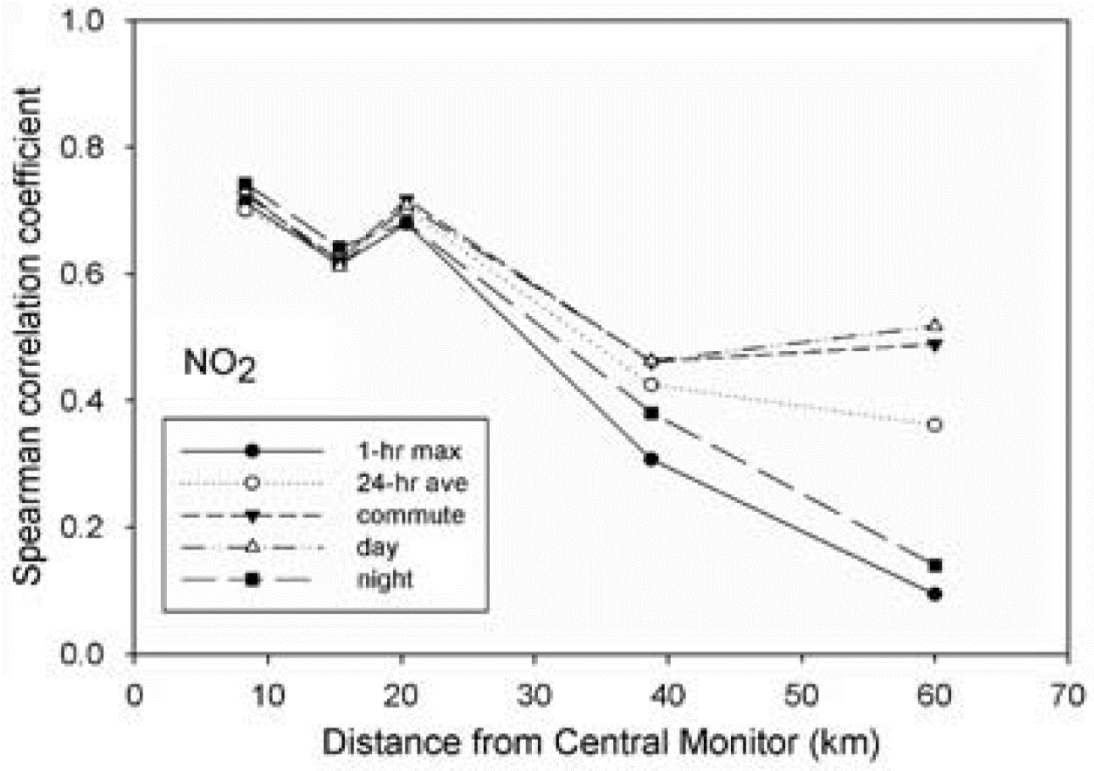
Sensitivity Analyses and Effect Modification of NO_2-Respiratory-Related ED Visits Relationship

Exposure Assignment

Questions often arise in air pollution epidemiologic studies with regard to the method used to assign exposure. Darrow et al. (2011a) and Strickland et al. (2011) in studies using ED visit data from Atlanta, GA assessed the effect of spatial variability of air pollutants and various exposure assignment approaches, respectively, on the relationship

between short-term NO_2 exposures and respiratory-related ED visits. Darrow et al. (2011a) in their analysis of multiple exposure metrics and respiratory ED visits also conducted an additional analysis to examine the spatial variability of each exposure metric (Figure 4-6).

Unlike O_3 and $PM_{2.5}$, which were found to be spatially homogenous, there was evidence that correlations for NO_2 metrics decreased dramatically as distance from the central monitor increased, especially for the 1-h max and night-time metrics (r<0.20) at 60 km. The 24-h avg metric was also reduced (r=~0.40), but not as dramatically as the 1-h max. Although reduced at greater distances, moderate correlations (r ~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.



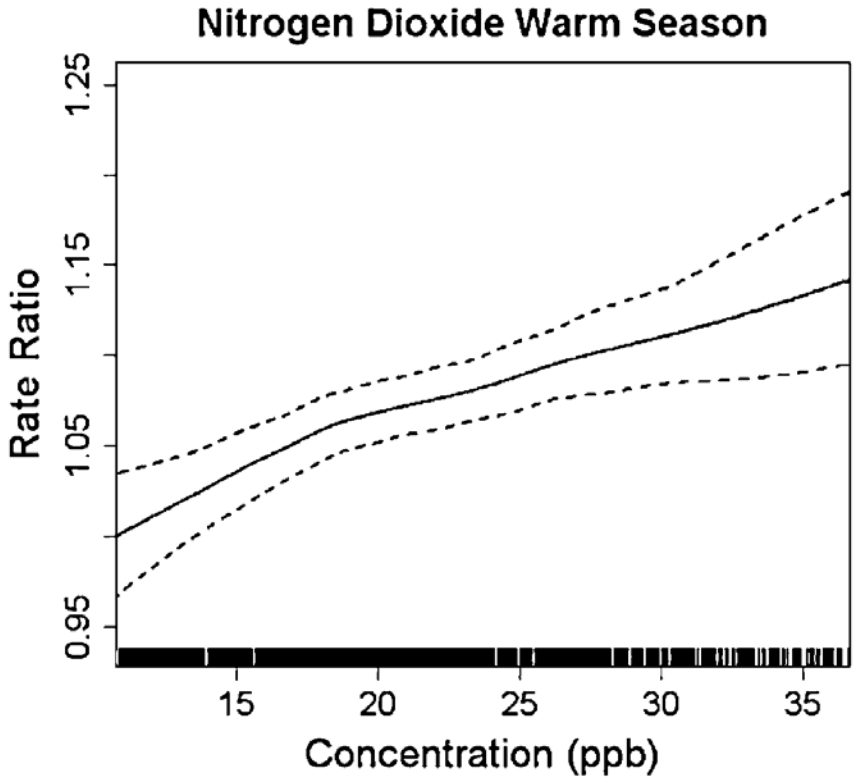
Source: Reprinted with permission of Nature Publishing Group Darrow et al. (2011a)

Figure 4-6 Spatial correlations for NO_2 metrics in the Atlanta, GA area.

Using data from the warm season from a previous analysis, Strickland et al. (2010) and Strickland et al. (2011) examined the relative influence of different exposure assignment approaches (i.e., central monitor, un-weighted average across available monitors, and population-weighted average) on the magnitude and direction of associations betwen NO_2 and pediatric asthma hospital admission. Strickland et al. (2011) reported that effect estimates per interquartile range (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). Therefore, Strickland et al. (2011) demonstrate that the different approaches used to assign exposure across the studies evaluated may alter the magnitude, but not direction, of the associations observed.

Concentration-Response (C-R) Relationship

To date, few studies have examined the 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 LOESS (locally weighted scatterplot smoothing) 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 pecentile (Figure 4-7). Collectively, these analyses do not provide evidence of a threshold.



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

Figure 4-7 LOESS 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) NO_2 concentrations and ED 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) examined 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]). It is important to note that the analysis that assumed a deviation in linearity did not assume zero risk below the inflection point. The focus of the analysis was on identifying whether risk increased above that 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.

Seasonal Differences

A number of the respiratory-related ED visit studies also conducted seasonal analyses. These studies found seasonal patterns similar to those observed in the hospital admission studies indicating larger effects during the warm or summer months.

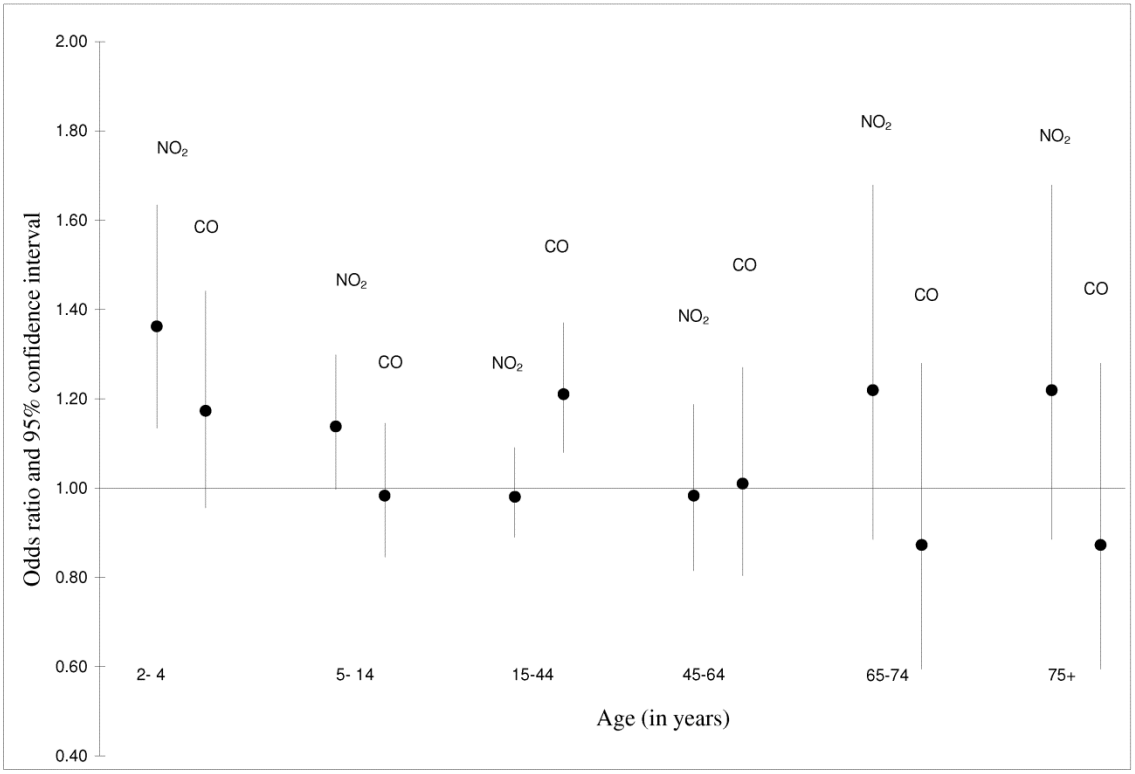
In the study of 6 Italian cities, Orazzo et al. (2009) reported slightly larger respiratory disease ED visits associations in the summer compared to the winter, but the confidence intervals were wide and overlapping (quantitative results not provided). Evidence of larger effects during warm or summer months was also found in studies of asthma ED visits. 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 in Edmonton, Canada. Additionally, Jalaludin et al. (2008) in a study conducted in Sydney, Australia found evidence of greater effects during the warm months (November-April) compared to the cold months (May-October). 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. Additionally, Zemek et al. (2010) in a study of otitis media ED visits in Alberta, Canada reported 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 for a 20-ppb increase in 24-h avg NO_2 concentrations.

In the study of 7 Canadian cities, Stieb et al. (2009) also conducted seasonal analyses, but did not present detailed results. However, the authors did state that no consistent associations were observed between any pollutants and the respiratory outcomes examined during the winter months (October-March).

Lifestage

A study that examined respiratory-related ED visits continues to provide evidence that children and older adults are at increased risk of NO_2-induced ED visits. Villeneuve et al. (2007) conducted an extensive analysis of the risk of NO_2-associated asthma ED visits across age ranges (i.e., 2-4, 5-14, 15-44, 45-64, 65-74, and 75+ years). In the warm season (April-September), where the greatest effects were estimated, across age ranges the risk of asthma ED visits was greatest for the ages 2-4 years and 75+ years, with elevated risks also observed for 5-14 years of age. There was no evidence of increased risk of NO_2-associated asthma ED visits in the population 45-64 years of age. Age-

specific risk estimates were also examined in copollutant models with CO and overall were relatively robust, except for the age range of 15-44 years (Figure 4-8).



Note: NO_2 odds ratios represent copollutant models with CO, while CO odds ratios represent copollutant models with NO_2.
Source: Reprinted with permission of Biomed Central Ltd (Villeneuve et al., 2007).

Figure 4-8 Age-specific NO_2 asthma ED visit effect estimates from copollutant models with CO in Edmonton, Canada.

Arbex et al. (2009) in a study in Sao Paulo, Brazil, examined respiratory ED visits in ages 40-64 and ≥ 65 years. Of the outcomes examined, NO_2 results were reported only for COPD and the population 65 years of age and older because larger effects were estimated for this age range compared to ages 40-64 years and ages >40 years.

Sex

Of the respiratory-related ED visit studies evaluated, only Zemek et al. (2010) examined whether there were differences in risk by sex. Focusing on the lag that showed the strongest association in the combined analysis (lag 2), Zemek et al. (2010) reported

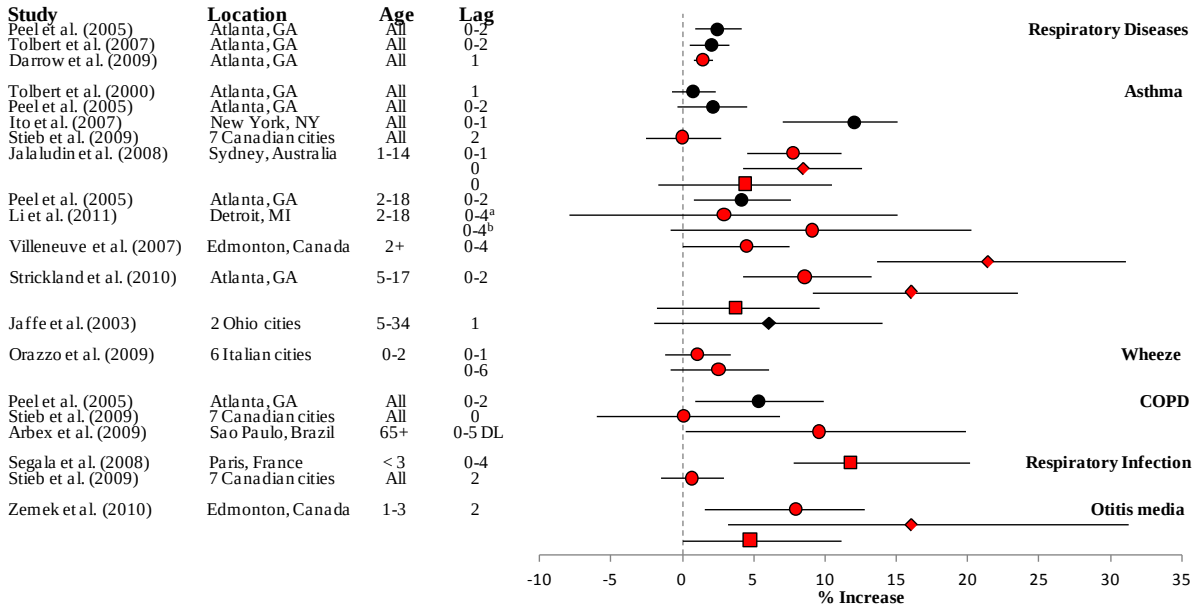
evidence of greater risk for NO_2-associated otitis media ED visits in females (9.5% [95% CI: 1.6, 17.1] for a 20-ppb increase in 24-h avg NO_2 concentrations) compared to males (4.7% [95% CI: -1.6, 12.80]). There especially was a dramatic difference in the warm months (32.9% for females compared to 6.3% for males).

Summary of ED Visit Studies

Recent respiratory-related ED visit studies build on the body of evidence from the 2008 ISA for Oxides of Nitrogen (Figure 4-9 and Table 4-22). These studies generally provide evidence of consistent positive associations, particularly for respiratory disease and asthma ED visits, with associations primarily observed at lags ranging from 0-2 days. In these studies copollutant confounding was not consistently examined. Of the studies that conducted copollutant modeling, NO_2 associations were consistently found to remain robust (i.e., similar in magnitude or attenuated slightly, but remaining positive). Overall, in the majority of studies NO_2 was not found to be highly correlated with other combustion-related pollutants, i.e., $r < 0.60$ for CO and $PM_{2.5}$.

In studies that examined various exposure-related issues and respiratory-related ED visits, it was identified that the 1-h max and 24-h avg exposure metrics for NO_2 result in similar associations (Darrow et al., 2011a). It was also found that the exposure assignment approach used can influence the magnitude, but not direction, of the NO_2-asthma ED visit risk estimate (Strickland et al., 2011). Additionally, it was demonstrated that the correlation between the NO_2 concentration for various exposure metrics and the NO_2 concentration at the monitor drops off as distance from monitor increases, which could lead to exposure misclassification (Darrow et al., 2011a). Although informative, it should be noted that all of these studies were conducted in one location (i.e., Atlanta, GA) using a similar dataset.

An examination of the C-R relationship for NO_2-respiratory-related ED visits provides evidence of a linear, no-threshold relationship. An examination of seasonal analyses indicates that associations between NO_2 and respiratory ED visits are greater in the warm or summer months, specifically in locations where hospital admissions studies provided similar evidence. Although limited in number, studies that examined potential effect modifiers of the NO_2-respiratory-related ED visits relationship continue to support larger effects in children and older adults.



Note: Black = U.S. and Canadian studies from the 2008 ISA for Oxides of Nitrogen, Red = recent studies. Circles = all-year, Diamonds = summer/warm, and Squares = winter/cold. a = time-series analysis results; and b = case-crossover analysis results.

Figure 4-9 Percent increase in respiratory-related ED visits for a 20-ppb increase in 24-h avg or 30-ppb increase in 1-h max NO_2 concentrations from U.S. and Canadian studies evaluated in the 2008 ISA for Oxides of Nitrogen and recent studies in all-year and seasonal analyses.

Table 4-22 Corresponding percent increase (95% CI) for studies presented in Figure 4-9.

Study	Location	Age	Avg Time	Season	Lag	% Increase (95% CI)
Respiratory Diseases						
Peel et al. (2005)[a]	Atlanta, GA	All	1-h max	All	0-2	2.4 (0.9, 4.1)
Tolbert et al. (2007)[a]	Atlanta, GA	All	1-h max	All	0-2	2.0 (0.5, 3.3)
Darrow et al. (2011a)	Atlanta, GA	All	1-h max	All	1	1.4 (0.8, 2.1)
					0-6	2.5 (-0.9, 6.0)
Asthma						
Tolbert et al. (2000)[a]	Atlanta, GA	All	1-h max	All	1	0.7 (-0.8, 2.3)
Peel et al. (2005)[a]	Atlanta, GA	All	1-h max	All	0-2	2.1 (-0.4, 4.5)
Ito et al. (2007)[a]	New York, NY	All	24-h avg	All	0-1	12.0 (7.0, 15.0)

Table 4-22 (Continued): Corresponding percent increase (95% CI) for studies presented in Figure 4-9.

Study	Location	Age	Avg Time	Season	Lag	% Increase (95% CI)
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)
Jalaludin et al. (2008)	Sydney, Australia	1-14	1-h max	Warm	0	8.4 (4.2, 12.5)
				Cold	0	4.4 (-1.7, 10.4)
Peel et al. (2005)[a]	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[b]	2.9 (-7.9, 15.1)
				All	0-4[c]	9.1 (-0.8, 20.2)
Villeneuve et al. (2007)	Edmonton, CAN	2+	24-h avg	All	0-4	4.5 (0.0, 7.5)
				Warm		21.4 (13.6, 31.0)
Strickland et al. (2010)	Atlanta, GA	5-17	1-h max	All	0-2	8.6 (4.2, 13.3)
				Warm		16.0 (9.1, 23.5)
				Cold		3.8 (-1.9, 9.6)
Jaffe et al. (2003)[a]	2 Ohio cities	5-34	24-h avg	Summer	1	6.1 (-2.0, 14.0)
Wheeze						
Orazzo et al. (2009)	6 Italian cities	0-2	24-h avg	All	0-1	1.1 (-1.2, 3.4)
COPD						
Peel et al. (2005)[a]	Atlanta, GA	All	1-h max	All	0-2	5.3 (0.9, 9.9)
Stieb et al. (2009)	7 Canadian cities	All	24-h avg	All	0	0.1 (-6.1, 6.8)
Arbex et al. (2009)	Sao Paulo, Brazil	65+	24-h avg	All	0-5 DL	9.6 (0.2, 19.9)
Respiratory Infection						
Ségala et al. (2008)	Paris, France	<3	24-h avg	Winter	0-4	11.8 (7.7, 20.1)
Stieb et al. (2009)	7 Canadian cities	All	24-h avg	All	2	0.7 (-1.5, 2.8)
Otitis media						
Zemek et al. (2010)	Edmonton, CAN	1-3	24-h avg	All	2	7.9 (1.6, 12.8)
				Warm		16.1 (3.1, 31.2)
				Cold		4.7 (0.0, 11.2)

Note:Studies correspond to studies presented in Figure 4-9.
[a]Studies evaluated in the 2008 ISA for Oxides of Nitrogen.
[b]Time-series analysis results.
[c]Case-crossover analysis results.

4.2.7.5 Outpatient and Physician Visit Studies

Several recent studies examined the association between ambient NO_2 concentrations and physician or outpatient (non-hospital, non-ED) visits for acute conditions in various geographic locations. Burra et al. (2009) examined asthma physician visits among patients aged 1-17 and 18-64 years in Toronto, Canada in a study focusing on differences by sex and income within age categories. 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 was found to be similar between sexes, income quintiles, both within and between ages. In a study conducted in Atlanta, GA, Sinclair et al. (2010) examined the association of acute asthma and respiratory infection (e.g., upper respiratory infections, lower respiratory infections) outpatient visits from a managed care organization. The authors separated the analysis into two time periods (the first 25 months of the study period and the second 28 months of the study period), in order to compare the air pollutant concentrations and relationships between air pollutants and acute respiratory visits for the 25-month time-period examined in Sinclair and Tolsma (2004) (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). 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 (both child and adult), as well as upper and lower respiratory infections. However, the confidence intervals for outcomes with smaller counts (i.e., approximately 12 per day for adult asthma and LRI, and 18 per day for child asthma compared to 263 per day for URI) were relatively large. An examination of potential seasonal differences in the association between air pollution exposures and child asthma visits produced evidence of larger risk estimates in the warm season at all lags, only in 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.

(Villeneuve et al., 2006b) examined the effect of short-term NO_2 exposure on allergic rhinitis physician visits among individuals aged 65 or older in Toronto, Canada. The authors reported a strong association between allergic rhinitis physician visits and ambient NO_2 concentrations at lag 0 in all-year and cold season (November-April) analyses (results not presented quantitatively), but not any other lag day. A similar

pattern of associations was observed with SO_2, but the authors did not examine correlations between pollutants or conduct copollutant analyses. Overall, the interpretation of these results is complicated because of the lack of a consistent association across the lags examined.

4.2.8 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 found consistent positive associations with respiratory mortality with some evidence indicating that the magnitude of the effect was larger compared to total and cardiovascular mortality. Recent multi-city studies conducted in Asia (Wong et al., 2008b), China (Chen et al., 2012b), and Italy (Chiusolo et al., 2011) add to the initial body of evidence indicating larger respiratory mortality effects (Section 4.4, Figure 4-17). However, an additional multi-city 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, inconsistent with the results of the original analysis and complicating interpretation of whether there is differential risk among mortality outcomes.

The initial observation of potentially larger respiratory mortality risk estimates was further examined in a few studies by examining the potential confounding effects of copollutants. Chen et al. (2012b) in the 17 Chinese cities 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 (10.1% [95% CI: 5.7, 14.5]; with PM_{10}: 6.9% [95% CI: 3.0, 11.0]; with SO_2: 7.2% [95% CI: 3.2, 11.3]; for a 20-ppb increase in 24-h avg NO_2 concentrations at lag 0-1 days). 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 11 Italian cities. In both an all year analysis of NO_2 with PM_{10} (NO_2: 14.1% [95% CI: 2.9, 26.4]; NO_2 + PM_{10}: 13.7% [95% CI: 3.0, 25.5]; 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: 42.4% [95% CI: 16.6, 73.9]; NO_2 + O_3: 44.6% [95% CI: 15.0, 81.9]; for a 20-ppb increase in NO_2 concentrations at lag 1-5 days) NO_2 associations with respiratory mortality were relatively unchanged. Overall, the limited number of studies that have examined the potential confounding effects of copollutants on the NO_2-respiratory mortality relationship indicate that associations remain robust.

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 1.5% to 9.4% 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 winter season analyses. 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 4.4, studies conducted in Asian cities observed much different seasonal patterns and it remains unclear if the seasonal patterns observed for total mortality would be similar to those observed for respiratory mortality in these cities.

4.2.9 Summary and Causal Determination

Evidence indicates that a causal relationship exists between short-term NO_2 exposure and respiratory effects based primarily on the coherence among multiple lines of evidence that indicate increases in asthma morbidity. There also is some support for NO_2-related effects on impaired host defense, COPD, and respiratory mortality, but coherence among various lines of evidence is limited. The determination of a causal relationship represents a change from the "sufficient to infer a likely causal relationship" determined in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c).

Consistent with previous findings, recent epidemiologic results continue to demonstrate associations between increases in ambient NO_2 concentrations and increases in hospital admissions and ED visits for asthma and respiratory symptoms and pulmonary inflammation in children with asthma. As in the 2008 ISA for Oxides of Nitrogen, biological plausibility is demonstrated by NO_2-induced AHR, pulmonary inflammation, and impaired host defenses in controlled human exposure and animal toxicological studies. However, recent studies address the key uncertainty identified in the 2008 ISA for Oxides of Nitrogen regarding the potential for NO_2 to serve as an indicator for another combustion-related pollutant or mixture. Recent results from copollutant models additionally demonstrate ambient NO_2-associated increases in asthma and respiratory effects in diverse geographic locations with adjustment for copollutants such as PM, PM components, O_3, SO_2, and CO. The evidence for a causal relationship between short-term exposure to NO_2 and respiratory effects 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 framework is presented in Table 4-23.

A causal relationship between short-term NO_2 exposure and respiratory effects is strongly supported by the coherence of evidence across related outcomes and disciplines indicating increases in asthma morbidity. Epidemiologic studies consistently demonstrate associations between increases in ambient NO_2 concentration and increases in asthma hospital admissions and ED visits (Son et al., 2013; Li et al., 2011b; Strickland et al., 2010; Ito et al., 2007; Ko et al., 2007b; Villeneuve et al., 2007) among subjects of all ages and in children. Risk estimates ranged from a 2.1% to 12% increase per 20-ppb increase in 24-h avg NO_2 or 30-ppb increase in 1-h max NO_2. These observations are supported by evidence in children and adults with asthma for increases in respiratory symptoms (Mann et al., 2010; Schildcrout et al., 2006; Gent et al., 2003; Mortimer et al., 2002), the major reason for seeking medical treatment. Recent epidemiologic evidence substantiates the robustness of NO_2-associated asthma morbidity with additional results from studies conducted in diverse locations in the U.S., Canada, and Asia, including several multicity studies. Individual epidemiologic studies examined multiple outcomes and lags of exposure; however, the pattern of association observed with NO_2 does not indicate that a higher probability of findings due to chance explains the evidence. The epidemiologic findings specifically for respiratory symptoms are only weakly supported by findings from controlled human exposure studies, as NO_2-induced (120-4,000 ppb) increases in respiratory symptoms were found in some but not all studies of adults with asthma and one study of adolescents with asthma (Section 4.2.6.2).

Key biological plausibility for NO_2-associated asthma morbidity is provided by findings of NO_2-induced increases in AHR in controlled human exposure studies of adults with asthma. AHR can be a key contributor to increases in respiratory symptoms such as wheeze. Controlled human exposure studies demonstrated increases in AHR in adults with asthma at rest with the lowest NO_2 exposures examined in experimental studies, i.e., 200 to 300 ppb NO_2 for 30 minutes and 100 ppb for 1 hour (Section 4.2.2.2). Exposures in this range and up to 4,000 ppb were not found consistently to have a direct effect on changes in lung function in controlled human exposure of adults with asthma, and epidemiologic evidence in adults also is inconsistent. However, epidemiologic studies of children with asthma found NO_2-associated decrements in lung function measured under supervised conditions. Several of these study populations had high prevalence of atopy (e.g., 53-90%). Allergen-induced airway obstruction is a mechanism that can lead to lung function decrements and respiratory symptoms. Thus, the epidemiologic evidence for NO_2-associated lung function decrements in children with asthma supports the evidence for increases in respiratory symptoms in children with asthma.

A causal relationship between short-term NO_2 exposure and respiratory effects also is supported by evidence characterizing potential mechanisms for NO_2-induced AHR and respiratory symptoms. Whereas changes in lung permeability were not consistently found

in experimental animals with ambient-relevant NO_2 exposures (Section 4.2.4.2), controlled human exposure studies and animal toxicological studies indicate the effects of NO_2 on pulmonary oxidative stress (Sections 4.2.4.1 and 4.2.4.2) and modification of adaptive and innate immunity (Sections 3.3.2.6 and 4.2.4.3). NO_2 exposures of 260-400 ppb enhanced allergic inflammation in humans with allergic asthma or animal models of allergic disease, as characterized by increases in Th2 cytokines, IgE, eosinophil activation, myeloperoxidase levels, and PMNs (Section 4.2.4.3). As allergic inflammation promotes bronchoconstriction and airway obstruction, the evidence describes key events to inform the mode of action for NO_2-associated increases in respiratory symptoms found in populations of children with asthma with atopy prevalence ranging from 53 to 100%. NO_2-related pulmonary inflammation also was demonstrated as increases in PMNs with 3- to 6-hour exposures to 1,500-3,500 ppb NO_2 in healthy adults (Section 4.2.4.1) and increases in eicosanoids (Section 4.2.4.1), which are involved in PMN recruitment. Several epidemiologic studies demonstrated associations between increases in ambient NO_2 concentrations and increases in pulmonary inflammation in individuals with asthma (Section 4.2.4.4). Recent epidemiologic studies in children added to the robustness of evidence by demonstrating associations with measures of NO_2 that account for spatial heterogeneity in ambient concentrations, including measures of personal and school outdoor exposures. Further, the recruitment of children from schools supports the likelihood that study populations were representative of the general population of children with asthma.

Additional evidence indicates that the respiratory effects of short-term NO_2 exposure extend beyond those specifically related to asthma morbidity. Epidemiologic studies demonstrate associations between ambient NO_2 concentrations and hospital admissions and ED visits for all respiratory causes combined (Faustini et al., 2013; Darrow et al., 2011a; Cakmak et al., 2006; Dales et al., 2006). Ambient NO_2-associated increases in pulmonary inflammation and respiratory symptoms (Sections 4.2.4.4 and 4.2.6.1) also were found in children in the general population. Ambient NO_2 concentrations were associated with respiratory infections, particularly in children; however, some effects were estimated with imprecision (Figure 4-4 and Figure 4-5, and Sections 4.2.5.1 and 4.2.7.3). There is clear evidence for impaired host defense demonstrated as mortality from bacterial or viral infection in animal models exposed to 1,500-5,000 ppb NO_2 (Section 4.2.5.1). Evidence also describes key events to inform the mode of action for impaired host defense, including NO_2-induced increases in pulmonary inflammation found in controlled human exposure and animal toxicological studies and diminished alveolar macrophage function found in some but not all studies (Section 4.2.5.3). Effects on pulmonary clearance were more variable (Section 4.2.5.2). Evidence supports associations of ambient NO_2 exposure with COPD exacerbations, primarily as increases in hospital admissions and ED visits for COPD (Sections 4.2.6.2 and 4.2.6.1). However,

in adults with COPD, NO_2 was not consistently related with increases in respiratory symptoms lung function decrements in epidemiologic or controlled human exposure studies. Epidemiologic studies also demonstrated NO_2-associated increases in respiratory mortality (Section 4.2.8). The spectrum of respiratory effects that can explain NO_2-related increases in respiratory mortality is not entirely clear. There is consistent evidence for the effects of NO_2 exposure on asthma, but limited coherence among outcomes and disciplines for effects on COPD and respiratory infections. Among the leading causes of mortality, COPD and respiratory infections are the ones related to respiratory causes (Hoyert and Xu, 2012).

Previous uncertainty regarding the independent effects of NO_2 exposure on respiratory outcomes is reduced by recent epidemiologic observations that NO_2 remains associated with respiratory effects with statistical adjustment for temperature, relative humidity, season, long-term time trends, and particularly copollutant concentrations. In a few studies, NO_2 associations were largely explained by copollutant exposure; however, among the studies that conducted copollutant modeling, most found that NO_2 associations persisted with adjustment for copollutants such as PM_{10}, $PM_{2.5}$, $PM_{10-2.5}$ (Figure 4-10), BC, EC, UFP, PNC (Figure 4-11), SO_2, O_3, and CO (Figure S4-1U.S. EPA, 2013d). Many NO_2 associations remained robust to copollutant adjustment, although confounding by any particular copollutant was examined to a limited extent and not all potentially correlated pollutants were examined. Further, the interpretation of copollutant models can be limited and methods to adjust for multiple copollutants simultaneously are not reliable. Thus, the potential for residual confounding is recognized (Section 1.5). In some cases, the loss of precision in NO_2 effect estimates was magnified because the increment used to standardize effect estimates is much larger than the variability in NO_2 concentrations reported in the study (Martins et al., 2012; Strak et al., 2012; Mann et al., 2010; Schwartz et al., 1994). There also was some evidence that NO_2 exposure confounded respiratory effects associated with copollutants. Among copollutants, NO_2 typically is more highly correlated with CO, BC, and UFP (Figure 2-20). Time series provided evidence for NO_2 associations independent of CO, and panel studies indicated associations independent of BC and UFP. There was previous evidence of NO_2-associated respiratory effects with adjustment for copollutants, but recent findings strengthen the evidence with additional studies of respiratory hospital admissions and ED visits conducted in diverse locations and additional panel studies with strong exposure assessment by modeling individual subjects' outdoor exposures or monitoring exposures during outdoor activity (Martins et al., 2012; Strak et al., 2012).

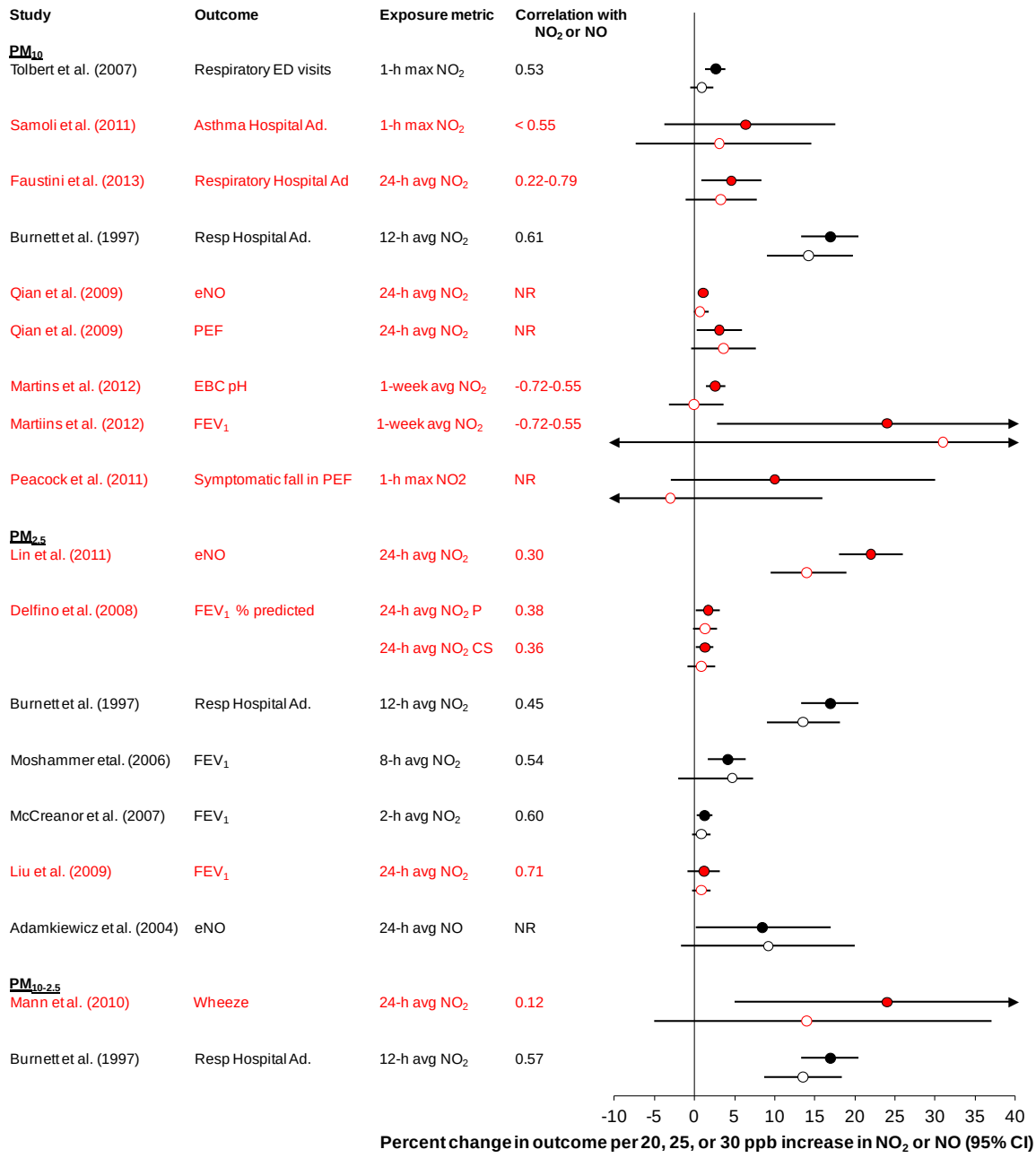
Other lines of evidence indicate that NO_2-associated respiratory effects are independent of copollutants. Larger ambient NO_2-associated increases in respiratory hospital admissions and ED visits were found in the warm season, during which the potential for

confounding by $PM_{2.5}$ may be lower because of lower NO_2-$PM_{2.5}$ correlations in the warm than cold season (Section 2.6.4.1). Correlations between NO_2 and O_3 remain low in the warm season. Recent studies continue to support associations between short-term increases in indoor NO_2 exposures and increases in respiratory effects in children with asthma (Sections 4.2.4.5 and 4.2.6.3). In schools in Ciudad Juarez, Mexico, correlations between NO_2 and copollutants such as BC, PM, and SO_2 differed between the indoor and outdoor environment, suggesting that NO_2 was part of a different pollutant mixture indoors and outdoors. Thus, the coherence of evidence for respiratory effects related to indoor and outdoor NO_2 exposure supports the independent effects of NO_2 exposure.

Most epidemiologic studies assessed NO_2 exposures from central monitoring stations; however, personal, outdoor school, and near-road exposures also were associated with respiratory effects in children and adults with asthma. Among studies that compared exposure assessment methods, results did not consistently estimate stronger respiratory effects for personal or outdoor school NO_2 concentrations than indoor or central site NO_2 (Greenwald et al., 2013; Sarnat et al., 2012; Delfino et al., 2008a; 2006). Most evidence for respiratory effects was related to multiday lags of NO_2 of 2 to 5 days, but associations also were found with single-day lags of 0 or 1 day. Comparisons among lags did not clearly indicate a stronger association for a particular lag. Respiratory hospital admissions and ED visits were associated with 24-h avg and 1-h max NO_2 whereas pulmonary inflammation and respiratory symptoms were associated primarily with 24-h avg NO_2. In the few studies that compared averaging times, no clear difference was found in the magnitude of association for respiratory hospital admissions or ED visits. 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 distribution of 24-h avg or 1-h max ambient NO_2 concentrations examined (Li et al., 2011b; Strickland et al., 2010).

In conclusion, multiple lines of evidence support a relationship between short-term NO_2 exposure and asthma morbidity, particularly epidemiologic evidence for increases in asthma hospital admissions and ED visits and respiratory symptoms and pulmonary inflammation in children with asthma. Findings also point to NO_2-related effects on impaired host defense, COPD, and respiratory mortality, but there is limited coherence among various lines of evidence. The evidence for asthma is substantiated by additional findings from recent multicity studies conducted in diverse geographic locations and recent panel studies with strong exposure assessment characterized by monitoring of personal, outdoor school, or outdoor near-road exposures. Associations are found primarily for NO_2, both 24-h avg and 1-h max concentrations. Respiratory effects were found in association with multiday averages of ambient NO_2 of 2 to 5 days and also single-day lags of 0 or 1 day. Across studies finding NO_2-associated respiratory effects,

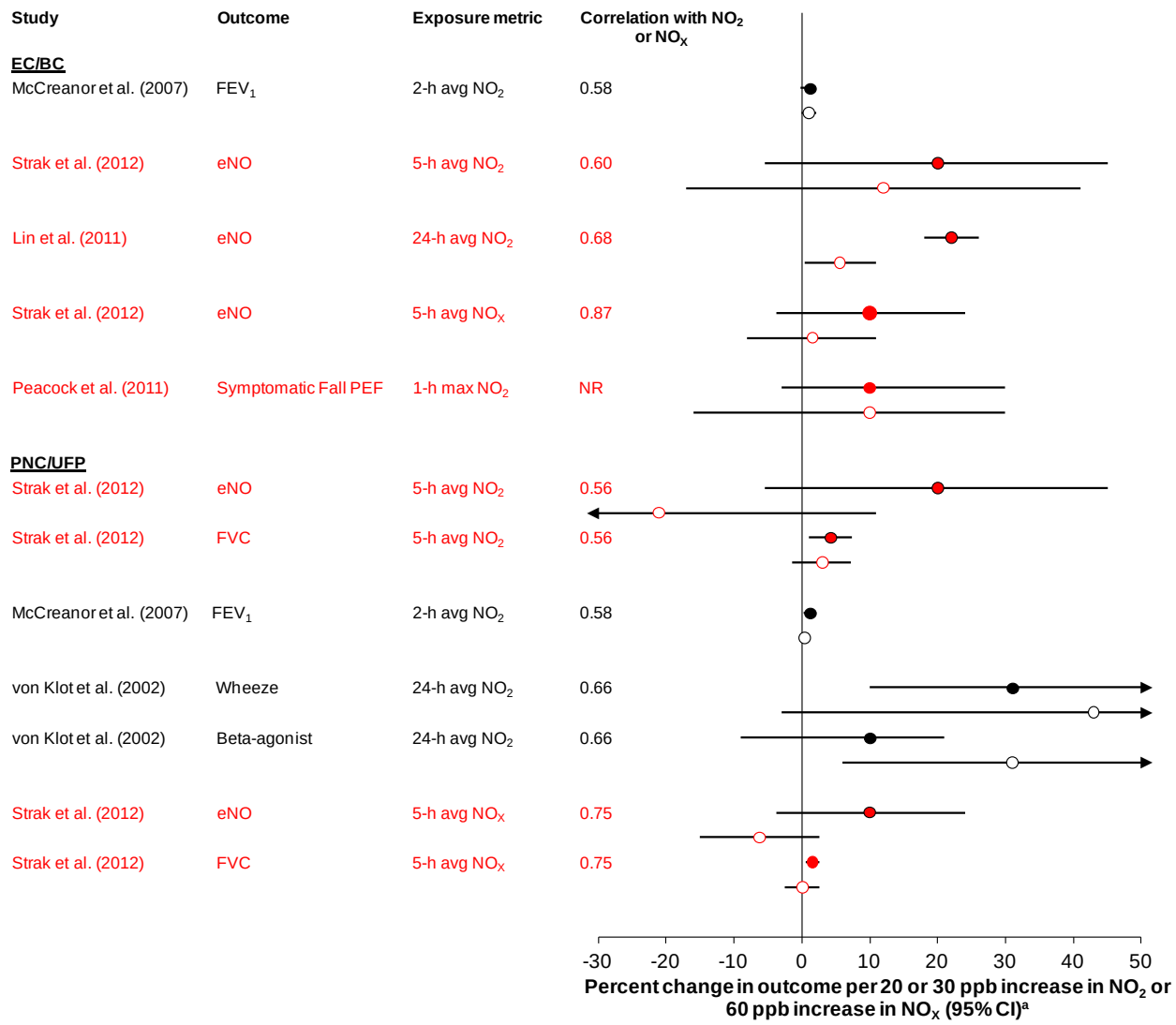
the range of mean ambient NO_2 concentrations was 11.7-36.9 ppb for 24-h avg NO_2 and 22.0-44.4 ppb for 1-h max NO_2. Associations of NO_2 with respiratory effects persist with adjustment for meteorological factors and for copollutants such as BC, EC, UFP, $PM_{2.5}$, PM_{10}, and CO. Because of limited analysis of potentially correlated copollutants and recognized limitations of copollutant models, the epidemiologic evidence combined with the biological plausibility provided by findings of NO_2-induced increases in AHR in adults with asthma in previous controlled human exposure studies provide compelling evidence for the independent respiratory effects of NO_2 exposure. NO_2-associated increases in oxidative stress and pulmonary inflammation, particularly allergic inflammation, describe key events to inform the modes of action for AHR, lung function decrements, and increases in asthma morbidity. The consistency and coherence of evidence for increases in asthma morbidity, including biological plausibility and copollutant-adjusted associations found for NO_2, with more limited evidence for COPD, impaired host defense, and respiratory mortality is sufficient to conclude that a causal relationship exists between short-term NO_2 exposure and respiratory effects.



Note: Magnitude and precision of effect estimates should not be compared among different outcomes. Results are organized by copollutant analyzed, then in order of decreasing correlation between NO_2 and copollutant. Studies not reporting correlations are presented thereafter. Range of correlations refers to correlations across cities or different times of outcome assessment. Percent change in FEV_1, PEF, or EBC pH refers to percent 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 effect estimate adjusted for a copollutant. ED = Emergency department, Resp Hospital Ad. = Respiratory-related Hospital Admission, eNO = Exhaled nitric oxide, PEF, Peak Expiratory Flow, EBC = Exhaled breath condensate, FEV_1 = Forced Expiratory Volume in 1 second, P = Personal NO_2, CS = Central site NO_2, NR = Not reported.

[a]Effect estimates standardized to a 20-ppb increase for 24-avg or 1-week average NO_2 or NO, a 25-ppb increase for 8-h or 12-h avg NO_2, and a 30-ppb increase for 1-h max or 2-h avg NO_2. Quantitative data are reported in Section 4.2.7.3 and Table 4-7 and Table 4-14.

Figure 4-10 Associations of ambient NO_2 or NO with respiratory outcomes adjusted for PM_{10}, $PM_{2.5}$, or $PM_{10-2.5}$.



Note: Magnitude and precision of effect estimates should not be compared among different outcomes. Results are organized by copollutant analyzed, then in order of decreasing correlation between NO_2 and copollutant. Studies not reporting correlations are presented thereafter. Percent change in FEV_1 refers to percent 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 effect estimate adjusted for a copollutant. FEV_1 = Forced Expiratory Volume in 1 second, PEF, Peak Expiratory Flow, eNO = Exhaled nitric oxide, NR = Not reported.

[a]Effect estimates standardized to a 20-ppb increase for 24-avg NO_2, a 30-ppb increase for 2-h avg or 5-h avg NO_2, and a 60-ppb increase for 2-h avg or 5-h avg NO_X. Quantitative data are reported in Table 4-7, Table 4-14, and Table 4-18.

Figure 4-11 Associations of ambient NO_2 or NO_X with respiratory outcomes adjusted for elemental carbon (EC) or black carbon (BC), ultrafine particles (UFP), or particle number concentration (PNC).

Table 4-23 Summary of evidence supporting a causal relationship between short-term 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 morbidity			
Consistent associations from multiple, high quality epidemiologic studies at relevant 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 in additional recent studies of all ages and children.	Strickland et al. (2010), Villeneuve et al. (2007), Ko et al. (2007b), Son et al. (2013), Ito et al. (2007), Li et al. (2011b) Sections 4.2.7.3, Figure 4-4	Overall study mean 24-h avg: 15.7-28.5 ppb Overall study mean 1-h max: 22.0-44.4 ppb
	No association in recent Canadian multicity study of all ages	Stieb et al. (2009)	Mean 24-h avg: 22.7 ppb
	Coherence with increases in respiratory symptoms in children with asthma in diverse populations in association with 24-h avg, 2-4 avg NO_2, 1-h max, lags 0, 3 to 6-day avg in previous and recent studies	U.S. multicity studies: Mortimer et al. (2002), Schildcrout et al. (2006) Gent et al. (2003), Mann et al. (2010), Section 4.2.6.1, Figure 4-3	Overall study mean 24-h avg: 14.2-28.6 ppb Overall study mean 1-h max: 37.4-66 ppb
	Decrements in lung function in children with asthma in recent studies; inconsistent results in adults with asthma. Associations with school outdoor, central site, personal NO_2.	O'Connor et al. (2008), Greenwald et al. (2013), Holguin et al. (2007), Delfino et al. (2008a), Section 4.2.3.1, Figure 4-1	Outdoor school-specific mean 1-week avg: 4.5-18.3 ppb Overall study mean 24-h avg personal: 26.8 ppb

Table 4-23 (Continued): Summary of evidence supporting a causal relationship between short-term NO_2 exposure and respiratory effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Chance, confounding, and other biases can be ruled out with reasonable confidence in part, by epidemiologic evidence	Across various outcomes and locations, NO_2 associations found with adjustment for weather, time trends in previous and recent studies Across various outcomes and locations, NO_2 associations persist in copollutant models adjusted for PM_{10}, $PM_{2.5}$, EC, BC, BS, UFP, CO, VOCs,O_3 Recent studies expand on evidence	Ko et al. (2007b), Strickland et al. (2010), Villeneuve et al. (2007), Jalaludin et al. (2008) Delfino et al. (2008a); Delfino et al. (2006), Martins et al. (2012), Qian et al. (2009a), Gent et al. (2003), Mann et al. (2010), von Klot et al. (2002) Figure 4-10 and Figure 4-11	Same as above
	Some associations were attenuated with adjustment for $PM_{2.5}$, SO_2, UFP	Samoli et al. (2011), Liu et al. (2009b),	
	Copollutants weakly-moderately correlated with NO_2 in many studies (r = -0.43 to 0.49)	Sarnat et al. (2012), Delfino et al. (2006), Martins et al. (2012), Gent et al. (2003)	
	Indoor NO_2 associated with increases in respiratory effects in children with asthma in previous and recent studies Consistent results across various lags of exposure and outcomes examined in previous and recent studies. Previous and recent panel studies of children examine representative populations recruited from schools.	Sarnat et al. (2012), Greenwald et al. (2013), Lu et al. (2013), Hansel et al. (2008)	
Chance, confounding, and other biases can be ruled out with reasonable confidence in part, by evidence from controlled human exposure studies	NO_2-induced increases in AHR in adults with asthma exposed at rest following nonspecific or allergen challenge in several individual previous studies and meta-analyses.	Folinsbee (1992) Section 4.2.2, Table 4-3, Table 4-4, Table 4-5	100 ppb for 1 h 200-300 ppb for 30 min

Table 4-23 (Continued): Summary of evidence supporting a causal relationship between short-term NO_2 exposure and respiratory effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Some evidence describes key events to inform mode of action			
Modification of innate and adaptive immunity	Increases in eosinophil activation, IgE, Th2 cytokines in adults and rats and guinea pigs	Barck et al. (2005a); Barck et al. (2002), Gilmour et al. (1996), Ohashi et al. (1994) Table 4-11, Table 4-12, Sections 3.3.2.6, and 4.2.4.3	Humans: 260 ppb 15-30 min Rats/guinea pigs: 3,000 ppb for 2 weeks, 5,000 ppb for 3 h
	No consistent effect on pulmonary clearance	Sections 4.2.5.2	1,500-3,500 ppb for 2-6 h
Initiation of inflammation	Increases in PMNs and prostaglandins in healthy adults	Section 4.2.4.1	5,000 ppb for 3 h
	Increases in eNO in children and adults with asthma in association with 2-h avg, 24-h avg NO_2 Recent studies expand on evidence.	Delfino et al. (2006), Sarnat et al. (2012), Martins et al. (2012), Qian et al. (2009a) Section 4.2.4.4	24-h avg: 26.8 ppb personal, 23.6 ppb ambient 1-week avg outdoor school: 4.5-18.3 ppb
Oxidative stress	Changes in antioxidants in experimental animals	Sections 3.3.2.3	
Alteration of epithelial barrier function	Increases in LDH, BALF protein, Clara cells in some but not all studies of humans, rats, guinea pigs	Sections 4.2.4.1, 4.2.4.2, 3.3.2.4	Animal models: 400 or 2,000 ppb for 1-3 weeks
COPD			
Inconsistent epidemiologic evidence	Increases in COPD hospital admissions and ED visits in additional recent studies	Faustini et al. (2013), Ko et al. (2007b), Arbex et al. (2009) Sections 4.2.7.2 and 4.2.7.5	Mean 24-h avg: 24.1-34.6 ppb Mean 1-h max: 63.0 ppb
	Lack of association with lung function decrements and symptoms in adults with COPD in previous and recent studies	Sections 4.2.3.1 and 4.2.6.1	
Inconsistent evidence from controlled human exposure studies	Increased inflammation in adults	See above for asthma morbidity	
	Inconsistent evidence for decreased lung function in adults with COPD in previous studies	Decreases found in: Morrow et al. (1992), Vagaggini et al. (1996), Section 4.2.3.2	300 ppb for 1 h, 4 h

Table 4-23 (Continued): Summary of evidence supporting a causal relationship between short-term NO_2 exposure and respiratory effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Impaired Host Defense			
Consistent animal toxicological evidence	Previous studies show 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 4.2.5.1	1,500-5,000 ppb for 1-8 h
Some epidemiologic evidence	Additional recent evidence for associations with hospital admissions/ED visits for respiratory infections and parental reports of infection, particularly in children. Some results have wide 95% CIs.	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 4.2.5.1, 4.2.7, 4.2.8	Mean 24-h avg: 11.7-34.6 ppb
Some evidence describes key events to inform mode of action			
Initiation of inflammation	See above for asthma morbidity		
Modification of innate and adaptive immunity	Diminished superoxide production in AM and bactericidal activity No consistent effect on pulmonary clearance	Section 4.2.5.2	
Respiratory Mortality			
Consistent epidemiologic evidence	Recent multicity studies add to evidence for associations of respiratory mortality with 24-h avg NO_2 at lag 0-1 days NO_2 results robust to adjustment for PM_{10}, SO_2, O_3	Wong et al. (2008b), Chen et al. (2012b), Chiusolo et al. (2011), Bellini et al. (2007), Biggeri et al. (2005)	Means across cities for 24-h avg: 13.5-55.5 ppb
Limited biological plausibility	Some evidence for asthma morbidity in adults but limited coherence among lines of evidence for COPD and respiratory infection. Uncertainty regarding spectrum of effects that can lead to respiratory mortality.		

[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, ≤ 5,000 ppb).

4.3 Cardiovascular Effects

4.3.1 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, 2008c). Specifically, the epidemiologic studies of heart rate variability (HRV), electrocardiographic markers of cardiac repolarization, and arrhythmic events among patients with implanted cardioverter defibrillators available at the time of the last review were inconsistent. Additionally, while multiple studies reviewed had found associations between NO_2 and rates of hospital admission or ED visits for cardiovascular diseases (CVDs), 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. Some evidence from toxicological studies was reported for effects of NO_2 on various hematological parameters in animals, but these studies were limited, inconsistent, and provided little biological plausibility for the cardiovascular effects observed in epidemiologic studies.

This section reviews the published studies pertaining to the cardiovascular effects of exposure to oxides of nitrogen in humans, animals, and cells. With the existing body of evidence serving as the foundation, where available, emphasis was placed on studies published since the 2008 ISA for Oxides of Nitrogen. As described in the following sections, the recent epidemiologic and toxicological publications strengthen the evidence for independent effects of NO_2 exposure on cardiovascular morbidity and mortality.

4.3.2 Arrhythmia and Cardiac Arrest

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, 2008c). There continues to be limited epidemiologic evidence for such an association, either from studies of patients with implantable cardioverter defibrillators (ICDs), studies of arrhythmias detected on ambulatory electrocardiographic (ECG) recordings, studies of out-of-hospital cardiac arrest, or studies of hospital admission with a primary discharge diagnosis related to arrhythmias (Table 4-24).

In terms of studies of patients with ICDs, Ljungman et al. (2008) found that NO_2 was positively associated with increased risk of confirmed ventricular tachyarrhythmias in a panel of patients with ICDs. 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 versus ventricular tachycardia), age, history of ischemic heart disease (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 ventricular tachyarrhythmias among the 22 subjects that were outdoors at the time of ICD activation. Because the study authors accounted for personal activity/behavior, exposure measurement error may have been reduced in the effect modification analysis (Section 2.6.5.2). 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) were able to review the electrocardiograms from only about 60% of ICD activations, 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 $\geq$ 30 seconds and NO_2 concentrations, though the effects associated with NO_2 were smaller than those observed for $PM_{2.5}$. The NO_2 associations were generally stronger when the authors used a 2-h lag compared to a 2-day lag. Finally, Metzger et al. (2007) observed generally null associations between NO_2 concentrations and ventricular tachyarrhythmic events over a 10-year period in Atlanta, GA. Using a different approach, 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 non-smoking patients with stable heart failure.

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. 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.

In summary, there is currently inconsistent epidemiologic evidence for an association between 24-h avg NO_2 or NO and risk of cardiac arrhythmias as examined in patients with ICDs, continuous ECG recordings, and out-of-hospital cardiac arrest. However, existing studies have focused almost exclusively on ventricular arrhythmias and are potentially limited by misclassification of the outcome.

Table 4-24 Epidemiologic studies of arrhythmia and cardiac arrest.

Study	Location (Sample Size)	Mean NO_2 (ppb)	Exposure assessment	Selected Effect Estimates[a] (95% CI)
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 seconds])	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 year: 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 year: 1.01 (0.94, 1.10) Events resulting in defibrillation: All year: 1.07 (0.93, 1.23)

Table 4-24 (Continued): Epidemiologic studies of arrhythmia and cardiac arrest.

Study	Location (Sample Size)	Mean NO_2 (ppb)	Exposure assessment	Selected Effect Estimates[a] (95% CI)
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)
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 0-3: 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

[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 and 1-h averaging times, respectively.

4.3.3 Heart Rate/Heart Rate Variability

HRV provides a non-invasive marker of autonomic nervous system function. 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). 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, an index influenced mainly by the parasympathetic nervous system) and low frequency (LF) components, as well as the ratio of the LF to HF components (LF/HF, an index of relative sympathovagal balance) (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Sympathetic stimulation increases the firing rate of pacemaker cells in the heart's sinoatrial node, thereby increasing heart rate (HR) as well as affecting the LF/HF ratio. On the other hand, parasympathetic stimulation decreases the firing rate of pacemaker cells and the HR and affects the HF component of HRV.

4.3.3.1 Epidemiologic Studies

The 2008 ISA for Oxides of Nitrogen found 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, 2008c). Additional studies are now available for review (Table 4-25) 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 multi-country ULTRA study assessed the longitudinal association between ambient pollution and HRV among elderly participants 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 18.8% (95% CI: -28.4%, -3.0%) decrease in LF/HF associated with a 20-ppb increase in 24-h NO_2 levels at lag 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 two-pollutant models, 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.

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. In this study, 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. In single-pollutant models, a 30-ppb increase in 1-h max NO_2 was associated with a 8.7% decrease (95% CI: −12.0%, −4.8%) in SDNN, a 20.5% decrease (95% CI: −26.7%, −9.2%) in LF. The association with SDNN was stronger among those with a higher 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. They observed increases in heart rate that were generally consistent in magnitude across lags from 0 to 6 days.

Several studies (Weichenthal et al., 2011; Laumbach et al., 2010; Suh and Zanobetti, 2010a) used exposure assessment techniques that would tend to reduce uncertainty in the exposure when compared to the use of central site monitors. Suh and Zanobetti (2010a) 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 personal exposures to NO_2 were associated with decreased HRV. Decreases in PNN50 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. Laumbach et al. (2010) studied the effects of in-vehicle exposure to traffic-related pollutants among a group of individuals with diabetes. The authors observed decreases in HF HRV about 1 day after the in-vehicle exposures, with effects that were similar, but smaller in magnitude, attributed to NO_2 concentrations. 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 levels 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 two-pollutant 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) found no association between NO_2 or NO

and indices of HRV in a repeated-measures study of non-smoking patients with stable heart failure. 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. Peel et al. (2011) examined data from 4,277 Atlanta-area infants prescribed home cardiorespiratory monitors and found a slightly elevated risk of bradycardia linked to 1-h maximum NO_2 concentrations over the past 2 days measured at a central site monitor. The clinical or public health significance of this finding is unclear.

The above studies have all focused on infants or participants with a documented history of heart disease. In contrast, HRV appears to be not 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). 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 from the multi-city Multi-Ethnic Study of Atherosclerosis (MESA), Park et al. (2010) found no association between NO_2 concentrations and indices of HRV. Participants in this study were 45–84 years old and free of cardiovascular disease. A cross-sectional study from Taipei also found no association between NO_2 and HRV among 46 elderly participants with cardiovascular disease risk factors (Chuang et al., 2007b). A cross-sectional study of 1,349 healthy participants in Taein Island, 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, HRV seems to be associated with changes in HRV 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.

Table 4-25 Epidemiologic studies of heart rate/heart rate variability.

Study	Location (Sample Size)	Mean NO_2 (ppb)	Exposure assessment	Selected Effect Estimates[a] (95% CI)	
Timonen et al. (2006)	Amsterdam, the Netherlands; Erfurt, Germany; Helsinki, Finland (n = 131)	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: -25.94 (-50.00, -1.88)
Huang et al. (2012a)	Beijing, China (n = 40)	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: -3.75 (-6.71, -0.59) 4-h: -8.54 (-12.48, -4.82) 12-h: -9.91 (-15.14, -4.40) r-MSSD (% Change) 1-h: 2.76 (-2.17, 7.70) 4-h: -4.82 (-12.48, 3.28) 12-h: -6.06 (-16.79, 5.50)	LF (% Change) 1-h: -10.66 (-18.36, -2.76) 4-h: -19.49 (-28.91, -9.42) 12-h: -21.74 (-35.23, -7.71) HF (% Change) 1-h: -6.91 (-16.18, 2.76) 4-h: -11.17 (-24.09, 2.85) 12-h: -10.18 (-28.62, 9.63)
Zanobetti et al. (2010)	Boston, MA (n = 46) (aged 43-75 years)	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	

Table 4-25 (Continued): Epidemiologic studies of heart rate/heart rate variability.

Study	Location (Sample Size)	Mean NO_2 (ppb)	Exposure assessment	Selected Effect Estimates[a] (95% CI)	
Barclay et al. (2009)	Aberdeen, Scotland, U.K. (n = 132)	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)	LF power NO_2: 2.353 (-1.052, 5.757) NO: 1.940 (-1.328, 5.207)
				SDNN (msec) NO_2: 0.619 (-0,588, 1.826) NO: 0.608 (-0.562, 1.778)	LF normalized NO_2: -0.857 (-2.817, 1.102) NO: -0.183 (-2.066, 1.699)
				SDANN (msec) NO_2: 0.512 (-0.865, 1.890) NO:0.570 (-0.766, 1.906)	HF power NO_2: 3.365 (-1.169, 7.900) NO: 2.895 (-1.457, 7.247)
				rMSSD (msec) NO_2: 0.398 (-0.003, 0.799) NO: 0.353 (-0.036, 0.742)	HF normalized NO_2: 0.722 (-1.554, 2.998) NO: 1.407 (-0.775, 3.558)
				PNN 50% NO_2: 1.568 (-3.851, 6.986) NO: 0.909 (-4.347, 6.165)	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)	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)	
Chuang et al. (2007a)	Taipei, Taiwan (n = 76)	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)	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	

Table 4-25 (Continued): Epidemiologic studies of heart rate/heart rate variability.

Study	Location (Sample Size)	Mean NO_2 (ppb)	Exposure assessment	Selected Effect Estimates[a] (95% CI)	
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)	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	
Chuang et al. (2007b)	Taipei, Taiwan (n = 46)	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)	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)

Table 4-25 (Continued): Epidemiologic studies of heart rate/heart rate variability.

Study	Location (Sample Size)	Mean NO_2 (ppb)	Exposure assessment	Selected Effect Estimates[a] (95% CI)	
Weichenthal et al. (2011)	Ottawa, Canada (n = 42)	1-h max NO_2 4.8	Central Monitor	ΔLF (ms^2)	ΔSDNN (msec)
				1-h: -532.5 (-2872.5, 1807.5)	1-h: -18.75 (-112.50, 72.00)
				2-h: 12.0 (-2467.5, 2490.0)	2-h: -75.0 (-150.0, -2.55)
				3-h: 577.5 (-2055.0, 3217.5)	3-h: -39.75 (-120.0, 40.50)
				4-h: -397.5 (-3532.5, 2025.0)	4-h: -12.00 (-82.50, 61.50)
				ΔHF (ms^2)	ΔrMSSD (msec)
				1-h: -420.0 (-1785.0, 952.5)	1-h: -12.00 (-48.75, 24.75)
				2-h: -487.5 (-1612.5, 637.5)	2-h: -12.00 (-41.25, 17.25)
				3-h: -24.0 (-1020.0, 975)	3-h: 2.33 (-30.0, 34.5)
				4-h: -247.5 (-1417.5, 922.5)	4-h: -2.10 (-33.0, 29.25)
				ΔLF:HF	ΔpNN50 (5)
				1-h: 5.70 (-2.10, 13.50)	1-h: -3.30 (-31.50, 24.75)
				2-h: 10.50 (2.63, 18.75)	2-h: -8.25 (-33.00, 15.75)
				3-h: 12.75 (4.20, 21.75)	3-h: -3.23 (-29.25, 22.50)
				4-h: 7.50 (-1.80, 17.25)	4-h: 1.28 (-26.25, 29.25)
Peel et al. (2011)	Atlanta, GA (n = 4,277)	1-h max NO_2 41.7 90th: 65.6 Max: 109.2	Central Monitor	Bradycardia (OR) 1.04 (1.00, 1.08)	
Williams et al. (2012b);	Detroit, MI (n = 65)	24-h avg NO_2 24.0 75th: 28.0 Max: 100.0	Personal Monitor	HR (bpm) bpm: -2.95 (-4.82, -0.80)	

Table 4-25 (Continued): Epidemiologic studies of heart rate/heart rate variability.

Study	Location (Sample Size)	Mean NO_2 (ppb)	Exposure assessment	Selected Effect Estimates[a] (95% CI)	
Suh and Zanobetti (2010a)	Atlanta, GA (n = 30)	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)
Laumbach et al. (2010)	New Brunswick, NJ (n = 21)	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)	
Rich et al. (2012)	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. Positive, but non-statistically significant increase in heart rate, generally consistent across lags from 0 to 6.	

[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 and 1-h averaging times, respectively.

4.3.3.2 Controlled Human Exposure Studies

HRV was not evaluated in previous controlled human exposure studies of NO_2, but was evaluated in two recent studies (Table 4-29). Huang et al. (2012b) recently 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, however 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 LFn (1 hour; $p= 0.021$) and cardiac t wave amplitude (18 hour; $p = 0.057$). CAPs exposure alone did not induce such changes. Vagal modulation of cardiac activity following NO_2 exposure was also observed in both a controlled human exposure and animal toxicological study (Section 3.3.2.8); however, at higher than ambient-relevant concentrations. Epidemiologic studies found NO_2-associated decreases in HRV primarily in adults with or at risk for cardiovascular disease. However, a recent study of adults with stable coronary heart disease and impaired left ventricular systolic function showed no statistically significant changes in HRV with 400 ppb NO_2 for exposure for 1 hour while seated and without exercise (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, 2008c, 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).

4.3.3.3 Toxicological Studies

Toxicology studies examining HRV changes were not available for review in the 2008 ISA for Oxides of Nitrogen. Consistent with controlled human exposure studies, a recent

study in rats found mixed evidence for changes in HR and HRV (Table 4-30). 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. 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 a pollutant are difficult to distinguish in a multipollutant model because of multicollinearity among pollutants.

4.3.4 ST-Segment Amplitude and QT-Interval Duration

ST-segment changes (either ST-segment elevation or depression) on the electrocardiogram are considered a nonspecific marker of myocardial ischemia. The QT interval provides an electrocardiographic marker of ventricular repolarization. Prolongation of the QT interval is associated with increased risk of life-threatening ventricular arrhythmias.

4.3.4.1 Epidemiologic Studies

The 2008 ISA for Oxides of Nitrogen did not review any epidemiologic studies of ambient oxides of nitrogen concentrations and markers of myocardial ischemia or ventricular repolarization (U.S. EPA, 2008c). A few recent studies examined these endpoints (Table 4-26). Chuang et al. (2008) conducted a repeated-measures study of Boston-area patients with a history of coronary heart disease and examined the association between ambient pollutants and ST-segment level changes. This study found an odds ratio of 3.29 (95% CI: 1.82, 5.92) for ST-segment depression of $\geq$ 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 two-pollutant model.

Delfino et al. (2011) used a similar design to study 38 elderly, nonsmoking residents of 4 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 to help address uncertainties related to exposure assessment. This study found an odds ratio of 10.13 (95% CI: 1.37, 74.23) for ST-segment depression $\geq$ 1.0 mm per 30-ppb increase in mean 1-h 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.

Within the context of the Veterans Administration Normative Aging Study, Baja et al. (2010) found that heart-rate corrected QT interval was not associated with the 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). The only prior study available for comparison found that 24-h avg NO_2 concentrations were positively associated with increased QT interval duration, but this association was imprecise, and the 6-hour moving average of NO_2 was not associated with QT interval duration (Henneberger et al., 2005).

In summary, a few available epidemiologic studies report an association between short-term exposure to NO_2 and ST-segment changes on the electrocardiogram of elderly participants 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 previous studies are available for comparison. Additionally, a recent study suggests a potential link between NO_2 and ventricular repolarization as assessed by QT interval duration, in contrast to a previous study.

Table 4-26 Epidemiologic studies of ST-segment amplitude and QT-interval duration.

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: 5.97 (2.45, 14.40)
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)
Baja et al. (2010)	Boston, MA (n = 580)	1-h max NO_2 19-21	City-wide avg	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)

[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 and 1-h averaging times, respectively.

4.3.4.2 Controlled Human Exposure Studies

Epidemiology studies report mixed results for an association between QT interval changes and NO_2 exposure. A recent controlled human exposure study (Huang et al., 2012b) also evaluated this endpoint (Table 4-29). The study found a borderline statistically significant decrease in QT interval corrected for heart rate (QTc) at 1 and 18 hours after a 2-hour exposure to 500 ppb NO_2 with exercise in healthy volunteers. In this study, NO_2 exposure also induced a 29.9% decrease ($p = 0.001$) in the QT variability index (an increase has been associated with arrhythmia). However, when volunteers were exposed to both $PM_{2.5}$ and NO_2 the QT variability index increased. Overall the various cardiovascular parameters examined in this study were mixed.

4.3.5 Blood Pressure

4.3.5.1 Epidemiologic Studies

The 2008 ISA for Oxides of Nitrogen did not review any epidemiologic studies of ambient oxides of nitrogen concentrations and blood pressure (BP) (U.S. EPA, 2008c). Several studies are now available for review (Table 4-27). There is little evidence from longitudinal studies of the association between NO_2 and BP.

In the Detroit area, Williams et al. (2012b) measured BP up to 10 times in each of 65 adult participants and found no association between BP and either personal or ambient NO_2 concentrations. Huang et al. (2012a) measured BP repeatedly in participants with pre-existing cardiovascular disease 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, as described in more detail in Section 4.3.3.1, above. Despite these large changes in NO_2 concentrations, this study found no association between NO_2 and either systolic or diastolic BP. Using a similar study design, Rich et al. (2012) also found no association between NO_2 and either systolic or diastolic BP among healthy young participants assessed before, during, and after the 2008 Beijing Olympics. In a repeated-measures study of pregnant women in France, Hampel et al. (2011) found that a 20-ppb increase in 24-h avg NO_2 at lag 1 was associated with a 0.8% decrease in systolic BP (95% CI: -1.3%, -0.3%). Similar associations were reported for lags 1, 5, and 6 days, as well as for the 7 day moving average. The magnitude of this association tended to be stronger in the cool months of the year and among non-smoking women. Diastolic BP was not associated with NO_2 levels.

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 1.1 mmHg (95% CI: 0.2, 2.0 mmHg) increase in systolic BP and a 2.06 mmHg (95% CI: 1.11, 3.17 mmHg) increase in diastolic BP per 20-ppb increase in 24-h avg NO_2. 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, subsequently, Chen et al. (2012c) used cross-sectional data from a different population-based health screening in adults across 6 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.

One available study has examined the association between NO_2 concentrations and other markers of vascular function. In an analysis of data from the U.S. EPA's Detroit Exposure and Aerosol Research Study (DEARS), Williams et al. (2012b) found that 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.

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. One large, repeated-measures study among pregnant women found pronounced decreases in systolic BP associated with ambient NO_2 concentrations (Hampel et al., 2011). In a recent epidemiologic study, Williams et al. (2012b) did not clearly indicate whether or not short-term exposure to NO_2 is associated with other markers of vascular function such as flow mediated dilation.

Table 4-27 Epidemiologic studies of blood pressure.

Study	Location (Sample Size)	Mean NO_2 (ppb)	Exposure assessment	Selected Effect Estimates[a] (95% CI)
Williams et al. (2012b)	Detroit, MI (n = 65)	24-h avg NO_2: 24.0 75th: 28.0 Max: 100.0		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)	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, including both statistically significant positive and negative associations across lags. Generally null and inconsistent associations with DBP across lags 0-6.
Hampel et al. (2011)	Nancy and Poitiers, France (n = 1,500)	24-h avg NO_2: 10.0 75th: 14.2 Max: 38.0	Residence within 20 km of one of 28 permanent background-monitoring sites	Change in SBP (mmHg) Lag 0: -0.58 (-0.86, -0.14) Lag 0-6 days: -0.72 (-1.14, 0.28) Change in DBP (mmHg) No quantitative results presented; "We detected no clear associations between air pollutants and diastolic BP"

Table 4-27 (Continued): Epidemiologic studies of blood pressure.

Study	Location (Sample Size)	Mean NO_2 (ppb)	Exposure assessment	Selected Effect Estimates[a] (95% CI)
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 Max: 34.3 to 49.1	Central Monitor	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 4-27 (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)

[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 and 1-h averaging times, respectively.

4.3.5.2 Controlled Human Exposure Studies

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) reviewed controlled human studies of cardiac output or BP (Table 4-29). Several of these studies also examined HR as described in Section 4.3.3.2, and similarly found no effects of NO_2 exposure on increasing 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 5 young healthy adult males to 620 ppb NO_2 for 2 hours with intermittent exercise. The authors reported no changes in HR, cardiac output, or BP. Drechsler-Parks (1995) exposed 8 older healthy adults to FA, 600 ppb NO_2, 450 ppb O_3, and NO_2 + O_3 for 2 hours with intermittent exercise. There was no change in HR, stroke volume, or cardiac output following exposure to NO_2 or O_3 alone compared to FA; however, a decrease in cardiac output was observed following NO_2 + O_3 exposure compared to O_3 and FA exposures ($p < 0.05$). Gong et al. (2005) reported a statistically nonsignificant increase in HR, but no change in BP after exposure to 400 ppb NO_2 for 2 hours with intermittent exercise in volunteers with COPD and healthy volunteers.

Exposures to higher concentrations of NO_2 have also been examined. Linn et al. (1985b) reported a small, but statistically significant decrease in BP after exposure to 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.

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. 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 xenobiotics.

In summary evidence suggests NO_2 does not alter cardiac output or vascular function. Collectively, a few observations from epidemiologic and controlled human exposure studies indicate NO_2-associated decreases in BP. However, most do not suggest an association between NO_2 exposure and increased BP.

4.3.6 Blood Biomarkers of Cardiovascular Effects

Several epidemiologic and toxicological studies have explored the potential association 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, 2008c) (Table 4-28). Such effects also have been examined in controlled human exposure and animal toxicological studies.

4.3.6.1 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, California and measured plasma biomarkers weekly over a 12 week period. They found that indoor and/or outdoor NO_2 concentrations measured at the retirement homes were associated with increases in interleukin-6 (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 C-reactive protein (CRP), P-selectin, D-dimer, TNFα, soluble intercellular adhesion molecule-1 (sICAM-1), or soluble vascular adhesion molecule-1 (sVCAM-1). In subsequent analysis, 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. Similarly, Ljungman et al. (2009) repeatedly measured plasma IL-6 in 955 myocardial infarction survivors from 6 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).

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 6 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. Among elderly men participating in the Veterans Administration 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 nonsmoking subjects that participated in a controlled-exposure study in Toronto, Canada. Importantly, the blood samples used in this study were collected before participants entered the exposure chamber. They 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. Increases in NO_2 concentrations during the Beijing Olympics were associated with increases in biomarkers indicative of the thrombosis-endothelial dysfunction mechanism (i.e., sCD62P) among healthy young adults (Rich et al., 2012). Among 3,659 individuals in Tel-Aviv, Steinvil et al. (2008) found null association between NO_2 levels 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 adverse health effects have also been studied. 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 levels, but not other markers of coagulation, but detailed results were not presented in the paper. In a cross-sectional analysis of pregnant women in Allegheny County, PA, there was no association between NO_2 and CRP (Lee et al., 2011c). 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.

In summary, there is some epidemiologic evidence to suggest the presence of an association between NO_2 concentrations and some markers of systemic inflammation among participants with a history of heart disease. This association is not consistently observed in healthy individuals. Other potentially at-risk populations have not been clearly identified.

Table 4-28 Epidemiologic studies of biomarkers of cardiovascular effects.

Study	Location (Sample Size)	Mean NO_2 (ppb)	Exposure assessment	Selected Effect Estimates[a] (95% CI)	
Delfino et al. (2008b)	Los Angeles, CA (n = 29)	1-h 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: 1,124.73 (-313.63, 2,565.25) Lag 0-2: 1,027.40 (-465.03, 2,519.83) Fibrinogen (µg/mL) Lag 0: -110.31 (-503.97, 283.35) Lag 0-2: -110.31 (-501.80, 281.18) 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: -493.15 (-9,387.17, -248.74) Lag 0-2: -3,211.97 (-7,788.75, 1,364.82) 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: 289.83 (-41.10, 622.93) Lag 0-2: 240.09 (-82.19, 562.36)	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: 53,733.96 (-11,381.40, 118,849.32) Lag 0-2: 18,266.04 (-45,532.08, 82,062.00) ICAM-1 (pg/mL) Lag 0: 5,381.40 (-8,987.02, 19,747.66) Lag 0-2: 575.34 (-13,494.59, 14,643.11) SOD (U/g Hb) Lag 0: -540.74 (-1,020.91, -62.73) Lag 0-2: -571.02 (-1,036.05, -105.98) 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)

Table 4-28 (Continued): Epidemiologic studies of biomarkers of cardiovascular effects.

Study	Location (Sample Size)	Mean NO_2 (ppb)	Exposure assessment	Selected Effect Estimates[a] (95% CI)	
Delfino RJ: Staimer et al. (2009)	Los Angeles, CA (n = 60)	1-h NO_2 Phase 1: 26.4 Phase 2: 28.3 1-h 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)
Delfino et al. (2010)	Los Angeles, CA (n = 60)	Warm season 1-h NO_2: 26.4 1-h NO_X: 37.2 Cool Season 1-h NO_2: 28.3 1-h 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)	
Ljungman et al. (2009)	Six European cities (n = 955) (total n = 5,539 measurements)	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)

Table 4-28 (Continued): Epidemiologic studies of biomarkers of cardiovascular effects.

Study	Location (Sample Size)	Mean NO_2 (ppb)	Exposure assessment	Selected Effect Estimates[a] (95% CI)
Brüske et al. (2011)	Augsburg, Germany (n = 200)	24-h avg NO_2 20.8 75th: 24.7 Max: 38.2 24-h NO 24.0 75th: 25.8 Max: 141.1	Central site monitor	Lp-PLA2 (% Change) NO_2 Lag 4: 7.28 (3.00, 11.56) NO Lag 4: 2.74 (-0.21, 5.70) "Inverse associations were observed for … NO_2 with Lp-PLA-2 at lag days 1-2 and positive associations were estimated …with Lp-PLA2 lagged 4 and 5 days."
Bind et al. (2012)	Boston, MA (n = 704)	24-h avg NO_2 18 95th: 35	City-wide avg	Fibrinogen (percent change) Lag 0-2: 8.18 (4.73, 11.64)
Ren et al. (2011)	Boston, MA (n = 320)	24-h avg NO_2 17.8	Central site Monitor	8-OHdG (% change) Lag 0: 28.48 (-19.39, 76.36) Lag 0-6: 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)	24-h avg NO_2 23.8	Central site monitor	Quantitative results not presented.

Table 4-28 (Continued): Epidemiologic studies of biomarkers of cardiovascular effects.

Study	Location (Sample Size)	Mean NO_2 (ppb)	Exposure assessment	Selected Effect Estimates[a] (95% CI)	
Rudez et al. (2009)	Rotterdam, the Netherlands (n = 40)	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 4-28 (Continued): Epidemiologic studies of biomarkers of cardiovascular effects.

Study	Location (Sample Size)	Mean NO_2 (ppb)	Exposure assessment	Selected Effect Estimates[a] (95% CI)	
Rudez et al. (2009) (Continued)	Rotterdam, the Netherlands (n = 40)	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 hrs: 0.42 (-27.08, 27.92); -0.18 (-19.64, 19.29) Lag 72-96 hrs: -19.17 (-50.00, 12.08); -12.32 (-30.71, 6.25)

Table 4-28 (Continued): Epidemiologic studies of biomarkers of cardiovascular effects.

Study	Location (Sample Size)	Mean NO_2 (ppb)	Exposure assessment	Selected Effect Estimates[a] (95% CI)	
Steinvil et al. (2007)	Tel Aviv, Israel (n = 3,659)	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 (% change) 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 (% change) 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)	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	
Chuang et al. (2007a)	Taipei, Taiwan (n = 76)	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	
Wellenius et al. (2007)	Boston, MA (n = 28)	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	
Baccarelli et al. (2007)	Lombardia, Italy (n = 1,213)	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, postmethionine-load (% change) Lag 24 h: 0.00 (-2.86, 2.86) Lag 0-6 days: 0.49 (-2.94, 4.17)

Table 4-28 (Continued): Epidemiologic studies of biomarkers of cardiovascular effects.

Study	Location (Sample Size)	Mean NO_2 (ppb)	Exposure assessment	Selected Effect Estimates[a] (95% CI)	
Barclay et al. (2009)	Aberdeen, Scotland (n = 132)	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)
Rich et al. (2012)	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. 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.	

[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 and 1-h averaging times, respectively.

4.3.6.2 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, 2008c) (Table 4-29). 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 found endothelial cell activation.

In healthy adults, 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 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 5.9% increase in high density lipoprotein (HDL) 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 1 at baseline and 4 and 6 hours post-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 following NO_2 exposure. Plasma samples were collected from healthy volunteers exposed to FA 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. Expression levels of endothelial cell adhesion molecules, VCAM-1 and ICAM-1, from hCAECs were elevated for both post-exposure time points compared to control. hCAECs treated with plasma (30%) collected immediately post NO_2 exposure had significantly 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.

Riedl et al. (2012) reported on the cardiovascular effects of healthy volunteers and individuals with asthma exposed to FA, 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, 2008c) 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 supports 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 2.5-3 hours with intermittent exercise. However, a recent study reported no change in hemoglobin levels 4 and 6 hours after exposure to 4,000 ppb NO_2 for 1 hour (Langrish et al., 2010).

To summarize results for biological markers of cardiovascular effects, also discussed in Section 3.3.2.8, the few available controlled human exposure studies from the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) demonstrated that short-term NO_2 exposure causes a slight reduction in hematocrit and hemoglobin levels associated with a decrease in RBC levels. The clinical significance of these findings is unknown (Section 3.3.2.8). The recent available studies demonstrate that NO_2 does not affect all measured cardiovascular biomarkers. For instance, evidence has not shown NO_2 to alter circulating blood coagulation factors or modify the body's response to vasodilators. However, some evidence suggests NO_2 exposure increases inflammatory mediators and induces endothelial cell activation, which has been linked to risk of atherosclerosis.

Table 4-29 Controlled human exposure studies of short-term NO_2 exposure and cardiovascular effects.

Study	Lifestage; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Channell et al. (2012)	Adult (25.3 ± 5.5 yr); M/F; n = 7 Primary human coronary artery endothelial cells (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 [body surface area]). 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 two 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 minutes; $\dot{V}_E$ = 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)	Elderly; 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 4-29 (Continued): Controlled human exposure studies of short-term NO_2 exposure and cardiovascular effects.

Study	Lifestage; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Langrish et al. (2011)	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)	Adults; M/F w/ asthma (18-34 yr) n = 23, w/o 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 was 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 diphospoglycerate, 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.

4.3.6.3 Toxicological Studies

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) reported on various hematological parameters in animals including oxidative stress, RBC 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 4-30).

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 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 GSH peroxidase 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 (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.

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 GSH peroxidase. GSH peroxidase levels in RBCs increased in both groups immediately and 48 hours after exposure; however, plasma levels were decreased in Se-L rats at both time points. RBC SOD activity also decreased in Se-L rats at both time points, but increased in Se-S rats 48 hours after exposure. Overall, NO_2 exposure stimulated oxidative stress protective mechanisms with high Se, but were mixed with low Se.

The effects of NO_2 on endothelial mediators, endothelin-1 (ET-1) and endothelial nitric oxide synthase (eNOS) were recently examined in two studies. ET-1 is a potent vasoconstrictor while the enzyme eNOS catalyzes the production of NO, which induces vasodilation. Campen et al. (2010), described above, did not see a significant increase in ET-1 expression level in the aorta after exposure of rats to 200 and 2,000 ppb NO_2. However, with exposure to higher NO_2 concentrations, ET-1 increased significantly in the heart at the mRNA (10,640 ppb) and protein level (5,320 and 10,640 ppb) (Li et al., 2011a). Furthermore, eNOS mRNA and protein levels were increased at both the 2,660

and 5,320 ppb doses and decreased to control levels at the 10,640 ppb dose. At more relevant concentrations 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 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 those from a controlled human exposure study (Channell et al., 2012) described in Section 4.3.6.2.

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) reported on several animal studies examining hematological parameters. Three studies indicate elevated levels of a younger population of RBC following NO_2 exposure. RBC 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 RBC 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 RBC 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.

In summary, there is some evidence from a few animal toxicological studies that short-term NO_2 exposure affects the cardiovascular system. Oxidative stress effects of NO_2 were evident in RBC, the heart, and aorta of rodents. In addition, an increase in inflammatory markers and adhesion molecules was also observed after exposure to NO_2. At higher concentrations, NO_2 was found to induce the expression and production of the vasoconstrictor ET-1. Other effects included changes in hematological parameters.

Table 4-30 Animal toxicological studies of short-term NO_2 exposure and cardiovascular effects.

Study	Species (Strain); Lifestage; 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	High (6 µg/day) or low (1.3 µg/day) selenium; 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 were 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 7days	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	Meta-hemoglobin 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 weeks	ECG (HR, SDNN, r-MSSD, TP, LF, HF, LH:HF), BW; Endpoints measured throughout the exposure.

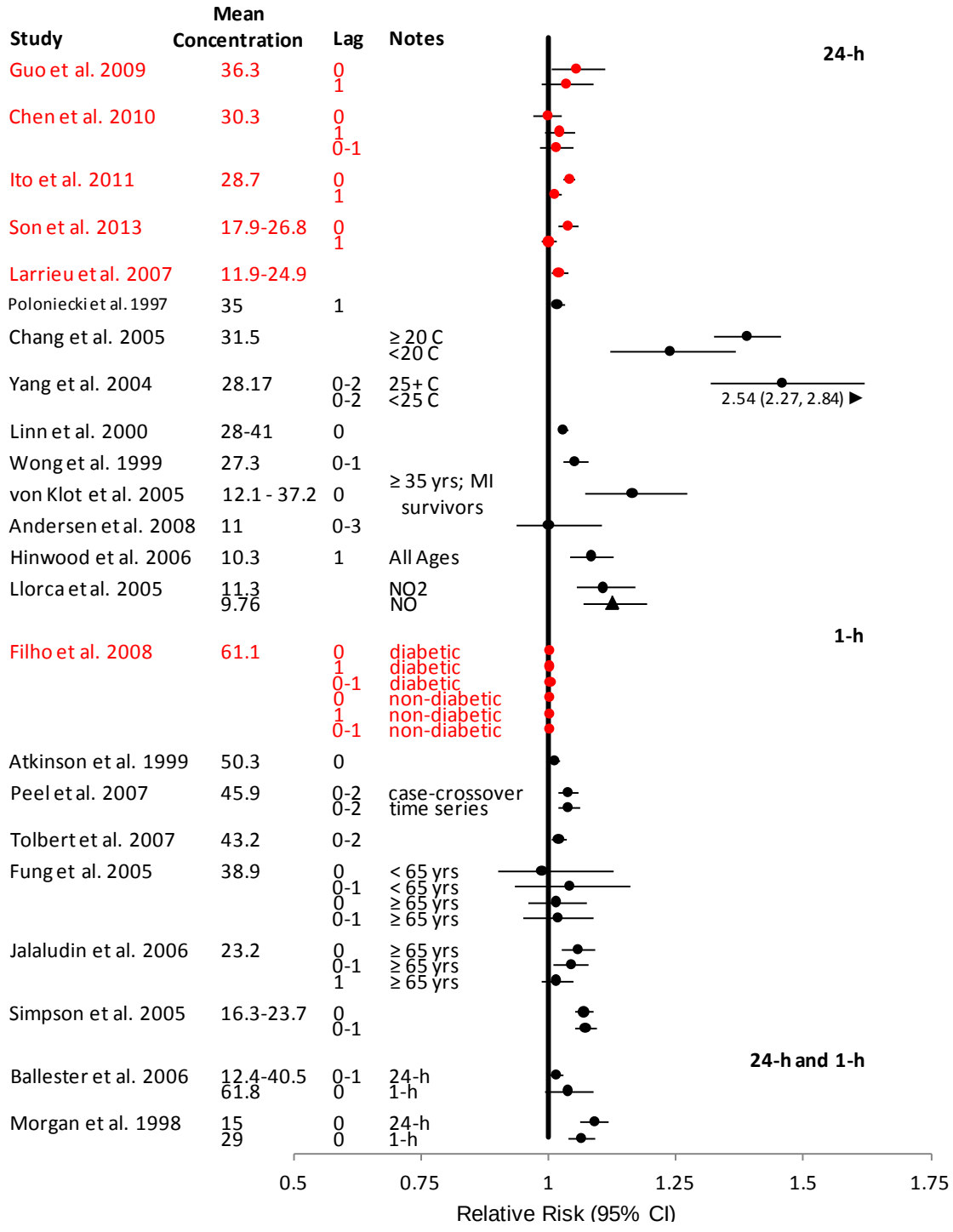
4.3.7 Hospital Admissions and Emergency Department Visits

4.3.7.1 All Cardiovascular Diseases

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, 2008c) (Figure 4-12 and Table 4-31). 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.

Ito et al. (2011) found that risk of CVD hospitalization was associated with NO_2 concentrations at lag 0 in New York City. Results from copollutant models were not reported. In Beijing, China, Guo et al. (2009) found an association between ambient NO_2 concentrations and risk of CVD hospital admissions at lag 0, but this association was attenuated and less precise in copollutants models adjusting for either $PM_{2.5}$ or SO_2, or both. In Shanghai, China, Chen et al. (2010b) 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. A study in Sao 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). Studies from Copenhagen, Denmark (Andersen et al., 2008b), Madrid, Spain (Linares and Diaz, 2010), and Taipei, Taiwan (Chan et al., 2008) reported null or negative associations between NO_2 concentrations and risk of hospital admission for CVD.

In summary, consistent evidence reported in the 2008 ISA for Oxides of Nitrogen combined with recent epidemiologic data available continues to support the presence of an association between ambient NO_2 levels and risk of hospital admission for cardiovascular diseases (Figure 4-12 and Table 4-31). Generally, the associations observed in these studies are robust in copollutant models that adjust for PM or gaseous pollutants.



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, 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 4-12 Results of studies of short-term exposure to oxides of nitrogen and hospital admissions for all cardiovascular disease.

Table 4-31 Corresponding effect estimates for hospital admissions for all cardiovascular disease studies presented in Figure 4-12.

Study	Location	Relative Risk[a] (95% CI)
Guo et al. (2009)	Beijing, China	Lag 0: 1.05 (1.00, 1.11) Lag 1: 1.03 (0.985, 1.09)
Chen et al. (2010b)	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)
Ito et al. (2011)	New York City, NY	Lag 0: 1.04 (1.03, 1.05) Lag 1: 1.01 (1.00, 1.02)
Son et al. (2013)	8 Korean Cities	Lag 0: 1.04 (1.02, 1.06) Lag 1: 1.00 (0.98, 1.01)
Larrieu et al. (2007)	8 French Cities	1.02 (1.00, 1.04)
Poloniecki et al. (1997)	London, U.K.	Lag 1: 1.02 (1.00, 1.04)
Chang et al. (2005)	Taipei, Taiwan	≥ 20 °C: 1.39 (1.32, 1.45) <20 °C: 1.23 (1.12, 1.37)
Yang et al. (2004)	Kaohsiung, Taiwan	≥ 25 °C: 1.46 (1.31, 1.62) <25 °C: 2.54 (2.27, 2.84)
Linn et al. (2000)	Los Angeles, CA	Lag 0: 1.03 (1.02, 1.04)
Wong et al. (1999)	Hong Kong, China	Lag 0-1: 1.05 (1.03, 1.08)
Von Klot et al. (2005)	Five European Cities	Lag 0: 1.16 (1.07, 1.27)
Andersen et al. (2008b)	Copenhagen, Denmark	Lag 0-3: 1.00 (0.93, 1.10)
Hinwood et al. (2006)	Perth, Australia	Lag 1: 1.08 (1.04, 1.13)
Llorca et al. (2005)	Torrelavega, Spain	NO_2: 1.11 (1.05, 1.17) NO: 1.13 (1.07, 1.19)
Filho et al. (2008)	Sao 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)
Atkinson et al. (1999)	London, U.K.	Lag 0: 1.01 (1.00, 1.02)
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)
Tolbert et al. (2007)	Atlanta, GA	Lag 0-2: 1.02 (1.01, 1.03)

Table 4-31 (Continued): Corresponding effect estimates for hospital admissions for all cardiovascular disease studies presented in Figure 4-12.

Study	Location	Relative Risk[a] (95% CI)
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)
Jalaludin et al. (2006)	Sydney, Australia	Lag 0: 1.06 (1.02, 1.09) Lag 1: 1.04 (1.01, 1.08) Lag 0-1: 1.01 (0.98, 1.05)
Simpson et al. (2005a)	4 Australian Cities	Lag 0: 1.07 (1.05, 1.09) Lag 0-1: 1.07 (1.05, 1.09)
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)
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)

Note: Studies correspond to studies presented in Figure 4-12.

[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 and 1-h averaging times, respectively.

4.3.7.2 Cardiac Causes (MI and Heart Failure)

The 2008 ISA for Oxides of Nitrogen found that the epidemiologic evidence consistently supported the associations between short-term changes in NO_2 concentrations and hospital admissions or ED visits for cardiac diseases (U.S. EPA, 2008c). This hypothesis continues to be supported by studies published since the 2008 ISA, as reviewed below (Figure 4-13 and Table 4-32).

Generally, studies based on clinical registries are less susceptible to misclassification of the outcome and exposure which may explain why they provide stronger evidence than those based on administrative data. The most convincing evidence of an association between ambient NO_2 and risk of myocardial infarction (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. The association with NO_2 was strengthened in a multipollutant model adjusted for PM_{10}, CO, SO_2, and

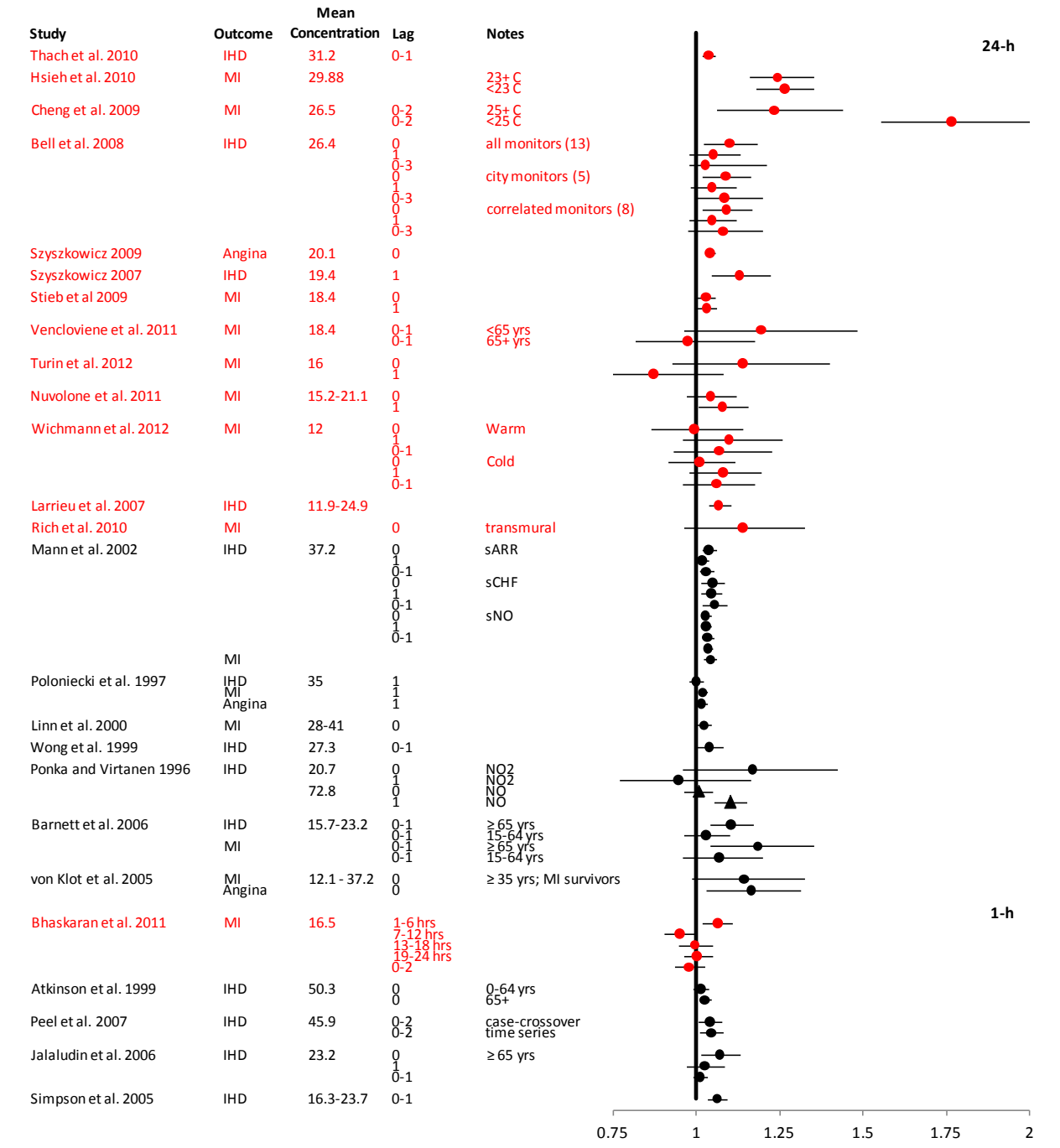
O_3; however, multipollutant model results are more difficult to interpret because of multicollinearity among pollutants. NO_2 results were robust to a number of sensitivity analyses that evaluated key aspects of study design and model specification (e.g., stricter diagnosis criteria, different time strata). 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. On the other hand, in a study of 429 MI events, Turin et al. (2012) did not observe any association using data from the Takashima County Stroke and AMI Registry in Central Japan.

A number of studies based on administrative data have also been published since the 2008 ISA for Oxides of Nitrogen. Using data from 14 hospitals in 7 Canadian cities, Stieb et al. (2009) found a 3.0% (95% CI: 0.2%, 5.8%) increase in risk of ED visits for the composite endpoint of angina or acute MI per 20-ppb increase in 24-h avg NO_2 on the previous day. However, the overall association was dominated by the association observed in Edmonton, and exclusion of the data from Edmonton from analyses attenuated the results. A related study in 6 Canadian cities found that NO_2 concentrations were associated with risk of ED visits for chest pain (Szyszkowicz, 2009). Larrieu et al. (2007) observed a positive association between hospital admissions for IHD and NO_2 concentrations in 8 French cities. The magnitude of the association was higher for older adults (i.e., $\leq$ 65 years) than for the general population. In 6 areas in central Italy, Nuvolone et al. (2011) found a 8% (95% CI: 0, 15%) increase in risk of hospitalization for MI per 20-ppb increase in 24-h avg NO_2 on the previous day. Similar associations were seen in relation to lags 1 to 4 days prior to hospital admission. The finding at lag 2 was robust to adjustment for PM_{10} in a two-pollutant model but remained positive, though somewhat attenuated, by adjustment for CO. The association with NO_2 was somewhat more pronounced among females and in the cold season. Szyszkowicz (2007b) and Thach et al. (2010) found that NO_2 was associated with increased risk of hospital admission for IHD in Montreal, Canada, and Hong Kong, China, respectively.

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. No results were reported for all MIs or for non-transmural infarcts. Wichmann et al. (2012) found that NO_2 was positively associated with risk of hospital admission in Copenhagen, Denmark, but only in the warm months of the year. NO_2 was positively associated with hospital admissions for MI (Hsieh et al., 2010; Bell et al., 2008) and for IHDs (Bell et al., 2008) in Taipei, Taiwan, and risk of hospital admissions for MI in Kaohsiung, Taiwan (Cheng et al., 2009a). The study by Bell et al. (2008) used three different exposure assessment techniques aimed at reducing uncertainty related to the use of central site monitors. NO_2 was not associated with risk of hospital admission for acute coronary syndrome in Lithuania (Vencloviene et al., 2011).

Two additional studies have considered hospital admissions or ED visits for heart failure. In the study of 7 Canadian cities described above, Stieb et al. (2009) found 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. 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 $\geq$ 20 °C.

In summary, the epidemiologic data available continue to support associations between ambient NO_2 concentrations and risk of hospital admission or ED visits for cardiac causes, particularly MI and IHD. Generally, the associations observed in these studies are robust in copollutant models that adjust for PM or other gaseous pollutants.



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, 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 4-13 Results of studies of short-term exposure to oxides of nitrogen and hospital admissions for cardiac disease.

Table 4-32 Corresponding risk estimates for hospital admissions for cardiac disease for studies presented in Figure 4-13.

Study	Location	Health Effect	Relative Risk[a] (95% CI)
Thach et al. (2010)	Hong Kong, China	IHD	Lag 0-1: 1.04 (1.02, 1.05)
Hsieh et al. (2010)	Taipei, Taiwan	MI	≥ 23 °C: 1.24 (1.16, 1.35) <23 °C: 1.26 (1.18, 1.35)
Cheng et al. (2009a)	Kaohsiung, Taiwan	MI	≥ 25 °C: 1.23 (1.06, 1.44) <25 °C: 1.76 (1.55, 2.02)
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) Lag 0-3: 1.08 (0.97, 1.20)
Szyszkowicz (2009)	6 Canadian Cities	Angina	Lag 0: 1.04 (1.03, 1.05)
Szyszkowicz (2007b)	Montreal, Canada	IHD	Lag 1: 1.13 (1.04, 1.22)
Stieb et al. (2009)	7 Canadian Cities	MI	Lag 0: 1.03 (1.00, 1.05) Lag 1: 1.03 (1.00, 1.06)
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)
Turin et al. (2012)	Takashima County, Japan	MI	Lag 0: 1.14 (0.92, 1.40) Lag 1: 0.87 (0.70, 1.08)
Nuvolone et al. (2011)	Tuscany, Italy	MI	Lag 0: 1.04 (0.97, 1.12) Lag 1: 1.08 (1.00, 1.15)
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)

Table 4-32 (Continued): Corresponding risk estimates for hospital admissions for cardiac disease for studies presented in Figure 4-13.

Study	Location	Health Effect	Relative Risk[a] (95% CI)
Larrieu et al. (2007)	8 French Cities	IHD	1.07 (1.03, 1.10)
Rich et al. (2010)	New Jersey, U.S.	MI	Lag 0: 1.14 (0.96, 1.32)
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)
		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)
Linn et al. (2000)	Los Angeles, CA	MI	Lag 0: 1.02 (1.00, 1.04)
Wong et al. (1999)	Hong Kong, China	IHD	1.04 (1.00, 1.08)
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)
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)
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)
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)

Table 4-32 (Continued): Corresponding risk estimates for hospital admissions for cardiac disease for studies presented in Figure 4-13.

Study	Location	Health Effect	Relative Risk[a] (95% CI)
Atkinson et al. (1999)	London, U.K.	IHD	Lag 0; 0-64 yrs: 1.01 (0.99, 1.04) Lag 0; 65+ yrs: 1.03 (1.01, 1.04)
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)
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)
Simpson et al. (2005a)	4 Australian Cities	IHD	Lag 0-1: 1.06 (1.03, 1.09)

Note: Studies correspond to studies presented in Figure 4-13.

[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 and 1-h averaging times, respectively.

4.3.7.3 Cerebrovascular Diseases and Stroke

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 evidence for an NO_2 effect (U.S. EPA, 2008c). Recent studies published since the 2008 ISA add to the evidence (Figure 4-14 and Table 4-33).

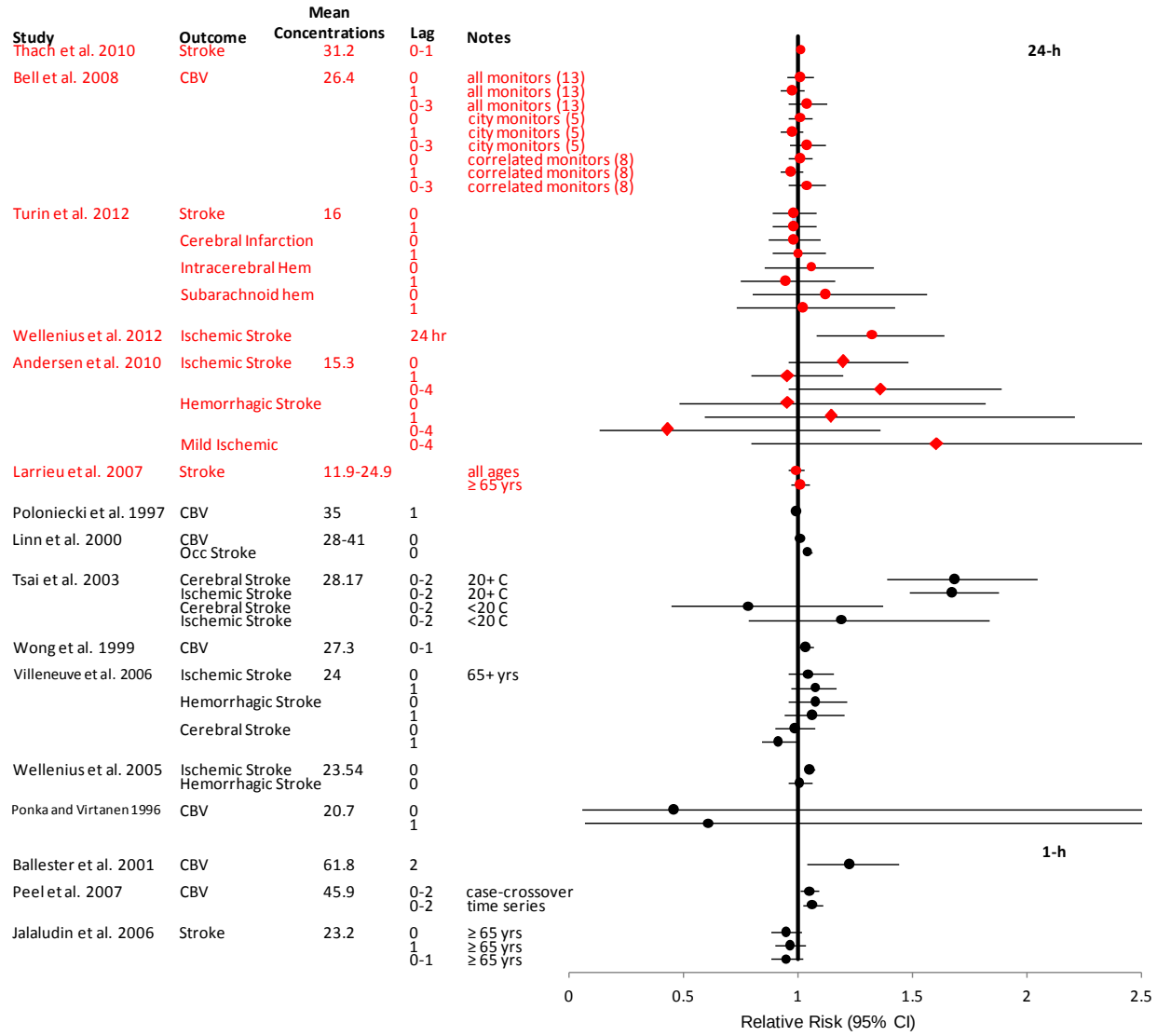
Generally, studies based on clinical registries are less susceptible to misclassification of the outcome and exposure, which may explain the stronger evidence provided by these studies than those based on administrative data. Wellenius et al. (2012) reviewed the medical records of 1,705 Boston-area patients hospitalized with neurologist-confirmed acute ischemic stroke and found an odds ratio for ischemic stroke onset of 1.32 (95% CI: 1.08, 1.63) per 20-ppb increase in mean NO_2 concentrations over the past 24 hours. A unique strength of this study was the availability of information on the date and time of stroke symptom onset in most patients, thereby substantially reducing misclassification of the exposure. Copollutant models were not considered.

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 seemed to be attenuated after adjustment for ultrafine particles. Using data from a stroke registry in Como, Italy, Vidale et al. (2010) found that NO_2 was associated with risk of ischemic stroke hospitalization. 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.

Additional studies based on administrative data are also available. Szyszkowicz (2008b) observed a positive association between NO_2 and emergency department visits for ischemic stroke in Edmonton, Canada, but only within specific subgroups according to sex, season, and age. Studies from Taipei, Taiwan (Bell et al., 2008) and Hong Kong (Thach et al., 2010) failed to find any associations with cerebrovascular disease.

In summary, the epidemiologic data available continues to support an association between ambient NO_2 concentrations and risk of hospital admission for cerebrovascular disease and stroke. Generally, the associations observed in these studies are robust in copollutant models that adjust for PM or other gaseous pollutants.



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 and 40 ppb or 60 ppb for NO_X concentrations for 24-h and 1-h averaging times, 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 4-14 Results of studies of short-term exposure to oxides of nitrogen and hospital admissions for cerebrovascular disease and stroke.

Table 4-33 Corresponding risk estimates for hospital admissions for cerebrovascular disease and stroke for studies presented in Figure 4-14.

Study	Location	Health Effect	Selected Relative Risks[a] (95% CI)
Thach et al. (2010)	Hong Kong, China	Stroke	Lag 0-1: 1.01 (1.00, 1.03)
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)
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)
Wellenius et al. (2012)	Boston, MA	Ischemic Stroke	Lag 24 h: 1.32 (1.08, 1.63)
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)

Table 4-33 (Continued): Corresponding risk estimates for hospital admissions for cerebrovascular disease and stroke for studies presented in Figure 4-14.

Study	Location	Health Effect	Selected Relative Risks[a] (95% CI)
Larrieu et al. (2007)	8 French Cities	Stroke	All ages: 0.99 (0.96, 1.03) ≥ 65 yrs: 1.01 (0.97, 1.05)
Poloniecki et al. (1997)	London, U.K.	Cerebrovascular disease	Lag 1: 0.99 (0.98, 1.00)
Linn et al. (2000)	Los Angeles, CA	Cerebrovascular disease Occlusive Stroke	Lag 0: 1.01 (0.99, 1.02) Lag 0: 1.04 (1.02, 1.06)
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)
Wong et al. (1999)	Hong Kong, China	Cerebrovascular disease	Lag 0-1: 1.03 (0.99, 1.07)
Villeneuve et al. (2006a)	Edmonton, Canada	Ischemic Stroke Hemorrhagic Stroke Cerebral Stroke	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)
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)
Pönkä and Virtanen (1996)	Helsinki, Finland	Cerebrovascular disease	Lag 0: 0.96 (0.87, 1.07) Lag 1: 0.98 (0.87, 1.09)
Ballester et al. (2001)	Valencia, Spain	Cerebrovascular disease	Lag 2: 1.22 (1.04, 1.44)
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)
Jalaludin et al. (2006)	Sydney, Australia	Stroke	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)

Note: Studies correspond to studies presented in Figure 4-14.

[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 and 1-h averaging times, respectively.

4.3.7.4 Other Cardiovascular Causes of Hospital Admission or ED Visit

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%) increased rate of hospital admission for venous thrombosis and pulmonary embolism, respectively, per 20-ppb increase in 24-h avg NO_2 concentrations (Dales et al., 2010). These associations were somewhat attenuated in copollutant models, but the associations remained positive.

In Beijing, China, Guo et al. (2010) found that NO_2 was associated with rates of ED visits for hypertension, and the association remained relatively unchanged in copollutant models adjusting for PM_{10} or SO_2. In contrast, 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.

Using data from 14 hospitals in 7 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 finding an association in Taipei, Taiwan.

In summary, few studies from single locations have documented associations with hospital admissions and ED visits for other cardiovascular causes including venous thrombosis, pulmonary embolism, and hypertension.

4.3.8 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 found 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 multi-city studies provide evidence that is consistent with those studies evaluated in the 2008 ISA for Oxides of Nitrogen (Section 4.4, Figure 4-17).

The NO_2-cardiovascular mortality relationship was further examined in a few studies through copollutants analyses. Chen et al. (2012b) in the 17 Chinese cities study (CAPES) found that NO_2 risk estimates for cardiovascular mortality were slightly attenuated, but remained positive in copollutant models with PM_{10} and SO_2 (7.1% [95%

CI: 3.9, 10.3] for a 20-ppb increase in 24-h avg NO_2 concentrations at lag 0-1; 4.7% [95% CI: 1.2, 8.2] with PM_{10}; 5.9% [95% CI: 2.6, 9.2] with SO_2). Chiusolo et al. (2011) also found evidence that associations between short-term NO_2 exposure and cardiovascular mortality remained robust in copollutant models in a study of 11 Italian cities. In an all-year analysis, a 20-ppb increase in NO_2 at lag 1-5 was associated with a 10.5% (95% CI: 6.0, 15.2) increase in cardiovascular mortality and a 10.3% (95% CI: 4.1, 16.8) increase adjusted for PM_{10}. In a warm season analysis (April-September), the NO_2 effect estimate was 19.6% (95% CI: 11.7, 28.1) and 19.3% (95% CI: 11.0, 28.2) 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 robust.

Of the 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). 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 1.6% to 7.4% 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 also 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 winter season analyses. Although the cardiovascular 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 4.4.3, studies conducted in Asian cities observed much 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.

4.3.9 Summary and Causal Determination

Evidence indicates that there is likely to be a causal relationship between short-term exposure to oxides of nitrogen and cardiovascular health effects based primarily on epidemiologic studies of adults that consistently demonstrate NO_2-associated hospitalizations and ED visits for cardiovascular effects and mortality from cardiovascular disease. This conclusion represents a change from the 2008 ISA for Oxides of Nitrogen that 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, 2008c). 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, 2008c). 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 high-quality epidemiologic studies have further evaluated this uncertainty using copollutant models and comparing associations of NO_2 with those of other criteria pollutants. These studies provide evidence for independent associations of NO_2 with cardiovascular effects, thus reducing the uncertainty from the 2008 ISA for Oxides of Nitrogen regarding the potential for NO_2 to serve as an indicator for another combustion-related pollutant or mixture. An uncertainty that remains from the 2008 ISA for Oxides of Nitrogen is the lack of mechanistic evidence to describe a role for NO_2 in the development of cardiovascular diseases, including key events that inform the mode of action. The evidence for cardiovascular effects, with respect to the causal determination for short-term exposure to oxides of nitrogen is detailed below using the framework described in Table II of the Preamble. The key evidence, supporting or contradicting, as it relates to the causal framework is summarized in Table 4-36.

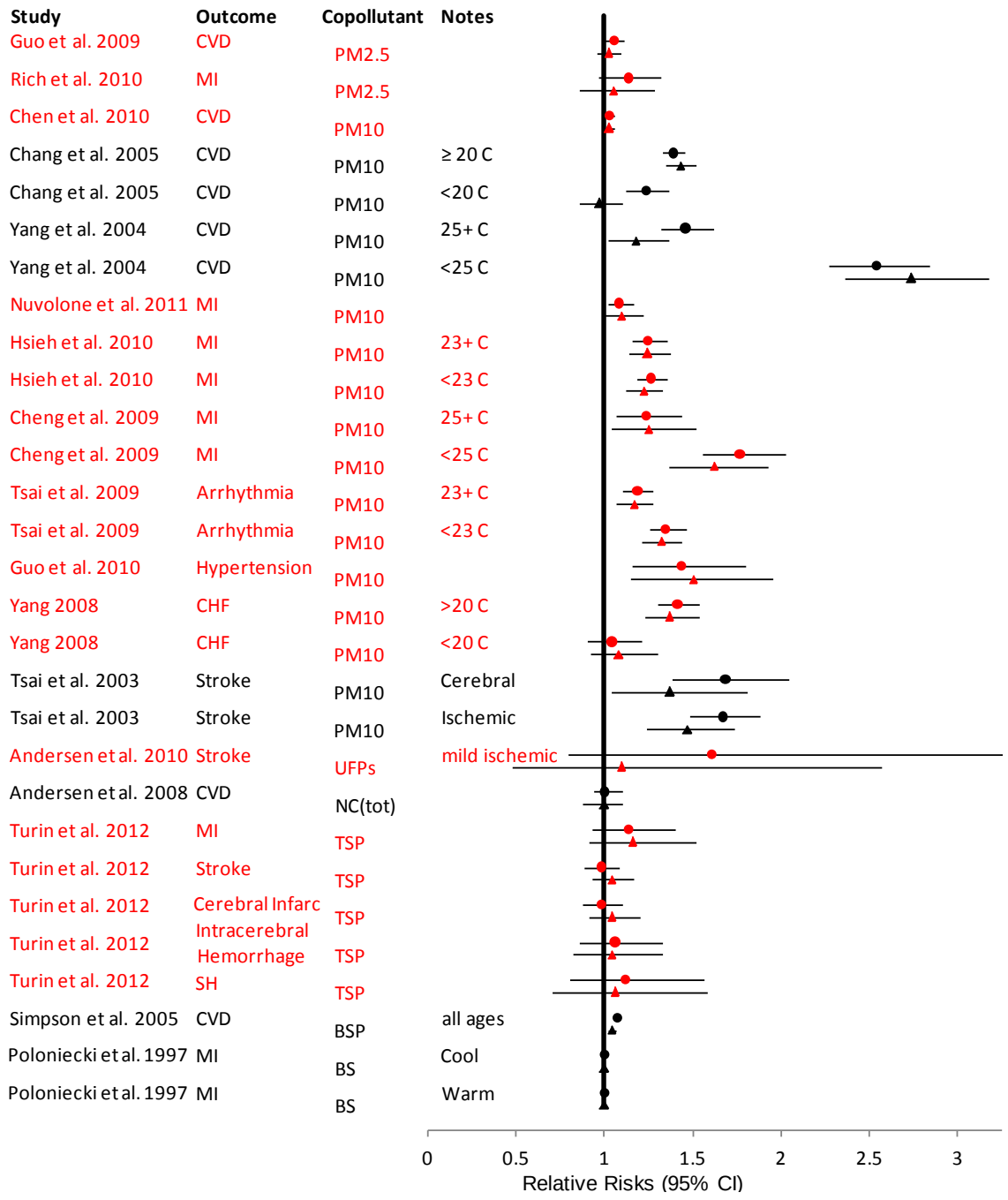
Time-series studies of adults in the general population consistently report positive associations between concentrations of oxides of nitrogen and hospital admissions and ED visits for all cardiovascular disease and, specifically, IHD. High-quality single-city studies from the U.S. (Ito et al., 2011; 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 hospitalizations in adults with adjustment for numerous potential confounding factors, including weather and time trends. The strongest evidence is for hospital admissions due to IHD (Stieb et al., 2009; Larrieu et al., 2007; Peel et al., 2007; Von Klot et al., 2005; Mann et al., 2002). Recent controlled human exposure and animal toxicological studies provide weak evidence for a potential biologically plausible mechanism leading to cardiovascular disease and IHD, with some studies reporting induction of systemic inflammation and oxidative stress (Channell et al., 2012; Huang et al., 2012b; Li et al., 2011a).

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. 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.

There is limited evidence from epidemiologic and controlled human exposure studies to suggest that NO_2 exposure results in alterations of cardiac autonomic control, which may trigger life-threatening cardiovascular events. Recent epidemiologic studies generally reported associations between ambient NO_2 levels and decreases in indices of HRV and changes in ventricular repolarization among populations with pre-existing or at elevated risk for cardiovascular disease. Since these effects are known indicators of myocardial ischemia and other cardiovascular events, the consistently observed associations with measures of altered autonomic control provide biological plausibility for the hospitalizations and mortality associations observed in the studies. 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 were inconsistent (Scaife et al., 2012). Evidence from epidemiologic and experimental studies for other cardiovascular effects, including blood pressure and arrhythmia, was mixed or limited in scope. Inconsistencies across studies and the limited evidence available to suggest NO_2-related subclinical and clinical cardiovascular effects represent a lack of coherence across all lines of evidence to support the effects observed in hospital admissions and ED visits, and cardiovascular mortality.

A key uncertainty discussed in the 2008 ISA for Oxides of Nitrogen was the potential for confounding by other correlated pollutants. Recent studies have evaluated this uncertainty using copollutant models and comparing associations of NO_2 with those of other pollutants. Figure 4-15 and Figure 4-16 present the single-pollutant and copollutant model results from studies that examined associations between short-term exposure to NO_2 and cardiovascular disease adjusted for PM or CO, respectively. Additional figures characterizing the copollutant models for NO_2 with SO_2 or O_3 can be found online in Supplemental Figures S4-2 and S4-3 (U.S. EPA, 2013c). Specifically, a number of studies found that associations of NO_2 and cardiovascular hospital admissions were stronger in magnitude than associations with other pollutants in copollutant models, including PM, O_3, CO, and SO_2 (Ito et al., 2011; Larrieu et al., 2007; Peel et al., 2007; Tolbert et al., 2007). Other studies found that estimates for NO_2 were robust to inclusion of copollutants in the models (Ballester et al., 2006; Von Klot et al., 2005). In addition, a limited number of studies that examined copollutant confounding on the NO_2-cardiovascular mortality relationship indicate that associations remain robust (Chen et al., 2012b; Chiusolo et al., 2011). However, not all analyses reported NO_2 as the strongest predictor of cardiovascular effects. One study reported that associations with cardiovascular hospitalizations were not robust in models matching on 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 (non-criteria) pollutants that may be potentially correlated with NO_2 were generally not examined in copollutant models, resulting in the potential for unmeasured confounding. Finally, while copollutant models are a common statistical tool used to evaluate the potential for copollutants confounding, their interpretation can be limited (Section 1.5). Until more reliable methods to adjust for multiple copollutants simultaneously become available, there is potential for residual confounding due to unmeasured copollutants (Section 1.5). Without consistent and reproducible experimental evidence that is coherent with the effects observed in epidemiologic studies, some uncertainty still exists concerning the role of correlated pollutants in the associations observed with oxides of nitrogen. However, recent epidemiologic studies examining the extent to which NO_2 is independently associated with cardiovascular effects have decreased the uncertainty that these associations are a result of NO_2 serving as a marker for effects of another traffic-related pollutant or mix of pollutants.



Note: Studies in red are recent studies. Studies in black were included in the 2008 ISA for Oxides of Nitrogen. Relative risks were standardized to a 20 ppb or 30-ppb increase in NO_2 concentration for 24-h and 1-h averaging times, respectively. Relative risks from Andersen et al. (2010) were standardized to a 40 ppb or 60-ppb increase in NO_X concentration for 24-h and 1-h averaging times, respectively. Model estimates are presented as pairs with the top estimate (circles) for the single pollutant model and the bottom estimate (triangles) for the copollutants model. Horizontal lines indicate 95% confidence intervals around the central estimate. Associated data presented in Table 4-34. BSP: black smoke particles; BS: black smoke; NC(tot): total number count.

Figure 4-15 Results of single-pollutant and copollutants models of short-term exposure to NO_2 or NO_X with and without PM and hospital admissions for cardiovascular disease.

Table 4-34 Corresponding risk estimates of ambient NO_2 or NO_X for hospital admissions for cardiovascular disease in studies conducting copollutant models with PM for presented in Figure 4-15.

Study	Location	Notes	Cause	Single Pollutant Relative Risk[a] (95% CI)	Copollutant Relative Risk[a] (95% CI)
Guo et al. (2009)	Beijing, China		CVD	1.05 (1.00, 1.11)	+$PM_{2.5}$: 1.02 (0.96, 1.09)
Rich et al. (2010)	New Jersey, U.S.		MI	1.14 (0.96, 1.32)	+$PM_{2.5}$: 1.05 (0.85, 1.28)
Chen et al. (2010b)	Shanghai, China		CVD	1.03 (1.00, 1.06)	+PM_{10}: 1.03 (1.00, 1.05)
Chang et al. (2005)	Taipei, Taiwan	≥ 20 °C	CVD	1.39 (1.32, 1.45)	+PM_{10}: 1.43 (1.35, 1.52)
Chang et al. (2005)	Taipei, Taiwan	<20 °C	CVD	1.24 (1.12, 1.37)	+PM_{10}: 0.97 (0.86, 1.10)
Yang et al. (2004)	Kaohsiung, Taiwan	≥ 25 °C	CVD	1.46 (1.32, 1.62)	+PM_{10}: 1.18 (1.02, 1.36)
Yang et al. (2004)	Kaohsiung, Taiwan	<25 °C	CVD	2.54 (2.27, 2.84)	+PM_{10}: 2.74 (2.36, 3.17)
Nuvolone et al. (2011)	Tuscany, Italy		MI	1.09 (1.02, 1.16)	+PM_{10}: 1.10 (1.00, 1.21)
Hsieh et al. (2010)	Taipei, Taiwan	≥ 23 °C	MI	1.24 (1.16, 1.35)	+PM_{10}: 1.24 (1.14, 1.37)
Hsieh et al. (2010)	Taipei, Taiwan	<23 °C	MI	1.26 (1.18, 1.35)	+PM_{10}: 1.22 (1.12, 1.33)
Cheng et al. (2009a)	Kaohsiung, Taiwan	≥ 25 °C	MI	1.23 (1.06, 1.44)	+PM_{10}: 1.25 (1.04, 1.51)
Cheng et al. (2009a)	Kaohsiung, Taiwan	<25 °C	MI	1.76 (1.55, 2.02)	+PM_{10}: 1.62 (1.36, 1.93)
Tsai et al. (2009)	Taipei, Taiwan	≥ 23 °C	Arrhythmia	1.19 (1.10, 1.27)	+PM_{10}: 1.16 (1.06, 1.27)
Tsai et al. (2009)	Taipei, Taiwan	<23 °C	Arrhythmia	1.34 (1.25, 1.46)	+PM_{10}: 1.32 (1.21, 1.44)
Guo et al. (2010)	Beijing, China		Hypertension	1.44 (1.15, 1.79)	+PM_{10}: 1.50 (1.15, 1.95)
Yang (2008)	Taipei, Taiwan	≥ 20 °C	CHF	1.41 (1.30, 1.53)	+PM_{10}: 1.37 (1.23, 1.53)
Yang (2008)	Taipei, Taiwan	<20 °C	CHF	1.04 (0.90, 1.21)	+PM_{10}: 1.08 (0.92, 1.30)
Tsai et al. (2003)	Kaohsiung, Taiwan		Cerebral Stroke	1.68 (1.38, 2.04)	+PM_{10}: 1.37 (1.04, 1.81)
Tsai et al. (2003)	Kaohsiung, Taiwan		Ischemic Stroke	1.67 (1.48, 1.87)	+PM_{10}:1.47 (1.24, 1.73)
Andersen et al. (2010)[b]	Copenhagen, Denmark		Mild Ischemic Stroke	1.61 (0.79, 3.30)[b]	+UFP: 1.09 (0.48, 2.56)[b]
Andersen et al. (2008b)	Copenhagen, Denmark		CVD	1.00 (0.93, 1.10)	+NC(tot): 1.00 (0.87, 1.10)

Table 4-34 (Continued): Corresponding risk estimates of ambient NO_2 or NO_X for hospital admissions for cardiovascular disease in studies conducting copollutant models with PM for presented in Figure 4-15.

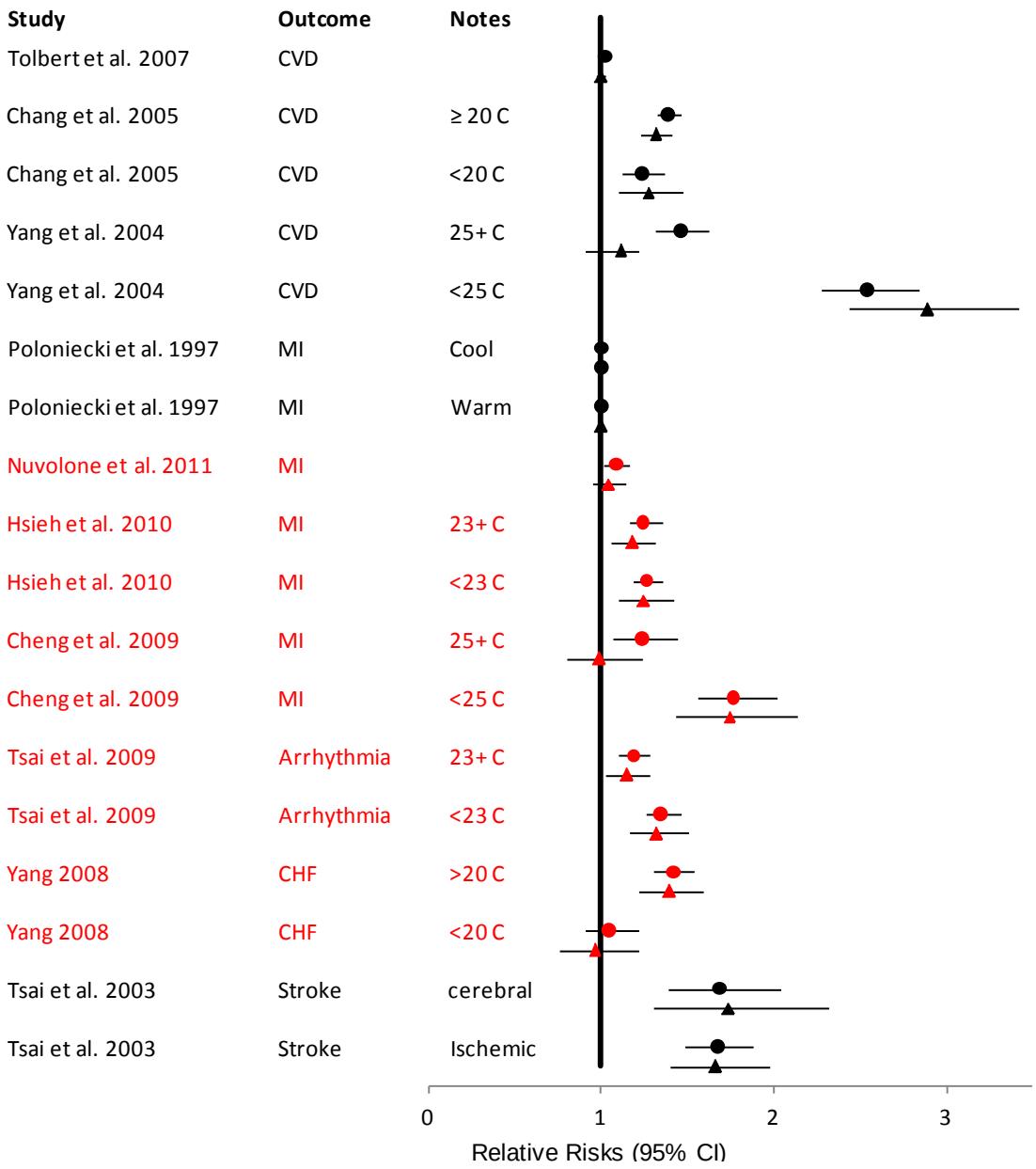
Study	Location	Notes	Cause	Single Pollutant Relative Risk[a] (95% CI)	Copollutant Relative Risk[a] (95% CI)
Turin et al. (2012)	Shiga, Japan		MI	1.14 (0.92, 1.40)	+TSP: 1.16 (0.91, 1.51)
Turin et al. (2012)	Shiga, Japan		Stroke	0.98 (0.89, 1.08)	+TSP: 1.04 (0.92, 1.16)
Turin et al. (2012)	Shiga, Japan		Cerebral Infarction	0.98 (0.87, 1.10)	+TSP:1.04 (0.91, 1.20)
Turin et al. (2012)	Shiga, Japan		Intracerebral Hemorrhage	1.06 (0.85, 1.33)	+TSP: 1.04 (0.82, 1.33)
Turin et al. (2012)	Shiga, Japan		Hemorrhage	1.12 (0.80, 1.56)	+TSP: 1.06 (0.70, 1.58)
Simpson et al. (2005a)	4 Australian Cities		CVD	1.07 (1.05, 1.09)	+BSP: 1.04 (1.02, 1.07)
Poloniecki et al. (1997)	London, U.K.	Cool	MI	1.00 (1.00, 1.00)	+BS: 1.00 (1.00, 1.00)
Poloniecki et al. (1997)	London, U.K.	Warm	MI	1.00 (1.00, 1.00)	+BS: 1.00 (1.00, 1.00)

Note: Studies correspond to studies presented in Figure 4-15.

[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 and 1-h averaging times, respectively.

[b]Effect estimates from Andersen et al. (2010) were standardized to a 40 ppb or 60-ppb increase in NO_X concentration for 24-h and 1-h averaging times, respectively.

BSP: black smoke particles; BS: black smoke; NC(tot): total number count.



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 for 24-h and 1-h averaging times, respectively. Model estimates are presented as pairs with the top estimate (Circles) for the single pollutant model and the bottom estimate (Triangles) for the copollutants model. Horizontal lines indicate 95% confidence intervals around the central estimate. Associated data presented in Table 4-35.

Figure 4-16 Results of single-pollutant and copollutants models of short-term exposure to NO_2 (withCO [triangles] and without CO [circles]) and hospital admissions for cardiovascular disease.

Table 4-35 Corresponding risk estimates of ambient NO_2 for hospital admissions for cardiovascular disease in studies conducting copollutant models with CO presented in Figure 4-16.

Study	Location	Notes	Mortality Cause	Single Pollutant Relative Risk[a] (95% CI)	Copollutant Relative Risk[a] (95% CI)
Tolbert et al. (2007)	Atlanta, GA		CVD	1.02 (1.01, 1.03)	0.99 (0.97, 1.02)
Chang et al. (2005)	Taipei, Taiwan	≥ 20 °C	CVD	1.39 (1.32, 1.45)	1.31 (1.22, 1.41)
Chang et al. (2005)	Taipei, Taiwan	<20 °C	CVD	1.24 (1.12, 1.37)	1.27 (1.09, 1.47)
Yang et al. (2004)	Kaohsiung, Taiwan	≥ 25 °C	CVD	1.46 (1.32, 1.62)	1.11 (0.91, 1.21)
Yang et al. (2004)	Kaohsiung, Taiwan	<25 °C	CVD	2.45 (2.27, 2.84)	2.89 (2.43, 3.42)
Poloniecki et al. (1997)	London, U.K.	Cool	MI	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)
Poloniecki et al. (1997)	London, U.K.	Warm	MI	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)
Nuvolone et al. (2011)	Tuscany, Italy		MI	1.09 (1.02, 1.16)	1.04 (0.94, 1.14)
Hsieh et al. (2010)	Taipei, Taiwan	≥ 23 °C	MI	1.24 (1.16, 1.35)	1.18 (1.06, 1.31)
Hsieh et al. (2010)	Taipei, Taiwan	<23 °C	MI	1.26 (1.18, 1.35)	1.24 (1.10, 1.42)
Cheng et al. (2009a)	Kaohsiung, Taiwan	≥ 25 °C	MI	1.23 (1.06, 1.44)	0.99 (0.80, 1.23)
Cheng et al. (2009a)	Kaohsiung, Taiwan	<25 °C	MI	1.76 (1.55, 2.02)	1.74 (1.42, 2.13)
Tsai et al. (2009)	Taipei, Taiwan	≥ 23 °C	Arrhythmia	1.19 (1.10, 1.27)	1.14 (1.02, 1.27)
Tsai et al. (2009)	Taipei, Taiwan	<23 °C	Arrhythmia	1.34 (1.25, 1.46)	1.32 (1.16, 1.51)
Yang (2008)	Taipei, Taiwan	≥ 20 °C	CHF	1.41 (1.30, 1.53)	1.39 (1.21, 1.58)
Yang (2008)	Taipei, Taiwan	<20 °C	CHF	1.04 (0.90, 1.21)	0.96 (0.76, 1.21)
Tsai et al. (2003)	Kaohsiung, Taiwan		Cerebral Stroke	1.68 (1.38, 2.04)	1.73 (1.30, 2.32)
Tsai et al. (2003)	Kaohsiung, Taiwan		Ischemic Stroke	1.67 (1.48, 1.87)	1.66 (1.40, 1.98)

Note:Studies correspond to studies presented in Figure 4-16,

[a]Effect estimates are standardized to a 20 ppb or 30-ppb increase in NO_2 concentration for 24- h and 1-h averaging times, respectively.

In conclusion, epidemiologic studies of adults consistently demonstrate NO_2-associated hospitalizations and ED visits for cardiovascular effects and mortality from cardiovascular disease. These high-quality studies have been replicated by different researchers in different locations and have adjusted for numerous potential confounding factors, thus limiting the level of uncertainty for bias from confounding. Due to limited analysis of potentially correlated non-criteria pollutants and recognized limitations of copollutant models, some uncertainty remains regarding the extent to which oxides of

nitrogen are independently associated with cardiovascular outcomes or if NO_2 serves as a marker for the effects of another traffic-related pollutant or mix of pollutants. Animal toxicological, controlled human exposure, and epidemiologic panel studies provide limited evidence for changes in measures of autonomic nervous system function to support the associations observed in epidemiologic studies of hospitalizations and cardiovascular mortality. Thus, the combined evidence from epidemiologic and experimental studies is sufficient to conclude that there is likely to be a causal relationship between short-term NO_2 exposure and cardiovascular effects.

Table 4-36 Summary of evidence supporting a likely to be a causal relationship between short-term NO_2 exposure and cardiovascular effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Consistent associations from multiple, high-quality epidemiologic studies at relevant NO_2 concentrations	Consistent evidence for 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 4.3.7	Mean 24-h avg: 11.9 – 40.5 ppb Mean 1-h max: 43.2 – 45.9 ppb
	Associations are strongest for hospitalizations for IHD.	Larrieu et al. (2007); Stieb et al. (2009); Peel et al. (2007); Von Klot et al. (2005); Mann et al. (2002) Section 4.3.7.2	Mean 24-h avg: 11.9 – 37.2 ppb Mean 1-h max: 45.9 ppb
Additional epidemiologic evidence help rule out chance, confounding, and other biases with reasonable confidence	Associations with ED visits, hospital admissions, and mortality found with adjustment for numerous potential confounding factors including meteorological factors and time trends.		
	Associations between ED visits and hospital admissions and NO_2 are generally robust in copollutant models containing PM, CO,O_3, or SO_2.	Figure 4-15, Figure 4-16, and Supplemental Figures S4-2, and S4-3 (U.S. EPA, 2013c)	
Consistent associations from multiple, high-quality epidemiologic studies at relevant NO_2 concentrations and cardiovascular mortality	Consistent evidence for increased risk of cardiovascular mortality in adults applying differing model specifications in diverse locations.	Bellini et al. (2007); Wong et al. (2008b); Chen et al. (2012b); Chiusolo et al. (2011) Section 4.3.8	Mean 24-h avg: 13.5 – 35.4 ppb

Table 4-36 (Continued): Summary of evidence supporting a likely to be a causal relationship between short-term NO_2 exposure and cardiovascular effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Uncertainty due to limited coherence with other lines of evidence	Limited evidence from epidemiologic panel studies and experimental studies for subclinical and clinical cardiovascular effects.		
Limited evidence from epidemiologic panel studies	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 (2010a); Zanobetti et al. (2010) Section 4.3.3.1 QT interval: Henneberger et al. (2005) Section 4.3.4.1	
Limited evidence from animal toxicological and controlled human exposure studies	Decrease in HF domain normalized to HR (i.e., HRV) but decrease in QTVI in controlled human exposure study	Huang et al. (2012b)	Healthy adults: 500 ppb NO_2
	Increase in a marker of endothelial dysfunction (i.e., ICAM-1) in rats and in cells treated with plasma from adults exposed to NO_2.	Li et al. (2011a) Channell et al. (2012)	Rats: 2,660 and 5,320 ppb NO_2 Human cells exposed to plasma from healthy adults: 500 ppb NO_2
Weak evidence to describe key events that inform the mode of action		Section 3.3.2.8	
Oxidative stress	Evidence of increased oxidative stress 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) Channell et al. (2012)	Rats: 5,320 ppb NO_2 Healthy adults: 500 ppb NO_2
Inflammation	Toxicological evidence of increased transcription of some inflammatory mediators in vitro (i.e., IL-8) and in rats (i.e., TNF-α).	Channell et al. (2012) Li et al. (2011a)	Human cells exposed to plasma from healthy adults: 500 ppb NO_2 Rats: 5,320 ppb NO_2
	Inconsistent epidemiologic evidence for changes in CRP, IL-6, and TNF-RII.	Section 4.3.6.1	

[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, 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.

4.4 Total Mortality

4.4.1 Introduction and Summary of 2008 ISA 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, 2008c). 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 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, 2008c) 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.

4.4.2 Associations between Short-term NO_2 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. 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., 2008b), 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 Europe, potentially limiting the generalizability of results from these studies to other cities.

Consistent with ISAs for other criteria pollutants, this section focuses primarily on multicity studies because they examine the association between short-term NO_2 exposure and a health effect over a large geographic area using a consistent statistical methodology (U.S. EPA, 2008c). Where applicable single-city studies are evaluated that encompass a long study-duration, provide additional evidence indicating that a specific population may be at increased risk of NO_2-related mortality, or address an uncertainty in the NO_2-mortality relationship not represented in other single-city or multicity studies. Other recent studies of mortality are not the focus of this evaluation because of inadequate study design or insufficient sample size. The full list of the studies can be found in Supplemental Table S4-2 (U.S. EPA, 2013f). Overall this section evaluates studies that

examined the association between short-term NO_2 exposure and mortality, and addresses the key uncertainties 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 or within a population), seasonal heterogeneity in NO_2 associations, and the NO_2-mortality concentration-response (C-R) relationship.

4.4.3 Associations between Short-term NO_2 Exposure and Mortality in All-Year Analyses

Multicity studies evaluated in the 2008 ISA for Oxides of Nitrogen reported consistent, positive associations between short-tem NO_2 exposure and mortality in all-year analyses (U.S. EPA, 2008c). 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 (Biggeri et al., 2005; Simpson et al., 2005b). 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 4-37.

Table 4-37 Air quality characteristics of studies evaluated in the 2008 ISA for Oxides of Nitrogen and recently published multicity and select single-city studies.

Study	Location	Years	Mortality Outcome(s)	Averaging Time	Mean Concentration (ppb)	Upper Percentile Concentrations (ppb)
Biggeri et al. (2005)[a]	8 Italian cities	1990-1999	Total, Cardiovascular, Respiratory	24-h avg	30.1 – 55.0	95th: 45.8 – 94.0 Max: 62.6 – 160.7
Brook et al. (2007)[a]	10 Canadian cities	1984-2000	Total	24-h avg	---	---
Burnett et al. (2004)[a]	12 Canadian cities	1981-1999	Total, Cardiovascular, Respiratory	24-h avg	10.0 – 26.4	---
HEI (2003)[a]	58 U.S. cities[b]	1987-1994	Total	24-h avg	9.2 – 39.4	---
Hoek (2003)[a]	the Netherlands	1986-1994	Total	24-h avg	---	---
Samoli et al. (2006)[a]	30 European cities	1990-1997	Total, Cardiovascular, Respiratory	1-h max[c]	24.0 – 80.5	90th: 33.1 – 132.5
Simpson et al. (2005b)[a]	4 Australian cities	1996-1999	Total, Cardiovascular, Respiratory	1-h max	16.3 – 23.7	Max: 96.0 – 111.5
Stieb et al. (2003)[a]	Meta-analysis	---	Total	---	---	---
Bellini et al. (2007)	15 Italian cities	1996-2002	Total, Cardiovascular, Respiratory	24-h avg	---	---
Berglind et al. (2009)	5 European cities	1992-2002	Total	24-h avg	11.0 – 35.4	---
Cakmak et al. (2011b)	7 Chilean cities[d]	1997-2007	Total	24-h avg	21.3 – 27.0	---
Chen et al. (2012b)	17 Chinese cities	1996-2010[e]	Total, Cardiovascular, Respiratory	24-h avg	13.5 – 34.8	Max: 55.1 – 132.1
Chiusolo et al. (2011)	10 Italian cities[f]	2001-2005	Total, Cardiovascular, Cerebrovascular, Respiratory	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	24-h avg	35.4	---

Table 4-37 (Continued): Air quality characteristics of studies evaluated in the 2008 ISA for Oxides of Nitrogen and recently published multicity and select single-city studies.

Study	Location	Years	Mortality Outcome(s)	Averaging Time	Mean Concentration (ppb)	Upper Percentile Concentrations (ppb)
Moolgavkar et al. (2013)	72 U.S. cities[g]	1987-2000	Total	24-h avg	---	---
Shin et al. (2012)	24 Canadian cities	1984-2004	Cardiopulmonary	24-h avg	8.7 – 25.0	---
Stieb et al. (2008)	12 Canadian cities	1981-2000	Total	3-h max	1981-1990: 24.7 – 42.6 1991-2000: 16.3 – 39.2	---
Wong et al. (2010); Wong et al. (2008b)	4 Asian cities	1996-2004[h]	Total, Cardiovascular, Respiratory	24-h avg	23.2 – 34.6	75th: 28.5 – 41.2 Max: 72.6 – 131.9

[a]Multicity studies evaluated in the 2008 ISA for Oxides of Nitrogen.

[b]Of the 90 cities included in the NMMAPS analysis only 58 had NO_2 data

[c]Samoli et al. (2006) estimated 1-h max concentrations for each city by multiplying 24-h avg concentrations by 1.64.

[d]Of the 7 cities only 4 had NO_2 data.

[e]Study period varied for each city and encompassed 2 to 7 years. Hong Kong was the only city that had air quality data prior to 2000.

[f]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.

[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.

As demonstrated in Figure 4-17 (Table 4-38), 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 (nonaccidental) 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), which both focused on the risk of mortality attributed to air pollution in the population $\geq$ 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).

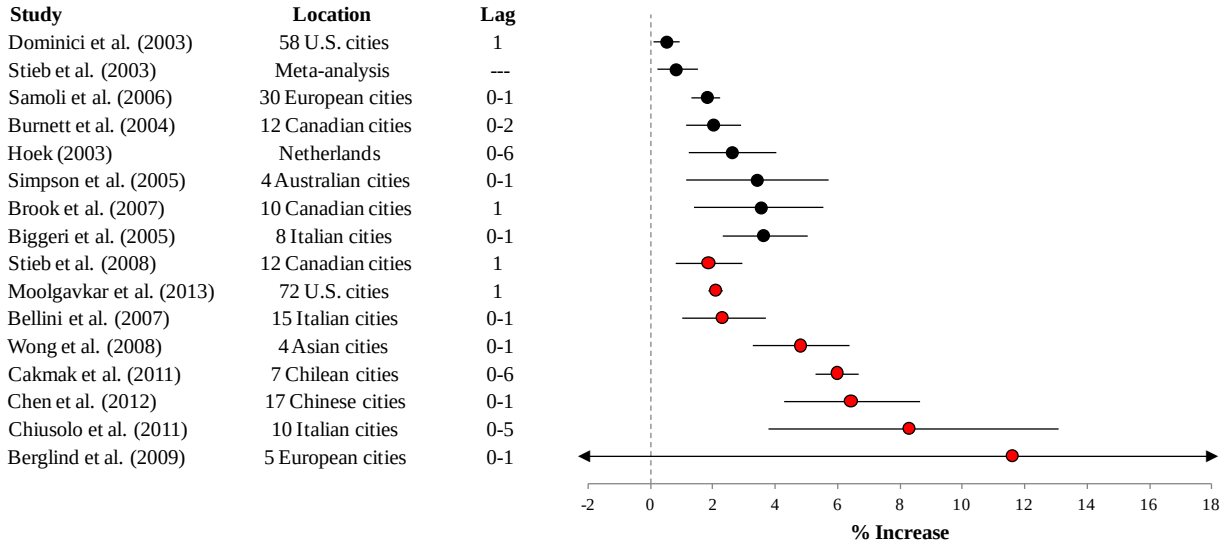


Figure 4-17 Summary of multicity studies evaluated in the 2008 ISA for Oxides of Nitrogen (black circles) and recently published (red circles) that examined the association between short-term NO_2 exposure and total mortality.

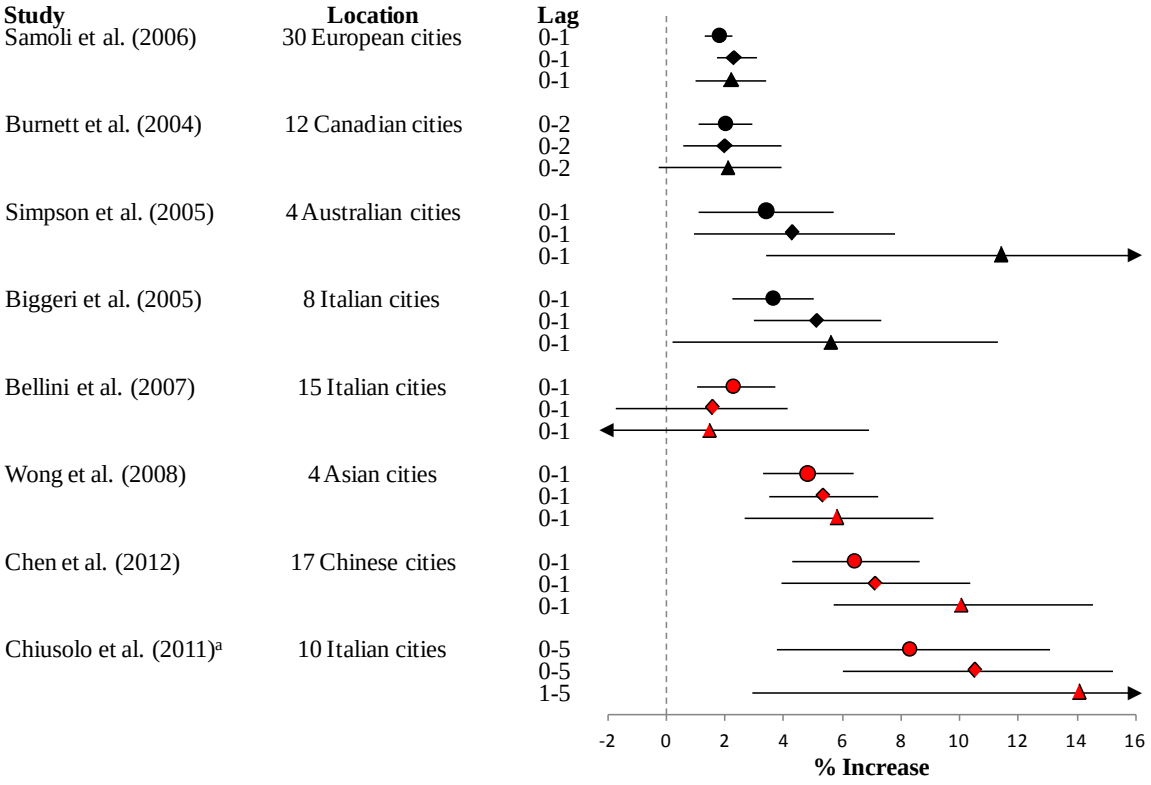
Table 4-38 Corresponding percent increase in total mortality (95% CI) for Figure 4-17.

Study	Location	Age	Lag	Averaging Time	% Increase (95% CI)
Dominici et al. (2003)[a]	58 U.S. cities	All	1	24-h avg	0.50 (0.09, 0.90)
Stieb et al. (2003)[a]	Meta-analysis	All	---	24-h avg	0.80 (0.20, 1.5)
Samoli et al. (2006)[a]	30 European cities	All	0-1	1-h max	1.8 (1.3, 2.2)
Burnett et al. (2004)[a]	12 Canadian cities	All	0-2	24-h avg	2.0 (1.1, 2.9)
Hoek (2003)[a]	the Netherlands	All	0-6	24-h avg	2.6 (1.2, 4.0)
Simpson et al. (2005b)[a]	4 Australian cities	All	0-1	1-h max	3.4 (1.1, 5.7)
Brook et al. (2007)[a]	10 Canadian cities	All	1	24-h avg	3.5 (1.4, 5.5)
Biggeri et al. (2005)[a]	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	2.3 (1.0, 3.7)
Wong et al. (2008b)	4 Asian cities	All	0-1	24-h avg	4.8 (3.3, 6.4)
Cakmak et al. (2011b)	7 Chilean cities	All	0-6	24-h avg	6.0 (5.3, 6.7)
Chen et al. (2012b)	17 Chinese cities	All	0-1	24-h avg	6.4 (4.3, 8.6)
Chiusolo et al. (2011)	10 Italian cities	≥ 35	0-5	24-h avg	8.3 (3.8, 13.1)
Berglind et al. (2009)	5 European cities	≥ 35	0-1	24-h avg	11.6 (-5.9, 32.4)

Note:Studies correspond to studies presented in Figure 4-17.

[a]Multicity studies evaluated in 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 4-18). 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.



Black symbols = multicity studies evaluated in the 2008 ISA for Oxides of Nitrogen; Red symbols = recent studies.
Circle = total mortality; Diamond = cardiovascular mortality; Triangle = respiratory mortality.
a = Study focused on individuals ≥ 35 years of age while the other studies focused on all ages.

Figure 4-18 Percent increase in total, cardiovascular, and respiratory mortality from multicity studies for a 20-ppb increase in 24-h avg or 30-ppb increase in 1-h max NO_2 concentrations.

Table 4-39 Corresponding percent increase (95% CI) for Figure 4-18.

Study	Location	Age	Lag	Averaging Time	Mortality	% Increase (95% CI)
Samoli et al. (2006)[a]	30 European cities	All	0-1	1-h max	Total	1.8 (1.3, 2.2)
					Cardiovascular	2.3 (1.7, 3.0)
					Respiratory	2.2 (0.98, 3.4)
Burnett et al. (2004)[a]	12 Canadian cities	All	0-2	24-h avg	Total	2.0 (1.1, 2.9)
					Cardiovascular	2.0 (0.53, 3.9)
					Respiratory	2.1 (-0.27, 3.9)
Simpson et al. (2005b)[a]	4 Australian cities	All	0-1	1-h max	Total	3.4 (1.1, 5.7)
					Cardiovascular	4.3 (0.90, 7.8)
					Respiratory	11.4 (3.4, 19.7)
Biggeri et al. (2005)[a]	8 Italian cities	All	0-1	1-h max	Total	3.6 (2.3, 5.0)
					Cardiovascular	5.1 (3.0, 7.3)
					Respiratory	5.6 (0.2, 11.3)
Bellini et al. (2007)	15 Italian cities	All	0-1	24-h avg	Total	2.3 (1.0, 3.7)
					Cardiovascular	1.6 (-1.8, 4.1)
					Respiratory	1.5 (-2.4, 6.9)
Wong et al. (2008b)	4 Asian cities	All	0-1	24-h avg	Total	4.8 (3.3, 6.4)
					Cardiovascular	5.3 (3.5, 7.2)
					Respiratory	5.8 (2.6, 9.1)
Chen et al. (2012b)	17 Chinese cities	All	0-1	24-h avg	Total	6.4 (4.3, 8.6)
					Cardiovascular	7.1 (3.9, 10.3)
					Respiratory	10.1 (5.7, 14.5)
Chiusolo et al. (2011)	10 Italian cities	≥ 35	0-5	24-h avg	Total	8.3 (3.8, 13.1)
					Cardiovascular	10.5 (6.0, 15.2)
			1-5		Respiratory	14.1 (2.9, 26.4)

Note:Studies correspond to studies presented in Figure 4-18.

[a]Multicity studies evaluated in the 2008 ISA for Oxides of Nitrogen.

4.4.4 Potential Confounding of the NO_2-Mortality Relationship

A key uncertainty of the NO_2-mortality relationship identified in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) 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 copollutants models 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).

In a study of 108 U.S. cities using data from the National Morbidity, Mortality, and Air Pollution Study (NMMAPS) for 1987-2000 (of which 72 had NO_2 data), Moolgavkar et al. (2013) used a subsampling 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. In a copollutant analysis, the NO_2-mortality risk estimate remained robust to the inclusion of PM_{10} (1.9% [95% CI: 1.2, 2.5]).

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; 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. However, the results of the copollutant and multipollutant analyses conducted by Stieb et al. (2008) indicate that the NO_2-mortality relationship remains robust when adjusted for other pollutants (quantitative results not presented).

Additional studies conducted in Europe and Asia also provide evidence indicating that NO_2-mortality associations remain robust in copollutants models. In a multicity study conducted with a time-stratified, case-crossover analysis of 10 Italian cities, which is part

of the Italian Epi Air multicenter study "Air Pollution and Health: Epidemiological Surveillance and Primary Prevention," Chiusolo et al. (2011) reported consistent, positive associations for total and cause-specific mortality (i.e., cardiac, cerebrovascular, and respiratory), ranging from an 8.3 to 14.1% increase for a 20-ppb increase in 24-h 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 with all-year data and with O_3 with data restricted to the summer season (i.e., April -September) (Table 4-40).

Table 4-40 Percent increase in total and cause-specific mortality for a 20-ppb increase in 24-h avg NO_2 concentrations in single- and copollutant 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)	8.3 (3.7, 13.1)
		With PM_{10} (lag 0-5)	7.7 (1.9, 13.9)
	April-September	NO_2 (lag 0-5)	18.3 (12.6, 24.2)
		With O_3 (lag 0-5)	18.7 (13.4, 24.2)
Cardiac	All-year	NO_2 (lag 0-5)	10.5 (6.0, 15.2)
		With PM_{10} (lag 0-5)	10.3 (4.1, 16.8)
	April-September	NO_2 (lag 0-5)	19.6 (11.7, 28.1)
		With O_3 (lag 0-5)	19.3 (11.0, 28.2)
Cerebrovascular	All-year	NO_2 (lag 0-5)	9.4 (-0.5, 20.2)
		With PM_{10} (lag 0-5)	10.2 (-2.7, 24.8)
	April-September	NO_2 (lag 0-5)	33.8 (19.7, 49.7)
		With O_3 (lag 0-5)	30.9 (14.2, 50.1)
Respiratory	All-year	NO_2 (lag 0-5)	14.1 (2.9, 26.4)
		With PM_{10} (lag 0-5)	13.9 (3.0, 25.5)
	April-September	NO_2 (lag 0-5)	42.4 (16.6, 73.9)
		With O_3 (lag 0-5)	44.6 (15.0, 81.9)

Note: Concentrations converted from $\mu g/m^3$ to ppb using the conversion factor of 0.532, assuming standard temperature (25 °C) and pressure (1 atm).

Source: Reprinted from Chiusolo et al. (2011), Environmental Health Perspectives.

The Public Health and Air Pollution in Asia (PAPA) study as well as the China Air Pollution and Health Effects Study (CAPES) collectively found that the NO_2-mortality association remains robust in copollutant models with other criteria air pollutants. 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., 2008b). 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. 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 robust to adjustment for copollutants (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 6.4% increase (95% CI: 4.3, 8.6) in total mortality, 7.1% increase (95% CI: 3.9, 10.3) for cardiovascular mortality, and 10.0% increase (95% CI: 5.7, 14.5) for respiratory mortality for a 20-ppb increase in 24-h average 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 remained robust across total, cardiovascular, and respiratory mortality with the percent increase in mortality ranging from 4.7-6.9% in copollutant models with PM_{10} and 5.3-7.2% in models with SO_2 for a 20-ppb increase in 24-h average 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 (APHENA) study, although 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. datasets, 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 dataset, 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 as the df per year increased.

Unlike Stieb et al. (2008) and Wong et al. (2010), which 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), the authors 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 1day in the 50 df model and 2.1% (95% CI: 1.8, 2.3) in the 100 df model.

4.4.5 Modification of the NO_2-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, 2008c), 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 Air Pollution and Health: A European Approach (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. Because these studies examine similar effect measure modifiers 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.

Lifestage

Each of the evaluated multicity studies examined whether older adults were at increased risk of mortality in response to short-term NO_2 exposures. These studies collectively

found evidence of increased risk in the population over the age of 65 years, but the exact age groups examined varied across studies. Comparing the risk of NO_2-related mortality across age groups in a multicity study in Italy, Chiusolo et al. (2011), reported that those over 85 years of age were at greatest risk compared with individuals 35-64 years old. Additionally, the authors observed that the risk of NO_2-associated mortality did not follow a consistent trend as age increased over the age of 64. These results are similar to those observed in a multicity study in Chile (Cakmak et al., 2011b). Cakmak et al. (2011b) reported that NO_2-mortality risk estimates increased in age groups $\geq$ 65 years of age, with the magnitude of the effect increasing as age increased over 75 years. Chen et al. (2012b) in CAPES did not examine individual age groups above 65 years of age, but reported increased risk in individuals older than 65 years of age (6.7% [95% CI: 3.5, 10.0] for a 20-ppb increase in 24-h average NO_2 concentrations at lag 0-1 days) compared to those less than 65 years (3.3% [95% CI: 1.3, 5.3]).

Sex

Among the studies that examined potential differences in the risk of NO_2-related mortality by sex, both Cakmak et al. (2011b) and Chen et al. (2012b) reported some evidence supporting increased risk of NO_2-associated mortality in females compared to males. Cakmak et al. (2011b) found NO_2 risk estimate for females (7.2%) were slightly higher than those for males (4.7%). Chen et al. (2012b) reported risk estimates similar in magnitude to those observed in Cakmak et al. (2011b) (males: 4.7% [95% CI: 2.3, 7.2]; females: 6.8% [95% CI: 2.9, 10.8] for a 20-ppb increase in 24-h avg NO_2 concentrations at lag 0-1). However, in Italy, Chiusolo et al. (2011) reported some evidence of effect measure modification of the NO_2-mortality relationship by sex with a slightly larger risk estimate found in males (9.4%) compared to females (6.7%).

Pre-existing Diseases

Of the multicity studies evaluated, only Chiusolo et al. (2011) examined whether pre-existing diseases increased the risk of NO_2-related mortality. The authors reported the greatest risk in highly diseased individuals (i.e., individuals with 3 or more chronic diseases or who had been hospitalized one month to 2 years prior to death for either chronic or acute cardiopulmonary conditions). In analysis of individual chronic diseases and individuals without the disease as the referent, the strongest evidence for increased risk of NO_2-related mortality was for individuals with pre-existing diseases that affect the cardiovascular system (i.e., diabetes, cardiac ischemic disease, diseases of the pulmonary circulation, heart conduction disorders, and heart failure). In a study conducted in 5 European cities, Berglind et al. (2009) reported additional evidence supporting this link

by demonstrating increased risk of NO_2-associated mortality in survivors of myocardial infarction. Although Chiusolo et al. (2011) examined only one pre-existing respiratory condition (i.e., chronic pulmonary diseases), it provided evidence that individuals with pre-existing respiratory conditions also may be at increased risk of NO_2-related mortality.

Socioeconomic Status

The potential modification of the NO_2-mortality relationship by SES was examined in a number of studies, but each used slightly different indicators for income/employment and educational attainment. Chiusolo et al. (2011) examined socioeconomic position and income (both measured as the median of the census block of residence) and reported inconsistent results: increased risk in the low and high socioeconomic position groups, and in the low and middle income groups. Cakmak et al. (2011b) examined the effect of income and employment on the risk of NO_2-related mortality using community income level and employment category (i.e., unemployed, blue-collar, and white-collar). Across levels of each of these indicators, increased risk of NO_2-related mortality was observed for those that were not white-collar workers or had a lower income (Cakmak et al., 2011b). These results are consistent with Wong et al. (2008b), which found that the most socially deprived areas of Hong Kong, as measured by a composite metric of socioeconomic status, had higher NO_2-mortality risks, especially for cardiovascular mortality.

Studies that examined the effect of educational attainment on the NO_2-mortality relationship consistently reported that low educational attainment increased risk. Although Cakmak et al. (2011b) divided educational attainment into more refined categories (i.e., primary school not completed, primary school graduation, high school graduation, some college, and university diploma), the authors only compared low versus high education groups. The authors reported evidence of increased risk in the low education group. Chen et al. (2012b) also reported that low educational attainment increased the risk of NO_2-related mortality using the crude high and low categories, where high corresponded to middle school education and above, and low corresponded to illiterate or primary school.

4.4.6 Potential Seasonal Differences in the NO_2-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 effects 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 to the summer months).

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 to 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 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 to 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 suggest 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=~0.35), which makes it less likely O_3 is a confounder of the NO_2-mortality relationship (Section 4.4.6).

To date, U.S.-based 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, 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 risk estimates in all-year (1.8% [95% CI: 0.17, 3.3] for a 20-ppb increase in 24-h avg NO_2 concentrations at lag 1 day) and seasonal (Warm: 1.8% [95% CI: -0.35, 3.9]; Cold: 2.3% [95% CI: 0.0, 4.7]) analyses.

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 to the winter (October-March) for total (6.54% versus 0.98% for a 20-ppb increase in 24-h NO_2 concentrations at lag 0-1 days), respiratory (9.4% versus -0.04%), and cardiovascular (7.4% versus -0.19%) 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 to all-year (Table 4-39) for total (non-accidental) 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 to the warm for total (nonaccidental) mortality (Cold: 4.9% versus Warm: 1.8% for a 20-ppb increase in 24-h avg NO_2 at lag 0-1 days), cardiovascular (Cold: 4.9% versus Warm: 1.2%), and respiratory mortality (Cold: 10.6% versus Warm: -5.2%). 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.

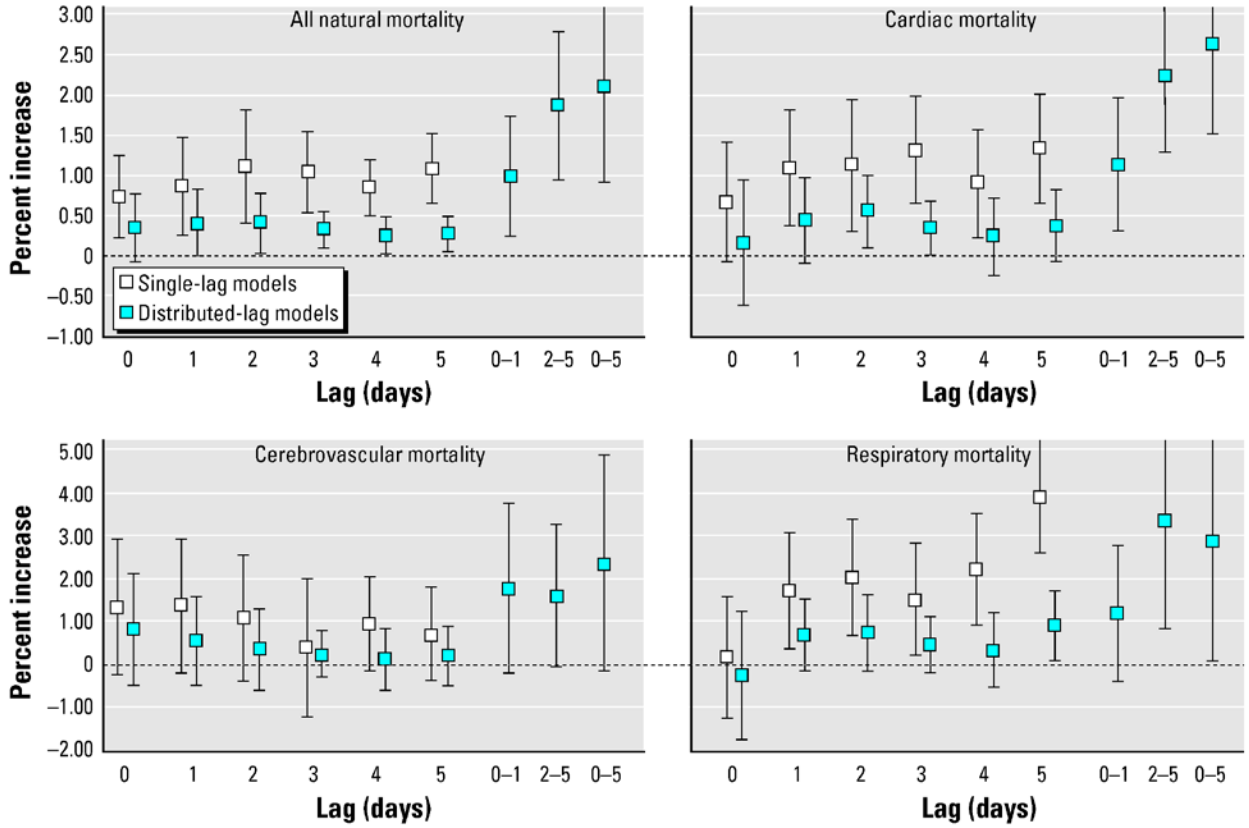
4.4.7 NO_2-Mortality Concentration-Response (C-R) Relationship and Related Issues

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, 2008c). 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.

Chiusolo et al. (2011) in the analysis of 10 Italian cities 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 delayed effect extending out to 5 days for all mortality outcomes (Figure 4-19).

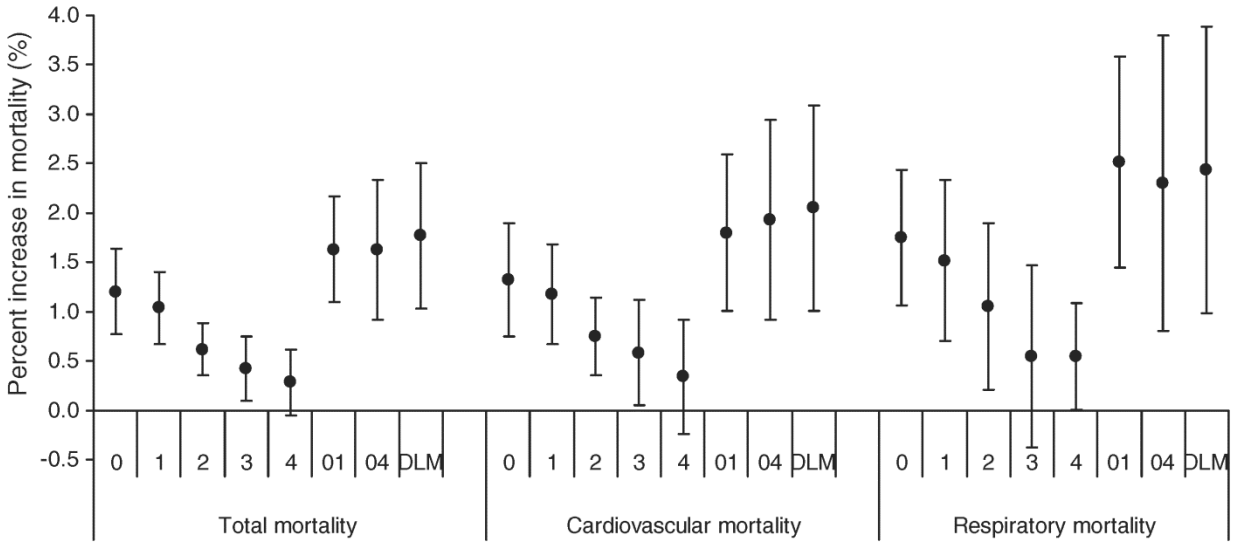


Source: Reprinted from Chiusolo et al. (2011) in Environmental Health Perspectives.

Figure 4-19 Percent increase in total and cause-specific mortality due to short-term NO_2 exposure at single day lags, individual lag days of a constrained polynomial distributed lag model, and multi-day 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 4-20). However, the similar or larger magnitude 0-4 day average 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 4-19).



Percent 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. Multi day average lags (01 [corresponds to 2-day moving average] and lag 04 [corresponds to 5-day moving average of NO2 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)

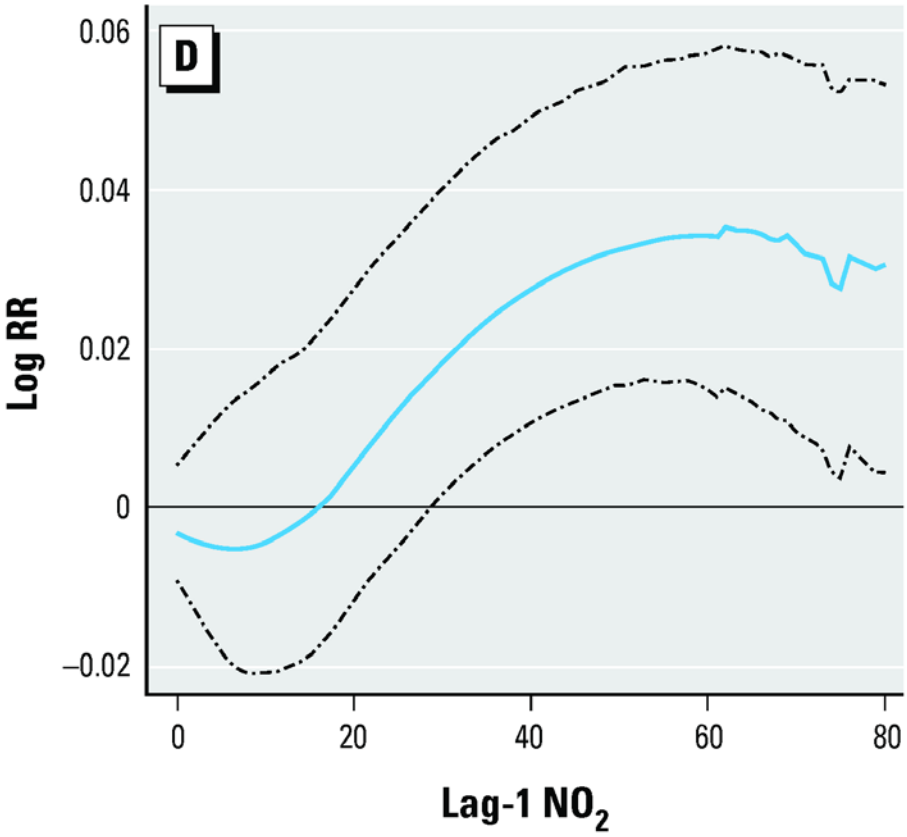
Figure 4-20 Percent increase in total and cause-specific mortality due to short-term NO_2 exposure in single- and multi-day lag models.

Additional studies that examined the effect of NO_2 on 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, 2008c), 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. (2008b) and Wong et al. (2010) examined single and multi-day lags in each individual city in the PAPA study. In the 3 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.

C-R Relationship

The studies evaluated in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) 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 dataset 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 4-21). 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 prevent a clear interpretation of the shape of the curve at the tails of the distribution 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.



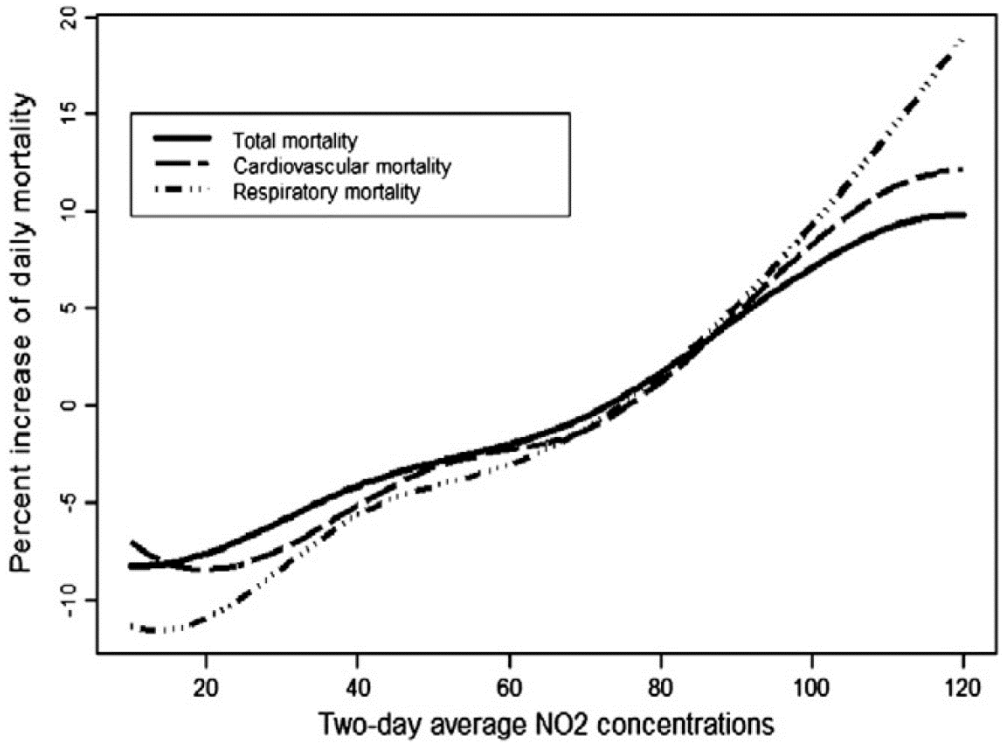
Source: Reprinted from Moolgavkar et al. (2013), Environmental Health Perspectives.

Figure 4-21 Flexible ambient C-R relationship between short-term NO_2 (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 (AIC). 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 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 μg/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 μg/m^3 [20.8 ppb] and 70 μg/m^3 [36.4 ppb] for total mortality, 50 μg/m^3 [26.0 ppb] and 70 μg/m^3 [36.4 ppb] for cardiovascular mortality, and 40 μg/m^3 [20.8 ppb] and 60 μg/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 4-22), which was confirmed by the lack of a statistically significant difference in the deviance between the spline and linear fit models.

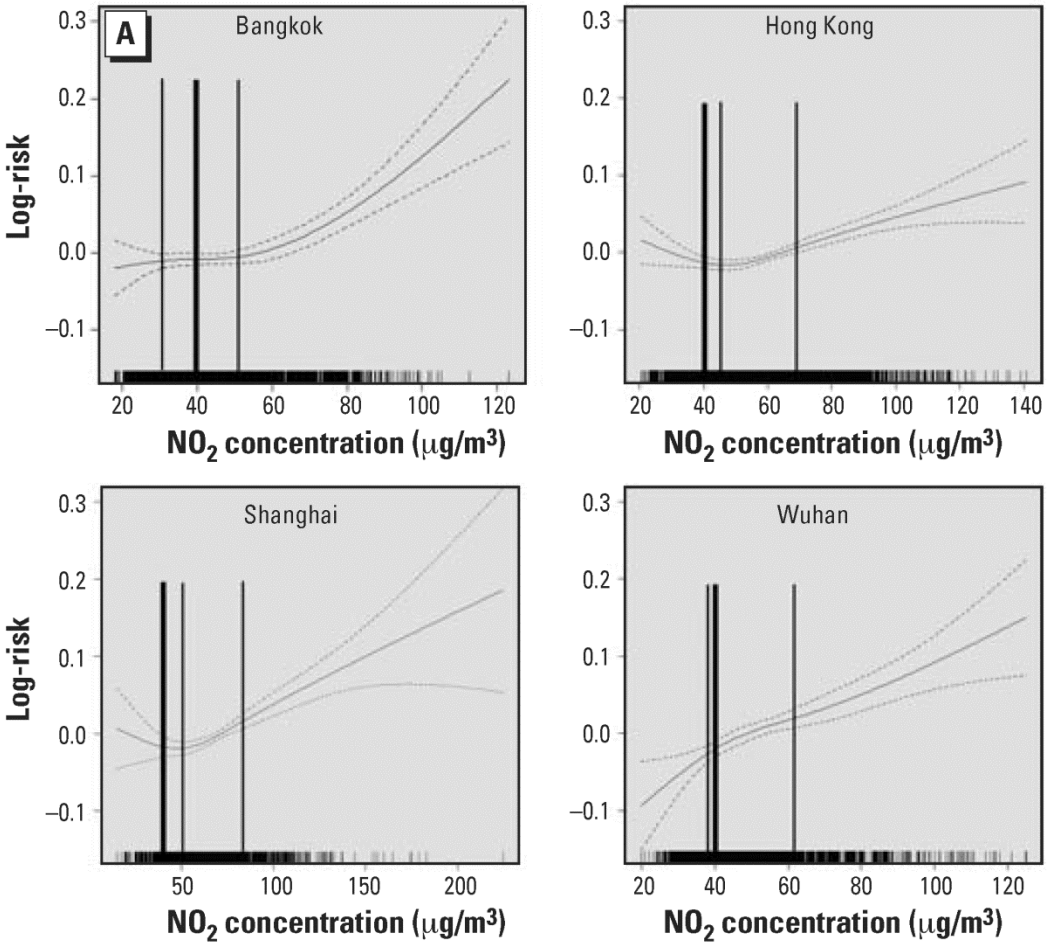


Source: Reprinted with permission of Elsevier Ltd. (Chen et al., 2012b),

Figure 4-22 CAPES C-R curve for the association between total and cause-specific mortality and 24-h avg NO_2 concentrations at lag 0-1 days. NO_2 concentrations on the x-axis are in the unit of μg/m^3.

The four-city PAPA study (Wong et al., 2010; Wong et al., 2008b) 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 supports 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 4-23). 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 non-linearity, 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. (2008b) to depict where the WHO 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: Reprinted from Wong et al. (2008b), Environmental Health Perspectives.

Figure 4-23 C-R curve for association between total mortality and 24-h avg NO_2 concentrations at lag 0-1 days in the four cities of the PAPA study.

4.4.8 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. This collective evidence indicates that there is likely to be a causal relationship between short-term NO_2 exposures and total mortality. This conclusion represents a change from the 2008 ISA for Oxides of Nitrogen that

concluded the evidence "was suggestive but not sufficient to infer a causal relationship" (U.S. EPA, 2008c). The recent multi-city studies evaluated inform key uncertainties and limitations 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. This section describes the evaluation of evidence for total mortality, with respect to the causal determination for short-term exposure to oxides of nitrogen using the framework described in Table II of the Preamble.The key evidence, as it relates to the causal framework, is summarized in Table 4-41.

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. However, it should be noted that it is difficult to disentangle the independent effects of NO_2 from those of other measured or unmeasured pollutants that also contribute to traffic-related pollution (Section 1.5), 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, 2008c). Additionally, recent studies examined the potential confounding effects of inadequate control for temporal trends and reported similar NO_2-mortality risk estimates across a range of degrees of freedom per year.

An examination of factors that may contribute to increased risk of NO_2-related mortality found evidence that older adults ($\geq$ 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 delayed 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, 2009a) and O_3 (U.S. EPA, 2013b)], 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., 2008b).

In conclusion, the recent epidemiologic studies build upon and support the consistent positive associations between short-term NO_2 exposures and total mortality presented in the 2008 ISA for Oxides of Nitrogen. These associations were found to remain generally robust (i.e., positive, and almost unchanged compared to single-pollutant model results) in the numerous studies that conducted copollutant analyses. However, studies that focused on PM and examined whether NO_2 modified the PM-mortality relationship suggest that NO_2 and PM may be effect modifiers of each other. Although the collective evidence supports an independent effect of short-term NO_2 exposures on all-cause mortality, 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 4.3 and respiratory effects in Section 4.2) 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 evidence for increases in cardiovascular-related hospital admissions and ED visits, specifically for IHD. However, there is limited evidence from experimental studies for NO_2-induced cardiovascular effects. Together, the evidence does not clearly describe a biologically plausible mechanism to support NO_2-induced cardiovascular mortality. There is stronger evidence for NO_2-induced respiratory effects with toxicological and controlled human exposure studies demonstrating AHR (Section 4.2.2) 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 4.2.6). However, the biological mechanism that explains the continuum of effects that could lead to respiratory-related mortality also remains unclear. The robust evidence observed across various multicity studies in combination with the uncertainty in the biological mechanism that could lead to NO_2-induced mortality forms a collective body

of evidence that is sufficient to conclude there is likely to be a causal relationship between short-term NO_2 exposure and total mortality.

Table 4-41 Summary of evidence supporting a likely to be a causal relationship between short-term 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 associations from multiple, high quality epidemiologic studies at relevant concentrations	Increases in mortality in multicity studies conducted in the U.S., Canada, South America, Europe, and Asia.	Section 4.4.3 Table 4-37	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 4-37
Additional epidemiologic evidence to reduce chance, confounding, and other biases	NO_2 associations remain robust with adjustment for other combustion-related pollutants in copollutant models.	Moolgavkar et al. (2013); Chen et al. (2012b); Chiusolo et al. (2011); Wong et al. (2010); Stieb et al. (2008); Wong et al. (2008b)	
	NO_2 and PM may be effect modifiers of each other.	Katsouyanni et al. (2009); Katsouyanni et al. (2003); Katsouyanni et al. (2001)	
Uncertainty due to limited coherence with morbidity evidence	Limited coherence and biological plausibility for NO_2-induced cardiovascular mortality (~35% total mortality[d]). Epidemiologic evidence for increases in cardiovascular-related hospital admissions and ED visits, specifically for IHD, but inconsistent epidemiologic and experimental evidence for other endpoints and weak biological plausibility for cardiovascular effects, including mode of action.	Section 4.3.9 Table 4-36	
	Limited coherence for NO_2-induced respiratory mortality (~8% total mortality[d]).Consistent evidence for increases in respiratory morbidity across disciplines, particularly for asthma morbidity. Less robust evidence for COPD and respiratory infection.	Section 4.2.9 Table 4-23	

[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 5 INTEGRATED HEALTH EFFECTS OF LONG-TERM EXPOSURE TO OXIDES OF NITROGEN

5.1 Introduction

This chapter summarizes, integrates, and evaluates the evidence for a broad spectrum of health effects associated with long-term exposure (i.e., 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 5.2), cardiovascular effects (Section 5.3), reproductive and developmental effects (Section 5.4), and mortality (Section 5.5). Chapter 5 concludes with a discussion of the evidence for the cancer effects of oxides of nitrogen (Section 5.6). In order to characterize the weight of evidence for the effects of oxides of nitrogen on reproductive and developmental effects in a cohesive manner, results from both short-term (i.e., up to 1 month) and long-term exposure studies are included in this chapter and are identified according to their exposure duration in the text and tables throughout Section 5.4.

Individual sections for major outcome categories (e.g., respiratory effects, cardiovascular effects) begin with a summary of conclusions from the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) followed by an evaluation of recent (i.e., published since the completion of the 2008 ISA for Oxides of Nitrogen) studies that is intended to build upon evidence from previous reviews. Within each of these sections, results are organized into smaller groups of endpoints (e.g., asthma incidence) then specific scientific discipline (i.e., epidemiology, toxicology).

Sections for each of the major outcome categories (e.g., respiratory effects, cardiovascular effects) conclude with an integrated summary of the assessment of evidence and conclusions regarding causality. A determination of causality was made for a major outcome category (e.g., respiratory effects) or smaller group of related outcomes (e.g., birth outcomes) by evaluating the evidence for each outcome category or group independently with the causal framework (described in the Preamble to this ISA). 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 considered relevant to this ISA, i.e., NO_2 concentrations up to 5,000 ppb as described in Section 1.1. Studies that examined higher NO_X or NO_2 concentrations were evaluated particularly to inform mode of action.

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_X or NO_2 exposures or ambient concentrations and relationship with the outcome), and other biases could be ruled out with reasonable confidence. This evaluation involved consideration of the strength of study design and analytical methodology as well as the potential for selection bias, publication bias, and confounding.

Epidemiologic studies of long-term NO_X or NO_2 exposure rely on between-subject differences in exposure, as a result of residence in locations (spatial differences) or examination in 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 correlated with NO_X or NO_2 exposure that vary between subjects. These potential confounding factors can include socioeconomic status, diet, smoking or exposure to environmental tobacco smoke, medication use, and copollutant exposures. 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 also is possible by factors that are poorly measured. The evidence was examined based on factors shown to be associated with NO_2 exposure and health outcomes. Epidemiologic studies present effect estimates for associations with health outcomes scaled to various changes in concentrations, e.g., interquartile range for exposures of the study population or an arbitrary unit such as 10 ppb. To increase comparability among studies, the ISA presents effect estimates for a given averaging time scaled to the same increment. Compared with short-term averages, long-term averages of ambient concentrations are lower, less variable across time, and do not differ widely among averages of multiple months, annual averages, or multiyear averages. Thus, for long-term exposure, 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, and they represent the approximate difference between the median and 95th percentile of annual average concentrations among monitors in the network.

Animal toxicological studies can provide direct evidence for health effects related to or NO_2 exposures. Results from these studies were also used to address uncertainties in the epidemiologic evidence, such as potential confounding. Experimental studies also provide biological plausibility by describing key events to inform modes of action for

health effects. Thus, the integration of evidence across a spectrum of related endpoints, including cause-specific mortality, and across disciplines was used to inform uncertainties for any particular endpoint or discipline due to factors such as publication bias, selection bias, confounding by copollutant exposures.

5.2 Respiratory Effects

5.2.1 Introduction

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) examined the epidemiologic and toxicological evidence for effects of long-term exposure to NO_2 on respiratory morbidity and concluded that the evidence was suggestive but not sufficient to infer a causal relationship. The key supporting epidemiologic evidence comprised positive associations between NO_2 and decrements in lung function and partially irreversible decrements in lung function growth, although toxicological studies provided little evidence for key events to inform the mode of action for these observations. Animal studies did, however, demonstrate that long term exposure to NO_2 resulted in permanent morphologic changes to the lung, particularly the centriacinar region and bronchiolar epithelium. Results from the Southern California Children's Health Study (CHS) indicated that decrements in lung function in children were associated with increasing ambient NO_2 concentrations (Gauderman et al., 2004), though similar associations were also found for PM, O_3, and proximity to traffic (<500 meters). Generally, the high correlation among traffic-related pollutants in these long-term exposure studies made it difficult to accurately estimate the independent effects of individual pollutants. Additional uncertainty was related to the inconsistent 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 outcomes. 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.

Recent prospective studies have evaluated the association between long-term exposure to NO_2 and various respiratory morbidity endpoints, including the reduced lung function growth and lung function in children, the development of asthma and bronchitis, and the role such exposure has in the aging respiratory system and the development of disease in adults.

In this section, the current body of evidence for associations between long-term exposure to NO_2 and respiratory morbidity is characterized. This includes respiratory morbidity studies in children such as asthma incidence, pulmonary function development, respiratory symptoms, and various indicators of inflammation and allergy. For adults, the endpoints evaluated include adult onset asthma, pulmonary function decrements, respiratory symptoms, hospital admissions for respiratory disease, and indicators of pulmonary inflammation. Individual studies are described and results are integrated within the epidemiologic evidence base. Exposure-related concerns relevant to all epidemiologic studies are discussed, including NO_2 exposure assessment methods, the potential role of NO_2 as a surrogate measure for another pollutant or pollutant mixture, and copollutant associations. Additionally, it should be noted that exposure measures characterized in epidemiologic studies in this section are annual averages unless stated otherwise. Epidemiologic study design characteristics are also considered in evaluating the evidence and include study type (i.e., longitudinal, prospective study versus cross-sectional study), the age at which exposure and/or respiratory endpoint is assigned, the allergic versus nonallergic status, the follow-up interval, the concentration-response relationship, and coherence and consistency within the epidemiologic evidence. 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, but previous studies are evaluated for lung host defense; pulmonary inflammation, injury, and oxidative stress; and respiratory morphology.

5.2.2 Asthma/Chronic Bronchitis Incidence

5.2.2.1 Children

Since the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), recent prospective longitudinal cohort studies provide a larger evidence base to evaluate the relationship between asthma incidence in children and long-term NO_2 exposure. Details from these prospective studies are presented in Table 5-1 and Figure 5-3 and in the text later in this section. Cross-sectional studies were reviewed and are discussed as appropriate to inform discussion of copollutants, genetic variants and other policy-relevant issues (Tung et al., 2011; Akinbami et al., 2010; Hwang and Lee, 2010; Kim et al., 2008; Morgenstern et al., 2008; Hwang et al., 2005) and other studies found in the Annex tables of the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c). Cross-sectional studies also reported associations between NO_2 and asthma. The prospective studies demonstrate a positive relationship. The evidence base includes studies from North America, Europe and Asia that use different designs and analyses.

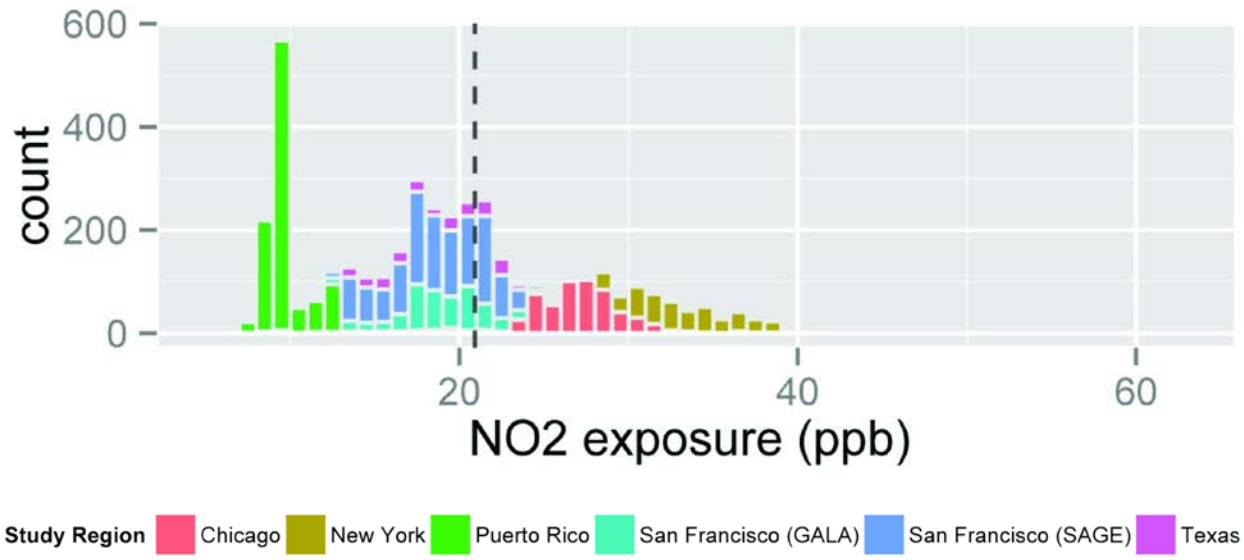
The results of individual studies are supported by recent meta-analyses that report positive associations (Gasana et al., 2012; Bråbäck and Forsberg, 2009), several of which adjust for or examine the influence of publication bias (Anderson et al., 2013; Gowers et al., 2012; Takenoue et al., 2012). Some of these meta-analyses included children and adults, and some included both cross-sectional and prospective studies.

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. 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 be provided by a physician making a diagnosis of asthma.

The relationship between traffic-related air pollution and asthma onset in children was evaluated in the prospective CHS cohort living in 11 communities with individual exposure measurement (Jerrett et al., 2008). The design of the study allowed the examination of the independent contributions of local versus regional NO_2 to the associations with asthma by modeling the effects of the within- and between-community variation in NO_2. Similar results were obtained in analyses of within-community variation and between-community variation in NO_2, providing evidence that both regional and local pollution contributed to the associations with asthma. However, these results were based on a 6.2-ppb increase in NO_2, which was the within-community interquartile range for NO_2. NO_2 concentrations showed less variation within than between communities. The hazard ratio (HR) for between-community effects increases to 3.25 if based on the average interquartile range across all measurements of 28.9 ppb for annual NO_2 and to 1.95 if based on the average within-community range of 16.4 ppb.

In a new CHS cohort recruited in 2002-2003 from 13 southern California communities, McConnell et al. (2010) prospectively evaluated childhood incident asthma (n = 120) and traffic-related pollutant (TRP) exposure based on estimates on a line source dispersion model of traffic volume, distance from home and school, and local meteorology. Ambient pollutants O_3, NO_2, and PM measured at central sites were also evaluated. Of the regional community central-site pollutants, a 10-ppb increase in ambient NO_2 measured at a central site in each community was associated with an HR for new onset asthma or 1.39 (95% CI: 1.07, 1.80). In models with both NO_2 and modeled TRP, there were independent associations of asthma with TRP at school and home, whereas the estimate for NO_2 was attenuated (HR 1.14 [95% CI: 0.85, 1.53]).

The GALA II (Chicago, IL; Bronx, NY; Houston, TX; San Francisco, CA; Puerto Rico) and SAGE II (San Francisco, CA) cohorts of Latino and African American children and young adults ages 8 to 21 were used to asses a relationship between air pollution and development of asthma (Nishimura et al., 2013a). A 10-ppb increase in average NO_2 during the first year of life was associated with physician-diagnosed asthma with an OR of 1.37 (95% CI: 1.08, 1.73). The different study regions had different concentrations and mixtures of pollutants reflecting differing geography, weather, and pollutant sources (Figure 5-1 for NO_2). Most regions showed a nominally positive association with very little between-study heterogeneity suggesting that the association between NO_2 and asthma is generalizable across geographic regions.



Note: Abbreviations: NO_2 = nitrogen dioxide, GALA = Genes-environments & Admixture in Latino Americans, SAGE = Study of African Americans, Asthma, Genes & Environments. Dotted line = World Health Organization air quality guideline.

Source:Reprinted with permission of the American Thoracic Society, Nishimura et al. (2013a), specifically Supplemental Material: (Nishimura et al., 2013b).

Figure 5-1 Distribution of NO_2 exposure in first year of life in GALA II/ SAGE.

All children born in southwestern British Columbia in 1999 and 2000 (n = 37,401) were assessed for incidence of asthma diagnosis up to 3–4 years of age using outpatient and hospitalization records and evaluated for a relationship to air pollution (Clark et al., 2010). A total of 3,482 children (9%) were classified as asthma cases, and a 10-ppb increase in NO_2 was associated with asthma with an OR of 1.24 (95% CI: 1.14, 1.35). Clougherty et al. (2007) used geographic information systems (GIS)–based models to

retrospectively estimate residential exposures to traffic-related pollution for 413 children in a community-based pregnancy cohort, recruited in East Boston, Massachusetts, between 1987 and 1993. Monthly NO_2 measurements for 13 sites over 18 years were merged with questionnaire data on lifetime asthma incidence. Univariate ORs for the seven candidate NO_2 exposure periods indicated that a 10-ppb increase in NO_2 was associated with asthma with an OR of 1.44 (95% CI: 0.87, 2.40).

A prospective study by Lee et al. (2012c) examined whether associations between high pulmonary function indices and incident respiratory diseases in Taiwan are modified by long-term ambient NO_2 exposure. Questions regarding respiratory symptoms and diseases were modeled after those used in the Children's Health Study in southern California. They report loss of protective effects by each pulmonary function index against bronchitis, chronic cough, and asthma in the communities with higher NO_2 concentrations compared to those with lower NO_2 concentrations. Per interquartile range increase in forced expiratory flow over the mid-range of expiration, the RR for asthma is 0.73 (95% CI: 0.64, 0.83) in the low NO_2 communities (<17.5 ppb) and 0.97 (95% CI: 0.85, 1.11) in the high NO_2 communities (>17.5 ppb). These results are consistent with the results of a similar analysis conducted by the Southern California CHS (Islam et al., 2007). For the purposes of presenting results in this ISA, the RRs reported above were combined to calculate the main effect of NO_2 exposure as RR: 1.33 (95% CI: 1.14, 1.52) in the high NO_2 community, with the low community serving as the reference. The main effect of NO_2 was calculated using the following formula:

RR for high NO_2 community versus low NO_2 community =

$$\exp[\log(\text{high } NO_2 \text{ RR}) - \log(\text{low } NO_2 \text{ RR})] = \exp[\log(0.97) - \log(0.73)] = 1.33.$$

Standard error (s.e.) for the high NO_2 community RR =

$$[\log(1.11) - \log(0.85)]/1.96 \times 2 = 0.07$$

s.e. for the low NO_2 community RR =

$$[\log(0.83) - \log(0.64)]/1.96 \times 2 = 0.07$$

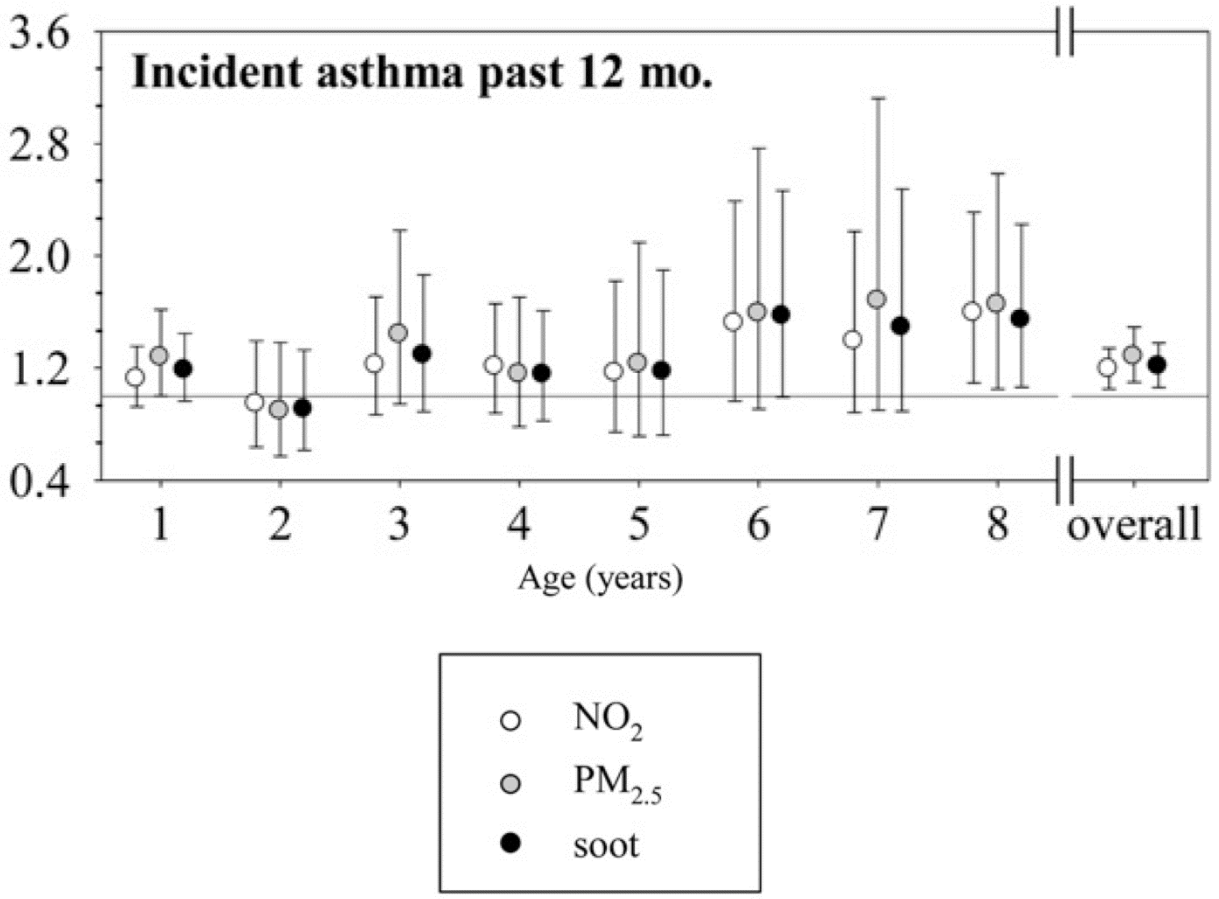
Lower 95% confidence limit =

$$1.33 - 1.96\sqrt{(s.e.\,high\ NO_2)^2 + (s.e.\,low\ NO_2)^2} = 1.14$$

Upper 95% confidence limit =

$$1.33 + 1.96\sqrt{(s.e.\,high\ NO_2)^2 + (s.e.\,low\ NO_2)^2} = 1.52$$

In the longitudinal analysis of the 8-year follow-up of the Dutch Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study in the Netherlands, Gehring et al. (2010) studied the association between traffic-related air pollution and the development of asthma, allergy and related symptoms. Preceding this analysis was Brauer et al. (2002) at the 2-year time period reviewed in the 2008 ISA for Oxides of Nitrogen and at the 4-year time period (Brauer et al., 2007); both presented cross-sectional analysis. Individual exposures to NO_2, $PM_{2.5}$, and soot at the birth address were estimated by land-use regression models. This prospective birth cohort (n ~ 3,100) study (Gehring et al., 2010) found positive associations to long-term NO_2 exposure in adjusted analysis for prevalent asthma, incident asthma, and wheeze. The results for incident asthma are shown in Figure 5-2. While the overall result is significant, the figure shows that for NO_2 significance is not achieved until age 8. Similar results are shown for $PM_{2.5}$ and soot. In this study no associations were found with atopic eczema, allergic sensitization, and bronchial hyperresponsiveness.



Note: Results are presented as adjusted except study region odds ratios (ORs) with 95% confidence intervals. Because study region is an important determinant of air pollution levels in the land use regression 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 Sociey, Gehring et al. (2010).

Figure 5-2 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.

The Swedish population-based birth cohort, BAMSE, examined development of asthma and related symptoms over a 12-year follow-up (Gruzieva et al., 2013). A 20-ppb increase in NO_X during the first year of life was associated with development of incident asthma at 12 years of age with an OR of 1.65 (95% CI: 1.0, 2.77). The Oslo Norway birth cohort was used to investigate the associations of long-term traffic-related exposures in early life and before onset with onset of doctor-diagnosed asthma assessed retrospectively in current 9- to 10-year-old children (Oftedal et al., 2009). The effect of annual averages pollutant levels over a 10-year period on asthmatic symptoms were

assessed in a prospective cohort study of 1,910 schoolchildren in 8 different communities in Japan (Shima et al., 2002). During the follow-up period, incidence rates of asthma were associated with NO_2.

A high-risk birth cohort for the risk of new onset asthma was considered in a population of children with first-degree relatives with asthma in Vancouver, Canada, that was studied by Carlsten et al. (2011c) to evaluate the association between early exposure to traffic related air pollution using land use regression. The OR associated with a 10-ppb increase in NO_2 in the total group was 2.88 (95% CI: 0.76, 10.94). Combined early exposure to dog (elevated Can-f1 levels or dog ownership) plus elevated NO_2 conferred an increased risk of incident asthma (Carlsten et al., 2011a).

In a cross-sectional analysis, Akinbami et al. (2010) examined the association between chronic exposure to outdoor pollutants (12-month avg levels by county) and asthma outcomes in a national sample of children ages 3-17 years living in U.S. metropolitan areas (National Health Interview Survey, N = 34,073). An increase in annual NO_2 concentration yielded a negative association both for children currently having asthma and for children having at least 1 asthma attack in the previous year. Models in which pollutant value ranges were divided into quartiles yielded adjusted odds for current asthma for the highest quartile (30.8-40.2 ppb) of estimated NO_2 exposure of 1.02 (95% CI: 0.71, 1.47).

The exposure methods in the above asthma incidence studies are listed in Table 5-1. Additionally, papers related to these studies that provide in depth discussion of the exposure methods are referenced in supplemental Table S5-1 (U.S. EPA, 2013e). The majority of these studies subjected methods to validation testing to ensure that the data were of sufficient quality. Of the exposure assessment methods employed, Palmes tubes are sometimes subject to positive biases in NO_2 (Section 2.6.3.1), dispersion models not employing NO_X chemistry would potentially overestimate NO_2 concentrations (Section 2.6.2.2), and central site NO_2 monitors may fail to capture spatial variability in concentrations and exposures influenced by time activity data (Section 2.6.5). All of these issues would have the effect of biasing the health effect estimate towards the null, so that reported effect estimates are conservative. LUR model predictions have been found to correlate well with outdoor NO_2 concentration measurements (Section 2.6.2.3) and so wouldn't be anticipated to result in substantial bias.

These studies differ in exposure period evaluated, age of asthma diagnosis, and length of follow-up time. Several involve birth cohorts up to an age of 8 to 12. Gruzieva et al. (2012) note that in general, the strongest effect was observed in relation to exposure during infancy, which may indicate that prenatal and early-life periods represent critical windows for the effects of exposure on development of childhood asthma and related

symptoms. Other studies found larger magnitudes of association with NO_2 exposure in the first 3 years of life (Nishimura et al., 2013a) or year of diagnosis (Clougherty et al., 2007), indicating other time periods of exposure also are important. Other studies evaluate children starting at age 8-10 and follow them until adolescence or young adulthood and report generally positive results. Age of asthma diagnosis is also a factor. 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. The results of this NO_2 asthma incidence evidence base are greater in magnitude and generally stronger at later age evaluation and longer follow-up time.

While model adjustment varied by study, the collective body of evidence adjusted for multiple potential confounding factors, including age, various SES indicators such as maternal education and household income, health indicators such as family history of asthma and atopic status, exposure to cigarette and wildfire smoke, housing characteristics, presence of a gas stove in the child's home, and meteorological conditions such as temperature and humidity.

Table 5-1 Prospective long-term NO_2 exposure new onset asthma in children cohort studies.

Study	Exposure	Pollutant Correlation	Statistical Methods	Comments	Results: (Results are standardized to 10 ppb)
Childrens Health Study (CHS), Southern California communities					
Jerrett et al. (2008) n = 217 children, 10-18 years of age enrolled in 1993 or 1996 in 11 Communities with 8 years of follow-up	Palmes tubes outside home, 2 weeks summer and winter to provide annual and seasonal levels	Moderate to high correlations between the measured residential NO_2 and various measures of traffic proximity or modeled concentrations	Random-effects Cox proportional hazards models. SEP measures of median household income, proportion of respondents with low education (i.e., no high school diploma), percent of males unemployed (as a marker for fulltime income instability), and percent living in poverty were tested as potential confounders. Meteorological conditions, temperature, and humidity were tested.	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 variation of NO_2 within communities was smaller than that between communities, and the HR values were smaller when scaled to the range within communities	Adjusted HR of 1.29 (95% CI: 1.07, 1.56) per the average within-community interquartile range of 6.2 ppb in annual residential NO_2. Based on the total interquartile range for all measurements of 28.9 ppb, the HR was 3.25 (95% CI: 1.35, 7.85)

Table 5-1 (Continued): Prospective long-term NO_2 exposure new onset asthma in children cohort studies.

Study	Exposure	Pollutant Correlation	Statistical Methods	Comments	Results: (Results are standardized to 10 ppb)
Childrens Health Study (CHS), Southern California communities (Continued)					
McConnell et al. (2010) n = 120 children 4.8 to 9.0 years of age enrolled into a new cohort during 2002-2003 in 13 communities with 3 years of follow-up	Community central site pollutant measurements and Line source dispersion model for residential and school TRP	Not Reported	Multilevel Cox proportional hazards model. Socio-demographic characteristics, exposure to cigarette and wildfire smoke, health insurance, housing characteristics, history of allergy, and parental asthma were assessed	--	Association of new-onset asthma with community central site pollutant measurements. HR: 1.39 (95% CI: 1.07, 1.80); p = 0.01 per 10 ppb across the 13 study communities at central sites. Modeled traffic-related pollution exposure from roadways near homes (HR: 1.67 [95% CI: 1.32, 2.12]) and near schools (HR 1.88 [95% CI: 1.10, 3.19]) per 10-ppb increase.

Table 5-1 (Continued): Prospective long-term NO_2 exposure new onset asthma in children cohort studies.

Study	Exposure	Pollutant Correlation	Statistical Methods	Comments	Results: (Results are standardized to 10 ppb)
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 years Brauer et al. (2007); Brauer et al. (2002)	Land-use regression models used to estimate annual concentrations for birth address of each participant	The estimated exposures for the various pollutants were highly correlated (r = 0.93, 0.96, and 0.97 for the correlation between NO_2 and $PM_{2.5}$, NO_2 and soot, and $PM_{2.5}$ and soot, respectively.	Generalized estimation equations Potential confounding variables included sex, study arm (intervention or natural history), 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.	--	Adjusted OR of 1.37 (95% CI: 1.09, 1.72) for a 10-ppb increase without adjustment for study region.
Vancouver High Asthma Risk Birth Cohort					
Carlsten et al. (2011c) n = 184 children at 7 years of age Carlsten et al. (2011a); Carlsten et al. (2011b)	Land use regression used to estimate annual concentrations of the birth address of each subject. Model validated.	Pearson correlations between pollutant measures were as follows: NO- NO_2, r = 0.8; NO_2- $PM_{2.5}$, r = 0.7; NO-$PM_{2.5}$, r = 0.5; BC-NO, r = 0.5; BC-NO , r = 0.3; and BC- $PM_{2.5}$, r = 0.2.	Multiple logistic regression analysis, potential confounders (maternal education, history of asthma (in mother, father or siblings), atopic status at 1 year)	High risk was defined as a child having, according to parental report, at least one first-degree relative with asthma or two first-degree relatives with other IgE-mediated allergic disease (atopic dermatitis, seasonal or perennial allergic rhinitis, or food allergy).	The risks associated with NO in both the total and control groups was: ORs: 1.8 (95% CI: 1.1, 2.9) and 2.5 (95% CI:1.2, 5.2), respectively. The risk associated with NO_2 in the total group was: OR 2.3 (95% CI: 1.0, 5.1); p = 0.05).

Table 5-1 (Continued): Prospective long-term NO_2 exposure new onset asthma in children cohort studies.

Study	Exposure	Pollutant Correlation	Statistical Methods	Comments	Results: (Results are standardized to 10 ppb)
Taiwan Children Health Study (TCHS)					
Lee et al. (2012c) 14 communities, n = 3,160, 12-14 years of age cohort entry 2007, mean follow-up 2 years	Average hourly levels of NO_2 measured in 14 monitoring stations used to compute annual average of ambient NO_2 levels between 2007 and 2009.	Poisson regression models, potential confounders, included in utero exposure to maternal smoking, family history of asthma, family history of atopy, and community	The two NO_2 strata were defined as less than and greater than the median level of 17.5 ppb: the mean annual ambient NO_2 level was 22.1 ppb in higher NO_2 communities and 14.0 ppb in lower NO_2 communities	--	For each inter-quartile range changes in maximal mid-expiratory flow (MMEF) the incidence rate ratio of asthma was lower in the lower NO_2 communities (RR: 0.73 [95% CI:0.64, 0.83]), compared with the effect in the higher NO_2 communities (RR: 0.97 [95% CI: 0.85, 1.11] (p for interaction = 0.04)
Children, Allergy, Milieu, Stockholm, Epidemiology Survey (BAMSE)					
Gruzieva et al. (2013) Swedish birth cohort followed up to 12 years of age enrolled between 1994 and 1996 n = 3,633 Nordling et al. (2008) Gruzieva et al. (2012)	Dispersion models were used to calculate NO_X for all addresses in the years 1994 to 2008 representing when the first child was born until the end of the 12-year follow-up.	r = 0.96 between NO_X and PM_{10} exposure levels during the first year of life	Multinomial regression/ GEE Potential confounders adjusted for include municipality, SES, year the house was built, and heredity.	Associations were stronger for the oldest children and for non allergic asthma. The authors investigated several time aspects of long-term exposure, including early-life exposure and current exposure (during the last year), and average exposure between follow-ups.	Association between NO_X during the first year of life and development of incident asthma at 12 years of age OR: 1.87 (95% CI: 1.0, 3.44). 46.8 $\mu g/m^3$ for NO_X.

Table 5-1 (Continued): Prospective long-term NO_2 exposure new onset asthma in children cohort studies.

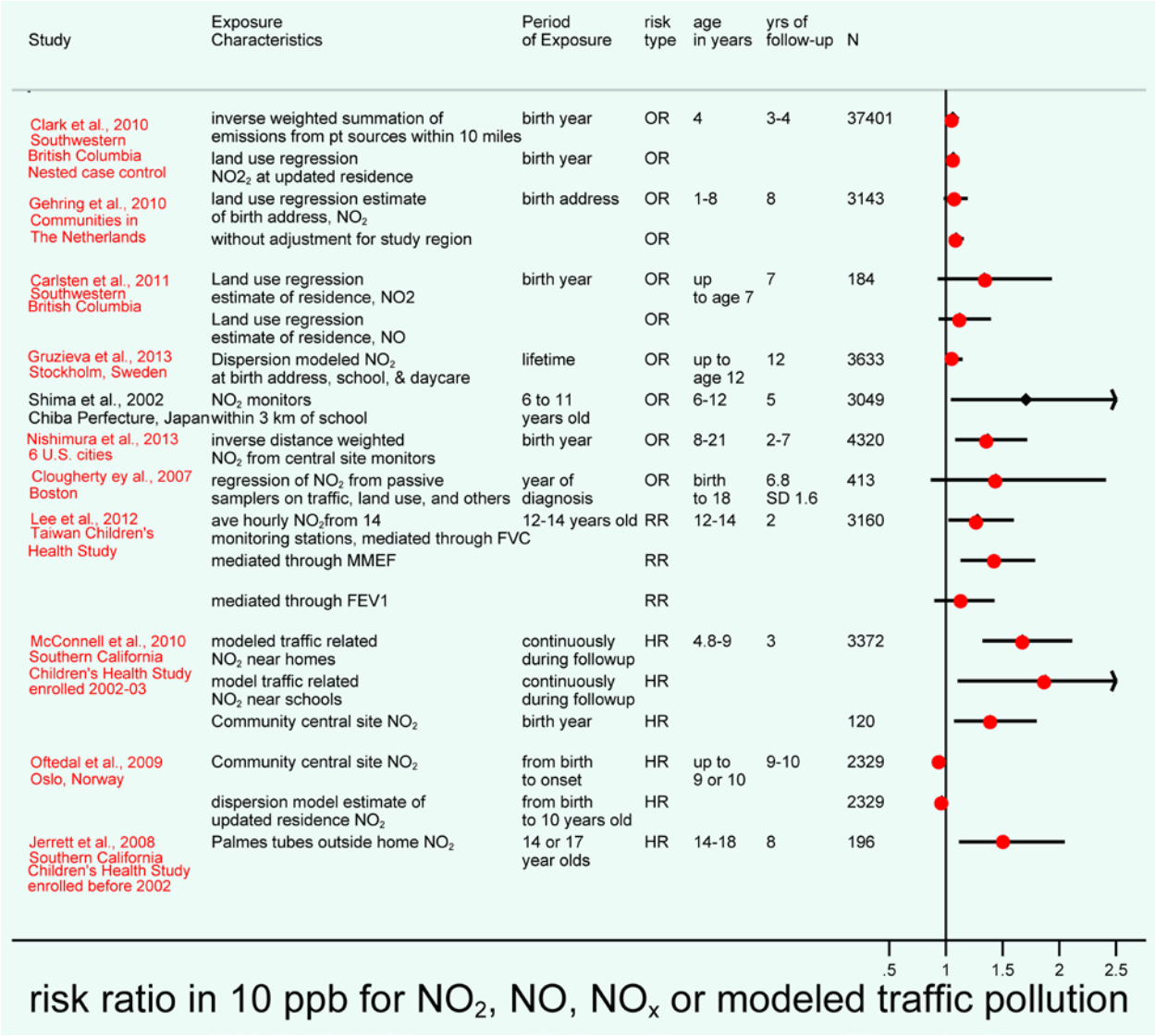
Study	Exposure	Pollutant Correlation	Statistical Methods	Comments	Results: (Results are standardized to 10 ppb)
Oslo Norway birth cohort					
Oftedal et al. (2009) n = 2,329 children born in Oslo in 1992–1993 were assessed when 9 to 10 years of age	NO_2 exposure was assessed by the EPISODE dispersion model and assigned at updated individual addresses during lifetime.	PM_{10} and $PM_{2.5}$ pollutants are correlated with NO_2 (r = 0.79-0.91).	Cox proportional hazard regression and logistic regression were used. Potential confounders considered included sex, parental atopy, maternal smoking in pregnancy, paternal education, and maternal marital status at the child's birth	Several long-term exposures were included: early exposure in first year of life, average exposure from birth to asthma onset, and previous year's exposure before completing the questionnaire.	Adjusted RR and 95% CI (per IQR for NO_2 varies between 14.5 and 10.4 ppb, decreasing over time) For NO_2 exposure 1st year of life was 0.82 (0.67, 1.02); early onset less than 4 years of age was 0.78 (0.62, 0.98) and for equal or later than 4 years of age was 1.05 (0.64, 1.72).
Chiba Prefecture, Japan Cohort					
Shima et al. (2002) n = 1,910 children in 8 communities at age 6 entered 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 in the vicinities of the study schools were used.	Not Reported	Logistic Regression Model Incidence rates were adjusted for sex, history of allergic diseases, respiratory diseases prior to age 2, parental history of allergic diseases, maternal smoking habits, type of heater used in winter in the home, and construction elements of the house.	--	The incidence of asthma was associated with increasing NO_2 concentrations (OR: 1.71 [95% CI:1.04, 2.79 for a 10-ppb increase).

Table 5-1 (Continued): Prospective long-term NO_2 exposure new onset asthma in children cohort studies.

Study	Exposure	Pollutant Correlation	Statistical Methods	Comments	Results: (Results are standardized to 10 ppb)
Genes-environment & Admixture in Latino Americans and the Study of African Americans, Asthma, Genes & Environments					
Nishimura et al. (2013a) The sample size analyzed was 4,320. Multi city study that includes: (Chicago, IL; Bronx, NY; Houston, TX; San Francisco Bay Area, CA) and Puerto Rico. Participants were 8-21 years old.	Exposures over the first three years of life were calculated by averaging all available pollutant values from birth to age three using residential histories using regional ambient pollutant data using inverse distance-squared weighted average from the four closest monitors within 50 km of the residence.	The different study regions had different levels and mixtures of pollutants, reflecting differing geography, weather, and pollutant sources.	Logistic regression models adjusted for age, sex, ethnicity, and composite SES were used. A sensitivity analysis examining additional potential covariates for maternal in utero smoking, environmental tobacco smoke in the household between 0 and 2 years old, and maternal language of preference (as an indicator of acculturation).	Region-specific results suggest that susceptibility to asthma due to air pollution may not be uniform throughout the nation and could be dependent on local characteristics, such as varying proportions of different racial/ethnic groups and differing pollution sources and/or weather patterns	After adjustment for confounders, a 10-ppb increase in average NO_2 during the first year of life was associated with an OR of 1.37 for physician-diagnosed asthma (95% CI: 1.08, 1.73) for the entire study.
British Columbia Birth Cohort					
Clark et al. (2010) N = 2,801, children mean age at follow-up 48 SD 7-mo, all 1999 and 2000 births in SW BC	Estimated using regulatory monitoring data, Land use regression models , and point source derived inverse distance-weighed (IDW) summation of emissions	Correlations between different pollutants were generally high, only the O_3 r = -0.7 to -0.9 was provided.	Covariate-adjusted conditional logistic regression. Covariates previously hypothesized to have an effect on asthma status (native status, breast-feeding, maternal smoking, income quartile, maternal age, birth weight, and gestational length were included.	The potential limitation of the young age of the children were wheezing is more common was addressed by restricting asthma cases to children with a hospital admission or at least two outpatient diagnosis of asthma which indicate severe ongoing symptoms.	An increased risk of asthma diagnosis with increased early life exposure to CO, NO, NO_2, PM_{10}, SO_2, and black carbon (BC) and proximity to point sources was found. Traffic-related pollutants were associated with the highest risks: Adjusted ORs: 1.15 (95% CI: 1.08, 1.24) for a 10-ppb increase of NO, and 1.24 (95% CI: 1.14, 1.35) for a 10-ppb increase in NO_2.

Table 5-1 (Continued): Prospective long-term NO_2 exposure new onset asthma in children cohort studies.

Study	Exposure	Pollutant Correlation	Statistical Methods	Comments	Results: (Results are standardized to 10 ppb)
Maternal-Infant Smoking Study of East Boston					
Clougherty et al. (2007) n = 413 full cohort/255 lifetime residents Pregnant women were recruited from East Boston between 1987 and 1993 and a questionnaire administrated in 1997 when the childrens average age was 6.8.	Estimated exposure using Land use regression based on predictive model using passive monitors, sites and traffic	Not Reported	Regression model examined for the main effects of NO_2. Potential confounders included maternal asthma, education, smoking before and after pregnancy, child's sex and age.	Found an association between traffic-related air pollution and asthma solely among urban children exposed to violence.	Univariate ORs for the seven candidate NO_2 exposure periods indicated that a 10-ppb increase showed the following association with asthma for the full cohort: OR: 1.44; (95% CI:0.87, 2.40).



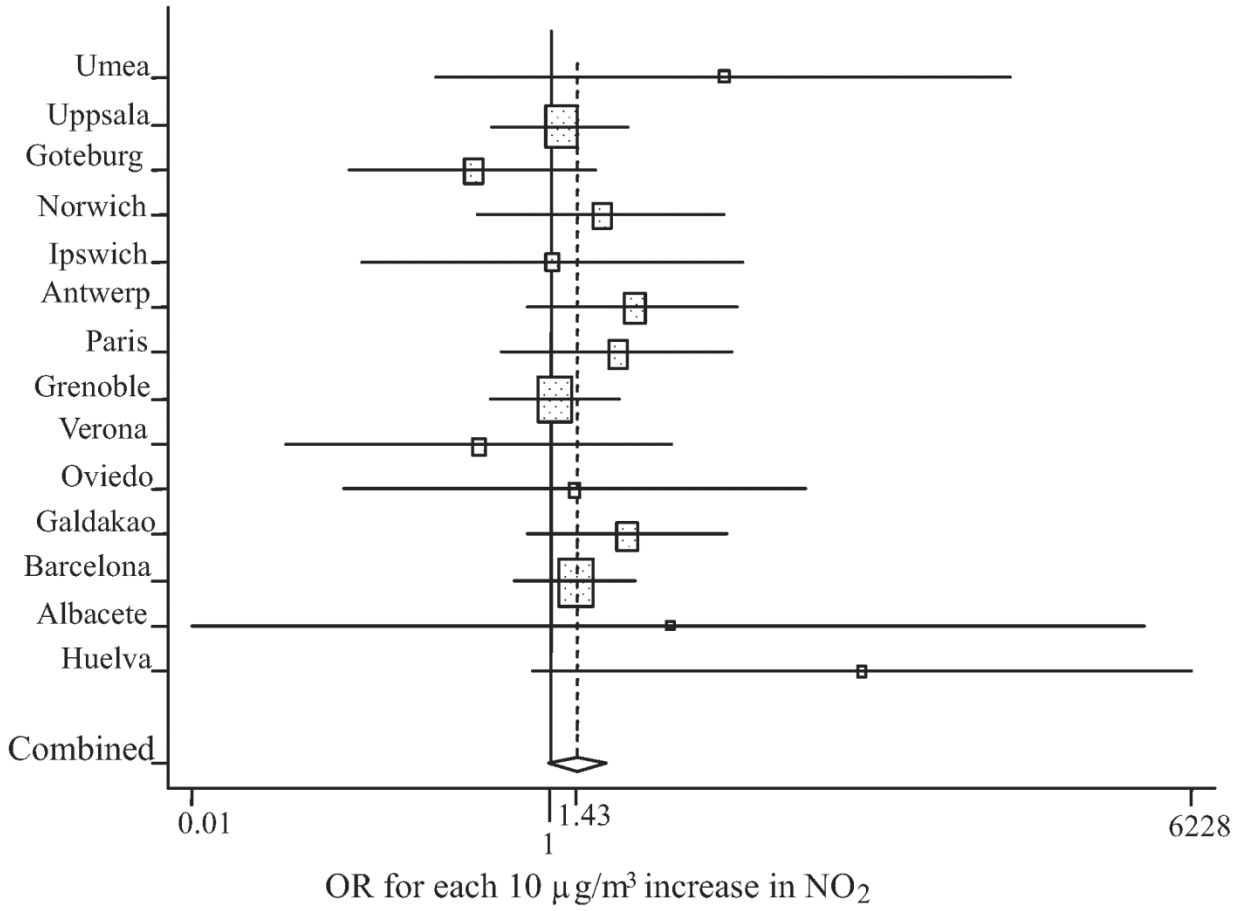
Note: These studies are presented by the statistical method used: first by odds ratio (OR), then relative risk (RR), and finally by hazard rate (HR).Then within the statistical method they are arranged by age.

Figure 5-3 Risk ratio estimates of asthma incidence from prospective studies.

5.2.2.2 Adults: Asthma-Chronic Bronchitis

The European Community Respiratory Health Survey (ECRHS), a European adult cohort study, assessed the relationship to long-term ambient pollution exposure and onset

asthma both via positively responding to the question “Have you ever had asthma?” (Jacquemin et al., 2009b) and via a continuous asthma score (Jacquemin et al., 2009a). Jacquemin et al. (2009b) analyzed 4,185 adults. Subjects’ home addresses were geocoded and linked to outdoor NO_2 estimates, as a marker of local traffic-related pollution using information from the 1-km background NO_2 surface modeled in APMoSPHERE (Air Pollution Modelling for Support to Policy on Health and Environmental Risk in Europe). Asthma incidence was defined as reporting asthma in the follow-up (1999 to 2001) but not in the baseline (1991 to 1993). They adjusted for center effects. A set of predefined covariates was included (sex, age, socioeconomic status, atopy, family history of asthma or atopy, and smoking). When assessing the risk of developing asthma using the 186 new cases, the adjusted OR for asthma onset was 1.96 (95% CI: 1.04, 3.70) for a 10 ppb change in NO_2. All the stratified associations between asthma incidence and NO_2 were higher than 1. Results were homogeneous among centers in both crude and the adjusted analyses (Figure 5-4) (Jacquemin et al., 2009b).



Note: P value for heterogeneity 0.594. Erfurt in Germany, and Pavia and Torino in Italy, were automatically dropped from the analysis due to empty cells. Boxes represent the OR per center, where the size of the box is proportional of the sample size of such center. Lines represent the 95% CI of the respective OR. Diamond represents the combined OR.

Locations in the Figure: Umea, Uppsla, and Goteburg, Sweden; Norwich and Ipswich, U.K.; Antwerp, Belgium; Paris and Grenoble, France; Verona, Italy; Oviedo, Galdakao, Barcelona, Albacte, and Huelva, Spain.

Source: Reprinted with permission of Wolters Kluwer Health, Jacquemin et al. (2009b).

Figure 5-4 Adjusted ratios of new asthma in ECRHS II (1999-2001) for every 10 µg/m^3 (5.3 ppb) NO_2 increase by center in subjects with no asthma in ECRHS I (1991-1993).

Defining asthma as a continuous trait using a grading scheme based on reported symptoms was used by Jacquemin et al. (2009a) to evaluate the relationship between long-term pollution exposure and asthma among adults with this novel measure in the ECRHS cohort. The asthma score was developed from the five symptoms: wheeze and breathlessness, feeling of chest tightness, attack of shortness of breath at rest, attack of shortness of breath after exercise, and woken by attack of shortness of breath during the last 12 months. Covariates evaluated included: sex, age, social class (in five groups based

on the International Standard Classification of Occupations coding of the occupational history at ECRHS II and derived from the longest-held job during the follow-up period between ECRHS I and II), family history of asthma or atopy, smoking (no, former or current), pack-years, exposure to second-hand tobacco smoke, any exposure to dust, fume or gases at work, gas cooking and season of the interview. The results are expressed as ratios of the mean asthma scores (RMS). A multivariate model and data pooling was adopted to analyze the association between the score and NO_2 concentration. Both were defined at follow-up, in a subpopulation reporting neither symptoms nor asthma at baseline. This population may be considered a sample being in all likelihood free of asthma at baseline. Thus, the occurrence of symptoms at follow-up may be interpreted as new onset of symptoms, which may ultimately reflect incidence of asthma. In the multivariate analysis, the RMS for each increase of 10 ppb of NO_2 was 1.48 (95% CI: 1.18, 1.85). The association was homogeneous among centers after excluding Turin, which had very large confidence intervals; the p-value for heterogeneity was still not significant. In participants with no asthma and no symptoms at baseline, the associations between NO_2 and asthma score were positive (RMS 1.25 [95% CI: 1.05, 1.50]). A high symptom score was shown to be strongly associated with doctor diagnosed asthma (Sunyer et al., 2007); thus, this study's finding may indicate a role of pollution in new onset of asthma in adults. This interpretation is consistent with previous finding based on the more traditional definition of ''asthma incidence'', using asthma at follow-up among those free of the disease at baseline (Jacquemin et al., 2009b) discussed in the above text.

In the prospective Respiratory Health in Northern Europe (RHINE) cohort study, Modig et al. (2009) assessed the relationship between traffic-related air pollution levels and two definitions of asthma among adults aged 20-44 at inclusion: the cumulative number of onset onset cases of asthma and incident cases of asthma. The RHINE cohort is based on the random sample of people receiving the first screening questionnaire sent out within the ECRHS stage 1 (Torén et al., 2004). The questionnaire was sent out in 1990 and included questions regarding respiratory symptoms such as wheezing, attacks of asthma and current use of asthma medication. All participants who answered the first questionnaire received the follow-up questionnaire in 1999. In contrast, in the ECRHS cohort, only a subsample of the participants that received the first survey was included in the follow-up. The study population consisted of 10,800 participants, born between 1945 and 1973. In order to be defined as an onset case of asthma observed during the follow-up period, the participant had to have negative answers to the questions on attacks of asthma during the last 12 months and current use of asthma medication in the first survey, followed by a positive answer to at least one of these questions at the follow-up: ''Do you have or have you ever had asthma?'' or ''Have you ever had asthma diagnosed by a doctor?" Adjustment was made for a predetermined set of potential confounding variables: body mass index (BMI), sex, age, smoking, water damage or mold in the home

at any time during the last 8 years, and city, simultaneously in the main analysis. Socioeconomic index (SEI) based on job title was used for 80% of the participants to classify five categories, and was only used for sensitivity analysis. The overall winter half-year mean NO_2 concentration was 9.6 ppb in total for Gothenburg and Umeå. The indoor/outdoor ratio for NO_2 in Umeå was 0.4/0.7. In the 3,824 participants, the analysis of the relationship between onset and incident cases of asthma and the levels of NO_2 outside the home showed a positive coefficient, indicating an increased risk of developing asthma among adults with increasing levels of NO_2 outside the home. The OR in the fully adjusted model was 2.04 (95% CI: 1.14, 3.65) for the onset definition and 2.25 (95% CI: 1.00, 5.07) for the incident definition of cases per 10-ppb increase in NO_2 levels. When NO_2 was grouped into tertiles there was a dose–response pattern, with higher estimates 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]), and with the first tertile used as a reference.

In 13 European cities, Castro-Giner et al. (2009) prospectively identified interactions between genetic variants and traffic-related pollution on asthma incidence and prevalence in the large (2,577 subjects at follow-up) adult population-based cohort – ECRHS. The genetic aspect of this study is discussed in Section 5.2.11. In an analysis of longitudinal data to evaluate the effect of NO_2 on new-onset asthma they observed an association between new-onset asthma and NO_2 levels for the 120 subjects who developed asthma during the follow-up period (OR = 2.20 [95% CI: 1.17, 4.10]). The authors restricted the analysis to those subjects who lived in the same home during follow-up (n = 1,348) to reduce exposure misclassification. Compared with subjects who changed homes, this group had an increased risk for main effects of exposure to NO_2 on asthma prevalence (movers OR: 2.53 [95% CI: 1.16, 5.56]; non-movers OR:1.04 [95% CI: 0.66, 1.64]; p-value for interaction = 0.03), whereas the effect on new-onset asthma was not different between movers and non-movers (movers OR: 2.09 [95% CI: 0.70-6.12], non-movers OR: 2.39 [95% CI: 1.10, 5.22], p-value for interaction = 0.81).

A separate analysis of the ECRHS cohort examined the relationship of chronic bronchitis and air pollution. Sunyer et al. (2006) used two definitions for symptoms of chronic bronchitis: (1) productive chronic cough for chronic cough and chronic phlegm (more than three months each year), and (2) chronic phlegm alone. Since the two definitions yielded similar results and given the higher frequency only the latter was used. The prospective nature of the study design allowed consideration of two potentially different symptomatic groups, namely those with symptoms at baseline, and those with symptoms only at follow up (‘‘new onset’’). The follow-up time period was 8.9 years. Potential confounding factors evaluated included smoking, age at end of education, occupational groups, and occupational exposures, respiratory infections during childhood, rhinitis,

asthma, and traffic intensity at household level. A home based measurement of NO_2 using Palmes tubes as a marker for local tail pipe emissions was implemented. At this individual level, outdoor (at the kitchen window) and kitchen indoor NO_2 concentrations were collected during a 14 day period in 16 centers involving 1,634 households of subjects who did not move house during the follow up. After about six months this procedure was repeated in 659 households (45%) who volunteered to repeat the measurement. A dose-response relationship with NO_2 using the GAM modeling after adjusting for variables was reported by the authors. The dose-response with NO_2 was linear in females but not in males (p for gain of non-linearity 0.15 and 0.03, respectively). Among females, the association remained when the outcome was chronic productive cough instead of chronic phlegm (the OR for a change of 10 ppb being 1.48 [95% CI: 0.99, 2.20]).

5.2.3 Pulmonary Function

5.2.3.1 Epidemiologic Studies

Children

Recent prospective cohort studies add to the evidence base that evaluates the relationship between supervised pulmonary function tests and long-term NO_2 exposure. These longitudinal prospective studies are summarized in Table 5-2 and the following text.

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) characterized the long-term prospective studies as observing a positive association for the relationship between NO_2 concentrations and decrements in lung function and reduction in growth. A key uncertainty associated with these studies was the high correlation of NO_2 concentrations with other ambient pollutants. In general the studies depicted in Figure 5-3 continue to show the relationship observed earlier, that is consistent decrements in lung function, especially as children reach later ages.

A recent study of the long-term relationship between exposure to air-pollution and lung function was examined in 1,924 school-age children in the Swedish birth cohort BAMSE (Schultz et al., 2012) that were followed with repeated questionnaires, dynamic spirometry and IgE measurements until 8 years of age. Exposure during the first year of life was associated with a deficit of 28.0 mL (95% CI: -64.0, 8.0) in FEV_1 for a 20 ppb difference in time-weighted exposure to traffic-NO_X, while the corresponding deficit was -79.2 mL (95% CI: -135.0, -22.8) among those sensitized at 8 years. The odds ratios

associated with 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 first year exposure to traffic-NO_X. No impact of short-term air pollution exposure on the estimates of the long-term effects of air pollution was found, but these analyses were limited to PM_{10}.

The long term effects of PM_{10} and NO_2 exposure on specific airway resistance (sRaw) and forced expiratory volume (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). Lifetime exposure to NO_2 was associated with less growth in FEV_1 (% 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]).

In the recent CHS study, Breton et al. (2011) examined the association between ambient air pollutant exposures and lung function growth in children. This study involves a second cohort of children in the CHS study started in 1996. Results are shown in Table 5-2. Gauderman et al. (2004) examined the 1993 CHS cohort. The results of Breton et al. (2011) are consistent with earlier results from the cohort (Gauderman et al., 2004).

In Mexico City, Mexico, Rojas-Martinez et al. (2007a, b) evaluated the association between long-term pollutant exposure and lung function growth in a prospective analysis in a cohort of 3,170 children aged 8 years at baseline. In multipollutant models presented in Section 1.5.2, the negative association of O_3, PM_{10}, and NO_2 with lung function growth persisted: 10-ppb increase in NO_2 was associated with an annual deficit in FEV_1 of 25 mL in girls and 20.8 mL in boys. A deficit in FVC and FEV_1 growth was observed for O_3, PM_{10}, and NO_2 after adjusting for the acute effect of these pollutants (previous-day concentrations) and for confounding factors. A cohort study in Oslo, Norway, examined short- and long-term NO_2 and other pollutant exposure effects on lung function (PEF, forced expiratory flow at 25% of forced vital capacity [$FEF_{25\%}$], and forced expiratory flow at 50% of forced vital capacity [$FEF_{50\%}$]) in 2,307 nine- and ten-year-old children (Oftedal et al., 2008). Examining short- and long-term NO_2 exposures simultaneously yielded only the long-term effects. Adjusting for a contextual socioeconomic factor diminished the association. The association between long-term exposure to NO_2 and decreased PEF was comparable to that found in the CHS, but associations with forced volumes were considerably weaker.

Generally, these findings provide further support that early life exposure has long-lasting impact on the lung function development. Effects were mainly on FEV_1 and FEV0.5, which reflect the mechanical properties of the airways and not as much on FVC, representing lung size.

Additionally, cross-sectional studies (Gao et al., 2013; Svendsen et al., 2012; Lee et al., 2011d; Rosenlund et al., 2009b; Tager et al., 2005; Sekine et al., 2004; Moseler et al., 1994) report associations between NO_2 exposure and lowered lung function in children.

Table 5-2 NO_2 long-term pulmonary function growth children cohort prospective studies.

Study	Exposure	Pollutant Correlation	Statistical methods	Comments	Results: FEV_1 and FVC (mL) MMEF, PEF, and FEF (mL/sec)
Children's Health Study (CHS) California Communities					
Breton et al. (2011) Two cohorts of fourth-grade children, one in 1993 (cohort 1, n = 1,759) and the second in 1996 (cohort 2, n = 2,004), mean age at baseline10.0, were enrolled and monitored for 8 years, through twelfth grade	Central monitoring stations in each of the original 12 study communities from 1994 to the present, average hourly levels of NO_2 used to compute annual averages	NO_2 and $PM_{2.5}$ = 0.79; NO_2 and O_3 = -0.11	Hierarchical mixed effects with adjustments for height, height squared, body mass index (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 year, glutathione S-transferase mu 1 (GSTM1) 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-year growth of FEV_1, FVC, and MMEF, respectively	Main study without genetic effect NO_2 (10 ppb) Change (95% CI); p=value FEV_1 -29.83 (-50.10, -9.57) 0.004; FVC -25.35 (-46.50, -4.20) 0.02; MMEF -54.38 (-90.80, -17.96); 0.003

Table 5-2 (Continued): NO_2 long-term pulmonary function growth children cohort prospective studies.

Study	Exposure	Pollutant Correlation	Statistical methods	Comments	Results: FEV_1 and FVC (mL) MMEF, PEF, and FEF (mL/sec)
Gauderman et al. (2004) 1757 children aged 10 to 18, 8 year follow-up see cohort one in Breton et al. (2011)	Air pollution monitoring stations in 12 study communities, beginning in 1994, measured average hourly levels NO_2 computed annual averages on the basis of average levels in a 24-h period calculated long-term mean pollutant levels (from 1994 through 2000) for use in the statistical analysis of the lung-function outcomes	Not Reported	2 stage linear regression adjusted for log values for height; body-mass index (the weight in kilograms divided by the square of the height in meters); the square of the body-mass index; race; the presence or absence of Hispanic ethnic background, doctor-diagnosed asthma, any tobacco smoking by the child in the preceding year, 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.	--	NO_2 (10 ppb) Change (95% CI); p=value FVC -27.5 (-54.7, -0.2); 0.05 FEV_1 -29.3 (-47.5, -11.1); 0.005 MMEF -61.0 (-109.1, -12.8); 0.02

Table 5-2 (Continued): NO_2 long-term pulmonary function growth children cohort prospective studies.

Study	Exposure	Pollutant Correlation	Statistical methods	Comments	Results: FEV_1 and FVC (mL) MMEF, PEF, and FEF (mL/sec)
Mexico City School Children Cohort					
Rojas-Martinez et al. (2007a, b) Cohort of 3,170 children aged 8 years at baseline in 31 schools from April 1996 through May 1999.	Ten air-quality monitoring stations within 2 km of the schools provided exposure data.	24-h avg NO_2 and 8-h avg O_3: 0.166 (p <0.001) NO_2 and 24-h avg PM_{10}: 0.250 (p = 0.001)	General linear mixed models were used to evaluate the association between air pollutant concentrations and deficits in lung function growth over time. Potential confounding factors adjusted for include age; body mass index; height; height by age; weekday time spent in outdoor activities; and environmental tobacco smoke.	A deficit in FVC and FEV_1 growth was observed for O_3, PM_{10}, and NO_2 after adjusting for the acute effect of these pollutants (previous-day concentrations) and for confounding factors	Annual change as percentage values per 10-ppb increase in 6-mo mean NO_2. Change (95% CI); p=value Multi-pollutant model (O_3, PM_{10}, NO_2) Girls FEV_1 -0.71 (-1.00, -0.42); <0.0001 Boys FEV_1 -0.64 (-0.92, -0.37); <0.0001 Girls FVC -1.05 (-1.32, -0.77); <0.0001 Boys FVC -1.09 (-1.36, -0.82) <0.0001 $FEF_{25-75\%}$ for 12-ppb increase in 6-mo concentration Girls 2.5 (-17.5, 12.5) Boys 6.7 (-20.8, 7.5)

Table 5-2 (Continued): NO_2 long-term pulmonary function growth children cohort prospective studies.

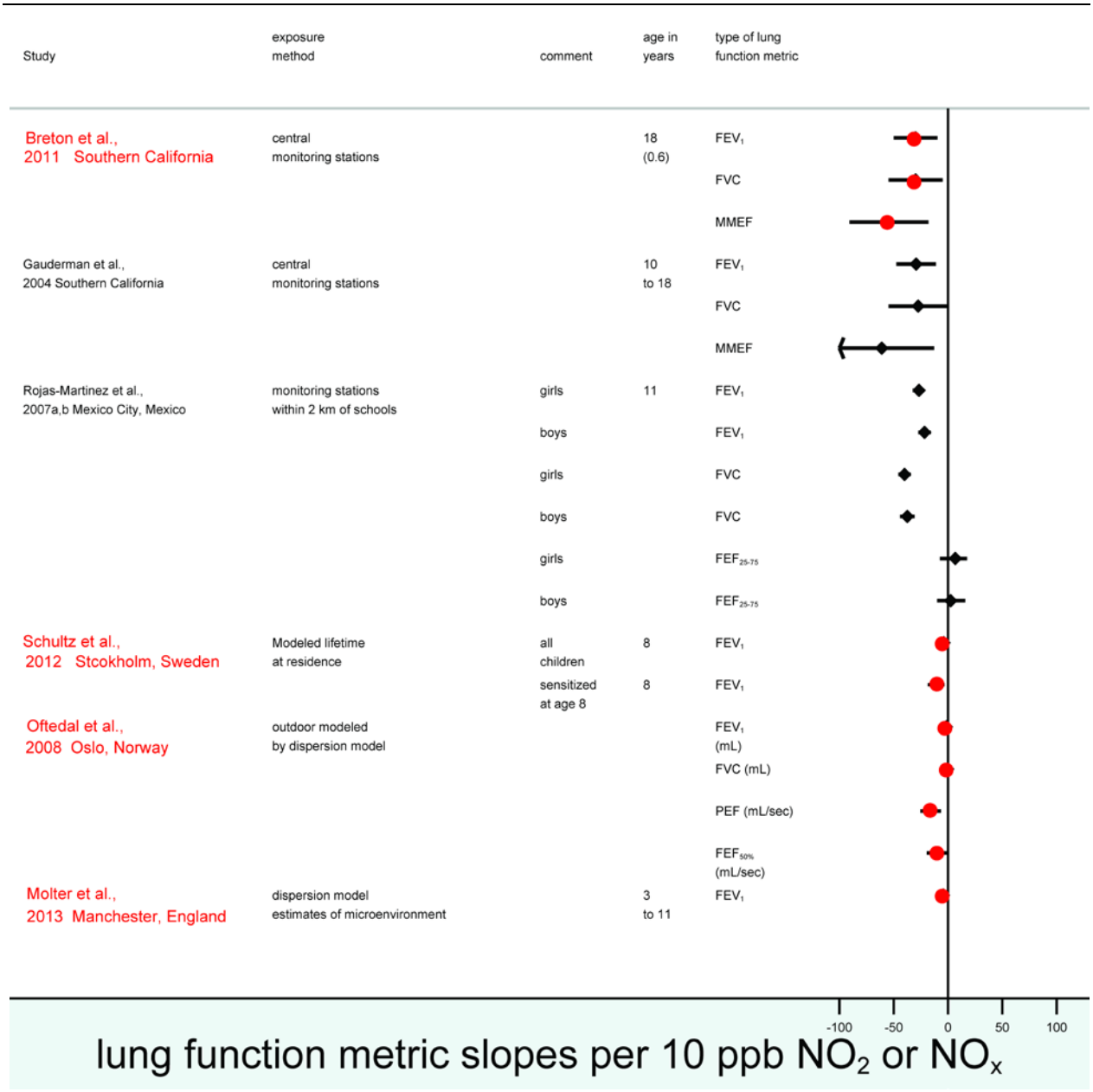
Study	Exposure	Pollutant Correlation	Statistical methods	Comments	Results: FEV_1 and FVC (mL) MMEF, PEF, and FEF (mL/sec)
Children, Allergy, Milieu, Stockholm, Epidemiology Survey (BAMSE)					
Schultz et al. (2012) In Swedish birth cohort, 1924 school-age children followed from birth until 8 years of age. Nordling et al. (2008)	The lifetime residential, day care, and school addresses were geocoded, and time-weighted average outdoor levels for the different time windows were calculated using emission inventories and a Gaussian air dispersion model. Short-term exposure was estimated using daily air quality measurements and meteorological data from urban background and rural monitoring stations		Linear regression. In addition to the chosen adjustment covariates (municipality, sex, age, height and heredity for asthma and/or allergy) the following potential confounders were evaluated: gestational age, birth weight, birth length, current passive smoking, maternal smoking during pregnancy or at birth of child, socioeconomic status of parents, possession of furred pets at birth and current, ethnicity, mold and moist in house during first year of life, year the house was built, but none of these were found to have any influence on the effect 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 year exposure to traffic-NO_X. Additional adjustment 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. Specific adjustment for NO_2 was not discussed. No impact of short-term air pollution exposure on the estimates of the long-term effects of air pollution was found, but these analyses were limited to PM_{10} (in Stockholm usually coarse) and O_3.	Exposure during the first year of life was associated with a deficit of -14.0 mL (95% CI: -32.0, 4.1) in FEV_1 for a 10 ppb difference in time-weighted exposure to traffic-NO_X, whereas the corresponding deficit was 39.6 mL (95% CI: -67.8, -11.4) among those sensitized against any common inhalant or food allergens, and those with asthma at 8 years. No clear effects on lung function seen after infancy.

Table 5-2 (Continued): NO_2 long-term pulmonary function growth children cohort prospective studies.

Study	Exposure	Pollutant Correlation	Statistical methods	Comments	Results: FEV_1 and FVC (mL) MMEF, PEF, and FEF (mL/sec)
Oslo Birth Cohort					
Oftedal et al. (2008) In 2001–2002, spirometry was performed in 2,307 9- and 10-year-old children who had lived in Oslo, Norway, since birth. Slørdal et al. (2003)	Outdoor air pollution was modeled by EPISODE, a dispersion model based on emissions, meteorology, topography, and background air pollution concentrations measured at regional background stations for which evaluation concluded that the modeled values represent exposure reasonably well.	NO_2 and PM r = 0.83 – 0.95	Multiple linear regression. Adjusted for height, age, body mass index, birth weight, temperature lags 1–3 days before the lung function test, current asthma, indicator for participation in the Oslo Birth Cohort, maternal smoking in early lifetime, parental ethnicity, education, and smoking. Models for all children are also adjusted for sex.	Early and lifetime exposures to outdoor air pollution were associated with reduced peak expiratory flow and reduced forced expiratory flow at 25% and 50% of forced vital capacity, especially in girls. Including short- and long-term NO_2 exposures simultaneously yielded only the long-term effect. No effect on forced volumes was found.	NO_2 in first year of life Change (95% CI) per 10 pb All children: FEV_1 6.0 (-18.0, 6.1) FVC 1.4 (-14.6, 11.7) PEF 57.9 (-92.5, -22.3) $FEF_{50\%}$ -37.3 (-71.1, -3.5)

Table 5-2 (Continued): NO_2 long-term pulmonary function growth children cohort prospective studies.

Study	Exposure	Pollutant Correlation	Statistical methods	Comments	Results: 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 = 1185. Manchester, U.K. Birth cohort recruited between 1995 and 1997 and evaluated at ages 3. 5. 8 and 11 years.	Land use regression model with modeled exposure at the individual level rather than community level. Using the Microenvironmental Exposure Model that incorporates children's time-activity patterns to predict personal exposure, the performance of which was found to be in agreement between modeled and measured NO_2 concentrations.	Not reported	Generalized estimating equations were used to analyze the association between lifetime exposure and the development of lung function. Potential confounding variables and covariates 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 two years of life, hospitalization during the first two years of life, presence of a gas cooker in the home, presence of a dog or cat in the home, visible signs of dampness or mould in the home, body height, body weight, body mass index, maternal age at birth, gestational age, duration of breast feeding, Tanner stage (age 11 only) and socioeconomic status (paternal income).	A negative association between post bronchodilator FEV_1 and PM_{10} and NO_2 exposure over time: (PM_{10}: β = -3.59 [95% CI: -5.36,-1.83]; NO_2: β = -1.20, [95% CI:-1.97, -0.43] per 1 μg/m^3). Based on the average predicted FEV_1 of 1.65 L (see above), these would be equivalent to a growth deficit in post bronchodilator FEV_1 of 59 mL from age 5 years to 11 years per unit increase in PM_{10}, and a growth deficit of 20 mL from age 5 years to 11 years per unit increase in NO_2.	For FEV_1 NO_2 exposures were associated with poorer lung function over time: (PM_{10}: β = -1.37 [95%CI: -2.52, -0.23]; NO_2: β = -0.83 [95%CI: -1.39, -0.28] per 1 μg/m^3). Based on the average predicted FEV_1 within MAAS at ages 5, 8 and 11 of 1.65 L, the model estimated that for each unit increase (1 μg/m^3) in PM_{10} exposure, the growth in FEV_1 from age 5 years to 11 years was 23 mL smaller, and for each 10-ppb increase in NO_2 exposure, the growth in FEV_1 was 263 mL smaller [ΔFEV_1 (mL) = β/100*1.65*1000].



Note The studies are presented by age. Follow-up was 3 years for Rojas-Martinez et al. (2007a), and 10 years for Oftedal et al. (2008). All other studies were 8 years of follow-up.

Figure 5-5 Associations of NO_2 or NO_X with lung function indices from prospective studies.

Adults

Prospective studies evaluating long-term NO_2 exposure and pulmonary function consist of a European multi city study and a study in the U.K. that are discussed next. Cross-sectional studies were reviewed but not presented here are (Forbes et al., 2009b; Sekine et al., 2004).

Götschi et al. (2008) examined the relationship between air pollution and lung function in adults in the European Community Respiratory Health Survey (ECRHS). 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). No statistically significant associations were found between city-specific annual mean NO_2 and average lung function levels which is in contrast to the results seen by Ackermann-Liebrich et al. (1997) (SAPALDIA) and Schikowski et al. (2005) (SALIA) which compared across far more homogenous populations than for the population assessed in the ECRHS. Misclassification and confounding may partially explain the discrepancy in findings.

A recent study Boogaard et al. (2013) evaluates 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 cites and other related policies. At 12 locations in the Netherlands, air pollution concentrations and respiratory health were measured in 2008 and 2010, indicating a reduction over that time period. NO_2 and NO_X concentrations were measured with Ogawa passive samplers. In regression analyses adjusted for important covariates, reductions in concentrations of soot, NO_2, NO_X, Cu, and Fe were associated with increases in forced vital capacity (FVC) (increase per interquartile range [IQR] decline). Airway resistance decreased with a decline in particulate matter ([PM_{10}] and $PM_{2.5}$ [per IQR]), although these associations were somewhat less consistent. No associations were found with exhaled NO. The response rate for participation in the study was around 10%. Over the two time periods 585 subjects were reevaluated for spirometry. This was a heterogeneous study population with both children and adults were 84% were above 30 years of age at baseline. 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.

Both (1) cross-sectional associations with bronchial hyperresponsiveness, FEV_1, spirometry defined COPD, skin test positivity, total IgE and questionnaire-reported wheeze, asthma, eczema and hay fever in 2,599 subjects, and (2) longitudinal associations with decline in FEV_1 in 1,329 subjects followed-up nine years later in 2000 were evaluated in a Nottingham U.K. cohort of adults aged 18-70 in relation to modeled outdoor NO_2 concentrations (Pujades-Rodriguez et al., 2009). There were no significant cross-sectional associations between home proximity to the roadside or NO_2 level with

any of the outcomes studied. Also, neither exposure was associated with a significantly greater decline in FEV_1 over time. Insufficient contrast in exposure may be a factor why this study was unable to detect any effects of localized traffic pollution markers in this study population were the modeled NO_2 variation ranged from values for IQR of 18.1 to 19.1 ppb.

5.2.3.2 Toxicological Studies

Studies included in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) showed minimal evidence of changes in lung function in animals after long-term exposure to concentrations of NO_2 relevant to ambient exposure.No recent studies were available. Arner and Rhoades (1973) published early studies that exposed rats to 2,900 ppb NO_2 continuously for 5 days per week for 9 months and reported changes in lipid composition in the airway and that could be related to observed functional consequences, including decreased lung volume and compliance and increased surface tension, though these changes have not been consistently observed in animals studies.

Tepper et al. (1993) exposed rats to a background concentration of 500 ppb NO_2 for 16 hours per 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, though changes were not 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, though mice tended to have slightly decreased vital capacity from 16 to 52 weeks of exposure.

Minimal effects were also described in studies of shorter, yet still long-term, NO_2 exposure. 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 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 hours per day, 5 days per week), but vital capacity and compliance were not affected.

Overall, these animal studies demonstrate minimal effects of long-term NO_2 exposure on pulmonary function in animals which is consistent with results from short-term

exposures; however, age may be an important factor that has not been adequately addressed by the existing body of toxicological evidence.

5.2.4 Hospital Admissions

Recent studies represent the first evaluation of long-term NO_2 exposure for respiratory hospital admissions. Studies include hospitalization for chronic obstructive pulmonary disease (COPD), asthma, and community-acquired pneumonia. The relationship between long-term pollutant exposures on the development of COPD was evaluated by Andersen et al. (2011) in a prospective cohort study. COPD incidence in relation to estimated modeled outdoor annual means of NO_2 and NO_X from residential address history since 1971 was studied with respect to the first admission to hospital for COPD in 57,053 participants (median age 56.1) in the Danish Diet, Cancer, and Health cohort in the Hospital Discharge Register between 1993 and 2006. The incidence (date of first admission) of COPD between baseline and 27 June 2006 was the main outcome. After adjustment for occupation, educational level, body mass index, and fruit intake, associations with COPD incidence remained for the 35- and 25-year mean levels 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 level of NO_X (1.16 [95% CI: 1.04, 1.31], per 20-ppb increase), whereas weak positive associations were observed with the 25-year mean level of NO_X, 15-year mean levels of NO_2 and NO_X, and baseline residence traffic proxies (major road within 50 meters, traffic load within 200 meters). The associations with NO_2 were stronger than those with NO_X. Additional adjustment for sex, physical activity, alcohol consumption, and vegetable consumption did not substantially change the risk estimates. The effect was stronger in people with diabetes and asthma compared to the rest of the cohort. The strongest association with COPD incidence was found with the longest exposure, reinforcing the conclusion that exposure over a long period, perhaps over a whole life, is relevant for the development of chronic lung diseases such as COPD. No evidence was found that the effect was modified by smoking or occupational exposure.

A study also examining long-term NO_2 exposure and COPD hospitalization was conducted in Canada. Gan et al. (2013) evaluated a population-based cohort 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). Residential exposures to traffic-related air pollutants (black carbon [BC], $PM_{2.5}$, NO_2, and NO) and wood-smoke were estimated using land-use regression models and integrated changes in residences during the exposure period. COPD hospitalizations during the follow-up period were identified from provincial hospitalization databases. Mortality data was also

studied and is discussed in Section 4.2. In unadjusted single-pollutant models, NO_2 and NO were associated with COPD hospitalization. However, after adjustment for covariates, the association of these air pollutants with COPD hospitalization was attenuated which may reflect the lack of spatial variability of these pollutants in this intra-urban study. Additionally, the shorter exposure period examined in this study compared with that in Andersen et al. (2011) may be a factor in their different results.

The relationship between long-term pollutant exposures on the risk for asthma hospitalizations in older people was also evaluated in the Danish Diet, Cancer and health cohort (Andersen et al., 2012). Associations between NO_2 level and first hospital admission were found in the full cohort (HR per IQR 10 ppb: 1.44 [95% CI: 1.14, 1.84]), which was insensitive to choice of potential confounders. The associations were similar for the first asthma hospitalization (1.36 [95% CI: 1.03, 1.80]), but markedly higher for re-hospitalization in people with a previous asthma hospitalization (3.05 [95% CI: 1.57-5.90], p = 0.05, Wald test for interaction). The risk for asthma hospitalization associated with NO_2 was four times higher in people with previous COPD hospitalizations (2.34 [95% CI: 1.25, 4.40], with effect modification [p = 0.04]). 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 dispersion model estimating NO_2 levels may have been poorer longer back in time, owing to the higher uncertainty of model input data such as emission factors and traffic counts. Thus, the stronger associations with NO_2 levels at follow-up, when compared with the 35-, 15- and 1-year means at baseline may reflect the relevance of more recent exposures for asthma hospitalization or merely the better performance of the dispersion model in more recent years.

In an ecological time series study, Delamater et al. (2012) explored the relationship between asthma morbidity, ambient levels of air pollutants, and weather conditions at a county level using a monthly time series analysis. In LA County, they found that asthma hospitalizations were associated with CO, NO_2, and $PM_{2.5}$ concentrations in single variable regression models and NO_2 + relative humidity, $PM_{2.5}$ +relative humidity, PM_{10} + relative humidity, and NO_2 + maximum temperature in multi-variable models. In a cross-sectional study, Meng et al. (2010) examined associations between air pollution and asthma morbidity in the San Joaquin Valley in California using the 2001 California Health Interview Survey data from subjects ages 1 to 65+ who reported physician-diagnosed asthma (n = 1,502). Subjects were assigned annual average concentrations for NO_2 based on residential ZIP code and the closet air monitoring station within 8 km but did not have data on duration of residence. No associations were found between average annual concentrations of NO_2 and odds of asthma-related ED visits or hospitalizations. No quantitative results were shown for NO_2.

In a population-based case-control study in Hamilton, Ontario, Canada, Neupane et al. (2010) examined the relationship between the previous 12 months exposure to ambient pollutants (NO_2, $PM_{2.5}$, SO_2) and hospitalization for community-acquired pneumonia in 345 hospitalized patients aged 65 years or more and 494 control participants aged 65 years and more. Control participants were randomly selected from the same community as cases from July 2003 to April 2005. Three methods were used to estimate the annual average NO_2 levels: daily ambient data, LUR models and inverse distance weighting. 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. Various interaction terms were evaluated but none significantly interacted with any air pollutant variant and therefore were not included in the logistic regression model. Covariates evaluated included: age, male sex, education, smoking, and history of exposure to fumes at work. NO_2 and $PM_{2.5}$ were associated with hospitalization for community-acquired pneumonia but SO_2 was not. For NO_2, all three exposure estimate methods indicated an association. While no mention of control or adjustment for short-term exposure effects was discussed, two recent unrelated short-term exposure studies of hospitalization for pneumonia (Chiu et al., 2009; Cheng et al., 2007) report smaller effect associations with NO_2 and pneumonia hospitalization than this long-term study which is as expected.

5.2.5 Respiratory Symptoms

5.2.5.1 Children

In the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), 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. That review considered the results to be inconsistent, mainly due uncertainties inherent to the cross-sectional studies. Recent prospective cohort studies evaluating the relationship between respiratory symptoms and long-term exposure to NO_2 are summarized in Table 5-3 and the following text. Cross-sectional studies were reviewed and are discussed in other sections as appropriate (Annesi-Maesano et al., 2012a; 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 in the Annex Table AX6.3-17 of the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c).

A number of studies have observed an association between different respiratory symptoms in children with asthma and long-term exposure to NO_2. McConnell et al.

(2003) examined children with asthma for bronchitic symptoms, including daily cough for 3 months in a row, congestive phlegm 3 months in a row, or bronchitis, all representing chronic indolent symptoms. The remaining studies focused on asthmatic symptoms. Belanger et al. (2013) recorded wheeze, night 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). Hansel et al. (2008) examined wheezing, coughing, chest tightness (activity limiting or while running), wheezing so bad that speaking is difficult, and nocturnal cough without a cold. Gehring et al. (2010) examined asthma symptoms (one or more attacks of wheeze, SOB, prescription of inhalation steroids), wheeze (transient, late onset, persistent), sneezing, hay fever, atopic eczema, and prevalence of asthma. Gruzieva et al. (2013) examined wheeze, categorized as either one or more episodes or three or more episodes in the past year. These approaches to examining respiratory symptoms in asthmatics provide different information from which to consider the impact of long-term NO_2 exposure on respiratory health.

It is clear that the respiratory symptoms examined vary among these studies and are measured in a different ways. McConnell et al. (2003) is unique in that it is the only prospective study examining bronchitic symptoms in children with asthma for which they report positive results that are stronger in their within community analysis as compared to the between community analysis. Hansel et al. (2008) finds positive results for all of the symptoms evaluated, and noted that symptoms of a more severe nature may produce stronger relationships to NO_2 exposure. In a threshold model, Belanger et al. (2013) report positive associations for the asthma severity score, wheeze, night symptoms, and rescue medication use. These results were generally larger than those reported in the other studies. Gehring et al. (2010) observed positive results for prevalent asthma, asthma symptoms, and wheeze; but not for hay fever or atopic eczema. Gruzieva et al. (2013) report a weak association with wheeze.

The respiratory symptoms evaluated in this group of studies vary and some health effect indicators involve several components. The ability of these various indicators to serve as a measure for respiratory health effects may differ. Some indicators may be better than others.

The collective evidence from this group of prospective studies provides results that could be cautiously viewed to be supportive of a relationship of long-term exposure to NO_2 and increased respiratory symptoms using various indicators in children with asthma. This is especially so, for studies that involve a group of health indicators, as they may be more able to inform the asthmatic relation as opposed to a more sparse approach, i.e., just

wheeze. Thus weighing the multiple approaches as potentially being more informative might indicate a stronger less uncertain relationship in this evidence base.

Some studies examined respiratory health to include symptoms and respiratory infection in infants and young children up to three years of age (Aguilera et al., 2013; Sonnenschein-Van der Voort et al., 2012; Raaschou-Nielsen et al., 2010b; Sunyer et al., 2004). Both Sunyer et al. (2004) and Raaschou-Nielsen et al. (2010b) found no associations in infants between indoor NO_2 exposure and respiratory infections and wheezing respectfully. Sonnenschein-Van der Voort et al. (2012) only found associations of increased risk in wheezing in children up to age three years exposed to tobacco smoke. Aguilera et al. (2013) related NO_2 exposure to increased risk of upper and lower respiratory tract infections in infants.

Table 5-3 Long-term NO_2 exposure prospective children studies: respiratory symptoms.

Study	Exposure	Pollutant Correlation	Statistical Methods	Comments	Results
Childrens Health Study (CHS) Southern California communities					
McConnell et al. (2003) Children with a history of asthma at study entry, who completed two or more follow-up questionnaires any time during the years 1996 to 1999 (n = 475) were included in the current analysis. Yearly follow-up for 4 years.	Air pollution monitoring stations were established in each of the 12 communities. Annual averages were computed of the 24-h NO_2. Four-year mean levels (1996–1999) in each community were computed for each pollutant metric.	The correlations of 4-year average pollution across the 12 communities were relatively high with each other (R >0.65) except with O_3 which was low. The within-community correlations differed in that NO_2 could be distinguished from most other major pollutants except OC and inorganic acid.	Examined the associations of bronchitic symptoms both with yearly variation in air pollution within communities and with the 4-year average of air pollutants across the 12 study communities. The modeling strategy can be conceptualized as a three-stage regression. OR adjusted for age, maternal and child's smoking history, sex, and race; within-community estimates were adjusted for between-community effects of the pollutant, and vice versa.	In two pollutant models, the effects of yearly variation in OC and NO_2 were only modestly reduced by adjusting for other pollutants, except in a model containing both OC and NO_2; McConnell et al. (2006) further found that this cohort result was modified by dog or cat ownership indicators or allergen and endotoxin exposure as the odds ratio for NO_2 was 1.49 (95% CI: 1.14, 1.95), indicating that dog ownership may worsen the relationship between air pollution and respiratory symptoms in asthmatic children,	Within communities NO_2 (OR: 1.97 [95% CI: 1.22, 3.18] per 10 ppb); ORs associated with yearly within-community variability in air pollution were larger than the effect of the between-community 4-year average concentrations. Between communities (NO_2 OR: 1.22 [95%CI: 1.00, 1.49] per 10 ppb).

Table 5-3 (Continued): Long-term NO_2 exposure prospective children studies: respiratory symptoms.

Study	Exposure	Pollutant Correlation	Statistical Methods	Comments	Results
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 years.	Land-use regression models used to provide annual levels for birth address of each participant.	The estimated exposures for the various pollutants were highly correlated (r = 0.93, 0.96, and 0.97 for the correlation between NO_2 and $PM_{2.5}$, NO_2 and soot, and $PM_{2.5}$ and soot, respectively.	Generalized estimation equations. Potential confounding variables included sex, study arm (intervention or natural history), 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, 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.	Adjusted OR for asthma symptoms of 1.17 (95% CI: 0.98, 1.39) per 10-ppb increase without adjustment for study region. For wheeze OR: 1.27 (95% CI: 1.07, 1.50) without adjustment for study region.
Children, Allergy, Milieu, Stockholm, Epidemiology Survey (BAMSE)					
Gruzieva et al. (2013) Swedish birth cohort followed up to 12 years of age enrolled between 1994 and 1996 n = 3,633. Nordling et al. (2008). Melén et al. (2008)	Dispersion models were used to calculate for all addresses in the years 1994 to 2008 representing when the first child was born until the end of the 12-year follow-up.	r = 0.96 between NO_X and PM_{10} exposure levels during the first year of life.	Multinomial regression/ GEE. Potential confounders adjusted for include municipality, SES, year the house was built, and heredity.		No overall association was observed between air pollution exposure after the first year of life and development of asthma symptoms. Wheeze at 12 years of age OR 3 or more episodes: 1.35 (95% CI: 0.79, 2.29) per 20 ppb for NO_X.

Table 5-3 (Continued): Long-term NO_2 exposure prospective children studies: respiratory symptoms.

Study	Exposure	Pollutant Correlation	Statistical Methods	Comments	Results
Longitudinal New England Indoor Children's Asthma Study					
Belanger et al. (2013) Connecticut/Massachusetts cohort , n = 1,642, age 5-10 followed for one year, asthma severity score from 2006 through 2009	Palmes tubes in bedrooms and dayroom for 4 weeks for 4 seasons	Not Reported	Adjusted, hierarchical ordered logistic regression models used Adjustments included 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	Included maintenance medication use as a covariate in models exploring associations between symptoms and NO_2 exposure. Because use of maintenance medication is also associated with socioeconomic status An alternative model that adds only "residual" amounts above what is measured indoors was considered. In this alternative model, where only "extra" NO_2 not accounted for in the indoor measurement is added, the OR for indoor NO_2 exposure on the asthma severity score is 1.52 (1.06–2.18), and the OR for outdoor NO_2 exposures is 1.20 (0.98–1.46).	Every 5-fold increase in NO_2 exposure above a threshold of 6 ppb was associated with a dose-dependent increase in risk of higher asthma severity score (ORs: 1.37 [95% CI: 1.01, 1.89]; Wheeze: 1.49 [95% CI: 1.09, 2.03]; Night symptoms: 1.52 [95% CI: 1.16, 2.00]; and Rescue Medication Use: 1.78 [95% CI: 1.33, 2.38]).

Table 5-3 (Continued): Long-term NO_2 exposure prospective children studies: respiratory symptoms.

Study	Exposure	Pollutant Correlation	Statistical Methods	Comments	Results
African-American Baltimore School					
Hansel et al. (2008) n = 150, 2-6 years of age with physician diagnosed asthma.	Indoor air was monitored over a 72-h period in the children's bedrooms at baseline and 3- and 6-mo.	There was minimal correlation (R^2 = 0.056, p <0.01) between ambient and indoor NO_2 concentrations.	Logistic regression models and GEE Multivariate models to adjust for potential confounders, including age, sex, race, caregiver education level, season of sampling, $PM_{2.5}$, secondhand smoke (SHS) exposure [defined as caregiver report of presence of a smoker in the home (yes vs. no)], distance from curb, and type of street in front of house.	This longitudinal study with repeated measures of NO_2 concentrations and respiratory symptoms improves on the ability to directly model individual response to changing NO_2 concentrations accounting for within-person correlations of asthma severity. The link between indoor NO_2 concentrations and asthma symptoms appears to be robust, because the associations were not affected by the potential confounders studied. This study was strengthened by its ability to adjust for other relevant copollutants. Although $PM_{2.5}$ was associated with increased asthma symptoms [(McCormack et al., 2008), data not shown], adjusting for other copollutants did not meaningfully alter the association between indoor NO_2 concentrations and asthma symptoms.	Adjusted IRR per 10-ppb increase in NO_2 exposure; Daytime wheezing, coughing, or chest tightness 1.02 (0.98, 1.06); Slowing activity due to asthma, wheeze, chest tightness, or cough: 1.04 (0.97, 1.11); Limited speech due to wheeze: 1.08 (1.04, 1.12); Wheeze, cough, or chest tightness while running: 1.04 (1.00, 1.08*); Coughing without a cold 1.07 (1.03, 1.11); Nocturnal awakenings due to cough, wheeze, shortness of breath, or chest tightness: 1.06 (1.02, 1.10)

5.2.5.2 Adults

Studies examining the relationship between long–term NO_2 exposure and respiratory symptoms in adults include prospective studies discussed under asthma incidence. 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." Results were homogeneous among the centers in both the crude and the adjusted analyses. Symptoms in the last 12 months at ECRHS II among people without asthma at baseline were also associated with NO_2. The authors note that these observations are, on the one hand, complementary to and in strong support of main findings on asthma incidence. On the other hand, due to the study design, the symptom results also call for a partly different interpretation. The standard questionnaire asks about symptoms during the last 12 months only. Thus, people with new asthma onset who did not suffer symptoms during the last 12 months (e.g., due to treatment) would not be captured. Instead, a subject without asthma who reported symptoms during the last 12 months (e.g., due to some infection) would be identified as an incident case. Moreover, air pollution is a known trigger of several asthma-related symptoms. Thus, reporting of symptoms at ECRHS II (but not at ECHRS I) may not necessarily reflect the onset of asthma due to air pollution, but represent the acute effects of air pollution exposure during the past 12 months. The main approach using asthma incidence is less affected by these methodological issues. Cases diagnosed during the entire follow-up period contributed to these findings, independent of symptom status during the last 12 months.

Zemp et al. (1999) report, in a cross-sectional study (SAPALDIA), an NO_2 association with high prevalence of respiratory symptoms in adults. Bentayeb et al. (2010) report the first baseline cross-sectional study results examining the respiratory symptoms cough and phlegm in adults ($\geq$ 65 years old, in Bordeaux, France) in relation to NO_2 exposure to be of borderline significance.

5.2.6 Allergic sensitization

5.2.6.1 Epidemiologic Studies

Children

Recent cross-sectional studies evaluate aspects of allergic responses and long-term exposure to NO_2. In the Munich metropolitan area, a study population consisting of two prospective birth cohort studies (German Infant Nutritional Intervention -GINI and Lifestyle-Related Factors on the Immune System and the Development of Allergies in Childhood study-LISA) was used to evaluate the relationship between individual-based exposure to traffic-related air pollutants and allergic disease outcomes during the first 6 years of life (Morgenstern et al., 2008). Exposure assessment was calculated at three different time points (birth, 2 or 3 years, and 6 years). Positive, but imprecise, associations were observed between doctor-diagnosed asthma and parental reporting of symptoms and long-term exposure to NO_2. Previous analyses of the LISA and GINI cohorts at age 1 reported positive associations between $PM_{2.5}$, $PM_{2.5}$ absorbance, and NO_2, and cough without infection, and dry cough at night. At age 2 years, these effects were attenuated (Morgenstern et al., 2007; Gehring et al., 2002). NO_2 was positively associated with eczema. For a 10-ppb increase in NO_2, the association for allergic sensitization at 6 years of age was 1.07 (95% CI: 0.72, 1.59) for any inhalant, 1.00 (95% CI: 0.72, 1.59) for outdoor allergens, and 0.89 (95% CI: 0.40, 2.00) for indoor allergens.

Annesi-Maesano et al. (2007) related individual data on asthma and allergy from 5,338 school children (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 the city level at fixed-site monitoring stations. NO_2 was positively associated with exercise-induced bronchial (EIB) reactivity, flexural dermatitis and skin prick test (SPT) to indoor allergens. Hwang et al. (2006) report the prevalence of allergic rhinitis (adjusted OR per 10 ppb NO_2 = 1.11 [95% CI: 1.08-1.15]) in a large cross-sectional study of school children in Taiwan. Parker et al. (2009) evaluated the association between air pollutants and childhood respiratory allergies in the U.S. using the 1999-2005 National Health Interview Survey of approximately 70,000 children and found no associations between NO_2 and the reporting of respiratory allergy/hay fever. Strong positive associations were found for O_3.

Nasal eosinophils, which participate in allergic disease, were observed to decrease by fourfold in 37 atopic mildly asthmatic children 7 days after relocation from a highly polluted urban area (NO_2 51.8 ± 6.3 ppb) in Italy to a rural location with lower NO_2

levels (NO_2 3.5 ± 0.27 ppb) (Renzetti et al., 2009). Living in a less-polluted environment was also associated with reduced eosinophilic inflammation of the lower airways, reflected by a decrease in mean FE_{NO} (fraction of NO in exhaled air) concentration and with consistent improvement in lower function reflected by an increase in mean PEF.

Nordling et al. (2008), discussed in Table 5-1, Table 5-2, and Table 5-3, reported that exposure to NO_2 from traffic during the first year of life was associated with IgE-antibodies for pollens (OR = 1.24 (1.04-1.49), per 10-ppb increase in NO_2) but other measures were not. The relationship between the development of allergic sensitization in children during the first 8 years of life and long-term exposure to NO_2 was evaluated in a prospective analysis of the BAMSE cohort (Gruzieva et al., 2012). There was no overall risk of sensitization at 4 years of age associated with traffic-related air pollution exposure. However, exposure during the first year of life was associated with an increased risk of sensitization to pollen (OR: 1.64 [95% CI: 1.02, 2.63, per 20-ppb increase) for traffic-related NO_X. On the other hand, there was no apparent effect of exposure to air pollution after the first year of life on the development of sensitization.

In a cross-sectional analysis in six French cites, Annesi-Maesano et al. (2012a) evaluated the relationship between indoor air quality in schools and the allergic and respiratory health of schoolchildren (mean age 10.4). For each pollutant, a 5-day mean concentration in the classroom was computed and a three-class variable of exposure (high, medium, or low) was defined with respect to the tertiles of concentration in the class room, independent of the city. Between-school and within-school variability of the measured indoor pollutants were estimated using linear mixed models for longitudinal data. Among atopic children (n = 1,719) NO_2 was related to past year allergic asthma after adjusting for the potential confounders age, sex, passive smoking and parental or maternal history of asthma and allergic diseases (OR: 1.40, p = 0.0514).

In a cross-sectional analysis of 30,139 Chinese children aged 3-to-12 years, Dong et al. (2011) evaluated the relationship between 3-year averages of ambient pollutants (PM_{10}, SO_2, NO_2, CO, and O_3) and asthmatic symptoms (persistent cough, persistent phlegm, doctor-diagnosed asthma, current asthma, current wheeze, and allergy rhinitis). The study examined 25 districts of seven cities in northeast China in 2009 and also investigated whether allergic predisposition modifies this relationship. The data confirm that ambient compound air pollution was associated with respiratory symptoms and diseases in young children. Among children without an allergic predisposition, males might be more susceptible to ambient air pollution than females; whereas among children with an allergic predisposition, more associations were detected in females in this group. Among children without allergic predisposition (n = 26,004) several NO_2 effects were obtained mainly in males in single pollutant models. In multipollutant models that evaluated the

five pollutants, persistent cough effects increased for NO_2 for both males and females; however, because of potential multicollinearity among more than two pollutants, these resutlts are difficult to interpret. Among children with allergic predisposition (n = 4,135), Several NO_2 analyses that were positive in the single pollutant models for both males and females were attenuated in the five pollutant model for those with allergic predisposition. This differs from the results without allergic predisposition as further discussed in Section 5.2.13.

Adults

Several recent studies examined the association between allergic responses and exposure to NO_2, and report generally inconsistent results. Castro-Giner et al. (2009), discussed in adult asthma incidence (Section 5.2.2.2), noted that stratification by atopic status showed that interaction between NO_2 and *NQO1* rs2917666 was more pronounced among carriers of *NQO1* rs2917666 C/C without atopy (OR: 5.10 [95% CI: 1.26, 20.70]; *p*-value for interaction = 0.01, per 10-ppb increase) compared with subjects with atopy (OR: 1.50 [95% CI: 0.72, 3.12]; *p*-value for interaction = 0.45). Pujades-Rodriguez et al. (2009) examined a cohort of 2,644 adults aged 18-70 living in Nottingham, U.K. to evaluate the relationship between NO_2 exposure and respiratory outcomes. They found no associations between NO_2 level and bronchial hyperresponsiveness, FEV_1, skin test positivity, total IgE and questionnaire-reported wheeze, asthma, eczema or hay fever in cross-sectional analyses. Further, they found no associations with decline in FEV_1 followed-up over nine years when the data were analyzed longitudinally. Total IgE levels were not related to NO_2 concentrations in 369 adult asthmatics in five French centers using generalized estimated equations (GEE) as part of the EGEA study (Rage et al., 2009) but were related to O_3 concentrations.

5.2.6.2 Toxicological Studies

Toxicological studies provide some experimental data which is coherent with the development of allergic responses seen in epidemiologic studies. One subchronic toxicological study showed that 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 in the same study. Furthermore, two shorter term studies provide evidence that exposure to NO_2 leads to Th2 skewing and/or allergic sensitization, as discussed in Sections 3.3.2.6 and 4.2.4.3 (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 epidemiologic evidence of the association of NO_2 exposure with the development of allergic responses.

5.2.7 Pulmonary Inflammation and Oxidative Stress

5.2.7.1 Epidemiologic Studies

Children

Inflammatory markers and peak expiratory pulmonary function were examined in 37 allergic children with physician-diagnosed mild persistent asthma in a highly polluted urban area in Italy. These 37 allergic children were evaluated again 7 days after relocation to a rural location with lower pollutant levels (Renzetti et al., 2009). The authors observed a 4-fold decrease in nasal eosinophils and a decrease in fractional exhaled nitric oxide along with an improvement in lower airway function. Several pollutants were examined, including PM_{10}, NO_2, and O_3, though pollutant-specific results were not presented. Exhaled NO (eNO) has been shown to be a useful biomarker for airway inflammation in large population-based studies (Linn et al., 2009). Thus, while the time scale of 7 days between examinations for eNO needs to be evaluated for appropriateness, the results suggest that inflammatory responses are reduced when NO_2 levels are decreased.

One epidemiologic study examined the relationship of airway inflammation (eNO) and pulmonary function and NO_2 in Windsor, Ontario (Dales et al., 2008). This cohort of 2,402 school children estimated NO_2 for each child's residence at the postal code level. The FEV_1 and FVC were approximately 40 mL less in the highest compared with the lowest tertiles of NO_2, but these differences were weak. NO_2 showed positive but weak associations with eNO. The LUR estimates for Windsor did not have a large degree of variability and thus may have had insufficient small-scale spatial variability. An eNO-roadway density association persisted after adjustment for air pollutant levels (NO_2, SO_2, $PM_{2.5}$) within the previous 24 and 48 hours of the eNO measure, indicating that it was not confounded by an unmeasured acute effect.

Adults

In a cross-sectional study, Wood et al. (2009) examined the association of outdoor air pollution with respiratory phenotype (PiZZ type) in alpha 1-antitrypsin deficiency

(α-ATD) from the U.K. α-ATD registry. This deficiency leads to exacerbated responses to inflammatory stimuli. In total, 304 PiZZ subjects underwent full lung-function testing and quantitative high-resolution computed tomography to identify the presence and severity of COPD – emphysema. Mean annual air pollution data for 2006 were matched to the location of patients' houses and used in regression models to identify phenotypic associations with pollution controlling for covariates. Regression models showed that estimated higher exposure to O_3 exposure was associated with worse gas transfer and more severe emphysema, albeit accounting for only a small proportion of the lung function variability. The positive association observed for NO_2, SO_2, and particles may most likely be attributable to an inverse correlation of their concentrations with those of O_3 or they may be insufficiently representative of long-term exposure to detect effects reliably.

5.2.7.2 Toxicological Studies

Similar to studies of short-term (minutes to weeks) NO_2 exposure, animal toxicological studies of long-term (months to years) exposure show increases in pulmonary inflammation and oxidative stress (Section 4.2.4.2). Compared with short-term exposure studies, long-term studies provide more evidence of NO_2-induced airway injury. Details from these studies, all of which were reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), are presented in Table 5-4.

Many studies investigating NO_2-induced injury and oxidative stress in the airway measure changes in lipid content, which is necessary for both lung function and defense. Sagai et al. (1982) and Ichinose et al. (1983) published studies showing that rats exposed to 40 or 120 ppb NO_2 for 9 or 18 months had increased ethane exhalation and 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 and altered lung mechanics.

Histopathological assessment of lung tissue showed that long-term exposure to NO_2 resulted in alveolar macrophage 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 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 TNF-α and IFN-γ at 8 weeks but not at 12 weeks, while in the weanling group, IFN-γ was increased only at 12 weeks.

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, 2008c). 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 non-protein sulfhydryl levels and glutathione S-transferase activity in adult male rats after 9 and 18 months of exposure to 400 ppb NO_2, and decreased glutathione peroxidase 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, or disulfide reductase after 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 though 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 across species do not consistently show this to be the case. Antioxidant enzymes are involved in response to NO_2 exposure, but this response is variable and transient. Overall, these findings are consistent with the reported effects from short-term exposures (Section 4.2.4.2).

Table 5-4 Animal Toxicological Studies of the Respiratory Effects of Long-term NO_2 Exposure.

Study	Species (Strain); Lifestage; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Arner and Rhoades (1973)	Rats (Long Evans); Male	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); Female; 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 (Fisher 344); 1-day or 6 weeks; Male, 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, Fisher 344); 6 week; Male	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); Female; 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; Male; 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

Table 5-4 (Continued): Animal Toxicological Studies of the Respiratory Effects of Long-term NO_2 Exposure.

Study	Species (Strain); Lifestage; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Furiosi et al. (1973)	Monkey (Macaca speciosa), Rat (Sprague-Dawley); Maturing (Monkey), Weanling (Rat); Male/female (Monkey), Male (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
Greene and Schneider (1978)	Baboons; 3 to 4 years; Males and Females; 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, BALF analysis (LDH, ALKP, glutathione peroxidase), antioxidant enzymes in lung homogenates
Hayashi et al. (1987)	Rat (Wistar); Male, 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; Male; n = 37	5,000 ppb continuously for 2 mo; Challenge with *Klebsiella pneumonia* or influenza after exposure	Infection resistance, mortality, peripheral blood markers, and respiratory function
Ichinose et al. (1983)	Rats (JCL, Wistar); 8 and 13 weeks; Male	(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; Female; n = 201	200, 500, or 2,000 ppb pre- and postnatal for up to 12 postnatal weeks	Immunologic evaluation (Alveolar macrophage activity)
Kubota et al. (1987)	Rat (JCL Wistar); 2 mo; Male, n = 3-4/group	40 ppb, 400 ppb, or 4,000 ppb continuously for 9, 18, and 27 mo	Serological examination and lung morphometry
Lafuma et al. (1987)	Hamster (Golden Syrian); Male, 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 (Fisher 344); 7 weeks; Male, n = 5/group	500 ppb continuously with 2 daily, 1 h peaks of 1,500 ppb for 9 weeks	Histopathologic evaluation and morphometry

Table 5-4 (Continued): Animal Toxicological Studies of the Respiratory Effects of Long-term NO_2 Exposure.

Study	Species (Strain); Lifestage; Sex; n	Exposure Details (Concentration; Duration)	Endpoints Examined
Miller et al. (1987)	Mice (CD-1); 4-6 weeks; Female; 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
Sagai et al. (1982)	Rats (JCL, Wistar); 8 weeks; Male	10,000 ppb continuously for 2 weeks	Histopathologic evaluation and morphometry
Sagai et al. (1984)	Rats (JCL Wistar); 8 weeks; Male; 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; Male, n = 30/group	340 ppb for 6 h/day for 5 days/week for 6 weeks	Type 2 pneumocytes in the lungs and alveolar wall area
Stevens et al. (1988)	Rat (Fischer 344); Young adult, neonate; Male, 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; Male; n = 11-16/group	500 ppb continuously 7 days/week with two daily, 2-h spikes of 1,500 ppb, 5days/week for up to 78 weeks	Pulmonary function and lung disease

5.2.8 Toxicological Studies of Airway Hyperresponsiveness

Animal toxicological studies have demonstrated that NO_2 exposure enhances responsiveness of airways to nonspecific and specific challenges. A subchronic study demonstrated dose-dependent increases in AHR to histamine in NO_2-exposed guinea pigs (U.S. EPA, 2008c; 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 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 hours per day, 5 days per week, 6 weeks) (Kitabatake et al., 1995). However, NO_2 exposure (4,000 ppb, 2 hours per 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). Studies of acute exposures to NO_2 are discussed in Section 4.2.2.2. Mechanisms underlying these responses are discussed in Section 3.2.5.

5.2.9 Toxicological Studies of Host Defense

Decrements in the 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 ranging from characteristics of alveolar macrophages (AMs) to increased infection-induced mortality. Details from these studies, which were reviewed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), are presented in Table 5-4.

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, though 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 one 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 functional effects of NO_2 exposure on AMs isolated from antigen-sensitized baboons exposed to 2,000 ppb NO_2 for 8 hours per day, 5 days per week for 6 months and found that they 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 also play an important role in host defense and inflammatory processes, and 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 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.

Effects of NO_2 on impaired host defense would be expected to contribute to increased susceptibility 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 pneumonia* 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 3 exposed monkeys died 5 days post infection. Ehrlich and Henry (1968) and Ehrlich (1980) also studied the effects of NO_2 on *Klebsiella pneumonia* infection in mice. Exposures were either continuous or intermittent (6 or 18 hours per day) at a concentration of 500 ppb NO_2 and bacterial challenge was done 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.

Taken together, these studies show that animals exposed to concentrations of NO_2 relevant to ambient exposure for periods greater than 6 weeks have modulated lung host

defense ranging from altered AM morphology and function to increased infection-induced mortality.

5.2.10 Toxicological Studies of Respiratory Morphology

While no recent studies are available, the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) 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 5-4. Studies have 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 have 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 2 cell hyperplasia and increases in fibroblasts, alveolar macrophages, and extracellular matrix. In the juvenile rats, effects of exposure were limited to thinning of Type 2 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 hours per day with two 30 minutes 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 hours per day, 5 days per 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 yield morphological changes that could be identified by microscopic techniques. Studies conducted at similar concentrations and duration have 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 2 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 2 cell hyperplasia in rats, and additional injury at 1,000 ppb included loss of cilia in the terminal bronchioles (Yamamoto and Takahashi, 1984). Type 2 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 high ambient levels of NO_2 can result in subtle changes in lung morphology including type 2 cell hyperplasia, loss of cilia in the bronchiolar region, and enlarged airspace.

5.2.11 Gene-Environment Interactions

Several recent studies evaluate long-term NO_2 exposure and health effects and consider the role that genetic variants may play in modifying risk of NO_2-associated respiratory effects. Such discussion provides information related to potentially at-risk populations. One used the indoor cohort study by Belanger et al. (2013) discussed in Section 5.2.5 to examine the association between NO_2 exposure, childhood asthma severity, and levels of methylation in the promoter region of the beta-adrenergic receptor (ADRB2), a target of beta-agonist bronchodilators, and found that higher NO_2 exposure and increased ADRB2

methylation in blood were associated with higher odds of asthma severity in children (Fu et al., 2012a).

Several studies examined interactions between variants in multiple genes and long term outdoor exposure to NO_2, increasing the possibility of finding associations by chance. In a separate analysis of the BAMSE cohort (discussed earlier in Section 5.2.2), Melén et al. (2008) assessed gene–environment interaction on multiple respiratory effects using exposure to traffic NO_X during the first year of life. Among multiple variants of ADRB2, TNF, and GSTP1, effect measure modification was only found for GSTP1 and allergic sensitization and PEF. Associations of these outcomes with NO_X were larger among children with Ile/Val or Val/Val genotypes for codon 105 (encodes enzyme with reduced oxidative metabolism) and Ala/Val or Val/Val genotypes for codon 114 (functional difference unknown). A three-way interaction was found with variants in TNF (inflammatory cytokine), but the odds of sensitization was estimated with large imprecision.

In the CHS (discussed earlier in Section 5.2.1), among the multiple glutathione genes (i.e., GSS, GSR, GCLC, and GCL) investigated, Breton et al. (2011) found that NO_2-associated lung function growth deficits were modified only by variants in GSS haplotype (combination of multiple alleles). Compared with children with other GSS haplotypes, children with the HO0100000 haplotype (48% of study population) had larger NO_2-associated decrement in growth of FEV_1 and MMEF but similar association with FVC growth. These interactions were found for NO_2 after adjusting for O_3, providing evidence for an independent association for NO_2. In the CHS, examination of community NO_2 concentrations as a modifier of associations between TNF-α 308 variants and bronchitic symptoms among children with asthma found no difference between children living in low (concentrations not reported) and high NO_2 communities (Lee et al., 2009). A study of children in Taiwan also examined NO_2 exposure as an effect measure modifier, and found the associations of the EPHX1 His/Arg or Arg/Arg genotype with lifetime asthma, early-onset asthma, and wheeze were larger among children who resided in higher NO_2 communities (Tung et al., 2011). Risk was elevated further if those children also had the GSTP1 Ile/Val or Val/Val genotype (reduced oxidative metabolism activity), but results were based on <1% of the study population. A three-way interaction was not consistently found with GSTM1 genotype variants.

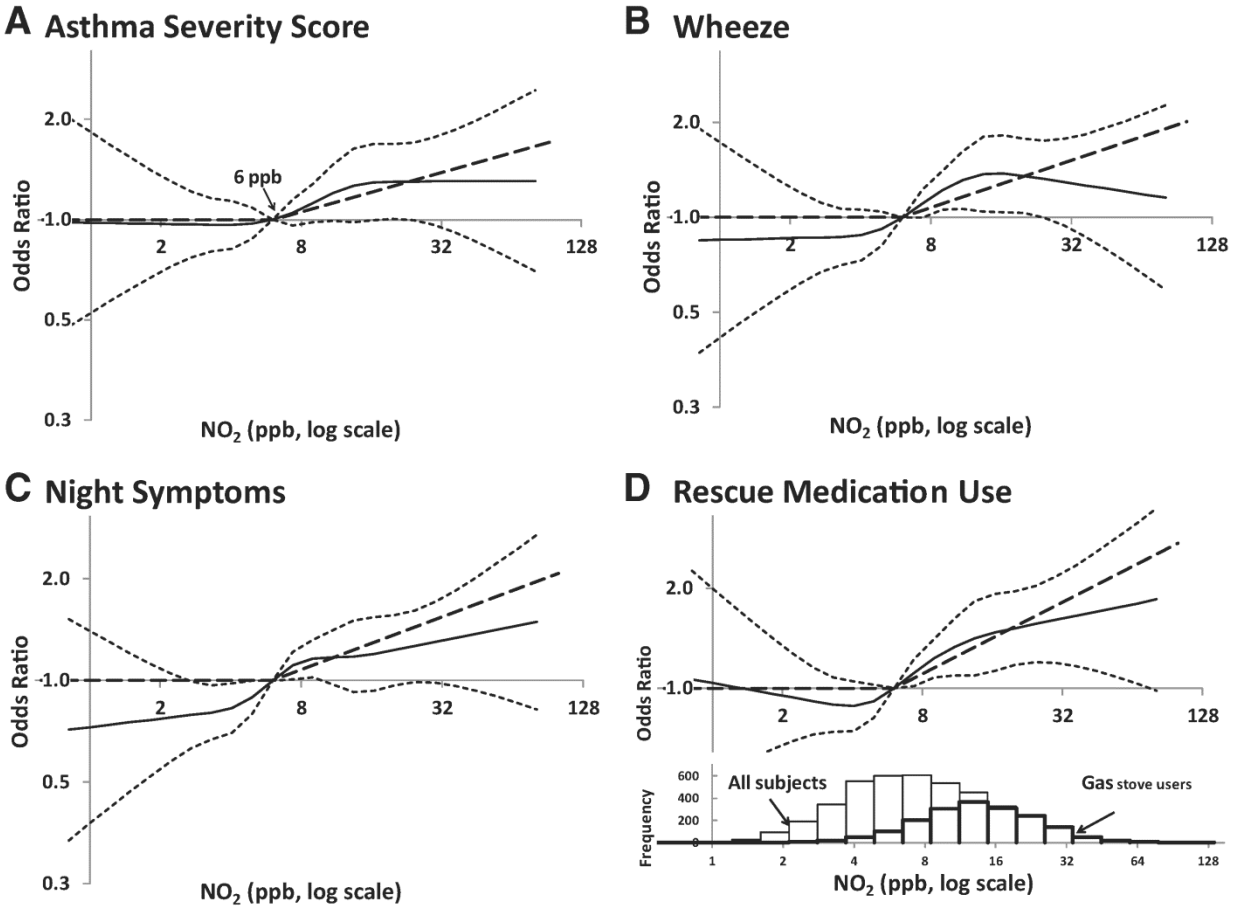
Castro-Giner et al. (2009) (Section 5.2.2.2) examined many variants of the same genes as the aforementioned studies but in adults. Associations between NO_2 and asthma prevalence were not modified by variants in GSTM1, TLR4 (involved in innate immunity), or most of the ADRB loci examined, although one TNF variant and several GSTP1 variants were found to modify the association between NO_2 and asthma

prevalence. In contrast, Melén et al. (2008) did not observe effect measure modification by variants in TNF, GSTP1, or ADRB2 for the association between NO_X and asthma in children. Also in contrast with Melén et al. (2008), a larger NO_2 effect was estimated for the GSTP1 Ile/Ile genotype. Castro-Giner et al. (2009) also found that associations between prevalence of asthma and concurrent NO_2 exposure were limited to adults with the C/C genotypes of various polymorphisms of NQO1, which also encodes an enzyme involved in oxidative metabolism. For example, adults with the C/C genotype for NQO1 rs291766 had a higher odds of asthma (OR: 3.75 [95% CI: 1.32, 10.64] per 10-ppb increase in NO_2) compared with those with CG/GG genotypes (OR: 1.54 [95% CI: 0.70, 3.38]).

Multiple recent studies examined genetic variants in ADRB2, GSTP1, and TNF, and results were not consistent in showing modification of the respiratory effects of long-term NO_2 exposure. A limitation of the collective body of evidence is the potentially increased probability of finding association by chance with multiple comparisons. Several studies examined genetic variants in oxidative metabolism enzymes, particularly those involving glutathione metabolism. For many variants, no interaction was found. However, there were a few observations of effect measure modification of lung function growth in children and asthma in adults by variants in GSS and NQO1.

5.2.12 Concentration-Response

Several studies report information examining the NO_2 exposure-response function. For the Connecticut/Massachusetts Childrens Asthma cohort discussed by Belanger et al. (2013), Figure 5-6 illustrates, for fully adjusted models, the exposure-response relationships between indoor NO_2 and health outcomes 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 examining quintiles of NO_2 exposure, levels >14.3 ppb compared with the reference level ($\leq$ 6 ppb, the threshold value) resulted in an increased risk of a one-level increase in asthma severity score (OR: 1.43 [95% CI: 1.08, 1.88]). Wide CI's were observed. These same exposures were also associated with increased risks of wheeze (1.53 [95% CI: 1.16, 2.02]), night symptoms (1.59 [95% CI: 1.24, 2.01]), and rescue medication use (1.74 [[95% CI: 1.34, 2.26]). In the fully adjusted threshold models, every 5-fold increase in NO_2 exposure >6 ppb was associated with a dose-dependent increase in asthma severity score (1.37 [[95% CI: 1.01, 1.89]) and asthma morbidity measured by wheeze (1.49 [[95% CI: 1.09, 2.03]), night symptoms (1.52 [[95% CI: 1.16, 2.00]), and rescue medication use (1.78 [[95% CI: 1.33, 2.38]).



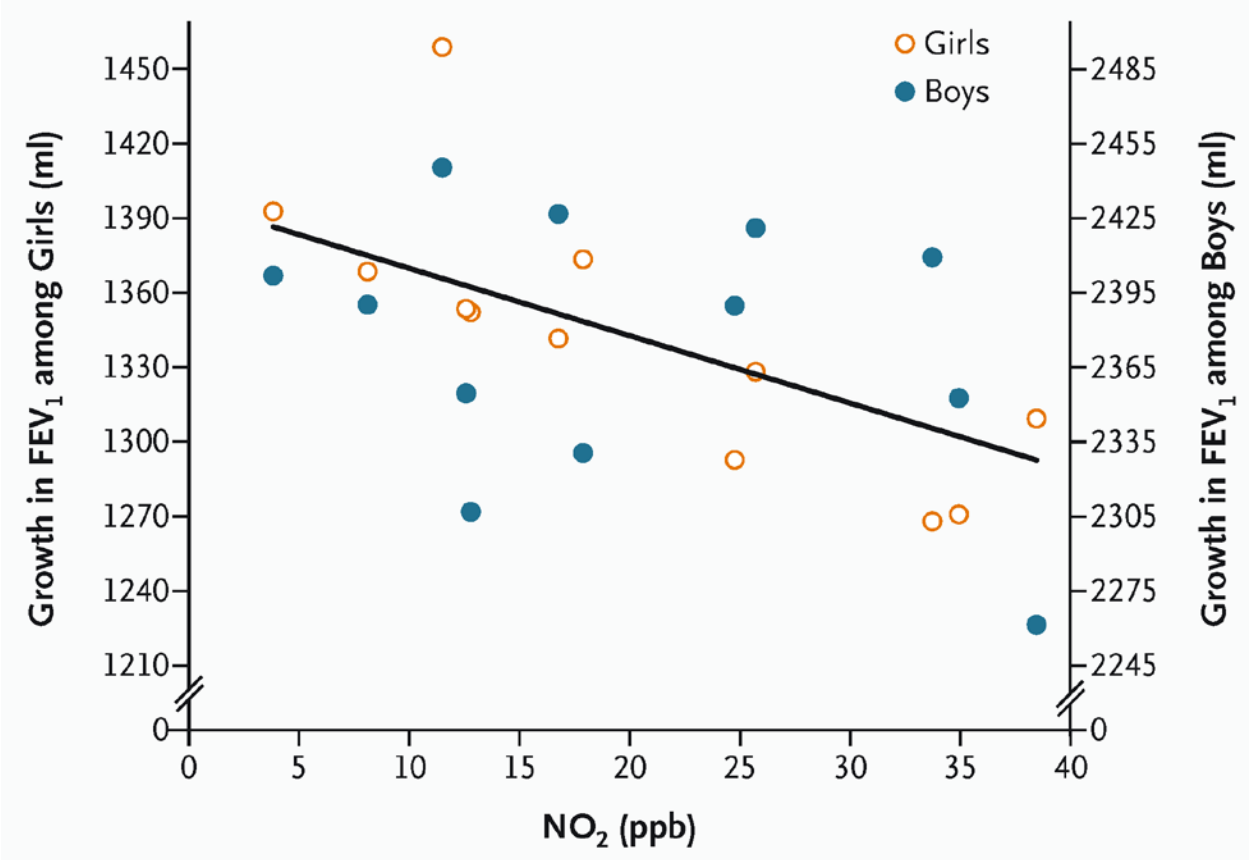
Note: (A) asthma severity score, (B) wheeze, (C) night symptoms, and (D) rescue medication use. Also shown is a histogram of NO_2 levels measured in subjects' homes (lower portion of panel D) for all observations (thin border) and observations taken in homes of gas stove users (bold border).

Source: Reprinted with permission of Wolters Kluwer Health, Belanger et al. (2013).

Figure 5-6 Concentration-response relationships between health outcome and NO_2 (log concentration as a continuous variable) illustrated with constrained, natural spline functions (solid lines) with 95% confidence limits (small dashed lines) and threshold function (bold dashed line) from fully adjusted, hierarchical ordered logistic regression models.

Gauderman et al. (2004) discussed in Section 5.2.1 states that although the average growth in FEV_1 was larger in boys than in girls, the correlations of growth with air pollution did not differ significantly between the sexes, as shown for NO_2 in Figure 5-7. The sex-averaged analysis, depicted by the regression line in the figure, demonstrated a significant negative correlation between the growth in FEV_1 over the eight-year period and the average NO_2 level ($p = 0.005$). The estimated difference in the average growth in

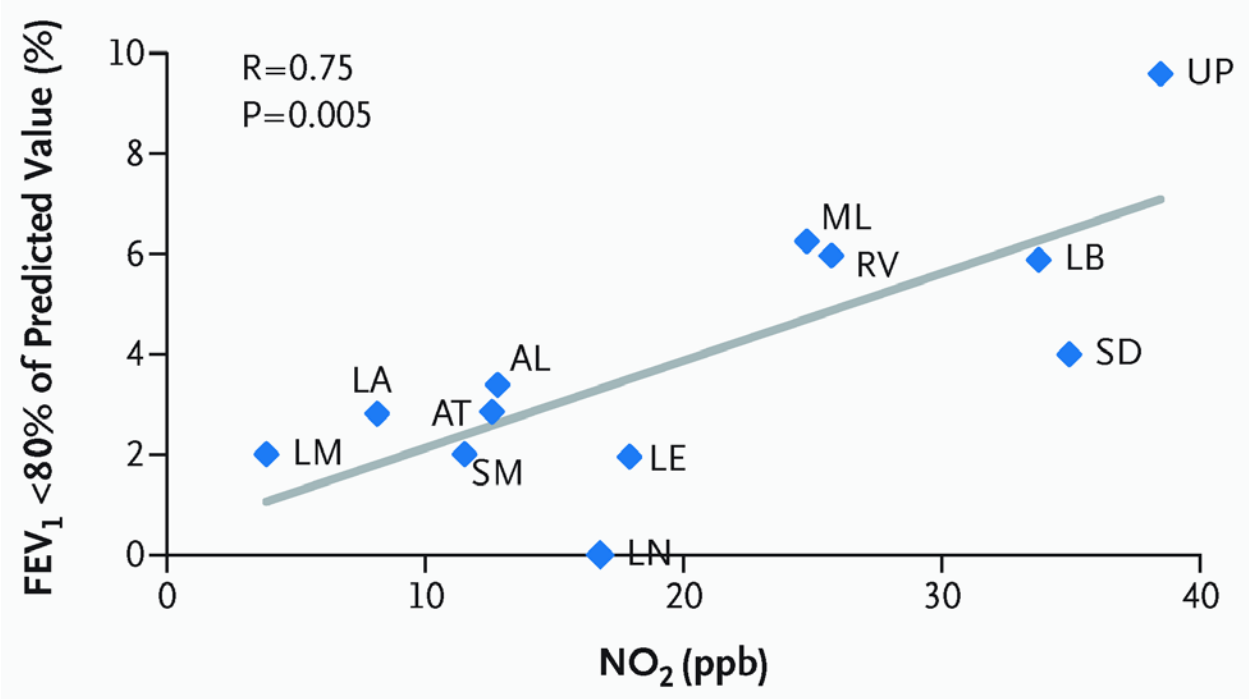
FEV_1 over the eight-year period from the community with the lowest NO_2 level to the community with the highest NO_2 level, represented by the slope of the plotted regression line in the Figure 5-7, was -101.4 mL.



Source: Reprinted with permission of the Massachusetts Medical Society, Gauderman et al. (2004).

Figure 5-7 Community-specific average growth in FEV_1 (mL) among girls and boys during the eight-year period from 1993 to 2001, plotted against average NO_2 levels from 1994 through 2000.

Gauderman et al. (2004) further observed that pollution-related deficits in the average growth in lung function over the eight-year period resulted in clinically important deficits in attained lung function at the age of 18 years (Figure 5-8). Across the 12 communities, a clinically low FEV_1 was positively correlated (r = 0.75) with the level of exposure to NO_2.



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.

Source: Reprinted with permission of the Massachusetts Medical Society, Gauderman et al. (2004).

Figure 5-8 Community-specific proportion of 18-year-olds with a FEV_1 below 80 percent of the predicted value, plotted against the average levels of NO_2 from 1994 through 2000.

Additional studies that demonstrate associations between long-term exposure to NO_2 and respiratory health outcomes have generally observed a linear relationship in the range of ambient NO_2 concentrations examined (Andersen et al., 2012; Lee et al., 2012c; Carlsten et al., 2011c; Modig et al., 2009; Islam et al., 2007; Rojas-Martinez et al., 2007a, b; Shima et al., 2002). Most of these studies did not conduct analyses to evaluate whether there is a threshold for effects.

5.2.13 Analysis of copollutants

Various studies provide information that informs the concept of copollutant aspects of effects that may be attributed to various NO_2 measures. Table 5-5 presents this information for the copollutants PM, O_3, SO_2, and CO showing the results of two pollutant models and those with three or more. Generally, these studies reported that

estimates from two-pollutant models were not substantially different from the estimates from models that just included NO_2 (Lee et al., 2012c; Rojas-Martinez et al., 2007a, b), (Hwang and Lee, 2010; Hansel et al., 2008; Hwang et al., 2005; McConnell et al., 2003).

Table 5-5 Studies that provide evidence for NO_2 and also provide analysis of copollutants (PM, O_3, SO_2, CO).

Study Design Health Effect Endpoint	Increament of NO_2 Exposure Examined	NO_2 single pollutant effect estimate	NO_2 with other pollutant effect estimate
Rojas-Martinez et al. (2007a) Prospective Pulmonary Function	10 ppb	**FEV_1, FVC (in mL)** **NO_2:** Girls FVC -1.26 (-1.51, -1.01) *** FEV_1 -0.70 (-0.96, -0.44) *** Boys FVC -1.18 (-1.42, -0.94) *** FEV_1 -0.56 (-0.81, -0.32) ***	**FEV_1, FVC-mL** **NO_2 and O_3:** Girls FVC -1.20 (-1.46, -0.94) *** FEV_1 -0.81 (-1.07, -0.54) *** Boys FVC -1.16 (-1.41, -0.91) *** FEV_1 -0.75 (-1.01, -0.50) *** **NO_2 and O_3 and PM_{10}:** Girls FVC -1.05 (-1.32, -0.77) *** FEV_1 -0.71 (-1.00, -0.42) *** Boys FVC -1.09 (-1.36, -0.82) *** FEV_1 -0.64 (-0.92, -0.37) ***

Table 5-5(Continued): Studies that provide evidence for NO_2 and also provide analysis of copollutants (PM, O_3, SO_2, CO).

Study Design Health Effect Endpoint	Increament of NO_2 Exposure Examined	NO_2 single pollutant effect estimate	NO_2 with other pollutant effect estimate
McConnell et al. (2003) Prospective Bronchitic symptoms in asthmatics in CHS OR	1 ppb	Within Communities **NO_2:** 1.07 (1.02, 1.13)**	Within Communities **NO_2 and O_3:** 1.06 NS **NO_2 and PM_{10}:** 1.07* **NO_2 and $PM_{2.5}$:** 1.05NS **NO_2 and $PM_{10-2.5}$:** 1.08** **NO_2 and inorganic:** 1.09** **NO_2 and organic:** 1.07* **NO_2 and EC:** 1.05* **NO_2 and OC:** 1.04 NS
		Between Communities **NO_2:** 1.02 (1.00, 1.03)*	Between Communities **NO_2 and O_3:** 1.02* **NO_2 and PM_{10}:** 1.01 NS **NO_2 and $PM_{2.5}$:**1.01 NS **NO_2 and $PM_{10-25.}$:** 1.02* **NO_2 and inorganic:** 1.02NS **NO_2 and organic:** 1.02 NS **NO_2 and EC:** 1.01 NS **NO_2 and OC:** 1.01 NS
Dong et al. (2011) Cross-sectional Respiratory Symptoms OR	5.3 ppb	**NO_2:** Without allergic predisposition **Persistent cough** Males 1.28 (1.16–1.41) Females 1.21 (1.09–1.33) **Persistent Phlegm** Males 1.16 (1.02–1.33) Females 1.13 (0.98–1.30) **Doctor-diagnosed asthma** Males 1.19 (1.06–1.34) Females 1.14 (0.99–1.30)	**NO_2 and PM_{10}, SO_2, O_3, CO:** **Persistent cough** Males 1.36 (1.19–1.56) Females 1.30 (1.13–1.50) **Persistent Phlegm** Males 1.26 (1.05–1.51) Females 1.15 (0.94–1.42) **Doctor-diagnosed asthma** Males 0.97 (0.80–1.16) Females 0.97 (0.74–1.26)

Table 5-5(Continued): Studies that provide evidence for NO_2 and also provide analysis of copollutants (PM, O_3, SO_2, CO).

Study Design Health Effect Endpoint	Increament of NO_2 Exposure Examined	NO_2 single pollutant effect estimate	NO_2 with other pollutant effect estimate
Hwang and Lee (2010) Cross-sectional Bronchitic Symptoms in children with asthma OR	8.79 ppb	**NO_2:** Bronchitis 1.83 (1.07, 3.14) Chronic Phlegm 1.52 (0.83, 2.78) Chronic Cough 1.12 (0.53, 2.4) Bronchitic symptoms 1.81 (1.14, 2.86)	**NO_2 and SO_2:** Bronchitis 1.99 (1.02, 3.87) Chronic Phlegm 1.27 (0.59, 2.74) Chronic cough 1.25 (0.49, 3.23) Bronchitic symptoms 1.76 (0.99, 3.14) **NO_2 and $PM_{2.5}$:** Bronchitis 2.04 (1.15, 3.63) Chronic Phlegm 1.51 (0.79, 2.89) Chronic cough 1.26 (0.57, 2.80) Bronchitic symptoms 2.01 (1.20, 3.36) **NO_2 and O_3:** Bronchitis 1.81 (1.06, 3.10) Chronic Phlegm 1.49 (0.81, 2.73) Chronic cough 1.10 (0.58, 2.07) Bronchitic symptoms 1.79 (1.12, 2.85)
Hwang et al. (2005) Cross-sectional Physician-diagnosed asthma OR	10 ppb	**NO_2:** 1.005 (0.954, 1.060)	**NO_2 and SO_2:** 1.048 (0.983, 1.117) **NO_2 and PM_{10}:** 1.065 (1.009, 1.123) **NO_2 and O_3:** 1.029 (0.973, 1.089) **NO_2, SO_2, and O_3:** 1.113 (1.038, 1.194) **NO_2, PM_{10} and O_3:** 1.152 (1.082, 1.227)

Table 5-5(Continued): Studies that provide evidence for NO_2 and also provide analysis of copollutants (PM, O_3, SO_2, CO).

Study Design Health Effect Endpoint	Increament of NO_2 Exposure Examined	NO_2 single pollutant effect estimate	NO_2 with other pollutant effect estimate
Lee et al. (2012c) Prospective Incident bronchitis/Pulmonary function differences	Two NO_2 strata defined as less than and greater than the median level of 17.5 ppb	Incidence rate ratio of bronchitis was 0.56 (95% CI: 0.49, 0.65) in the lower NO_2 communities, whereas the effect was 1.10 (95% CI: 0.96, 1.24) in the higher NO_2 communities (p for interaction = 0.005).	Found no statistical significant differences on the effects of pulmonary function indices for incident bronchitis in relation to exposure to the other air pollutants in TCHS ($PM_{2.5}$, PM_{10} and 8-h O_3).
Hansel et al. (2008) Prospective Asthma symptoms	20 ppb	In example Cough without cold: OR 1.10 (95% CI: 1.02, 1.18)**	Adjusting for other copollutants did not meaningfully alter the association between indoor NO_2 concentrations and asthma symptoms.

***p <0.0001,
**p <0.01,
*p <0.05,
NS p >0.05).

5.2.14 Mixtures: Traffic-related Pollutants

Studies that inform measures of NO_2 and estimates for traffic related pollutant are discussed next and are presented in Table 5-6. Several studies conducted in California evaluated the effects of exposure to NO_2 and an indicator of traffic related pollution on respiratory health effects. Each of the studies observed an independent effect between NO_2 and respiratory health effects; however it was not always simple to disentangle this effect from that observed for traffic related pollution. For example, McConnell et al. (2010) observed an association between ambient NO_2 measured at a central site and new-onset asthma. In models with both NO_2 and modeled traffic exposures, there were independent associations of asthma with traffic-related pollution (TRP) at school and home, whereas the estimate for NO_2 was attenuated. Associations with asthma were positive for TRP exposure estimates modeled from local non-freeway roadway proximity, traffic volume, and meteorology. There was little evidence for an effect of major roadway proximity alone, for traffic density, or for pollution from freeways. An important distinction between the TRP and simpler traffic metrics is the inclusion of meteorology (average annual wind speed and direction and height of the mixing layer) in addition to proximity and volume. The contribution that NO_2 may or may not make to TRP measures directly or indirectly is not clear. The modeled TRP may reflect the mixture of multiple pollutants from nearby traffic, and the high correlation of pollutants in the mixture may preclude identifying the effect of any specific pollutant in the mixture

as a causative agent. Similarly, Gauderman et al. (2007) noted that reduced lung-function growth was independently associated with both freeway distance and with regional air pollution. Positive associations were observed in joint models of regional pollution with distance to freeway and NO_2, acid vapor, EC, and PM_{10} and $PM_{2.5}$. Ozone was not associated with reduced lung-function growth. There was no evidence of effect modification (interaction) of local traffic effects with any of the regional pollutants. In a cross-sectional analysis of residential traffic and children's respiratory health (current asthma) in San Francisco, Kim et al. (2008) found that when outdoor school-based NO concentration was added to multivariate models containing residential-based traffic, the effect estimate for residential traffic was mildly attenuated. Effect estimates for residential traffic were essentially unchanged with the addition of NO_2, PM_{10}, or $PM_{2.5}$. Traffic density and maximum annual average daily traffic (AADT) were correlated with pollutants and explained between 35% and 60% of the variability in NO_X and NO. The traffic metrics used in these studies are surrogates for a complex mixture of traffic pollutants composed of reactive gases and PM, not just NO_X. Many constituents of traffic exhaust may contribute to toxicity.

Table 5-6 Studies reporting NO_2 results and results for traffic measures.

Study Design; Health effect	NO_2 Exposure	NO_2 Single pollutant Effect Estimate; Other TRP Measures	NO_2 and Indicator of Traffic
McConnell et al. (2010) Prospective Incident Asthma	Range of exposure over the 13 communities for central site NO_2 23.6 ppb	Single NO_2 HR 2.17 (1.18, 4.00) Other TRP measures: Non-freeway TRP Combined Home and School 1.61 (1.29, 2.00) Freeway TRP combined Home and School 1.12 (0.94, 1.35) Total TRP Combined 1.34 (1.07, 1.68) Traffic Density Combined 1.09 (0.99, 1.19) Distance to Major Road Combined 0.85 (0.68, 1.07) Distance to Freeway Combined 0.89 (0.76, 1.05)	NO_2 adjusted for nonfreeway TRP at Home and School HR 1.32 (0.69, 2.71) HR for NO_2 adjusted for Total TRP at Home and School 1.79 (0.91, 3.52)
Gauderman et al. (2007) Prospective Pulmonary function	Range of 12 community means 34.6 ppb	FEV_1 growth -109 mL p 0.003	FEV_1 Growth Local freeway distance (in meters) <500: -80 p 0.012 500-1000: -41 (p = 0.166) 1000-1500: -33 (p= 0.279)
Kim et al. (2008) Cross-sectional Current asthma and Bronchitis	Quantitative data not reported.	No direct NO_2 or NO_X effect reported	When school-based concentration of NO was added to multivariate models containing residential-based traffic, the effect estimate for residential traffic was mildly attenuated. School-based NO had borderline significance in the models (p <0.12). Effect estimates for residential traffic were essentially unchanged with the addition of the school pollutant NO_2, PM_{10}, or $PM_{2.5}$.

5.2.15 Indoor Studies

In the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), the intervention study by Pilotto et al. (2004) found that exposure to NO_2 from an indoor combustion source is associated with respiratory effects. In this study NO_2 effects would not be confounded by other motor vehicle emission pollutants, though potential confounding by other pollutants from gas stove emissions, such as UFP, could occur. This was an important study that helped reduce the uncertainty for NO_2 providing direct health effects. The two recent indoor studies (Belanger et al., 2013; Hansel et al., 2008) discussed in Section 5.2.5.1 provide evidence that supports this notion in that they are not the same air mixtures as in the ambient air and indicate strong positive relationships for long-term NO_2 exposure and respiratory symptoms in asthmatic children.

5.2.16 Surrogate for ambient NO_2 or other pollutants as a mixture

The above discussion of studies of potential health effects related to measures of ambient NO_2 used various methods to estimate levels of NO_2: (1) Palmes tubes outside the homes of the study subjects; (2) community pollutant monitoring cites; (3) data from indoor monitoring and activity patterns; and (4) various land use regression models most with an element of validation of the model. It is with this backdrop that we discuss NO_2 as an independent agent related to health effects observed or as discussed next a surrogate for a mixture or other role related to the observed health effects.

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) concluded that "The errors and uncertainties associated with the use of ambient NO_2 concentrations as a surrogate for personal exposure to ambient NO_2 generally tend to reduce rather than increase effect estimates, and therefore are not expected to change the principal conclusions from NO_2 epidemiologic studies." and further concluded that "It is difficult to determine from these new studies the extent to which NO_2 is independently associated with respiratory effects or if NO_2 is a marker for the effects of another traffic-related pollutant or mix of pollutants".

In evaluating the potential relationships between long-term exposure to NO_2 and respiratory effects, it is important to note the interrelationships between NO_2 and other pollutants, and the potential for NO_2 to serve as a marker for a pollutant mixture, particularly traffic-related pollution. This includes consideration of potential pathways, such as the direct causal pathway for effects, mediation of effects, the pollutant acting as a surrogate for a pollutant mixture, mixtures that share the same source (e.g., motor vehicles, electricity generation), or confounding between pollutants. As observed above,

associations with NO_2 were often robust to adjustment for traffic-related pollutants (e.g., PM and CO),

Although this complicates the efforts to disentangle specific long-term NO_2-related health effects, the evidence summarized in this assessment indicates that NO_2 associations generally remain robust in multipollutant models as discussed above in Section 5.2.13 which supports a direct effect of long-term NO_2 exposure on respiratory morbidity at ambient concentrations. The robustness of epidemiologic findings to adjustment for copollutants, coupled with limited data from animal and human experimental studies, inform the strength of evidence for a relationship between NO_2 and respiratory morbidity. In addition, the short-term exposure intervention study of indoor NO_2 exposures by Pilotto et al. (2004) discussed in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) found that exposure to NO_2 from indoor combustion sources were associated with respiratory effects. In Pilotto et al. (2004) NO_2 effects would not be confounded by other motor vehicle emission pollutants, though potential confounding by other pollutants from gas stove emissions, such as UFP, could hypothetically occur but data is limited in support of this notion. Support for long-term NO_2 exposure effects of indoor NO_2 are provided by the prospective studies Hansel et al. (2008) and Belanger et al. (2013), which demonstrate respiratory morbidity effects related to long-term NO_2 exposure which are unlikely to be confounded by other motor vehicle emission pollutants. Hansel et al. (2008) noted that adjusting for copollutants did not alter the relationship for indoor NO_2 and respiratory symptoms and Belanger et al. (2013) reported concentration response data.

Human clinical and toxicological study findings also provide support for independent effects of NO_2 on respiratory health. Limited short-term exposure evidence from human clinical studies indicated that NO_2 may increase susceptibility to injury by subsequent viral challenge; toxicological studies show that lung host defenses are sensitive to NO_2 exposure. The epidemiologic and experimental evidence together show coherence for effects of NO_2 exposure on host defense or immune system effects providing plausibility and mechanistic support for respiratory morbidity.

In regard to the question on surrogates, the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) evaluated the available evidence base. Recently, Meng et al. (2012b) further inform this question and note that it is necessary to examine personal-ambient associations of NO_2 in a multipollutant environment. The issue raised in the epidemiologic studies is whether ambient NO_2 is a surrogate of personal exposure to ambient NO_2 or a surrogate of personal exposure to other ambient pollutants, such as fine particles ($PM_{2.5}$). Meng et al. (2012b) conducted a quantitative research synthesis on studies of the associations between personal exposures and ambient concentrations of

NO_2 reported in peer-reviewed publications. Random-effects meta-analysis was conducted to estimate the strength of the associations between personal exposures and ambient concentrations of NO_2 across the studies. Ambient NO_2 was found to be significantly associated with personal NO_2 exposures, with overall correlation coefficient estimates of 0.42, 0.16, and 0.72 for pooled, longitudinal, and daily average correlation coefficients across studies. This conclusion was robust to correction for publication bias. Random effects meta-regressions were also conducted to examine factors affecting the heterogeneity in the reported correlation coefficients across studies. They reported that personal-ambient associations of NO_2 depend on various factors, including season, age of the study population, pre-existing disease, and possibly indoor and local sources and sampling aspects. The dependence of the personal-ambient associations on these factors complicates the interpretation of ambient NO_2 as a surrogate of personal NO_2 exposure of ambient origin. Ambient NO_2 might be a good surrogate for personal NO_2 exposure of ambient origin for some subpopulations but not for others, even though the collective evidence suggests that ambient NO_2 is a good surrogate for personal exposure of ambient origin. Furthermore, measured personal exposures could be influenced by sampling artifacts. More accurate real-time exposure measurements will help improve the interpretation of personal-ambient associations. Caution needs to be exercised when comparing personal ambient associations obtained with different study designs. It should be noted that the number of studies in this analysis was relatively small. More meaningful and rigorous comparisons would be possible if greater detail were published on study design and data quality.

The HEI (2010) review of traffic-related pollutants viewed the alternative hypothesis surrogate issue from a different perspective: surrogates for TRP. They noted that none of the surrogates considered met all the criteria for an ideal surrogate. Data are not available on the ratios of the surrogates to the complex pollutant mixtures emitted by traffic and how these ratios have varied over time. CO, benzene, and NO_X (in this case NO_2), found in on-road vehicle emissions, are components of emissions from all sources. All also have significant ambient and microenvironmental sources, making it difficult to disentangle the contributions from motor vehicles. Primary on-road emissions of PM represent a small contribution to emissions from all sources. The quality of the surrogates (i.e., their degree of association with “true” traffic exposure) therefore depends very much on the understanding of the contributions from other sources. Thus in the discussion of NO_2 representing various mixtures here, a surrogate, one should be cautious in considering the ideal surrogate that may represent the alternative hypothesis under consideration.

5.2.17 Summary and Causal Determination

Evidence indicates that there is likely to be a causal relationship between long-term NO_2 exposure and respiratory effects based on multiple lines of evidence indicating increases in asthma incidence in children, decrements in lung function, and partially irreversible decrements in lung function growth in children. There is supporting evidence for increases in respiratory symptoms in children with asthma, increases in asthma incidence in adults. Evidence from toxicological studies provide biological plausibility for the associations observed between long-term exposure to NO_2 and asthma incidence and demonstrate impaired lung host defense and increased infection mortality. This conclusion 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, 2008c). Consistent with previous findings, recent epidemiologic results continue to support associations between increases in ambient NO_2 concentrations and pulmonary function decrements. The recent epidemiological evidence base evaluating long-term NO_2 exposure and asthma incidence in children now includes several prospective longitudinal studies examining asthma incidence. Incontrast, the 2008 ISA for Oxides of Nitrogen which had a limited number of cross-sectional studies available to consider. The key uncertainty identified in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) (related to the high correlations among traffic-related pollutants which made it difficult to accurately estimate the independent effects of long-term NO_2 exposures) was the potential for NO_2 to serve as mainly an indicator for another combustion-related pollutant or mixture. This uncertainty is informed with recent studies, but remains as a key uncertainty. The evidence for respiratory effects with respect to likely to be a causal relationship with long-term NO_2 exposure is detailed below using the framework described in Table II of the Preamble to this ISA. The key evidence, supporting or contradicting, as it relates to the causal framework is presented in Table 5-9.

The strongest evidence is provided by recent studies of asthma incidence in children where previous evidence was inconsistent. Multiple longitudinal, prospective studies (Table 5-9) have demonstrated 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 have been replicated by investigators in different locations using various study designs and cohorts (Gruzieva et al., 2013; Nishimura et al., 2013a; Lee et al., 2012c; Carlsten et al., 2011c; Clark et al., 2010; Gehring et al., 2010; Mcconnell et al., 2010; Oftedal et al., 2009; Jerrett et al., 2008; Clougherty et al., 2007; Shima et al., 2002).

Another line of evidence supporting NO_2-related respiratory effects is multiple, high-quality longitudinal studies finding associations between long-term NO_2 exposure and

decrements in lung function and partially irreversible decrements in lung function growth in children which has added additional longitudinal prospective studies since the 2008 ISA (Figure 5-5) (Schultz et al., 2012; Breton et al., 2011; Oftedal et al., 2008; Rojas-Martinez et al., 2007a; Gauderman et al., 2004). Some studies found an NO_2 concentration-dependent decrement in lung function and lung function growth (Rojas-Martinez et al., 2007a; Gauderman et al., 2004). The toxicological evidence regarding NO_2 -induced changes in lung function and growth is limited and thus does not provide clear biological plausibility for epidemiologic observations. A few studies demonstrated effects of long-term NO_2 exposure on lung function in rats exposed to a base 500-2,000 ppb for 6-78 weeks (Tepper et al., 1993; Lafuma et al., 1987), and slight decrements in lung function resulting from short-term exposure were found to resolve with continued NO_2 exposure (1,000-3,000 ppb for 6 weeks) (Stevens et al., 1988). A number of studies have demonstrated that long-term exposure to NO_2 alters lung morphology in experimental animals, though these changes do not appear to contribute to altered lung function (Hayashi et al., 1987; Kubota et al., 1987). Additionally, these effects were not clearly demonstrated in juvenile animals (Chang et al., 1986; Furiosi et al., 1973).

These observations are supported by evidence from several longitudinal studies consistently demonstrating increases in respiratory symptoms in children with asthma with increasing ambient NO_2 concentrations (Table 5-3) (Belanger et al., 2013; Gruzieva et al., 2013; Gehring et al., 2010; Hansel et al., 2008; McConnell et al., 2003). Also supporting a relationship between long-term NO_2 exposure and respiratory effects is new evidence from several multicity studies demonstrating increases in asthma incidence in adults (Table 5-8) (Castro-Giner et al., 2009; Jacquemin et al., 2009a; Jacquemin et al., 2009b; Modig et al., 2009; Sunyer et al., 2006).

Evidence for asthma incidence, pulmonary function, and respiratory symptoms in children is substantiated by the prospective design of studies, which better characterizes the directionality between exposure and development of respiratory morbidity. Also, associations were found with adjustment for several well-characterized potential confounding factors such as SES, smoking exposure, housing characteristics, and meteorological conditions. In addition, studies characterized the concentration-response for the relationship between NO_2 and respiratory outcomes, generally observed a linear relationship (Andersen et al., 2012; Carlsten et al., 2011c; Modig et al., 2009; Islam et al., 2007; Rojas-Martinez et al., 2007a, b; Shima et al., 2002). Evidence for an increase in respiratory symptoms related to NO_2 indoors and the related indoor mixture is provided by (Belanger et al., 2013). These observations for long-term exposure are supported by evidence for short-term NO_2 increases in exposure increasing pulmonary inflammation and respiratory symptoms in children in the general population and increasing respiratory symptoms in children with asthma (Section 4.2). Recent meta-analysis of asthma

incidence also informs the evidence base (Anderson et al., 2013; Gasana et al., 2012; Gowers et al., 2012; Takenoue et al., 2012; Bråbäck and Forsberg, 2009).

Evidence from toxicological studies provides biological plausibility for the associations observed between long-term exposure to NO_2 and asthma incidence. Increases in AHR were reported following 6-12 weeks of exposure to NO_2 (1,000-4,000 ppb) (Kobayashi and Miura, 1995). Studies of short-term exposure and long-term exposure provide evidence of key events to inform the mode of action for development of asthma, including oxidative and nitrative stress, altered regulation of inflammation (Th2 cytokines), airway remodeling, and enhanced allergic sensitization (Sections 3.3.2 and 5.2.6). Associations between long-term NO_2 exposure and allergic sensitization in children and adults have been described (Section 5.2.6.1). There is additional evidence indicating oxidative stress may underlie the observed associations between long-term ambient NO_2 exposure and asthma incidence as some studies have found that individuals with variant genotypes for enzymes with antioxidant activity (i.e., NQO1, EPHX), are at greater risk for asthma incidence and symptoms. Long-term exposure of rodents to ambient-relevant concentrations of NO_2 resulted in increased lipid peroxidation in lung tissue and exhaled ethane (Kumae and Arakawa, 2006), though only slight, transient effects of NO_2 on antioxidant enzymes were observed (Sagai et al., 1984; Gregory et al., 1983).

The strongest evidence of effects of long-term NO_2 exposure (500-2,000 ppb for 1 month up to 1 year) in toxicological studies demonstrates impaired lung host defense and increased infection mortality. Alterations to alveolar macrophage function and morphology have been reported (Gregory et al., 1983; Aranyi et al., 1976) and increases in mortality following bacterial challenge have been documented in rodents and squirrel monkeys exposed to NO_2 compared to air controls (Miller et al., 1987; Henry et al., 1970). These findings provide support for the limited available epidemiologic evidence showing increases in respiratory infections, respiratory hospital admissions, or mortality in association with long-term NO_2 exposure. Neupane et al. (2010) demonstrated that NO_2 was associated with increases in community-acquired pneumonia using 3 different models to estimate annual NO_2 exposure. Although the models included covariates, they did not adjust for short-term NO_2 exposure. In addition, there is evidence for associations of long-term ambient NO_2 exposure and respiratory mortality as discussed in Section 5.5.2.

Several lines of epidemiologic evidence support a relationship between long-term ambient NO_2 exposure and increased asthma incidence and respiratory symptoms, and decrements in lung function growth in children. However, uncertainty remains from limited supporting toxicological evidence to provide biological plausibility and the

possibility that the observed effects could result from exposure to other pollutants or a mixture of NO_2 and other pollutants. Rojas-Martinez et al. (2007a) and McConnell et al. (2003) found a robust relationship for NO_2 with decrements in pulmonary function and bronchitic symptoms, respectively, with adjustment for O_3, PM_{10}, $PM_{2.5}$, or EC. However, analysis of copollutant models is limited. Several studies show associations with copollutants such as $PM_{2.5}$, CO, BC, and SO_2 , which tend to show high correlations with NO_2. Studies providing results for pollutants other than NO_2 show similar results for the other pollutants or in the case of Nishimura et al. (2013a) and McConnell et al. (2010) show NO_2 effects that are larger than those for the other pollutants.

Taken together, the recent epidemiologic studies of asthma incidence, decrements in lung function growth, increased respiratory symptoms, and toxicological studies of lung host defense and increased susceptibility to respiratory infections provide evidence that there is likely to be a causal relationship between long-term NO_2 exposure and respiratory effects (Table 5-9). The strongest evidence is provided by studies that demonstrated increases in asthma incidence with NO_2 exposure in children and decrements in pulmonary function in children. Supporting evidence is provided by evidence of asthma incidence in adults and increases in respiratory symptoms in children with asthma. Biological plausibility for epidemiologic evidence is provided by toxicological evidence for development of AHR and Th2 phenotype. Several studies characterize a linear relationship. The majority of studies adjust for potential confounding by SES, smoking exposure, housing characteristics, and meteorological conditions. While studies show associations with NO_2 to be robust with adjustment for O_3, PM_{10}, $PM_{2.5}$, or EC, analysis of potential copollutant confounding is limited, and NO_2 is often highly correlated with copollutants. Based on this small group of studies and limited evidence from experimental studies to provide biological plausibility, it is difficult to determine the extent to which long-term NO_2 exposure is independently associated with respiratory effects or if NO_2 is a marker for the effects of another traffic-related pollutant or mix of pollutants. Overall, the evidence for asthma incidence, respiratory symptoms, and decrements in lung function and lung function growth and epidemiologic and toxicological evidence for impaired host defense but some uncertainty regarding the independent effects of ambient NO_2 exposure is sufficient to conclude that there is likely to be a causal relationship between long-term NO_2 exposure and respiratory effects.

Table 5-7 Annual ambient NO_2 concentrations in prospective studies examining relationships in children with respiratory health effects in children.

Study-Cohort or Location	Annual Mean NO_2 Concentration, and/or Range, and/or IQR (ppb)
Asthma Incidence	
Jerrett et al. (2008) CHS	Within community IQR 6.2 Between community IQR 28.9 Annual mean range across communities 9.6 to 51.3
McConnell et al. (2010) CHS	Mean 20.4 Range 23.6
Gehring et al. (2010) PIMA/ the Netherlands	Mean 13.5 Range 6.7 to 31.1 IQR 5.53*
Carlsten et al. (2011c) Vancouver High Risk Birth Cohort	Mean (SD) 17.3 (3.1)*
Lee et al. (2012c) TCHS	High Mean 22.1 Low mean 14.0 Approximate overall range 10 to 25
Gruzieva et al. (2013) BAMSE	Mean NO_2 decrease over time from 11.4 to 4.1 5th to 95th% 24.9*
Oftedal et al. (2009) Oslo Norway cohort	1st year of Life mean 20.9 Min/Max 0.8 to 44.7 IQR decrease over time from 155.5 to 10.4*
Shima et al. (2002)	Mean range over study communities 7.3 to 31.4
Clark et al. (2010)	First year: 15.9 (2.9) ppb; 25% to 75%: 13.9 to 17.6 ppb
Clougherty et al. (2007)	Full cohort; 14.6 (2.3) ppb
Nishimura et al. (2013a)	Mean range across all communities 9.9 to 32.1 ppb; All 19.3 (8.0) ppb
Pulmonary Function	
Breton et al. (2011) CHS	Range 33.9 Over approximately 3 to 40
Gauderman et al. (2004) CHS	Range 34.6
Rojas-Martinez et al. (2007a) Mexico City School children	Mean (SD) range across communities 27.2 (10.9) to 42.6 (13.2) Overall mean 34.4 IQR 12

Table 5-7 (Continued): Annual ambient NO_2 concentrations in prospective studies examining relationships in children with respiratory health effects in children.

Study-Cohort or Location	Annual Mean NO_2 Concentration, and/or Range, and/or IQR (ppb)
Schultz et al. (2012) BAMSE	5th to 95th percentile: 25*
Mölter et al. (2013)	Range 9.0 to 11.7
Oftedal et al. (2009) Oslo Norway Cohort	Mean 1st year life 20.8 Mean lifetime 15.4 Annual mean 14.4 IQR 14.6*
Respiratory Symptoms	
McConnell et al. (2003) CHS	Between Communities Mean(SD) 19.4 (11.3) Range 4.2 to 38.0 Within Communities Mean (SD) 4.9 (4.0) Range 1.1 to 12.8
Gehring et al. (2010) PIMA	Annual mean 13.5 Range 6.7 to 31.0 IQR 5.53*
Gruzieva et al. (2013) BAMSE	5 to 95 % 24.9 Mean decrease 11.4 to 4.1*
Hansel et al. (2008) African-American Baltimore School	Indoor mean (SD) 30.0 (33.7) Range 2.9 to 394.0 Study mean 25.7
Belanger et al. (2013) New England Indoor Children's Asthma Study	Indoor mean 10.6 (SD = 9.4) ppb IQR 4.5 – 12.5 ppb

*Estimated using land use regression.

Table 5-8 Annual ambient NO_2 concentrations prospective studies examining relationships with respiratory health effects in adults.

Study Health Endpoint Cohort or Location	Annual Mean NO_2 Concentration, and/or Range, and /or IQR (ppb)
Jacquemin et al. (2009b) Asthma incidence ECRHS Europe	Medians by Center range from 6.4 to 30.3 Effects determined for a 5.3 change*
Jacquemin et al. (2009a) Asthma Incidence ECHRS	Medians by Center range from 6.4 to 30.3 Effects determined for a 5.3 change*
Modig et al. (2009) Asthma Incidence Rhine cohort	Overall mean Winter 9.6 for 3 cities Effects determined for a 5.3 change*
Castro-Giner et al. (2009) Asthma Incidence ECRHS	Median by center ranges from 6.4 to 30.3 Effects determined for a 5.3 change*
Sunyer et al. (2006) Chronic Bronchitis ECRHS	Mean 20.2 Range by center 3.28 to 40.5 Effects per increase of 15.9*
Gotschi et al. (2008) Pulmonary Function ECRHS	Medians by Center range from 6.4 to 30.3 Effects determined for a 5.3 change*
Pujades-Rodriguez et al. (2009) Pulmonary Function Nottingham U.K.	IQR Differences compared 18.1 to 19.1*
Andersen et al. (2011) COPD First hospital admission Danish cohort	Range <5.3 to 21.3* Median 8.1 IQR 3.1
Gan et al. (2013) COPD First hospital admission Vancouver Canada Cohort	Mean 17 (SD 4.3) IQR 4.47 Range 8.0 to 30.1
Andersen et al. (2012) Asthma Hospital Admission Danish cohort	Range <5.3 to 21.3* Median 8.1 IQR 3.1
Neupane et al. (2010) Hospitalization for community acquired pneumonia Hamilton, Ontario	Mean (SD) 15.25 (2.7) Range 8.79 to 25.67

*Estimated with land use regression

Table 5-9 Summary of evidence supporting a likely to be a causal relationship between long-term NO_2 exposure and respiratory effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Consistent associations from multiple, high quality epidemiologic studies with relevant exposures	Consistent evidence for increases in asthma incidence in children in U.S. multicity cohort and diverse cohorts in Europe, Canada, and Asia. Asthma ascertainment by parental report of doctor diagnosis. Associations found with NO_2 measured outside children's homes, at central monitoring sites, modeled using land-use regression, inverse distance-squared we Epidemiologic evidence for decrements in lung function and partially irreversible decrements in lung function growth in children	Jerrett et al. (2008), McConnell et al. (2010), Gehring et al. (2010), Carlsten et al. (2011c), Gruzieva et al. (2013), Oftedal et al. (2009), Shima et al. (2002), Nishimura et al. (2013a), Clark et al. (2010), Clougherty et al. (2007) Table 5-1, Figure 5-3. Breton et al. (2011), Gauderman et al. (2004), Rojas-Martinez et al. (2007a), Schultz et al. (2012), Oftedal et al. (2008)	Overall annual means from studies: 13.5-20.0 ppb Community-specific means: 9.6-51.3 ppb. Table 5-7
	Coherence with evidence for increases in respiratory symptoms in children with asthma.	McConnell et al. (2003), Gehring et al. (2010), Gruzieva et al. (2013), Hansel et al. (2008), Belanger et al. (2013) Table 5-2, Figure 5-5, Table 5-3.	
	Supporting epidemiologic evidence for asthma incidence in adults and respiratory hospital admissions, respiratory symptoms, respiratory mortality.	Jacquemin et al. (2009b), Jacquemin et al. (2009a), Modig et al. (2009), Castro-Giner et al. (2009), Sunyer et al. (2006) Hospital admissions: COPD Andersen et al. (2011), Gan et al. (2013) Asthma Andersen et al. (2012) And community – acquired pneumonia Neupane et al. (2010)	Overall annual means from studies: 8-20 ppb Community-specific annual means: 3-40 ppb Table 5-8

Table 5-9 (Continued): Summary of Evidence Supporting a Likely to be Causal Relationship between Long-term NO_2 Exposure and Respiratory Effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Uncertainty remains regarding independent effects of NO_2	Associations with respiratory symptoms, pulmonary function remain robust with adjustment for O_3, SO_2, PM_{10}, $PM_{2.5}$, $PM_{10-2.5}$, EC, or OC but analysis is limited When reported correlations between copollutants were often high Across outcomes, associations found with adjustment for various SES indicators, family history of asthma, smoking exposure, housing characteristics, presence of gas stove, temperature, and humidity	McConnell et al. (2003), Hwang and Lee (2010), Dong et al. (2011), Hansel et al. (2008); Hwang et al. (2005), Lee et al. (2012c), Rojas-Martinez et al. (2007b) See Table 5-5	
	Several studies indicate increases in respiratory symptoms related to indoor NO_2 exposure	Belanger et al. (2013), Hansel et al. (2008)	
Coherence with respiratory effects of short-term exposure	Across disciplines, results consistently demonstrate increases in asthma morbidity. Particular coherence with effects in children with asthma.	Table 4-23	
Limited biological plausibility for effects on asthma provided by toxicological evidence	Increased AHR in a few studies of 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
Toxicological evidence for impaired host defense	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
Toxicological evidence for changes in lung morphology	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

Table 5-9 (Continued): Summary of Evidence Supporting a Likely to be Causal Relationship between Long-term NO_2 Exposure and Respiratory Effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Some evidence for key events to inform mode of action			
Modification of innate and adaptive immunity	Increased IgE-mediated mast cell histamine release in guinea pigs with long-term exposure	Fujimaki and Nohara (1994), Gregory et al. (1983), Kumae and Arakawa (2006)	4,000 ppb for 12 weeks
	Increased macrophage infiltration to lung tissue or increased # of lymphocytes in BALF of experimental animals		200, 500, 2,000 pp
	Epidemiologic evidence for allergic sensitization in children		
	With short-term exposure: Upregulation of inflammatory cytokines (IL-10, IL-5, IL-13) and inflammatory adhesion molecule ICAM-1 in healthy humans and increased numbers of eosinophils in nose in animals.	Pathmanathan et al. (2003), Ohashi et al. (1994)	2,000 ppb over 4 consecutive days; 3,000 ppb NO_2 for 2 weeks
Inflammation	Epidemiologic evidence for increases in increases in eNO, elevated number of nasal eosinophils)	(Renzetti et al., 2009), (Dales et al., 2008)	
Oxidative Stress	Animal toxicology models: Increased lipid peroxidation	Arner and Rhoades (1973), Sagai et al. (1984), Gregory et al. (1983), Ayaz and Csallany (1978)	2,900 ppb
	Alterations in the glutathione antioxidant pathway		400-4,000 ppb

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to 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).

5.3 Cardiovascular Effects

5.3.1 Introduction

The 2008 ISA for Oxides of Nitrogen concluded that "the available epidemiologic and toxicological evidence was inadequate to infer the presence or absence of a causal relationship" for cardiovascular effects related to long-term NO_2 exposure.

This section reviews the published studies pertaining to the cardiovascular effects of NO_X exposure in humans, animals, and cells; study details can be found in Table 5-10 and Table 5-11. With the limited existing body of evidence serving as the foundation, emphasis was placed on studies published since the 2008 ISA for Oxides of Nitrogen. The recent epidemiologic and toxicological publications add to the evidence for independent effects of NO_X exposure on cardiovascular morbidity. For epidemiologic studies, emphasis was placed on longitudinal analyses of incident cardiovascular diseases with consideration of multiple potential confounding factors. With regard to animal toxicological studies, studies with relevant NO_2 exposure concentrations (i.e., less than 5,000 ppb) were included.

5.3.2 Cardiovascular Diseases

At the completion of the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) only one epidemiologic study had examined the association of cardiovascular disease with long-term exposure to NO_2. In this study, Miller et al. (2007) (see Table 5-10 for further details about this and other epidemiologic studies) included 65,893 postmenopausal women, between the ages of 50 and 79 years, without previous CVD residing in 36 U.S. metropolitan areas from 1994 to 1998. Exposures to air pollution were estimated by assigning the annual mean levels of air pollutants in 2000 measured at the monitor nearest the subject's residence, based on its five-digit ZIP Code centroid. In the single-pollutant model results, $PM_{2.5}$ showed the strongest associations with the CVD events (MI, revascularization, angina, CHF, CHD death) among pollutants, followed by SO_2. NO_2 was not associated with the overall CVD events (hazard ratio [HR]: 0.98 [95% CI: 0.89, 1.08] per 10-ppb increase in the annual average) when the dataset was restricted to those who were not missing exposure data. Several recent studies of the association of long term NO_2 exposure with preclinical and clinical cardiovascular outcomes add to the available body of evidence.

Lipsett et al. (2011) used Cox proportional hazards regression to analyze the association of incident stroke and MI with long-term exposure to NO_2, NO_X, other gases (CO, O_3, SO_2) and PM. These authors studied a cohort of California public school teachers aged 20-80 years old (n = 124,614). Approximately 40% of those initially contacted participated in the study, with approximately 80% continuing their participation during subsequent follow-up contacts (Bernstein et al., 2002). Each participant's geocoded residential address was linked to pollutant surfaces that were determined by inverse distance weighted (IDW) interpolation of pollutant concentrations measured at fixed site monitors during the period 1996-2005. The average of monthly NO_2 concentrations was modeled as a time dependent function for subjects with at least 12 months of exposure. Those living outside the radial range for which the monitor was intended to provide representative data were excluded from the analysis. This "representative range" was 3 km for neighborhood NO_X and NO_2 monitors and 5 km for the urban/regional NO_2 and NO_X monitors. The authors observed a positive association between NO_2 and incident MI (HR: 1.06 [95%CI: 0.88, 1.27] per 10-ppb, respectively) and a weak association with incident stroke (HR: 1.02 [95%CI: 0.90, 1.115] per 10-ppb, respectively). Point estimates for the association of other pollutants ($PM_{2.5}$, SO_2 and O_3) with incident stroke were increased and the association between PM_{10} and incident stroke was significantly increased. Fewer observations were available for the NO_X compared to PM analyses because the requirements for the participants' proximity to the monitor were more stringent for NO_X (residing within 5 km as opposed to 20 km for PM).

Gan et al. (2011) used Cox proportional hazards regression to examine the association of long-term exposure to black carbon, $PM_{2.5,}$ NO_2, and NO with CHD hospitalization and mortality among participants (45-85 year-olds) in the universal health insurance system residing in Vancouver, Canada (n = 418,826). In this study, land use regression was used to predict 5-year average concentrations at a resolution of 10 meters. These predicted concentrations were adjusted using factors derived from regulatory monitoring data and linked to each participant's postal code of residence. After adjustment for sex, age, comorbidity, and SES, 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 5.5.2).

Atkinson et al. (2013) applied Cox proportional hazards regression to examine 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. The authors report that approximately 98% of the population is registered with a general practitioner minimizing the potential for selective participation. 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, stroke, arrhythmias, and heart failure. 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, stroke 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, MI and stroke were null while the association of PM_{10} with heart failure was increased). Rosenlund et al. (2009a) conducted a case control study of first MI reported between 1985 and 1996 using the registry of hospital discharges and deaths for Stockholm County, Sweden and randomly selected population-based controls. Predicted 5-year average NO_2 concentration was determined and linked to each participant's geocoded address using dispersion models. The resolution of the predicted concentrations corresponded to 500 meters in the countryside, 100 meters in urban areas and 25 meters in the inner city. Multinomial logistic regression was performed to obtain association of most 5-year average NO_2 concentration with incident MI. This metric, which was designed to capture traffic exposure, was associated with fatal MI (OR: 1.14 [95%CI: 1.09, 1.19] per 10 ppb) but not with non-fatal 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.

Epidemiologic studies using a variety of exposure assessment methods (e.g., dispersion modeling, land use regression and central site monitor concentration nearest to the subject's residence) provide some evidence that long term exposure to NO_2 may be associated with the risk of cardiovascular diseases including MI and heart failure. The association with heart failure is reported in one large, well conducted study and was robust to adjustment for multiple potential confounding factors including age, sex, smoking, BMI, and pre-existing medical conditions (Atkinson et al., 2013). The association of long term NO_2 exposure with incident MI is also reported in one well conducted study after adjustment for a similar array of confounding factors (Lipsett et al., 2011); however associations of NO_2 with MI were not observed consistently across generally comparable studies (Atkinson et al., 2013; Rosenlund et al., 2009a). Further, the observed associations of MI and heart failure with long term NO_2 exposure are not supported by a study of CHD hospital admissions (Gan et al., 2011) nor are they supported by Miller et al. (2007) who found a null association between NO_2 and cardiovascular events combined (MI, revascularization, angina, CHF, CHD death) among post-menopausal women (a positive association with $PM_{2.5}$ was observed in this study).

Table 5-10 Epidemiologic studies of long-term exposure to NO_2 or NO_X and effects on the cardiovascular system.

Study	Cohort (location) Study Period	Mean (ppb)	Exposure Assessment	Effect Estimates (95% CI)
Miller et al. (2007)	WHI Cohort (U.S.) 1994-1998	NR	Annual avg (2000): nearest monitor to residence ZIP code centroid	CVD Events HR*: 0.98 (0.89, 1.08) per 10 ppb Covariates: age, ethnicity, education, household income, smoking, diabetes, hypertension, systolic blood pressure, BMI, and hypercholesterolemia *HR for subjects with non-missing exposure data
Lipsett et al. (2011)	CTS Cohort (CA, U.S.) June 1996-Dec 2005	NO_2 IQR: 10.29 Mean: 33.59 NO_X IQR: 58.31 Mean: 95.6	Geocoded residential address linked to pollutant surface developed using IDW (fixed site monitors concentrations from 1995-2005 used to model exposure as a time dependent function)	MI Incidence NO_X: HR 1.01 (0.01, 1.11) NO_2: HR 1.06 (0.88, 1.28) Stroke Incidence NO_X: HR 1.02 (0.96, 1.09) NO_2: HR 1.02 (0.90, 1.16) per 10 ppb NO_2 and 20 ppb NO_x Covariates: age, race, smoking second-hand smoke, BMI, lifetime physical activity, nutritional factors, alcohol, marital status, menopausal status, l hormone therapy, hypertension medication and aspirin, family history of MI/Stroke
Atkinson et al. (2013)	National GP Patient Cohort (U.K.) 2003	IQR: 5.7 ppb Mean (SD): 12.0	Annual average NO_2 concentration for 2002 at a 1 by 1 km resolution derived from emission-based models and linked to residential post codes.	MI Incidence HR: 0.97 (0.90, 1.04) Stroke Incidence HR: 0.98 (0.91, 1.06) 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, index of multiple deprivation
Rosenlund et al. (2009a)	SHEEP Study (Stockholm, Sweden) 1985-1996	5th-95th 15.9 cases: Median (cases); 6.9 Median: 6.3 (controls)	5-yr average NO_2 concentration assessed by dispersion modeling	First Nonfatal MI OR: 0.96 (0.93, 1.00) per 10 ppb Covariates: age, sex, calendar year and SES

Table 5-10 (Continued): Epidemiologic studies of long-term exposure to NO_X and effects on the cardiovascular system.

Gan et al. (2011)	Population based cohort (Vancouver, Canada) 1999-2002	Mean (NO_2): 16.3 IQR (NO_2): 4.5 Mean (NO): 26.1 IQR (NO): 10.8	LUR, 5-yr average concentration (1995-1998) and 4 yr avg concentration (1999-2002)	CHD Hospitalization: (ICD9 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, preexisting diabetes, COPD, hypertension, SES

WHI = Women's Health Initiative;
CTS = California Teachers Study;
GP=General Practice;
IDW = Inverse Distance Weighted;
SHEEP=Stockholm Heart Epidemiology Program

5.3.3 Markers of Cardiovascular Disease Risk

Epidemiologic and toxicological studies have also investigated the effects of long-term NO_2 exposure on risk factors and markers of cardiovascular disease risk, such as arterial stiffness, circulating lipids, and HRV. A recent experimental animal study also reported changes in markers that are characteristic of vascular disease and progression (see Table 5-11 for toxicology 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 3 predictors for ET-1 and TIMP-2 (Seilkop et al., 2012).

Hyperlipidemia is recognized as a risk factor for cardiovascular disease. Takano et al. (2004) reported that obese rats (Otsuka Long-Evans Tokushima Fatty) had elevated levels of triglycerides and decreased 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 non-obese rats (Long-Evans Tokushima). The authors suggested that obese animals were at greater risk of dyslipidemia following NO_2 exposure.

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 the participant's residential address using dispersion model predictions supplemented with land use and meteorological data. Annual average NO_2 concentration was associated with decreased SDNN, nighttime LF, and LF/HF ratio in women. No associations with other parameters of HRV were observed in these data.

The 1993 NO_X Air Quality Criteria Document (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 concentration 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).

A number of null findings related to changes in hematological parameters were reported in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c). Hematocrit and hemoglobin levels were unchanged in squirrel monkeys (Fenters et al., 1973), rats (Suzuki et al., 1981), or dogs exposed to $\leq$ 5,000 ppb NO_2 (Wagner et al., 1965). However, Furiosi et al. (1973) did report 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.

Overall, a limited number of epidemiologic and toxicological studies have evaluated long-term NO_X exposure on risk factors and 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.

5.3.4 Inflammation and Oxidative Stress

Inflammation and oxidative stress have been shown to play a role in the progression of chronic cardiovascular disease. A limited number of studies have evaluated markers of inflammation and oxidative stress. Forbes et al. (2009a) examined the association of predicted annual average NO_2 concentration with CRP and fibrinogen among the 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 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-year average

traffic-related NO_2 concentration estimated using dispersion models with increases in IL-6 and CRP; however, NO_2 was not associated with TNF-α, fibrinogen or PAI-1 in this population (Panasevich et al., 2009). Associations between several metrics of SO_2 exposure and increased IL-6 and CRP were observed in this study.

de Burbure et al. (2007) examined oxidative stress markers in rats on a low (Se-L) or sufficient 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 following a 48 hour recovery period. Glutathione peroxidase (GPx), of which Se is an integral component, also decreased immediately and 48 hours after exposure only in the plasma of Se-S rats. However, GPx levels increased in RBC of Se-L 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 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 TBARS immediately after exposure.

As discussed in Section 5.5.3, 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 thiobarbituric acid reactive substances [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 responses. Although HO-1 was not highly correlated with NO_2; NO_2, SO_2, and NO ranked among the top 3 predictors for TBARs.

Table 5-11 Study details for toxicological studies examining cardiovascular effects from long-term NO_2 exposure

Study	Species (Strain); Lifestage; 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 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
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, MMP3, MMP7, MMP9, TIMP2, 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 = NR	1,000 or 5,000 ppb NO_2; continuously for 18 mo	Hemoglobin and hematocrit levels were measured quarterly throughout exposure.

5.3.5 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 all cause 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 5.5.2, Figure 5-10, and Table 5-17). Specifically, the strongest evidence comes from a number of recent studies that have observed positive associations between exposure to NO_X and NO_2 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., 2008b), 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 4.3).

5.3.6 Summary and Causal Determination

Overall, the evidence is suggestive of a causal relationship between long-term exposure to NO_2 and cardiovascular effects. This conclusion is based on the consideration of recent prospective epidemiologic studies reporting associations of NO_2 or NO_X with CHF, MI and stroke although associations with these cardiovascular outcomes were not consistently observed across studies. 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. Although cardiovascular morbidity effects are not consistently observed across epidemiologic studies, 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. 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 framework is summarized in Table 5-12.

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. Miller et al. (2007) found no association between long-term NO_2 exposure and cardiovascular events among post-menopausal women enrolled in the WHI study, although an association with $PM_{2.5}$ was observed. Several studies evaluating hematological parameters reported mixed results that included no changes in hematocrit or hemoglobin and increased erythrocyte count.

Recent, large and well conducted prospective epidemiologic studies provide some evidence that long-term exposure to NO_2 is associated with heart failure, MI and stroke. The association with heart failure was reported in a large study with high participation rates using a validated database of doctor diagnosed cardiovascular outcomes and persisted after adjustment for multiple potential confounding factors including age, sex, smoking BMI, SES and pre-existing medical conditions (Atkinson et al., 2013). The association of long term NO_2 exposure with incident MI and long-term NO_X exposure with stroke was reported in another large study with adjustment for a similar array of potential confounding factors and which employed a refined exposure assessment strategy (i.e., residence with 3-5 km of a monitor) (Lipsett et al., 2011). An association between NO_2 exposure and MI were not consistently reported across studies of generally comparable quality (Atkinson et al., 2013; Rosenlund et al., 2009a). Additionally, studies examining associations of cardiovascular effects with other pollutants (i.e., PM_{10}, $PM_{2.5}$, CO, SO_2, O_3), in addition to NO_2 often reported associations with these other pollutants.

Because IHD includes MI and can lead to heart failure there is some coherence across the cardiovascular endpoints associated with NO_2 exposure in the epidemiologic studies described above. Additionally, consistent associations from multiple, high-quality epidemiologic studies of cardiovascular mortality support findings for cardiovascular morbidity effects. Studies in human populations offer limited support that long term NO_2 may be associated with arterial stiffness (Lenters et al., 2010) and decreased HRV (Felber Dietrich et al., 2008). Experimental animal studies report that NO_2 exposure was associated with dyslipidemia and increases in some markers of oxidative stress and vascular function, but each has been evaluated each in one study. There is weak evidence to describe a biologically plausible mechanism for the NO_2 related cardiovascular effects observed, potentially through the induction of oxidative stress and systemic inflammation. Evidence for these events is provided by both animal toxicological studies that report increased markers of oxidative stress and inflammatory markers as well as cytokines after short-term NO_2 exposure and human epidemiologic studies that report increased CRP and IL-6 after long-term NO_2 exposure. Overall, the evidence from some epidemiologic studies of cardiovascular morbidity, in consideration of the consistent associations observed between NO_2 exposure and cardiovascular mortality and the limited evidence demonstrating biological plausibility, is suggestive of a causal relationship between long term NO_2 exposure and cardiovascular morbidity.

Table 5-12 Summary of evidence supporting a suggestive relationship between long-term NO_2 exposure and cardiovascular effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Cardiovascular Morbidity – Suggestive			
At least one well conducted study reporting associations with heart failure and MI	Evidence from prospective studies in New England and California for heart failure, MI, and stroke in association with long term modeled NO_2 concentration.	Atkinson et al. (2013)	Mean annual avg (2002): 12.0 ppb
		Lipsett et al. (2011)	Mean avg (1996-2005): 33.59 ppb
	But, positive associations with MI not observed consistently	Atkinson et al. (2013)	Mean annual avg (2002): 12.0 ppb
		Rosenlund et al. (2009a)	Median of 5-yr avg: 6.9 ppb (cases) 6.3 ppb (controls)
Consistent associations from multiple, high-quality epidemiologic studies of cardiovascular mortality	Consistent evidence for increases in risk of cardiovascular mortality in adults in diverse populations and applying diverse methods Strongest evidence of mortality from IHD, CHD, and circulatory diseases	Section 5.5.2	--
Supporting evidence of decreased markers of cardiovascular disease risk	Increased arterial stiffness measured by pulse wave velocity and augmentation index among U.S. young adults.	Lenters et al. (2010)	Mean: 18.3 ppb
	Decreased HRV (SDNN, LF) among Swiss cohort study participants.	Felber Dietrich et al. (2008)	Mean: 12.1 ppb
Adequate consideration of potential confounding	Although epidemiologic studies generally considered important confounders, studies with similar designs and approaches for control of confounding did not consistently report associations with cardiovascular effects	Miller et al. (2007)	Annual svg (2000): NR
		Atkinson et al. (2013)	Mean annual avg (2002): 12.0 ppb
		Lipsett et al. (2011)	Mean avg (1996-2005): 33.59 ppb
		Rosenlund et al. (2009a)	Median of 5-yr avg: 6.9 ppb (cases) 6.3 ppb (controls)
Limited toxicological evidence with relevant exposures	Increased triglycerides and decreased HDL in rats.	Takano et al. (2004)	160 ppb

Table 5-12 (Continued): Summary of evidence supporting a suggestive relationship between long-term NO_2 exposure and cardiovascular effects.

Rationale for Causal Determination[a]	Key Evidence[b]	Key References[b]	NO_2 Concentrations Associated with Effects[c]
Weak evidence of key events that inform mode of action			
Oxidative Stress	Evidence of increased oxidative stress in rats with relevant NO_2 exposures (i.e., MDA, TBARS, GPx, GST).	Li et al. (2011a) de Burbure et al. (2007)	Rats: 5,320 ppb NO_2 Rats: 1,000 ppb NO_2
	Evidence of increased oxidative stress in plasma from NO_2-exposed humans (i.e., LOX-1).	Channell et al. (2012)	Healthy adults: 500 ppb NO_2
Inflammation	Toxicological evidence of increased transcription of some inflammatory mediators in vitro (i.e., IL-8) and in rats (i.e., TNF-α).	Channell et al. (2012)	Human cells exposed to plasma from healthy adults: 500 ppb NO_2
		Li et al. (2011a)	Rats: 5,320 ppb NO_2
	Limited epidemiologic support for increases in CRP and IL-6 in adults	Panasevich et al. (2009)	Mean: 12.6 ppb

[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 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).

5.4 Reproductive and Developmental Effects

5.4.1 Introduction

The body of literature characterizing the health effects associated with exposure to NO_2 is large and continues to grow, including research focusing on birth outcomes, for which the body of evidence has grown considerably since the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c). Among the epidemiologic studies, 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; [PTB]) have received more attention in air pollution research, while congenital malformations are less studied. The toxicological studies of similar outcomes measured litter size and birth weight. Nervous system and respiratory outcomes after early life exposures to NO_2 are examined in the developmental toxicology and epidemiology literature.

A major issue in studying environmental exposures and reproductive and developmental effects (including infant mortality) is selecting the relevant exposure period, since the biological mechanisms leading to these outcomes and the critical periods of exposure are poorly understood. To account for this, many epidemiologic studies evaluate multiple exposure periods, [including long-term (months to years) exposure periods, such as 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 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, an epidemiologic study that uses the entire pregnancy as the exposure period is considered to have a long-term exposure period (about 40 weeks, on average), while a toxicological study conducted with rats that also uses the entire pregnancy as the exposure period is considered to have a short-term exposure period (about 18-24 days, on average). In order to characterize the weight of evidence for the effects of 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 poorly understood 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 physical mechanisms are not fully understood, several hypotheses have been proposed for the effects of NO_2 on reproductive and developmental effects; these include: oxidative stress, systemic inflammation, vascular dysfunction, and impaired immune function. 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, 2008c) examined impacts on birth-related endpoints, including intrauterine, perinatal, postneonatal, and infant deaths; premature births; intrauterine growth retardation; very low birth weight (weight <1,500 grams) and low birth weight (weight

<2,500 grams); and birth defects. However, in the limited number of studies 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.

Several recent articles have reviewed methodological issues relating to the study of outdoor air pollution and adverse birth outcomes (Chen et al., 2010a; Woodruff et al., 2009; Ritz and Wilhelm, 2008; Slama et al., 2008). Some of the key challenges to interpretation of these study results include the difficulty in assessing exposure as most studies use existing monitoring networks to estimate individual exposure to ambient air pollution; the inability to control for potential confounders such as other risk factors that affect birth outcomes (e.g., smoking); evaluating the exposure window (e.g., trimester) of importance; and limited evidence on the physiological mechanism of these effects (Ritz and Wilhelm, 2008; Slama et al., 2008).

Overall, the 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 datasets from different countries (Woodruff et al., 2010). Initial results from this collaboration have examined PM and birth weight (Parker et al., 2011); work on 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 birth outcomes.

5.4.2 Fertility, Reproduction, and Pregnancy

5.4.2.1 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 exposures (~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). Overall, there is no epidemiologic evidence for an association between exposure to ambient NO_2 concentrations and effects on sperm.

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 male 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. Overall, there is no toxicological evidence of effects of NO_2 exposure on sperm or semen quality.

5.4.2.2 Effects on Reproduction

Evidence suggests that exposure to air pollutants during pregnancy may be associated with 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 non-smokers (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 from oocyte retrieval to embryo transfer, and embryo transfer to 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 significantly 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 contrast, NO_2 exposure has not been shown to induce such effects in animals. Breeding studies by Shalamberidze and Tsereteli (1971a, b) with exposures of animals to 67 ppb or 130 ppb NO_2 12h/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 un-exposed male. At the higher dose, Shalamberidze and Tsereteli (1971a, b) 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.

5.4.2.3 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 C-reactive protein (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 week, 2 weeks, and 4 weeks prior to delivery, but was strongest when exposure to NO_2 was measured over the entire pregnancy. Similarly, Lee et al. (2011c) observed generally null associations between exposure 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 Section 4.3.5). 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. (2012b) 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 very imprecise and was reduced when exposure was averaged over the second and third trimesters. The same pattern was observed when analyses were restricted to non-obese women, but among obese women, the effect estimate was below 1.00 for each trimester.

New-onset gestational hypertension can contribute to pre-eclampsia, a common complication of pregnancy diagnosed after 20 weeks of pregnancy. Wu et al. (2009) observed a 44% increase 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. van den Hooven et al. (2011) did not observe an association for NO_2 exposure and risks of pregnancy-induced hypertension or pre-eclampsia.

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). Key studies examining the association between exposure to NO_2 and pregnancy-related effects can be found in Table 5-13. A supplemental Table S5-2 (U.S. EPA, 2013g) provides an overview of all of the epidemiologic studies of pregnancy-related health effects.

Toxicological Evidence

Evidence from animal toxicological studies suggests that exposure to NO_2 may affect pregnancy. Maternal toxicity was reported in pregnant rats with inhalation exposure to 5,300 ppb NO_2 for 6 h/day throughout gestation (21 days). Deficits in maternal weight gain during gestation were also reported at 5,300 ppb NO_2 (Tabacova et al., 1984).

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. Mechanisms related to oxidative stress have been proposed (Section 3.3.2.8), but not specifically in relation to reproductive effects. Shalamberidze and Tsereteli (1971a, b) reported decreased litter sizes (fewer pups born) to dams that received 1,300 ppb NO_2 12h/day for 3 months during pregnancy.

5.4.3 Birth Outcomes

5.4.3.1 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. Low birth weight (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 small-for-gestational-age (SGA), which is defined as a birth weight <10th percentile for gestational age (and often sex and/or race), and intrauterine growth retardation (IUGR) are often used interchangeably. However, this definition of SGA does have limitations. For example, using it for IUGR may overestimate the percentage of "growth-restricted" neonates as it is unlikely that 10% of neonates have growth restriction (Wollmann, 1998). On the other hand, when the 10th percentile is based on the distribution of live births at a population level, the percentage of SGA among PTB is most likely underestimated (Hutcheon and Platt, 2008). Nevertheless, SGA represents a statistical description of a small neonate, whereas the term IUGR is reserved for those with clinical evidence of abnormal growth. Thus all IUGR neonates will be SGA, but not all SGA neonates with be IUGR (Wollmann, 1998). In the following section the terms SGA and IUGR are referred to as each cited study used the terms.

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, 2008c), 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 potentially transient effects of early exposure on fetuses. 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.

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 (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), and Kashima et al. (2011) 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).

When evaluating the association between fetal growth and exposure to NO_2, many studies relied on modeled concentrations of NO_2 coming from land use regression 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 modeled estimates of NO_2 were not substantially different from those that used measured NO_2 concentrations. However, in a study that assigned exposure to NO_2 using both a land use regression model and inverse distance weighting 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 land use regression model (OR 0.94 [95%CI: 0.80, 1.10]).

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 5-13. A supplemental Table S5-3 (U.S. EPA, 2013h) provides an overview of all of the epidemiologic studies of fetal growth effects.

5.4.3.2 Preterm Birth

Preterm birth (PTB) is a syndrome (Romero et al., 2006) that is characterized by multiple etiologies. It is therefore unusual to be able to identify an exact cause for each PTB. In addition, PTB is not an adverse outcome in itself, but an important determinant of health status (i.e., neonatal morbidity and mortality). Although some overlap exists for common risk factors, different etiologic entities related to distinct risk factor profiles and leading to different neonatal and postneonatal complications are attributed to PTB and measures of fetal growth. Although both restricted fetal growth and PTB can result in LBW, prematurity does not have to result in LBW or growth restricted babies.

A major issue in studying environmental exposures and PTB is selecting the relevant exposure period, since the biological mechanisms leading to PTB and the critical periods of vulnerability are poorly understood (Bobak, 2000). Short-term exposures proximate to the birth may be most relevant if exposure causes an acute effect. However, exposure occurring in early gestation might affect placentation, with results observable later in pregnancy, or cumulative exposure during pregnancy may be the most important determinant. The studies reviewed have dealt with this issue in different ways. Many have considered several exposure metrics based on different periods of exposure. Often the time periods used are the first month (or first trimester) of pregnancy and the last month (or 6 weeks) prior to delivery. Using a time interval prior to delivery introduces an additional problem since cases and controls are not in the same stage of development when they are compared. For example, a preterm infant delivered at 36 weeks is a 32-week fetus 4 weeks prior to birth, while an infant born at term (40 weeks) is a 36-week fetus 4 weeks prior to birth.

Recently, investigators have examined the association of PTB with both short-term (i.e., hours, days, or weeks) and long-term (i.e., months or years) exposure periods. Time-series studies have been used to examine the association between air pollution concentrations during the days immediately preceding birth. An advantage of these time-series studies is that this approach can remove the influence of covariates that vary across individuals over a short period of time. Retrospective cohort and case-control studies have been used to examine long-term exposure periods, often averaging air pollution concentrations over months or trimesters of pregnancy.

Studies of PTB fail to show consistency in 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, than it may be difficult to demonstrate additional risk associated with a higher quartile. Thus negative studies must be interpreted with caution.

Preterm birth occurs both naturally (idiopathic PTB), and as a result of medical intervention (iatrogenic PTB). Ritz et al. (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.

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 (Trasande et al., 2013; Olsson et al., 2012; Wu et al., 2011a; 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 (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 six 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., 2011a; Wu et al., 2009).

When evaluating the association between PTB and exposure to NO_2, several studies relied on modeled concentrations of NO_2 coming from land use regression models (Gehring et al., 2011a; Gehring et al., 2011b; Kashima et al., 2011; Wu et al., 2011a;

Llop et al., 2010; Brauer et al., 2008) and dispersion models (Wu et al., 2011a; 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 land use regression model and inverse distance weighting of measured NO_2 concentration from monitors Brauer et al. (2008) found generally comparable risk estimates for VPTB using the monitoring data (OR: 1.24, [95%CI: 0.80, 1.88]) and NO_2 estimates from the land use regression model (OR: 1.16 [95%CI: 0.93, 1.61]).

In summary, the evidence is generally inconsistent, with some studies observing associations between NO_2 exposure and PTB while other studies observe no consistent pattern of association. These studies are characterized in supplemental Table S5-4 (U.S. EPA, 2013i).

5.4.3.3 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 a significant determinant of postneonatal mortality and morbidity. Studies report that infants who are smallest at birth have a higher incidence of diseases and disabilities, which continue into adulthood (Hack and Fanaroff, 1999).

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 (Darrow et al., 2011b; 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 (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). When evaluating the risk of

having a baby weighing less than 2,500 g, the study results remained inconsistent, with some authors observing an association between LBW and exposure to NO_2 (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 (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). 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. 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. 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 modeled concentrations of NO_2 coming from land use regression 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 modeled estimates of NO_2 were similarly inconsistent, and not substantially different from those that used measured NO_2 concentrations. Several studies compared the use of a 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).

In animal toxicological studies by Shalamberidze and Tsereteli (1971a, b), albino rats with exposures to 67 or 130 ppb NO_2 12h/day for 3 months prior to breeding produced pups with significantly decreased birth weights. These body weight decrements continued to be significantly decreased at PND 4 and PND 12.

In summary, the evidence is generally inconsistent, with some studies observing associations between NO_2 exposure and birth weight while other studies observing no consistent pattern of association. Key studies examining the association between

exposure to NO_2 and birth weight can be found in Table 5-13. A supplemental Table S5-5 (U.S. EPA, 2013j) provides an overview of all of the epidemiologic studies of birth weight.

5.4.3.4 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 non-cardiac 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 general, however, studies of birth defects have focused on cardiac and oral cleft defects, and the results from these studies are not entirely consistent. This inconsistency could be due to the absence of true associations between NO_2 and risks of cardiovascular malformations and oral cleft defects; 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 or oral cleft defects might be due to issues relating to statistical power or measurement error. A recent meta-analysis of air pollution and congenital anomalies concluded that there was no statistically significant increase in risk of congenital anomalies and NO_2 (Vrijheid et al., 2011). These authors note that heterogeneity in the results of these studies may be due to inherent differences in study location, study design, and/or analytic methods, and comment that these studies have not employed some recent advances in exposure assessment used in other areas of air pollution research that may help refine or reduce this heterogeneity. These studies are characterized in supplemental Table S5-6 (U.S. EPA, 2013k).

5.4.4 Postnatal Development

The issue of prenatal exposure has assumed increasing importance since ambient air pollution exposures of pregnant women have been shown to lead to adverse pregnancy outcomes, as well as to respiratory morbidity and mortality in the first year of life.

Extensive growth and development of the nervous and respiratory systems take place during the prenatal and early postnatal periods. This early developmental phase is thought to be very important in determining long-term lung growth. Shalamberidze and Tsereteli (1971a, b) showed decrements in postnatal body weight at PND 4 and 12 in albino rats with prenatal exposures to 67 ppb or 1,300 ppb NO_2 12h/day for 3 months prior to breeding.

5.4.4.1 Developmental Nervous System Effects

Central nervous system effects were not evaluated in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c). Several recent studies have been performed examining the NO_2 effects on the central nervous system in children, with a more extensive examination of cognitive function and additional studies on attention-related behaviors, motor function, psychological distress, and autism. This section is organized by outcome category, and key studies examining the association between exposure to NO_2 and developmental nervous system effects can be found in Table 5-13. Supplemental Table S5-7 (U.S. EPA, 2013l) provides an overview of all of the epidemiologic studies of developmental nervous system effects.

Cognitive Function

Most of the studies examining cognitive function included here are on school children, employed widely used structured neuropsychological tests, assessed ambient exposure by modeling, and examined potential confounding by multiple SES indicators. While some studies considered birth outcomes and noise exposure, none of the studies considered polycyclic aromatic hydrocarbons or lead (Pb), both of which are well-characterized risk factors for neurodevelopmental decrements.

School Children

Studies of schoolchildren examined the effects of concurrent exposure on cognitive function were studied; other exposure periods were not examined.

van Kempen et al. (2012) and Clark et al. (2012) studied children who were part of the Road Traffic and Aircraft Noise Exposure and Children's Cognition and Health (RANCH) project to determine if there was a relationship between cognitive performance and air pollution, road traffic noise, and aircraft noise in home and school settings. van Kempen et al. (2012) used Neurobehavioral Evaluation System (NES) tests to evaluate 485 Dutch children at home and school (9-11 years old), while Clark et al. (2012) tested 719 children (9-10 years old) at school using the Suffolk Reading Scale, Child Memory

Scale, and a modified version of the Search and Memory Task. Both studies used modeling to predict annual mean ambient NO_2 concentrations, then linked the exposures to home and school addresses. A strength of the van Kempen et al. (2012) study that Clark et al. (2012) did not have was that they looked at exposure assessment both in the home and school settings. van Kempen et al. (2012) found a negative association between NO_2 exposure at school and memory (digit span length), but perceptual coding was not associated with school NO_2 after additional adjustment for road traffic noise and aircraft noise. No associations were found between home NO_2 exposure and cognition before or after noise adjustment. Home and school noise exposure were not associated with memory and perceptual coding before or after NO_2 adjustment. Cognitive function was not associated with combined exposure to air pollution and either road or aircraft noise and memory and perceptual coding at home or school. Clark et al. (2012) found that NO_2 at school was not associated with reading comprehension, recognition memory, information recall, conceptual recall, and working memory before or after adjustment for noise.

Freire et al. (2010) evaluated 210 four-year old boys (mean age: 51.3 months; part of the Environment and Childhood [IMNA] study) in the Granada province of southern Spain using a standardized Spanish adaptation of the McCarthy Scales of Children's abilities (MSCA) to determine if there was an association between NO_2 exposure (surrogate for traffic-related air pollution) and cognitive development. Using land use regression to assign exposure, predicted NO_2 levels were higher in urban settings (15.8 ppb) than non-urban settings (4.9 ppb). In the fully adjusted model, a negative although imprecise association was found between general cognitive score and NO_2 (β [95% CI]: 8.2-13.2 ppb: -1.07 [-9.99, 7.85] points; >13.2 ppb: -4.19 [-14.02, 5.64] points, <8.2 ppb as reference). Negative fully adjusted associations (sometimes imprecise) were also found in both the lower and higher predicted NO_2 exposure categories for perceptual-performance, verbal score, quantitative score, memory, executive function, memory span, verbal memory, and working memory.

Wang et al. (2009a) evaluated whether traffic-related air pollution exposure affected neurobehavioral function on 861 children aged 8-10 years old in Quanzhou, China. Their observations were consistent with those of other studies (communities with higher air pollution concentrations related to traffic exhaust was associated with a decrease in scores/NAI). However, a major limitation of the study was that it did not conduct a direct analysis of only NO_2 effects. Rather, the independent variable of the study was the location of the schools, not NO_2 exposure.

Morales et al. (2009) conducted a longitudinal study observing associations of indoor NO_2 from household gas appliances (exposure during first 3 months of life) with

cognitive functioning in 398 four-year-old children from Menorca, Spain. Morales et al. (2009) found that as the number of gas appliances increased, NO_2 concentrations increased (mean [SE]: 0 appliances: 6.10 [0.5] ppb; 1 appliance: 16.7 [1.0] ppb; 2 appliances: 25.7 [2.1] ppb). General cognitive, verbal, memory, and executive function scores were negatively associated with the number of gas appliances at home, and indoor NO_2 exposures were also associated with general cognitive, verbal, and executive function scores through a negative dose-response. Among children with any GSTP1 Val-105 allele, gas stove and fire exposure and indoor NO_2 was associated with a decrease in general cognitive score and general cognitive, verbal, and executive function scores, respectively, but children with the Ile/Ile genotype did not show any cognitive decrements. These results provide coherence with effects on other neurodevelopmental outcomes after exposure to ambient NO_2.

Infants

The Bayley Scales of Infant Development is a widely used test for infant mental development as it is a reliable indicator of current development and cognitive functioning in infants since it tests for markers such as motor function, memory, and early language skills. However, the Bayley Scales of Infant Development is not necessarily correlated with development of children at older ages, and the 1 year Bailey test does not have many outcomes tested that are analogous to those tested at 2 and 3 years old.

Guxens et al. (2012) investigated whether or not residential air pollution exposure during pregnancy affected mental development in 1,889 children (mean age 14.8 months) and whether or not antioxidant and detoxification factors reduced the effect. Women from four regions of Spain (Valencia, Sabadell, Asturias, Gipuzkoa; part of the Infancia y Medio Ambiente [INMA] project) were recruited during their first trimester of pregnancy, and children's mental development was tested around 14 months of age (using the Bayley Scales of Infant Development. An overall inverse, but imprecise, association with mental development was observed for NO_2 exposure in adjusted models (combined region β [95% CI]: -0.95 [-3.90, 1.89] per NO_2 doubling), as well as for benzene exposure. The strongest association was observed in the Gipuzkoa region (β [95% CI]: -5.15 [-8.04, -2.27] and -5.49 [-9.21, -1.76] per NO_2 doubling, respectively). When stratified by antioxidant and detoxification variables, Guxens et al. (2012) concluded that a higher consumption of fruits and vegetables (>405 g/day) during the first trimester of pregnancy may have reduced the effect of air pollutants on infant mental development, while higher maternal circulating Vitamin D levels and longer breast feeding duration had less of an effect, and parental education level and social class had no effect on infant mental development.

Attention-related Behaviors

In their study of children 9-11 years old, van Kempen et al. (2012) found that none of the NES test outcomes related to attention (e.g., Simple Reaction Time Test [SRTT], Switching Attention Test [SAT]) were associated with concurrent school NO_2. Additionally, no associations were found with home NO_2, with or without adjustment for road traffic and aircraft noise. The combined exposure to NO_2 and road traffic noise was negatively associated with the reaction times measured during the SAT 'block' condition in the school setting and the reaction times measured during the SRTT and the SAT 'arrow' condition in the home setting. At both school and home, high NO_2 concentrations had more of an effect on the reaction time in high road traffic noise than in low traffic noise. There was not strong evidence of associations of other attention tests with school or home air pollution, school or home road traffic noise, or school or home air pollution and road traffic noise combined exposure.

Morales et al. (2009) observed the effects of indoor NO_2 exposure during the first three months of life on ADHD-related symptoms tested by psychologists in 365 children (at 4-years old). Exposure to gas appliances and higher indoor NO_2 increased the risk of ADHD symptoms and inattention in children, but hyperactivity risk was not increased in association with either exposure. Children with any GSTP1 Val-105 allele showed a higher risk of ADHD symptoms and inattention in association with exposure to gas appliances and indoor NO_2, but children with the Ile/Ile genotype did not show any negative ADHD associations with either exposure. Morales et al. (2009) concluded that early-life exposure to gas appliances in the home and higher concentrations of indoor NO_2 had a higher risk of developing ADHD symptoms in 4-year-olds, and that those with the GSTP1 Val-105 allele had a higher risk of ADHD symptoms. These findings are coherent with associations found between other neurodevelopmental outcomes in association with exposure to ambient NO_2.

Motor function

van Kempen et al. (2012) used NES tests in 9-11 year old children to determine if there was a relationship between concurrent air pollution and noise exposure and motor function in home and school settings. Based on the Hand Eye Coordination Test (HECT), there was not strong evidence of association between locomotion and NO_2 exposure in either the school or home setting, with or without adjustment for road traffic and aircraft noise. There were also no associations found between locomotion and road traffic noise or aircraft noise after adjusting for NO_2, in the school or home settings, or when a combination of NO_2 and noise was considered.

Freire et al. (2010) used a standardized Spanish adaptation of the McCarthy Scales of Children's Abilities (MSCA) to test motor abilities of four-year-old children to determine if there was an association between concurrent NO_2 exposure and motor function. The predicted NO_2 levels were higher in urban settings (15.8 ppb) than non-urban settings (4.9 ppb). A negative association was found with both the lower and higher predicted NO_2 exposures in fully adjusted motor function and gross motor function, but fine motor skills showed a positive association (β [95% CI]: lower NO_2: 3.28 [-6.83, 13.40]; higher NO_2: 0.91 [-10.22, 12.05]). In contrast, Morales et al. (2009) found no association between indoor NO_2 exposure during the first three months of life and motor function in 398 four year old children.

Tabacova et al. (1985) examined postnatal development of pups from dams that were exposed to 50, 500, or 5,300 ppb NO_2 (5h/day, gestation day 0-21). Neuromotor deficits in the righting reflex and postural gait were also seen in pups with 50, 500 and 5,300 ppb NO_2. Comparison of the animal toxicology studies and the epidemiologic findings points to multiple sensitive windows of NO_2 exposure with potential effects on motor function.

Psychological distress

Clark et al. (2012) measured psychological distress in 9-10 year old children using the parental version of the Strengths and Difficulties Questionnaire, and measured aircraft noise, road traffic noise, and NO_2 at school. Clark et al. (2012) found that psychological distress showed no associations with noise exposures before or after adjustment for NO_2 and no substantial associations with NO_2 before or after adjustment for noise.

In an animal toxicological study, Di Giovanni et al. (1994) reported developmental neurobehavioral decrements, i.e., decreased pup vocalization, in males removed from the nest at PND5, PND10, or PND15 (3,000 ppb continuous dam NO_2 exposure, GD0-GD21).

Autism

Autism is a neurodevelopmental disorder characterized by impaired social interaction, verbal and non-verbal communication deficits, and repetitive or stereotypic behavior. Although the causes of autism are not fully understood, many potential factors have been implicated.

Becerra et al. (2013) and Volk et al. (2013) evaluated whether prenatal and first year of life exposure to NO_2 increased the risk of developing autism in children. Becerra et al. (2013) observed 7,603 autistic disorder (AD) and 75,782 control children born in Los

Angeles County, California at 36 to 71 months old. AD children (diagnosis based on the Diagnostic and Statistical Manual of Mental Disorders and reported on the Client Development Evaluation Report) were selected from seven regional centers contracted by the California Department of Developmental Services. Control children were selected at random without replacement from birth certificates and had no documentation of autism. NO_2 estimates were extracted from LUR model surfaces at each residential location to classify traffic-related exposure (LUR based), and ambient NO_2 was measured from the nearest monitoring stations to the residence (monitor-based). Relative increases in risk of AD diagnosis were seen in unseasonalized LUR (U-LUR) and seasonalized LUR (S-LUR) for NO_2 and for monitor-based NO_2 during entire pregnancy exposure (adjusted OR [95% CI]: U-LUR: 1.13 [1.06, 1.23] per 10 ppb; S-LUR: 1.05 [0.98, 1.12] per 10 ppb; Monitor-based: 1.04 [0.98, 1.10] per 10 ppb), but consistent patterns were not observed across trimester-specific effect estimates. The least educated mothers had the strongest association between AD and LUR-based estimates compared to mothers with the highest education. Adjusted two-pollutant models with U-LUR-NO_2 and O_3, NO, CO, PM_{10}, and $PM_{2.5}$ showed an increased risk of 13-17% per 10 ppb for AD in entire pregnancy. Volk et al. (2013) studied 524 children (279 with autism, 245 control, 24-60 months old; part of the Childhood Autism Risks from Genetics and the Environment [CHARGE] study) in California. Autistic children were evaluated using the Autism Diagnostic Observation Schedules (parents were given the Autism Diagnostic Interview-Revised), while those with a developmental delay and control children were given the Social Communication Questionnaire to screen for autistic features. Motor skills, language, socialization, and daily living skills were assessed using the Mullen Scales of Early Learning and the Vineland Adaptive Behavior Scales, and regional NO_2 data was collected from the U.S. EPA AQS. A 10-ppb increase in regional NO_2 exposure was associated with an increased risk of autism during the first year of life (adjusted OR [95% CI]: 1.67 [1.25, 2.23]), the entire pregnancy (adjusted OR [95% CI]: 1.52 [1.16, 2.00]), and each of the 3 trimesters (adjusted OR [95% CI]: 1st trimester: 1.30 [1.04, 1.14]; 2nd trimester: 1.40 [1.10, 1.78]; 3rd trimester: 1.42 [1.12, 1.80]).

Nervous system histopathology: Animal toxicology evidence

A recent study examined nervous system effects in adult rats which were exposed over a short-term period to NO_X. The evidence is included in this chapter (long-term) because there are no other studies of nevous system effects examined in relation to short-term NO_2 exposure (Chapter 4). Adult male Wistar rats were exposed for seven days (6 hours per day) to 2,500-10,000 ppb NO_2 (Li et al., 2012a). Brain tissue was collected 18 hours following the last exposure. NO_2 exposure had no effect on body weight, however concentration-dependent reductions in brain to body weight ratios were observed, with

statistical significance reached at 5,000 and 10,000 ppb NO_2. Histopathologic 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. Oxidative stress biomarkers were also measured, as well as gene expression and protein levels of oncogenes and apoptosis-related genes. Statistically significant changes in antioxidant enzyme activities (Cu/Zn SOD, MnSOD and glutathione peroxidase), protein carbonyls and malondialdehyde were observed in response to 5,000 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.

Summary of studies of Neurodevelopment

In summary, several studies found an association between long term exposure to NO_2 and cognitive function in schoolchildren and infants, but the results were inconsistent. Clark et al. (2012) found that air pollution had no effect on cognitive outcomes in children 9-10 years old and saw little evidence that air pollution moderated the association of noise exposure on cognition, but consuming more fruits and vegetables during pregnancy, higher maternal circulating Vitamin D levels, and longer breast feeding duration may reduce the effect of air pollution on infant mental development (Guxens et al., 2012). While some studies considered birth outcomes and noise exposure, none of the studies considered other pollutants associated with neurodevelopmental decrements, such as lead (Pb) and PM (U.S. EPA, 2013a, 2009b). Generally inconsistent results were found for several attention-related behaviors and motor function with exposure to indoor NO_2 or ambient NO_2 alone and in combination with noise exposure from road or air traffic. Psychological distress was found to have no substantial associations with NO_2 before or after adjustment for noise. Autism risk increased with exposure to NO_2 during pregnancy and the first year of life, with more pronounced risk found for late gestation and early life exposure. Homes that had children with autism had higher regional NO_2. Consistent patterns were not seen in trimester-specific exposure effects, and children with mothers with the least education showed the strongest association between NO_2 and autism.

5.4.4.2 Developmental Respiratory Effects

Several high-quality longitudinal epidemiologic studies have consistently found associations with long-term NO_2 exposure and decrements in lung function growth and are described in detail in Section 5.2.3.1. Briefly, studies included children from the U.S., Mexico, and Europe followed from birth or age 8 years for periods of 8 to 11 years. NO_2 exposures were assessed from central site measurements, dispersion modeling, and land use regression modeling. Results consistently demonstrated associations of NO_2 exposure in the first year of life or change in the annual average with decrements in lung function and partially irreversible decrements in lung function growth in children (Figure 5-5) (Mölter et al., 2013; Schultz et al., 2012; Breton et al., 2011; Oftedal et al., 2008; Rojas-Martinez et al., 2007a; Gauderman et al., 2004). Some studies found an NO_2 concentration-dependent decrement in lung function and lung function growth (Rojas-Martinez et al., 2007a; Gauderman et al., 2004). A number of studies have demonstrated that long-term exposure to NO_2 alters lung morphology in experimental animals, though these changes do not appear to contribute to altered lung function (Hayashi et al., 1987; Kubota et al., 1987). Additionally, these effects were not clearly demonstrated in juvenile animals (Chang et al., 1986; Furiosi et al., 1973). Thus, the animal toxicological evidence does not provide clear biological plausibility for epidemiologic observations.

5.4.4.3 Early Life Mortality

During the neonatal and post-neonatal periods, the developing lung is highly sensitive to environmental toxicants. The lung is not well developed at birth, with 80% of alveoli being formed postnatally. An important question regarding the association between 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); postneonatal (1 month to 1 year); and an overall interval for infants that includes both the neonatal and postneonatal periods (<1 year). The studies reflect a variety of study designs, exposure periods, regions, and adjustment for potential confounders. As discussed below, a handful of studies have examined the effect of ambient air pollution on neonatal and postneonatal mortality, with the former the least studied. These studies varied somewhat with regard to the outcomes and exposure periods examined and study designs employed.

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 postnatal development of pups from dams that were exposed to 50, 500, or 5,300 ppb NO_2 (5h/day, gestation day GD0-GD21). Significantly decreased pup viability was seen at PND21 with

5,300 ppb NO_2. Recent epidemiologic studies have examined the association between long-term exposure to NO_2 and stillbirths, with one study (Faiz et al., 2012) observing an association and another (Hwang et al., 2011) observing associations near the null value. One study investigated the association between short-term exposure to NO_2 and mortality during the neonatal period (Lin et al., 2004a), and did not observe a positive association. More studies have examined the association between exposure to NO_2 and mortality during the postneonatal 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 postneonatal 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 (SIDS). 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 S5-8 (U.S. EPA, 2013m) provides a brief overview of the epidemiologic studies of infant mortality.

Table 5-13 Key reproductive and developmental epidemiologic studies for NO_2.

Study	Location (Sample Size)	Mean NO_2 (ppb)	Exposure assessment	Selected Effect Estimates[a] (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	CALINE4 dispersion model to estimate local traffic-generated pollution	*Pre-eclampsia* Entire pregnancy: 1.44 (1.23, 1.68)
Malmqvist et al. (2013)	Sweden (n = 81,110)	NO_X 7.5	Modeled NO_X with data from emission database and AERMOD with a spatial resolution of 500 × 500 meters	*Pre-eclampsia* *Third trimester* Q1: ref 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: Ref Q2: 1.19 (0.99, 1.44) Q3: 1.52 (1.28, 1.82) Q4: 1.69 (1.41, 2.03)
Legro et al. (2010)	Northeastern U.S. (n = 7,403)	NO_2 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)

Table 5-13 (Continued): Key Reproductive and Developmental Epidemiologic Studies for NO_2.

Birth Outcomes				
Aguilera et al. (2010)	Catalonia, Spain (n = 562)	16.9-17.2	Land-use Regression	*Fetal Length* T1: -2.04 (-7.01, 2.95) T2: -1.69 (-7.05, 3.69) T3: 0.33 (-4.06, 4.72) *Head Circumference* T1: 0.25 (-5.42, 5.91) T2: 1.70 (-3.69, 7.07) T3: 0.23 (-4.32, 4.77) *Abdominal Circumference* T1: -2.82 (-8.24, 2.59) T2: -0.13 (-5.64, 5.38) T3: 0.74 (-3.926, 5.40) *Biparietal Diameter* T1: 3.87 (-2.04, 9.75) T2: 4.90 (-0.34, 10.11) T3: 1.48 (-3.41, 6.35) *Estimated Fetal Weight* T1: -2.22 (-7.39, 2.98) T2: 0.46 (-5.82, 6.72) T3: 0.91 (-3.65, 5.45)
Ballester et al. (2010)	Valencia, Spain (n = 785)	19.1-20.2	Land-use Regression	*Head Circumference* Entire pregnancy: -0.11 (-0.25, 0.03) *Birth Length* 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	Land-Use Regression	Birth Length Entire Pregnancy: -1.69 cm (-0.34, -0.02) Head Circumference Entire Pregnancy: -0.01 cm (0.13, 0.11)
Hansen et al. (2007)	Brisbane, Australia (n = 26,617)	Median: 7.8 75th: 11.4 Max: 24.2	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)

Table 5-13 (Continued): Key Reproductive and Developmental Epidemiologic Studies for NO_2.

Hansen et al. (2008)	Brisbane, Australia (n = 15,623)	9.8	Closest monitor (Within 2-14 km of one of 17 monitors)	*Head Circumference* 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* 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* 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* 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)
Iñiguez et al. (2012)	Valencia, Spain (n = 818)	Median: 20.2	Land-use Regression	*Fetal Length* T1: 0.97 (0.92, 1.02) T2: 0.96 (0.92, 1.00) T3: 0.97 (0.92, 1.02) *Abdominal Circumference* T1: 0.96 (0.92, 0.99) T2: 0.98 (0.94, 1.02) T3: 0.98 (0.94, 1.03) *Biparietal Diameter* T1: 0.96 (0.92, 1.00) T2: 0.97 (0.92, 1.01) T3: 0.98 (94, 1.02) *Estimated Fetal Weight* 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, the 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: Ref 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)
Bell et al. (2007)	Connecticut and Massachusetts (n = 358,504)	17.4	County-level avg	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)

Table 5-13 (Continued): Key Reproductive and Developmental Epidemiologic Studies for NO_2.

Darrow et al. (2011b)	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				
Freire et al. (2010)	Spain (n = 210)	11.1	Land-use regression	High exposure (>13.2 ppb) General cognitive model: -4.19 (-14.02, 5.64) Perceptual-performance: -2.17 (-12.76, 8.41) Verbal: -3.09 (-13.31, 7.13) Quantitative: -6.71 (-17.91, 4.49) Memory: -5.52 (-16.18, 5.13) Motor function: -5.30 (-15.96, 5.36) Executive function: -4.93 (-14.90, 5.05) Memory span: -3.46 (-13.93, 7.01) Verbal memory: -2.71 (-14.02, 8.59) Working memory: -7.37 (-18.96, 1.74) Gross motor function: -8.61 (-18.96, 1.74) Fine motor skills: 0.91 (-10.22, 12.05)

Table 5-13 (Continued): Key Reproductive and Developmental Epidemiologic Studies for NO_2.

van Kempen et al. (2012)	the Netherlands (n = 485)	School: 16.5 School: 16.9 Home: 16.4	Modeled data linked to home and school address	School - β (95% CI) *NO_2 (adjusted for noise)* Memory: -0.30 (-0.55, 0.04) SRTT, reaction time: -2.23 (-22.13, 17.66) SAT, block, no. of errors: -0.02 (-0.42, 0.38) SAT, block, reaction time: 13.92 (-16.70, 43.92) SAT, arrow, no. of errors: -0.30 (-0.92, 0.30) SAT, arrow, reaction time: 21.55 (-19.72, 62.81) SAT, switch, no. of errors: -1.19 (-3.62, 1.26) SAT, switch, reaction time: 21.5 (-45.17, 88.19) Locomotion: 0.08 (-0.08, 0.25) Perceptual coding: 0.04 (-0.21, 0.30) Home - β (95% CI) *NO_2 (adjusted for noise)* Memory: 0.17 (-0.08, 0.42) SRTT, reaction time: -2.11 (-20.96, 16.72) SAT, block, no. of errors: -0.04 (-0.40, 0.32) SAT, block, reaction time: 15.85 (-11.28, 42.96) SAT, arrow, no. of errors: -0.34 (-0.94, 0.26) SAT, arrow, reaction time: -3.32 (-42.96, 36.34) SAT, switch, no. of errors: -1.23 (-3.32, 0.87) SAT, switch, reaction time: -20.21 (-74.92, 34.51) Locomotion: 0.06 (-0.08, 0.21) Perceptual coding: -0.06 (-0.26, 0.15)
Clark et al. (2012)	U.K. (n = 719)	22.7	Modeled data linked to home and school address	*NO_2 (adjusted for traffic noise)* Reading comprehension: 1.078 (0.844, 1.404) Recognition memory: 1.254 (0.673, 2.294) Information recall: 1.327 (0.537, 3.221) Conceptual recall: 1.004 (0.797, 1.278) Working memory: 1.058 (0.004, 292.728) Physiological distress: 1.603 (0.537, 4.788)

Table 5-13 (Continued): Key Reproductive and Developmental Epidemiologic Studies for NO_2.

Guxens et al. (2012)	Spain (n = 1,889)	Overall: 15.4 Valencia: 19.6 Sabadell: 17.1 Asturias: 12.3 Gipuzkoa: 10.7	Passive Samplers; Land-use Regression	Mental Development - β [95% CI][b] 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) Breast feeding duration None: -3.47 (-7.82, 0.98) <6 mo: -0.71 (-4.06, 2.65) ≥ 6 mo: -0.61 (-2.97, 1.75) 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)	NR	CALINE4 line-source air quality dispersion model (within 5 km of child's home) Monitoring stations (within 50 km of home) Inverse distance-squared weighting	*Autism* Traffic-related exposure *First year* Q2: 0.91 (0.56, 1.47) Q3: 1.00 (0.62, 1.62) Q4: 3.10 (1.76, 5.57) *All pregnancy* Q2: 1.26 (0.77, 2.06) Q3: 1.09 (0.67, 1.79) Q4: 1.98 (1.20, 3.31)
Becerra et al. (2013)	California (n = 83,385)	30.8	Land-use regression Nearest monitoring station to birth residence	Autism Entire pregnancy: 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) *Monitor-based* Entire pregnancy: 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)

[a]Relative risk per 10 ppb change in NO_2, or 20 ppb change in NO_X, unless otherwise noted.
[b]Per NO_2 doubling
T1 = First Trimester, T2 = Second Trimester, T3 = Third Trimester
M1 = Month 1, M2 = Month 2, M3 = Month 3, M4 = Month 4
NR: No quantitative results reported

Table 5-14 Reproductive and developmental toxicological studies for NO_2.

Reference	Concentration NO_2 (ppb)	Strain, Age, Sex (n)	Exposure conditions	Endpoints Examined
Tabacova et al. (1985)	25, 50, 500, or 5,300 ppb (0, 50, 100, 1,000, or 10,000 µg/m^3)	Wistar rat,Adult, F (20)	Developmental exposure with postnatal neurotoxicity testing. 0, 25, 50, 500, or 5,300 ppb (0, 50, 100, 1,000, or 10,000 µg/m^3), 5 h/day during gestational days 0 through 21; progeny followed for up to PND 60	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	Albino rat, NR, Adult, F (7)	1,300 ppb for 12 h/day for 3 mo (further specifics unavailable)	Litter size, birth weight, postnatal weight gain (body weight).
Di Giovanni et al. (1994)	1,500 or 3,000 ppb	Wistar rat, M pups (7)	Direct exposure of dams at 0, 1,500, or 3,000 ppb continuously throughout gestational days 0–21, male offspring tested for vocalizations on PND5, PND10, and PND15.	Neurobehavior (ultrasonic vocalization)
Kripke and Sherwin (1984)	1,000 ppb	LEW/f mai rat, young adults, M (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.

5.4.5 Summary and Causal Determination

Overall, the evidence is suggestive of a causal relationship between exposure to NO_2 and all three of the reproductive and developmental outcomes: (1) fertility, reproduction and pregnancy, (2) birth outcomes, and (3) postnatal development. Separate conclusions are made for these three smaller groups of outcomes because they are likely to have different etiologies and exposure patterns over different lifestages. In past reviews, a limited number of epidemiologic 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. All available evidence, including more than 100 recent 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 framework is summarized in Table 5-15.

Fertility, Reproduction, and Pregnancy

A number of studies examined the association between exposure to measured concentration of NO_2 or modeled predictions of NO_X concentration 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. A single 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. There is emerging evidence for an association between modeled predictions of NO_X concentrations during the third trimester and pre-eclampsia; however, evidence for pregnancy induced hypertension, gestational diabetes, and reduced placental growth and function is limited and inconsistent. Collectively, the limited and inconsistent evidence is suggestive of 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 generally inconsistent, 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. Similarly, several high-quality studies observed associations between decreases in birth weight and NO_2 concentrations (Darrow et al., 2011b; Bell et al., 2007). Studies that examined PTB and birth defects (using both measured NO_2 concentrations and modeled predictions of NO_X concentrations) 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. Collectively, the limited and inconsistent evidence is suggestive of a causal relationship between exposure to NO_2 and effects on birth outcomes.

Postnatal Development Effects

There is limited though positive evidence from both epidemiologic and animal toxicological studies for an association between prenatal and early life NO_2 exposure and postnatal development effects, including decrements in cognitive function in humans and neurobehavioral development of pup vocalization and decreases in lung function growth in children. Evidence does not indicate that prenatal or early life NO_2 exposures are associated with infant mortality. Collectively, the limited and inconsistent evidence is suggestive of a causal relationship between exposure to NO_2 and effects on postnatal development.

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Table 5-15 Summary of evidence supporting a suggestive of a causal relationship between long-term 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 - Suggestive			
At least one high-quality epidemiologic study shows an association with pre-eclampsia	Consistent associations, especially with exposure during the third trimester, between NO_2 concentration and pre-eclampsia, after adjustment for common potential confounders. . Limited evidence for a linear concentration-response relationship. Associations not evaluated in copollutants models. Generally high correlations between exposures to NO_X and $PM_{2.5}$ ($R^2 \geq 0.7$)	Wu et al. (2009) Pereira et al. (2013) Malmqvist et al. (2013)	Mean: 7.2 ppb Mean: 23 ppb Mean: 7.5 ppb
Limited and inconsistent evidence for other pregnancy-related health effects	Limited and inconsistent epidemiologic evidence for associations with pregnancy-induced hypertension, gestational diabetes, and placental growth and function Limited toxicological evidence for deficits in maternal weight gain during pregnancy, rats.	Hampel et al. (2011), Lee et al. (2012b), Mobasher et al. (2013), Malmqvist et al. (2013), van den Hooven et al. (2012c) Tabacova et al. (1984)	Means: 8.7-28.6 ppb 5,300 ppb
At least one high-quality epidemiologic study shows an association in vitro fertilization failure	Decreased odds of live birth associated with higher NO_2 concentrations during ovulation induction and the period after embryo transfer	Legro et al. (2010)	19 ppb
Lack of evidence from available toxicological and epidemiologic studies to support an association of NO_2 exposure with detrimental effects on sperm quality	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 7h/day, 5 days/week Mean exposure averaged over 90-days:16.8 ppb Mean daily concentration: 30.1 ppb

Table 5-15 (Continued): Summary of evidence supporting a suggestive of a causal relationship between long-term 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]
Birth Outcomes – Suggestive			
At least one high-quality epidemiologic study shows an association with fetal growth restriction and decreased birth weight	Strong evidence from a well-conducted Spanish cohort studies that observes associations with NO_2 concentrations and fetal growth restriction. Supported by consistent evidence, SGA and IUGR. Outcomes assessed with anthropometric fetal measurements, Well-conducted studies show association with lower birth weight, though the body of evidence is generally inconsistent.	Section 5.4.3.3 Darrow et al. (2011b), Bell et al. (2007)	Mean exposure averaged over trimesters: 7.8-36.1 ppb Mean exposure averaged over 3rd trimester of pregnancy: 23.8 ppb Mean exposure averaged over entire pregnancy: 17.4 ppb
Evidence for other birth outcomes generally inconsistent	Some studies observe an association between NO_2 exposure and PTB or birth defects while other studies observe no consistent pattern of association	Section 5.4.3.2 Section 5.4.3.4	Mean exposure averaged over trimesters: 8.8-37.6 ppb Mean exposure averaged over early pregnancy (e.g., weeks 3-8): 8.2-28.0 ppb
Limited toxicological evidence with relevant NO_2 exposures	Evidence for decreased litter size and late embryonic lethality in rats.	Shalamberidze and Tsereteli (1971a), Shalamberidze and Tsereteli (1971b)	1,300 – 5,300 ppb
Limited evidence for key events informing 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
Postnatal Development - Suggestive			
At least one high-quality animal toxicological study shows an association with postnatal development	Impairments in postnatal development including pup viability, postnatal eye opening, and incisor eruption	Tabacova et al. (1985)	50, 500, 5,300 ppb
Consistent epidemiologic evidence for developmental respiratory effects	Consistent associations in children with decrements in lung function growth. Lack of analogous toxicological evidence	Section 5.2.3.1	Mean annual avg: 14-21 ppb Mean 6-mo avg: 34 ppb

Table 5-15 (Continued): Summary of evidence supporting a suggestive of a causal relationship between long-term 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]
Limited but supporting evidence for neurodevelopmental effects	Some epidemiologic studies showed cognitive function decrements in infants and schoolchildren in association with NO_2 exposure	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	Clark et al. (2012), Freire et al. (2010),	
	More limited and inconsistent epidemiologic evidence for attention-related behaviors, motor function, psychological distress.		
	Impaired vocalization and neuromotor function (righting reflex and postural gait) in pups after in utero NO_2 exposure.	Di Giovanni et al. (1994)	3,000 ppb
	Prenatal NO_2 exposure associated with autism in first year of life or at ages 3-6 years in California	Volk et al. (2013), Becerra et al. (2013)	Mean: 30.8 ppb
Results of epidemiologic studies for an effect on infant mortality generally inconsistent	Evidence for a positive association between exposure to NO_2 and infant mortality is inconsistent across studies and across post-natal periods	Section 5.4.4.3	Mean daily concentrations: 20.3-50.3 ppb

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to causal determination and where applicable to uncertainties and 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).

5.5 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, 2008c). In the current ISA, findings for cause-specific mortality (i.e., respiratory, cardiovascular) are used to assess the continuum of effects and inform the causality determinations for respiratory and cardiovascular effects. The causality determination for total mortality contained herein (Section 5.5) is based primarily on the evidence for non-accidental 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.

5.5.1 Review of Mortality Evidence from 2008 ISA 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 re-analyses and have reported extended results including additional years of follow-up. The initial reports from the ACS (Pope et al., 1995) and the HCS (Dockery et al., 1993) did not include results for gaseous pollutants. However, as reported in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), in re-analyses 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 their reanalyses of the ACS cohort data, Krewski et al. (2000) long-term exposure to NO_2 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 up through 2001. In the earlier studies, the authors reported increased risk of mortality associated with both concurrent and delayed 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. (2006a); Lipfert et al. (2006b) reported that traffic density was a better predictor of mortality than ambient air pollution variables, though they still observed a positive and statistically significant 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.

[1] Quantitative effect estimates from studies reviewed in the 2008 NO_X ISA (U.S. EPA, 2008c) can be found alongside effect estimates from more recent studies in Figure 5-9, Figure 5-10, and Figure 5-11 and the corresponding Tables (Table 5-16, Table 5-17, and Table 5-18, respectively).

In another cohort conducted in the U.S. (the California Seventh-day Adventist cohort [AHSMOG]), Abbey et al. (1999) enrolled young adult, non-smoking Seventh-day Adventists throughout California. Generally, NO_2 was not associated with all-cause, 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 females and 18 for males); 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 PAARC survey (Air Pollution and Chronic Respiratory Diseases) 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 wells 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, 2008c). The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) 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 high correlations between NO_2 and PM indices. The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) 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.

5.5.2 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 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 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 exposure to NO_X 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 NCLS 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 and respiratory mortality; this association is stronger than any observed with the traffic variables and total or cause-specific mortality.

In an update to a cohort of women in Germany (Gehring et al., 2006), 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 and all-cause and cardiopulmonary mortality. The effect estimates for lung cancer or respiratory mortality were positive, though less precise and not statistically significant. The effect estimates were highest for women living within 50 meters of a road with median daily traffic volume of 5,000 cars or greater. The effect estimates for the associations between all-cause and cardiopulmonary mortality and NO_2 were generally lower for the follow-up period compared to the original analysis.

In a recent U.S. cohort study, Hart et al. (2010) examined the association between residential exposure to NO_2 and mortality among men in the U.S. trucking industry. 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 all-cause 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, but were not statistically significant. 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 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 all-cause) were less consistent and generally close to the null value.

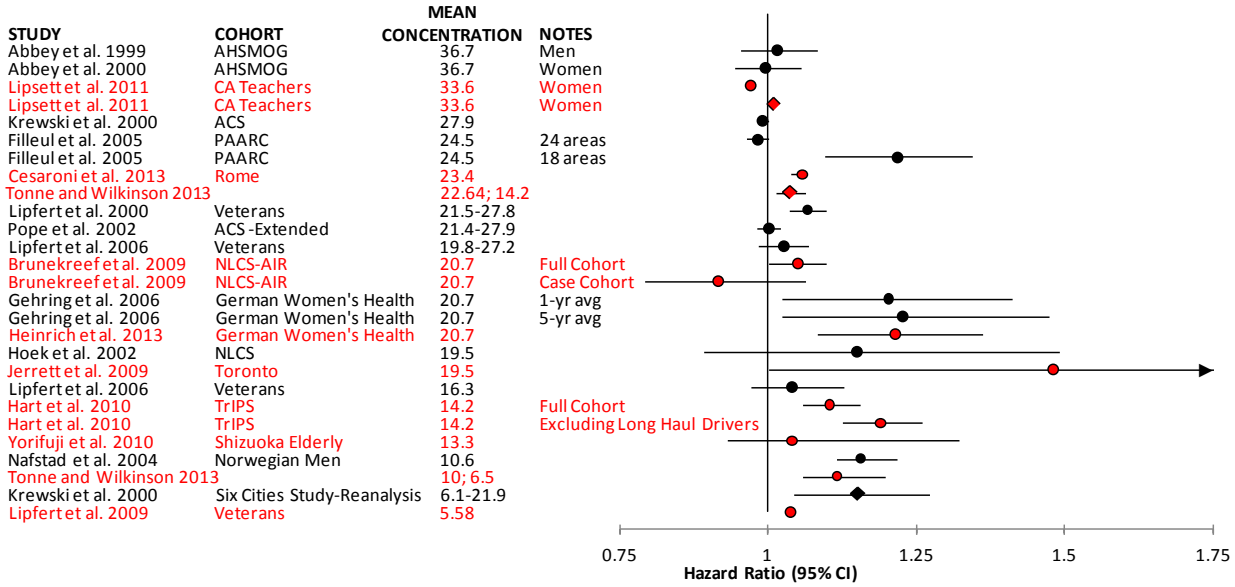
A number of recent studies have examined the association between long-term exposure to NO_2 and mortality in Canadian cities. Chen et al. (2013) conducted a cohort study of air pollution and cardiovascular mortality in three cities in Ontario. They used land-use regression models to assign exposure to 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 and all-cause mortality among subjects from a respiratory clinic. The authors observed positive associations with all-cause 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. (2011) conducted a population-based cohort study to evaluate the association between traffic-related pollutants and risk of mortality due to CHD. Land-use regression models were used to estimate exposure over a 5 year period (1994-1998) and the cohort was followed up 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.

In a large cohort study in Rome, Italy, Cesaroni et al. (2013) observed positive associations between long-term exposure to NO_2 and total, cardiovascular, IHD, respiratory and lung cancer mortality among the adult population. These associations were robust to the inclusion of $PM_{2.5}$ in the model. Tonne and Wilkinson (2013) evaluated the association between long-term exposure to NO_2 and NO_X among survivors of hospital admissions for acute coronary system in England and Wales and observed

evidence of a null association after adjustment for $PM_{2.5}$. Rosenlund et al. (2008b) conducted a cohort study in Rome, Italy to investigate the effects of long-term exposure to NO_2 and cardiovascular deaths, including mortality among previous myocardial infarction (MI) survivors. The authors observed a positive association between long-term exposure to NO_2 and fatal coronary events, though they did not observe an association with mortality among survivors of a first coronary event. In Brisbane, Australia, Wang et al. (2009b) examined the association between long-term exposure to NO_2 and cardio-respiratory mortality. The relative risk for NO_2 and cardio-respiratory mortality was near the null value and not statistically significant, indicating no association.

A number of studies were conducted in Asian countries to evaluate the association between long-term-exposure to NO_2 and mortality. Dong et al. (2012) observed a strong, positive association between long-term exposure to NO_2 and respiratory mortality in a cohort study conducted in Shenyang, China. In Shizuoka, Japan, Yorifuji et al. (2010) observed positive associations between NO_2 and all-cause, cardiopulmonary, IHD, and respiratory disease mortality, with the strongest effects observed for IHD mortality. When the analysis was restricted to non-smokers, 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. (2009a) also observed a positive association between long-term exposure to NO_2 and bladder cancer.

A supplemental Table S5-9 (U.S. EPA, 2013n) provides an overview of the epidemiologic studies of long-term exposure to NO_X and mortality. These studies are also characterized in Figure 5-9, Figure 5-10, and Figure 5-11; and Table 5-16, Table 5-17, and Table 5-18.



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 or NO_X concentration. 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; Diamonds = NO_X.

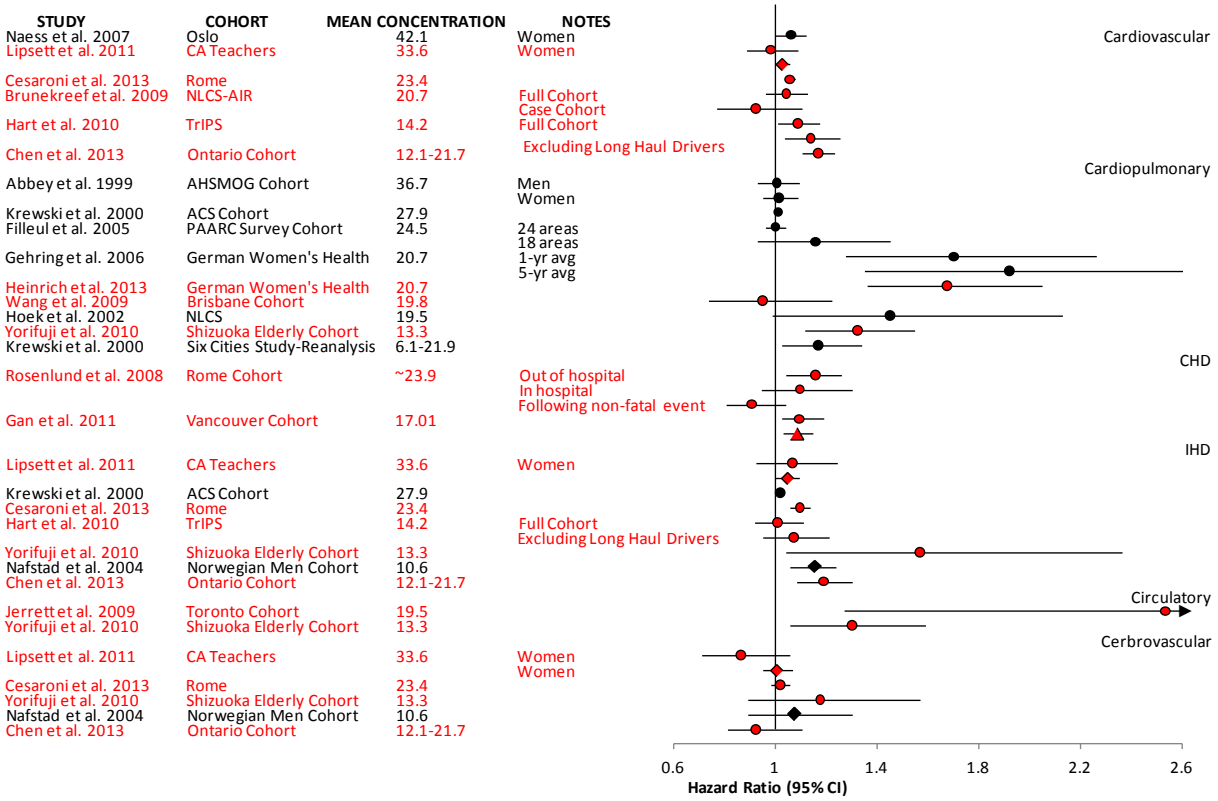
Figure 5-9 Results of studies of long-term exposure to NO_2 or NO_X and all-cause mortality.

Table 5-16 Corresponding risk estimates for Figure 5-9.

Study	Location	Notes	Relative Risk[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	1.01 (0.99, 1.03)
Krewski et al. (2000)	U.S.		0.99 (0.99, 1.00)
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)
Cesaroni et al. (2013)	Italy		1.06 (1.04, 1.06)
Tonne and Wilkinson (2013)	England and Wales	NO_X	1.04 (1.01, 1.06)
Lipfert et al. (2000)	U.S.		1.07 (1.04, 1.10)
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)
Yorifuji et al. (2010)	Japan		1.04 (0.93, 1.32)
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)

Note: Studies correspond to studies presented in Figure 5-9.

[a] Effect estimates are standardized to a 10-ppb increase in NO_2 or NO_X concentration.



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, NO_X or NO concentration. 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; Diamonds = NO_X; Triangles = NO.

Figure 5-10 Results of studies of long-term exposure to NO_2, NO, or NO_X and cardiovascular mortality.

Table 5-17 Corresponding risk estimates for Figure 5-10.

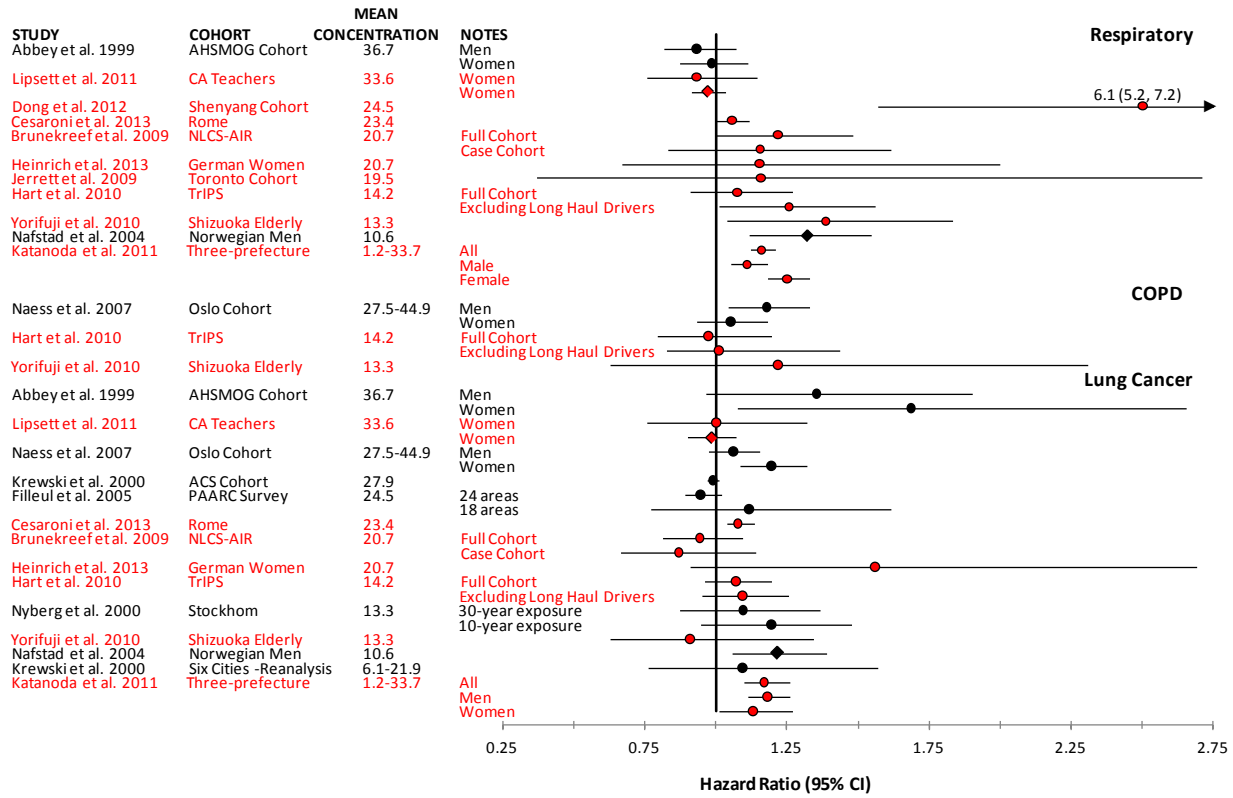
Study	Location	Notes	Relative Risk[a] (95% CI)
Cardiovascular Disease			
Naess et al. (2007)	Norway	Women	1.06 (1.00, 1.12)
Lipsett et al. (2011)	California	Women, NO_2	0.98 (0.88, 1.09)
Lipsett et al. (2011)	California	Women, NO_X	1.03 (1.00, 1.06)
Cesaroni et al. (2013)	Italy		1.06 (1.04, 1.08)
Brunekreef 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)
Chen et al. (2013)	Canada		1.17 (1.10, 1.23)
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)
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)
Coronary Heart Disease (CHD)			
Rosenlund et al. (2008b)	Italy	Out of hospital	1.16 (1.04, 1.26)
Rosenlund et al. (2008b)	Italy	In hospital	1.10 (0.94, 1.30)
Rosenlund et al. (2008b)	Italy	Following non-fatal 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)
Ischemic Heart Disease (IHD)			
Lipsett et al. (2011)	California	Women, NO_2	1.07 (0.92, 1.24)
Lipsett et al. (2011)	California	Women, NO_X	1.05 (1.00, 1.09)
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)

Table 5-17 (Continued): Corresponding risk estimates for Figure 5-10.

Study	Location	Notes	Relative Risk[a] (95% CI)
Nafstad et al. (2004)	Norway	NO_X	1.16 (1.06, 1.24)
Chen et al. (2013)	Canada		1.19 (1.08, 1.30)
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 (0.95, 1.07)
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)	Norway	NO_X	1.08 (0.89, 1.30)
Chen et al. (2013)	Canada		0.92 (0.81, 1.10)

Note: Studies correspond to studies presented in Figure 5-10.

[a]Effect estimates are standardized to a 10-ppb increase in NO_2, NO_X or NO concentration.



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 or NO_X concentration. 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; Diamonds = NO_X.

Figure 5-11 Results of studies of long-term exposure to NO_2 or NO_X and respiratory mortality.

Table 5-18 Corresponding risk estimates for Figure 5-11.

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	0.97 (0.91, 1.03)
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)
Nafstad et al. (2004)	Norway	NO_X	1.32 (1.12, 1.54)
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)
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)
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)
Filleul et al. (2005)	France	24 areas	0.94 (0.89, 1.02)

Table 5-18 (Continued): Corresponding risk estimates for Figure 5-11.

Study	Location	Notes	Hazard Ratio[a] (95% CI)
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-year Exposure	1.10 (0.87, 1.36)
Nyberg et al. (2000)	Sweden	10-year Exposure	1.20 (0.94, 1.48)
Yorifuji et al. (2010)	Japan		0.91 (0.63, 1.34)
Nafstad et al. (2004)	Norway	NO_X	1.22 (1.06, 1.39)
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)

Note: Studies correspond to studies presented in Figure 5-11.

[a] Effect estimates are standardized to a 10-ppb increase in NO_2 or NO_X concentration.

5.5.3 Summary and Causal Determination

Collectively, the evidence is suggestive of 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. and Europe, which show consistent, positive associations with total mortality, as well as deaths due to respiratory and cardiovascular disease (Lipsett et al., 2011; Hart et al., 2010; Brunekreef et al., 2009; Beelen et al., 2008b; 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., 2012; 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.

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, 2008c). Recent studies provide

evidence for an association between long-term exposure to 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. 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 (Krewski et al., 2009; Pope et al., 2002; Abbey et al., 1999). All available evidence for mortality due to long-term exposure to NO_X was evaluated using the framework described in Table II of the Preamble. The key evidence as it relates to the causal framework is summarized in Table 5-19. The overall evidence is suggestive of a causal relationship between long-term exposure to NO_2 and mortality among adults.

Table 5-19 Summary of evidence supporting a suggestive of a causal relationship between long-term 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]
At least one high-quality epidemiologic study shows an association	Positive association between long-term exposure to NO_2 and mortality in the Harvard Six Cities (HSC) Cohort, with effect estimates similar in magnitude to those observed with $PM_{2.5}$, even after adjustment for common potential confounders. Associations generally not evaluated in copollutants models.	Krewski et al. (2000)	Mean concentrations across cities (1980): 6.1-21.9 ppb
	Updated results from the Netherlands Cohort Study (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), Brunekreef et al. (2009)	Mean (1987-1996): 20.7 ppb Max: 35.5 ppb
	Recent cohort studies in the U.S. observe increases in 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; Max: 67.2 ppb
Some studies show no association	No association in several re-analyses of the American Cancer Society (ACS) cohort	Krewski et al. (2000) Pope et al. (2002)	Mean (1982-1998): 21.4-27.9 ppb
		Krewski et al. (2009)	Mean (1982-1998) 27.9; Max 51.1 ppb
	No association with total, cardiopulmonary or respiratory mortality in the California Seventh-day Adventists cohort (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. (2012)	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; Max: 67.2 ppb
		Atkinson et al. (2013)	Mean: 12.0; Max: 32.3 ppb

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to 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_X or NO_2 concentrations with which the evidence is substantiated (for experimental studies, below 5,000 ppb).

5.6 Cancer

The 1993 Oxides of Nitrogen AQCD and the 2008 ISA for Oxides of Nitrogen reported that there was no clear evidence that NO_2 or 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. 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, 2008c) 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, 2008c), additional studies have been published exploring this relationship. In addition, 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 S5-10 (U.S. EPA, 2013o).

5.6.1 Lung Cancer Incidence

Two previous studies included in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) reported positive associations between oxides of nitrogen 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-year average 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 correlation coefficient between NO_X and SO_2 was 0.63 and no association was observed between SO_2 concentration and cancer.

An HEI Research Report 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 unadjusted and adjusted 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 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 and no differences were observed between the groups. One of these cohorts was used in another study where the follow-up period was extended five years to include more cases (Raaschou-Nielsen et al., 2011). 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 study using the GEN-AIR case-control data reported on non-smokers 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.

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 due to the inclusion of other pollutants in the models nor does it appear to relate to the length of the exposure or follow-up period. Potentially important confounders, such as smoking status, were included in the analyses. Variables examined as effect measure modifiers, such as education, may be important in further understanding the association.

5.6.2 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 Netherlands Cohort Study (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.

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. 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 four 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 non-parametric 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 non-smokers 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. 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 values (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 non-respiratory 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. The inconsistency appears unrelated to exposure assessment or length of follow-up periods. Most of these studies controlled for confounders, such as smoking.

5.6.3 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 at a distribution of age and sex that matched that of the cases (Amigou et al., 2011). NO_2 concentration over 6.5 ppb were positively associated with the odds of leukemia. 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). 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 death related to leukemia.

5.6.4 Bladder Cancer Mortality

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., 2009a). 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. (2009a) further examined a three-level variable, with the lowest level being individuals in the lowest tertile of SO_2 and NO_2 concentrations ($\leq$ 4.32 ppb and $\leq$ 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 S5-10, (U.S. EPA, 2013o)]), 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.

5.6.5 Breast Cancer Incidence

A Canadian study of post-menopausal breast cancer incidence using a hospital-based case-control study design estimated NO_2 concentrations using two methods: extrapolating data from fixed-site monitoring stations or extrapolating data from predicted concentrations determined with land-use regression 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. 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. This study suggests a possible association between post-menopausal breast cancer incidence and NO_2 concentration.

An ecologic study was performed by Wei et al. (2012) using data from the Surveillance, Epidemiology, and End Results (SEER) program to determine the breast cancer incidence rate of various states and metropolitan areas and data from the US 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 rate of breast cancer incidence 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.

5.6.6 Prostate Cancer Incidence

Men enrolled in the Prostate Cancer and Environment Study (PROtEuS) 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 indentified through electoral lists and frequency matched by five-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 ten 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.

5.6.7 Animal and In Vitro Carcinogenicity and Genotoxicity Studies

Animal toxicology studies characterizing the carcinogenicity and genotoxicity of NO_2 follow. 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. This is consistent with mechanistic evidence of observed hyperplasia of the lung epithelium with NO_2 exposure (see Section 5.2.10). 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-hour exposure to 100 ppb NO_2 (Koehler et al., 2011). Percent 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 (2008c), Annex Table 4-12, Table 4-13, and Table 4-11, 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 h) and negative findings of genotoxicity seen in tests employing Drosophila recessive lethals (500,000-7,000,000 ppb NO_2, 1 h), Drosophila wing spot test (50,000-280,000 ppb NO_2, 2 days), mouse bone marrow micronuclei (20,000 ppb, 23 h), and mouse spermatocyte and lymphocyte chromosomal aberrations (100-10,000 ppb NO_2, 6 h). 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 h).

5.6.8 Animal Toxicology Studies of 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. 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 non-statistically significant five-fold increase in incidence of adenomas or adenocarcinomas 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 co-exposure 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 co-exposed to DEcCBP and NO_2 and/or SO_2 but not in those with DEcCBP 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 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.

5.6.9 Studies in Animals with Spontaneous 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 hours/day, 5days/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 to control; this finding was no longer significant at 14 or 16 months exposure.

5.6.10 Facilitation of 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 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.

5.6.11 Production of N-Nitroso Compounds and other Nitro Derivatives

Daily chemical transformations involving UV, 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 previous NO_X ISA U.S. EPA (2008c).

Table 5-20 Animal toxicological studies of carcinogenicity and genotoxicity with exposure to NO_2.

Reference	Concentration NO_2	Strain, Age, Sex (n)	Exposure conditions	Endpoints Examined
Koehler et al. (2013)	10 ppb	Human	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	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	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 3h	COMET assay, Micronucleus formation, proliferation assay, apoptosis, necrosis, cytotoxicity
Ohyama et al. (1999)	1,000, 5,000, 6,000 ppb	F344 rats, male,	Exposure to diesel exhaust particle extract-coated carbon black particles (DEPcCBP) and NO_2. IT installation of DEPcCBP 1x/week for 4 weeks. 6,000 ppb NO_2 was administered 16h/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	Strain A/J mice,	Exposure of mice with spontaneous high tumor rates to NO_2 for 6h/day, 5 days/week for 6 mo	Lung tumor multiplicity (pulmonary adenomas)
Richters and Damji (1990)	250 ppb	AKR/cum mice	Exposure of mice intermittently (7 h/day, 5days/week) to NO_2 for up to 26 weeks	Rodent survival rate.
Wagner et al. (1965)	5,000 ppb	CAF1/Jax mice	Continuous exposure to 5,000 ppb NO_2	Lung tumor multiplicity at 12, 14 and 16 mo.
Richters and Kuraitis (1981)	400 or 800 ppb	Swiss Webster mice, males (24); C57BL/6J males (90)	NO_2 exposure 8h/day, 5days/week for 10weeks (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)

Table 5-20 (Continued): Animal toxicological studies of carcinogenicity and genotoxicity with exposure to NO_2.

Reference	Concentration NO_2	Strain, Age, Sex (n)	Exposure conditions	Endpoints Examined
Richters and Kuraitis (1983)	300, 400 or 500 ppb	C57BL/6J mice (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	C57BL/6J mice	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	Adult Rats	Co-exposure with carcinogen NHPN and NO_2. NO_2 exposure for 17 mo.	Incidence of NHPN-induced lung tumors (adenoma or adenocarcinomas).
Ichinose and Sagai (1992)	500 ppb NO_2; 50 ppb NO_2 + 400 ppb O_3	Adult rats	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 NHPN-induced lung tumors (adenoma or adenocarcinomas).

5.6.12 Summary and Causal Determination

The overall evidence for long-term NO_2 exposure and cancer is suggestive of a causal relationship. This conclusion is based on evidence from some prospective epidemiologic studies reporting associations between NO_2 or NO_X exposure and 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, 2008c). 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 framework is summarized in Table 5-21.

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. Most studies of NO_2 or NO_X and lung cancer mortality reported no association, but there are some studies reporting positive associations. 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. 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 US 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 5.2.10), or promote

regenerative cell proliferation. Genotoxic and mutagenic studies with NO_2 have mixed results. Some studies with co-exposure 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, high-quality toxicological and epidemiologic studies is suggestive of a causal relationship between long-term exposure to NO_2 and cancer incidence and mortality.

Table 5-21 Summary of evidence supporting a suggestive of a causal relationship between long-term 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]
Cancer – Suggestive			
Some high-quality, epidemiologic studies provide evidence of associations.	Positive associations were observed between overall cancer incidence and mortality in multiple studies conducted in Europe and Asia	Nafstad et al. (2003), Nyberg et al. (2000), Raaschou-Nielsen et al. (2010a), Raaschou-Nielsen et al. (2011), Cesaroni et al. (2013), Filleul et al. (2005), Katanoda et al. (2011), Liu et al. (2008), Naess et al. (2007) Sections 5.6.1-5.6.6	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 32.4 ppb.
	Positive associations were also observed in studies of NO_2 concentrations and leukemia, bladder cancer, and prostate cancer.	Amigou et al. (2011), Weng et al. (2008), Liu et al. (2009a), Parent et al. (2013) Sections 5.6.1-5.6.6	Associations observed at levels as low as 6.5-8.6 ppb for leukemia
Some high-quality, epidemiologic studies demonstrate no associations.	No associations were observed between overall cancer incidence and mortality in multiple studies conducted in the United States, Europe, and Asia.	Brunekreef et al. (2009), Beelen et al. (2008a), Papathomas et al. (2011), Brunekreef et al. (2009), Beelen et al. (2008a), Cao et al. (2011), Hart et al. (2011), Heinrich et al. (2013), Krewski et al. (2009), Yorifuji et al. (2010) Sections 5.6.1-5.6.6	Means varied with estimated concentrations of NO_2 or NO_X ranging from 13.3 to 27.9 ppb.

Table 5-21 (Continued): Summary of evidence supporting a suggestive of a causal relationship between long-term 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 evidence from high-quality, toxicological studies	Studies of facilitation of metastasis and co-exposures with known carcinogens show NO_X related effects. Studies of NO_X 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 5.6.8 and 5.6.10	10,000 ppb 250 ppb 5,000 ppb 400, 800 ppb 300, 400, 500 ppb 400 ppb 4,000 ppb 500 ppb
Limited evidence for key events to inform mode of action	Finding of mutagenicity and micronucleus formation in ex vivo culture of primary human nasal epithelial cells exposed to NO_2. Mixed findings of mutagenicity and carcinogenicity in various models of NO_2 exposure in older studies, mainly in non-human species.	Koehler et al. (2013), Koehler et al. (2011), Koehler et al. (2010) Section 5.6.7 [U.S. EPA (2008c), Annex Table AX4-11, Table AX 4-12, and Table AX 4-13]	100, 1,000, 10,000 ppb

[a]Based on aspects considered in judgments of causality and weight of evidence in causal framework in Table I and Table II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to 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).

References for Chapter 5

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CHAPTER 6 POPULATIONS POTENTIALLY AT INCREASED RISK FOR HEALTH EFFECTS RELATED TO EXPOSURE TO OXIDES OF NITROGEN

6.1 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 provide an adequate margin of safety for both the population as a whole and those potentially at increased risk for health effects in response to ambient air pollution exposure (see Preface to this ISA). To facilitate the identification of populations and lifestages at greater risk for air pollutant related health effects, this chapter evaluates studies that examine factors that may contribute to the susceptibility and/or vulnerability of an individual to air pollutants. The definitions of susceptibility and vulnerability have been found to vary across studies, but in most instances "susceptibility" refers to biological or intrinsic factors (e.g., lifestage, sex, pre-existing disease/conditions) while "vulnerability" refers to non-biological or extrinsic factors (e.g., socioeconomic status) (Sacks et al., 2011; U.S. EPA, 2010b, 2009a). In some cases, the terms "at-risk" and "sensitive" populations have been used to encompass these concepts more generally. The main goal of this evaluation of evidence in this chapter is to identify and understand those factors that may result in a population or lifestage being at increased, or in some cases decreased, risk of health effects related to exposure to oxides of nitrogen, and not to categorize the factors by definition.

Individuals, and ultimately populations, could experience increased, or in some instances decreased risk, for air pollutant-induced health effects via multiple avenues. As discussed in the Preamble, risk may be modified by intrinsic or extrinsic factors, differences in dose/exposure, or differences in exposure to air pollutant concentrations. It is important to note that the emphasis of this chapter is to identify and understand the factors that potentially increase or decrease the risk of health effects related to exposure to oxides of nitrogen, regardless of whether the change in risk is due to intrinsic factors, extrinsic factors, increased dose/exposure, or a combination. The following sections examine factors that potentially lead to increased or decreased risk of health effects related to exposure to oxides of nitrogen and characterize the overall weight of evidence and the magnitude of effect, when possible, for each factor.

Approach to Classifying Potential At-Risk Factors

The systematic approach used to classify potential at-risk factors is described in more detail in the Preamble. The evidence evaluated includes recent studies discussed in Chapter 4 and Chapter 5 of this ISA building on the evidence presented in the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) and the 1993 Air Quality Criteria for Oxides of Nitrogen (U.S. EPA, 1993) and using the current framework to systematically classify at-risk populations or lifestages that has been presented in past ISAs (U.S. EPA, 2013a, b). In general, the current approach builds on the causal framework used throughout the ISA; conclusions made regarding the strength of evidence are based on evaluation and synthesis across scientific disciplines for each factor that may contribute to increased or decreased risk of a health effect related to exposure to oxides of nitrogen. Important considerations in the evaluation of stratified results include a priori versus post-hoc analyses, multiple comparisons, and small sample sizes in individual strata. These factors can increase the probability of finding associations by chance or reduce power to detect associations in subgroup analyses. Thus, coherence and biological plausibility from other lines of evidence are important to inform these potential uncertainties in epidemiologic results. As discussed in the Preamble, this evaluation focuses on epidemiologic studies that conducted stratified analyses to compare populations or lifestages exposed to similar air pollutant concentrations within the same study design in addition to controlled human exposure and toxicological studies in animals examining effects various at-risk factors (e.g., genetic background or pre-existing disease) on response to exposure to oxides of nitrogen. More detailed discussions of these individual studies are presented in Chapter 4 and Chapter 5 as the objective of this chapter is to evaluate and categorize the evidence for each factor as adequate, suggestive, inadequate, or no effect. These categories are described in more detail in Table 6-1, and a summary of the classification of evidence for the factors considered for increased risk of health effects related to exposure to oxides of nitrogen is presented in Section 6.6.

Table 6-1 Classification 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 or decreased risk of air pollutant-related health effect(s) relative to some reference population or lifestage. Where applicable this includes coherence across disciplines. Evidence includes multiple high-quality studies.
Suggestive evidence	The collective evidence suggests that a factor results in a population or lifestage being at increased or decreased risk of an air pollutant-related health effect relative to some reference population or lifestage, but the evidence is limited due to some inconsistency within a discipline or, where applicable, a lack of coherence across disciplines.
Inadequate evidence	The collective evidence is inadequate to determine if a factor results in a population or lifestage being at increased or decreased risk of an air pollutant-related health effect relative to some reference population or lifestage. The available studies are of insufficient quantity, quality, consistency, and/or statistical power to permit a conclusion to be drawn.
Evidence of no effect	There is substantial, consistent evidence within a discipline to conclude that a factor does not result in a population or lifestage being at increased or decreased risk of air pollutant-related health effect(s) relative to some reference population or lifestage. Where applicable this includes coherence across disciplines. Evidence includes multiple high-quality studies.

6.2 Genetic Factors

Genetic variation in the human population is known to contribute to numerous diseases and differential physiologic responses. Furthermore, genetic background has been considered as a response modifying factor in studies examining air pollution-related health effects, including NO_2. Studies included in this ISA that evaluate genetic factors have used a targeted approach, focusing on specific genes that are suggested to have a role in signaling pathways involved in biological responses to air pollutants. In particular, most studies examined variants for genes encoding antioxidant enzymes (glutathione S-transferases [GSTM1 and GSTP1], glutathione synthetase [GSS], glutathione reductase [GSR], and NADPH reductase quinone 1[NQO1]) and mediators of immune response (tumor necrosis factor [TNF] and toll-like receptor 4 [TLR4]). Modification by gene variants has been examined primarily for NO_2-associated respiratory outcomes, although a few studies examined other health outcomes (i.e., cognitive function, heart rate variability). It is important to note that the functional or biological consequence of some of the gene variants examined in the literature is unknown; however, when available, the variant effect is described (Table 6-2).

Oxidative stress has been described as a key process underlying the respiratory effects of NO_2 exposure (Section 3.3.2.1); however, studies did not find NO_2-associated respiratory effects to be modified consistently by variants in GSTM1 or GSTP1, which encode enzymes with altered oxidative metabolizing activity. Romieu et al. (2006) found

associations of short-term NO_2 exposure with respiratory symptoms and asthma medication to be larger for children with the GSTM1 positive genotype compared to children who were GSTM1 null. GSTM1 positive is associated with normal antioxidant activity and characterized 62% of the study population. In contrast, Castro-Giner et al. (2009) did not observe the association between long-term NO_2 exposure and asthma to differ by GSTM1 genotype. For the GSTP1 variant at codon 105, short-term NO_2 exposure was associated with larger risk of respiratory symptoms and medication use among children with asthma with the Ile/Ile or Ile/Val genotype (Romieu et al., 2006), which is not associated with reduced antioxidant activity. However, studies of long-term exposure found no difference between the group having the Ile/Ile genotype and the group with Ile/Val or Val/Val genotypes for associations of NO_2 with risk of asthma or wheeze (Castro-Giner et al., 2009; Melén et al., 2008). Melén et al. (2008), however, did find evidence for increased NO_2-related risk of asthma in children with the GSTP 114Val genotype compared to children having the GSTP Ala114Ala genotype.

Variant genotypes in other glutathione metabolism pathway genes (GSS, GSR, GCLM, and GCLC) were evaluated for potential effect modification of impaired lung function growth in children and exposure to oxides of nitrogen (Breton et al., 2011). Among the multiple comparisons made, only variation in the GSS haplotype containing a polymorphism of unknown function (rs1801310) was associated with differences in lung function growth (FEV_1 and MMEF) attributable to NO_2 exposure. Baja et al. (2010) was the only study to report on differences in non-respiratory effects (heart rate-corrected QT interval) of NO_2 exposure across glutathione genotypes. A strength of this study was analysis of genetic variants as a composite rather than performing multiple comparisons of individual variants. A genetic susceptibility score was determined for each subject based on genotypes for 10 different genes involved in oxidative stress responses; subjects with a high genetic susceptibility score had mostly unfavorable genotypes while subjects with a low genetic susceptibility score had mostly genotypes associated with protection against oxidative stress. The association between heart-rate-corrected QT interval and NO_2 was greater among subjects with a high genetic susceptibility score (Baja et al., 2010).

The enzyme NADPH dehydrogenase (quinone-1) (NQO1) is also associated with oxidative metabolism, and adults homozygous for the major allele of NQO1 rs2917666 were found to have higher NO_2-associated prevalence of asthma and bronchial hyperresponsiveness (Castro-Giner et al., 2009), though the functional consequence of this polymorphism is unknown. Associations between NO_2 and asthma prevalence did not vary for other genotypes of NQO1 (rs1800566 and rs10517). Further, this study examined several genetic variants, and the prevalence of any given variant was 15% or less.

Mediators of the immune response including TNF and TLR4 are known to have a role in oxidant-induced inflammation and asthma pathogenesis and as such they have been examined as potential factors that may increase the risk of NO_2-related health effects, but evidence does not clearly demonstrate effect modification by TNF variants. Castro-Giner et al. (2009) evaluated associations between NO_2 and asthma prevalence across three polymorphisms in TNF, comparing homozygotes for the major allele to heterozygotes and homozygotes for the minor allele. Subjects homozygous for the major allele of TNFA rs2844484 had higher odds of NO_2-associated asthma prevalence compared to other genotypes; however, no differences were observed for other polymorphisms, including the common TNF 308 variant (rs1800629). Melén et al. (2008) found that this TNF 308 variant genotype in combination with GSTP Val/Val increased risk for NO_2-associated sensitization to allergens in children relative to other diplotypes. This analysis was based on small numbers, and the association in the group with both variants was estimated with large imprecision. Risk in individuals with polymorphisms in TLR4 was also evaluated, but no genotype differences were observed for NO_2-associated asthma (Castro-Giner et al., 2009).

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 induce AHR in adults (Section 4.2.2.1), providing a plausible role for variants in this gene in increasing the risk of NO_2-associated respiratory effects. However, evidence for effect modification is inconsistent. Castro-Giner et al. (2009) found that variant genotypes for several ADRB2 polymorphisms did not modify odds of NO_2-associated asthma prevalence. In contrast, Fu et al. (2012a) demonstrated that the association between indoor NO_2 exposure and severe childhood asthma was stronger among children with higher methylation of the ADRB2 promoter, which is associated with reduced expression of the receptor. Coherent with the mixed evidence for effect modification by ADRB2 variants, there is mixed evidence for bronchodilator use modifying NO_2-associated respiratory effects (Section 4.2.2.2).

Antioxidant and immune modulation have been described as key events to inform the mode of action underlying the health effects associated with NO_2 exposure (Sections 3.3.2.1 and 3.3.2.6). The epidemiologic evidence demonstrates that some antioxidant and immune-related gene variants can modify response to NO_2 exposure, but there are inconsistent results for the modification by any particular gene variant of associations with respiratory outcomes across studies. Several results are based on post-hoc analyses comprising small proportions of study populations and multiple comparisons. Further, there are no controlled human exposure or toxicological studies comparing effects of NO_2 across different genotypes. However, a role for variants in enzymes involved in

oxidative metabolism is supported by findings in animals that dietary antioxidant vitamin levels influence NO_2-related oxidative stress (Section 6.5.1). Overall, the collective evidence suggests that genetic factors modify risk for NO_2-related asthma outcomes, based on evidence from variant genotypes in glutathione metabolism.

Table 6-2 Summary of epidemiologic studies evaluating effect modification by genetic variants.

Gene variant	Referent genotype	Variant effect	Direction of effect modification by variant	Health outcome/ Population	Reference
GSTM1 null	GSTM1 positive	Null oxidant metabolizing capacity	↓	Respiratory symptoms and medication use in asthmatic children	Romieu et al. (2006)
			↔	Asthma prevalence in adults	Castro-Giner et al. (2009)
GSTP1 Val105Val (rsID 947894)	Ile105Ile	Reduced oxidant metabolizing capacity (Val/Val)	↓	Respiratory symptoms and medication use in asthmatic children	Romieu et al. (2006)
			↔	Respiratory symptoms and asthma prevalence in children	Melén et al. (2008)
			↔	Asthma prevalence in adults	Castro-Giner et al. (2009)
GSTP Ala114Val or Val114Val (rs1799811)	GSTP Ala114Ala	Unknown	↑	Asthma prevalence in children	Melén et al. (2008)
GSS haplotype (0100000; rs1801310)	Other haplotypes	Unknown	↑	Lung function growth in children	Breton et al. (2011)
GSR Various SNPs	Other haplotypes	Unknown	↔	Lung function growth in children	Breton et al. (2011)
GCLM Various SNPs	Other haplotypes	Unknown	↔	Lung function growth in children	Breton et al. (2011)
GCLC Various SNPs	Other haplotypes	Unknown	↔	Lung function growth in children	Breton et al. (2011)
NQO1 CC (rs2917666)	GC or GG	Unknown	↑	Asthma prevalence in adults	Castro-Giner et al. (2009)
TNF 308 308 GA/AA	GG	Altered TNF expression	↔	Asthma prevalence in adults	Castro-Giner et al. (2009)

Table 6-2 (Continued): Summary of epidemiologic studies evaluating effect modification by genetic variants.

Gene variant	Referent genotype	Variant effect	Direction of effect modification by variant	Health outcome/ Population	Reference
GSTP1 105 Ile/Val or Val/Val + TNF 308 GA/AA	Any other diplotype	Lower oxidant metabolizing capacity and altered TNF expression	↑	Sensitization and lung function in children	Melén et al. (2008)
ADRB2 Intermediate or high levels of methylation	Low levels of methylation	Reduced expression of ADRB2	↑	Asthma severity in children	Fu et al. (2012a)
ADRB2 rs1042713 GG rs1042714 C/C rs1042718 C/C rs1042719 G/G	G/A or AA C/G or G/G C/A or A/A G/C or C/C	Unknown	↔	Asthma prevalence in adults	Castro-Giner et al. (2009)

6.3 Pre-existing Disease/Conditions

Individuals with pre-existing disease are often considered as a subpopulation at greater risk for air pollution-related health effects because they may be at a compromised biological state depending on the disease and severity. The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) concluded that those with pre-existing pulmonary conditions were likely to be at greater risk for NO_2-associated health effects, especially individuals with asthma. The majority of recent studies examining effect modification by pre-existing disease continued to focus on asthma, though some studies provide evidence for COPD, cardiovascular disease, and diabetes. Sections 6.3.1, 6.3.2, 6.3.3, and 6.3.4 discuss these studies and draw conclusions regarding risk related to each pre-existing disease. Table 6-3 presents the prevalence of these diseases according to the CDC'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. Data for children, when available, are discussed within the relevant sections.

Table 6-3 Prevalence of respiratory diseases, cardiovascular diseases, and diabetes among adults by age and region in the U.S. in 2010.

	Adults (18+)	Age (%)[a]				Region(%)[b]			
Chronic Disease/ Condition	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									
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

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

[b]Percent 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 CDC report.

6.3.1 Asthma

Approximately 8.2% of adults and 9.5% of children in the United States currently have asthma (Schiller et al., 2012; Bloom et al., 2011), and it is the leading chronic illness of children. A variety of factors affecting the health of individuals with asthma have been identified including ambient air pollution. The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) concluded that individuals with pre-existing pulmonary conditions are likely at greater risk for ambient NO_2-related health effects, with the strongest evidence for individuals with asthma. This conclusion was based on evidence for NO_2-related increases in asthma-related hospital admissions and emergency department (ED) visits and AHR and respiratory symptoms in people with asthma. A number of studies discussed in this ISA have evaluated the potential for increased risk of NO_2-related respiratory effects among individuals with asthma through comparisons of individuals

with or without asthma, comparisons among groups varying in asthma severity, and comparisons among groups varying in asthma medication use.

Among epidemiologic studies comparing children with and without asthma, a few studies that defined comparisons a priori found larger NO_2-related increases in respiratory symptoms (Patel et al., 2010), or decrements in lung function (Timonen and Pekkanen, 1997) in children with asthma. However, other studies reported similar (Lin et al., 2011; Gauderman et al., 2004) or larger NO_2-related respiratory effects in children without asthma (Berhane et al., 2011; Barraza-Villarreal et al., 2008) or null associations in either group (Flamant-Hulin et al., 2010; Holguin et al., 2007). In most studies, asthma was ascertained as self or parental report of physician-diagnosed asthma; however, children with asthma were not at increased risk of NO_2-related respiratory effects even with asthma assessed by a pediatric allergist (Barraza-Villarreal et al., 2008). Barraza-Villarreal et al. (2008) found that associations of interleukin 8 and forced vital capacity with NO_2 were actually stronger in children without asthma, though the majority of those children had positive atopy as defined by having a positive skin prick test to an allergen. Berhane et al. (2011) found that associations were stronger in children with a history of respiratory allergies compared to those without. These results suggest increased risk associated with allergy, which is supported by evidence for NO_2-induced increases in allergic inflammation in controlled human exposure and animal toxicological studies (Sections 3.3.2.6.2, and 4.2.4.3). However, comparisons of children with and without atopic asthma were inconsistent, with Mann et al. (2010) finding larger NO_2-related increases in wheeze among children with asthma sensitized to cat or fungal allergens but other studies finding no difference in associations of NO_2 with eNO or lung function decrements in children with and without atopic asthma (Sarnat et al., 2012; Ranzi et al., 2004).

Among studies, there was heterogeneity in asthma severity and asthma medication use, and there was some evidence for these factors contributing to heterogeneity in NO_2-related respiratory effects. Mann et al. (2010) examined asthma severity and found larger NO_2-related increases in wheeze among boys with mild, intermittent asthma. There were several studies examining medication use, and overall, results for effect modification were mixed. Some studies found larger NO_2-related increases in pulmonary inflammation or oxidative stress among ICS users (Qian et al., 2009a; Delfino et al., 2006) while others reported stronger associations with pulmonary inflammation among ICS nonusers (Sarnat et al., 2012; Hernández-Cadena et al., 2009; Liu et al., 2009b). Similarly, larger NO_2-related lung function decrements were found among bronchodilator users in one study (Qian et al., 2009b) and among those not using bronchodilators in another (Delfino et al., 2008a). It is difficult to interpret these findings without a consistent definition of asthma

and medication use across studies. For example, ICS use could possibly represent different subgroups of asthma (i.e., severe or well-controlled). Furthermore, most comparisons for medication use were not specified a priori, so these observations could be attributable to a higher probability of finding differences by chance or a lower probability of finding differences because of insufficient statistical power in subgroup analyses.

Consistent with epidemiologic studies, controlled human exposure studies provide some evidence that asthma status may be related to health outcomes associated with NO_2 exposure, particularly for airway hyperresponsiveness (AHR), but there are inconsistencies in results across studies and outcomes. Across studies, short-term exposure to oxides of nitrogen was found to induce AHR in adults with and without asthma. Although no individual study compared adults with and without asthma, increased risk of adults with asthma was indicated by lower concentrations of NO_2 exposure inducing nonspecific AHR in adults with asthma (200-300 ppb for 30 minutes, 100 ppb for 1 h, Section 4.2.2.2) than healthy adults without asthma (1,500-2,000 ppb for 1-3 hours, Section 4.2.2.1). A few controlled human exposure studies did compare subjects with asthma and healthy controls with respect to other respiratory outcomes, and NO_2-induced decreases in FEV_1 were found in subjects with asthma but not healthy subjects (Jörres et al., 1995). However, other studies did not find NO_2-related effects on lung function or inflammation among subjects with asthma or healthy subjects (Vagaggini et al., 1996); Linn et al. (1985b). Most studies that examined only subjects with asthma did not find NO_2-induced decrements in lung function (Jenkins et al., 1997; Jörres and Magnussen, 1991; Kleinman et al., 1983). In contrast, Bauer et al. (1986) reported that NO_2 exposure with exercise in subjects with asthma yielded a significant reduction, approximately 10%, in forced expiratory volume in 1 second (FEV_1) and partial expiratory flow rates at 60% of total lung capacity. Studies of allergic responses in adults with asthma also were mixed in reporting NO_2-related effects on Th2 cytokines, eosinophil activation, and pulmonary neutrophilia (Riedl et al., 2012; Witten et al., 2005; Barck et al., 2002).

As described in Section 4.2.9, the strongest evidence for a causal relationship for respiratory effects of short- and long-term NO_2 exposure is that for asthma morbidity. Compelling evidence indicating increased risk of individuals with asthma is provided by controlled human exposure studies showing increased sensitivity of adults with asthma to NO_2-induced AHR. While some epidemiologic studies with a priori-defined comparisons of children with and without asthma indicated larger NO_2-associated respiratory effects in children with asthma, other studies did not indicate increased risk of children with asthma. Some studies showed effect modification by atopy, asthma severity, or asthma

medication use, whereas others did not. Controlled human exposure studies in adults with asthma indicated greater NO_2-related effects for some outcomes but not others, but the lack of comparison to healthy adults limits interpretation. Despite some inconsistencies across disciplines, the collective evidence suggests that asthma may increase risk for NO_2-related health effects, particularly AHR, as demonstrated in controlled human exposure studies and some epidemiologic studies.

6.3.2 Chronic Obstructive Pulmonary Disease (COPD)

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 4.3% and 1.9% of the U.S. adult population (Schiller et al., 2012). Given that people with COPD have compromised respiratory function, they may be at increased risk of NO_2-related health effects.

Of the epidemiologic studies evaluated in this ISA, only Suh and Zanobetti (2010b) conducted stratified analyses to examine the potential of differential risk in people with and without COPD, albeit in relation to cardiovascular rather than respiratory-related health effects. Further, associations were compared between people with COPD and people with previous MI. NO_2-associated decreases in PNN50, a measure of heart rate variability were larger among people with COPD than those with MI; however, associations with other measures of heart rate variability were similar between groups or larger among people with MI. In contrast, a previous study found with larger NO_2-related cardiovascular-related ED visits among people with COPD than people without COPD (Peel et al., 2007).

Unlike the epidemiologic study discussed above, controlled human exposure studies that examined NO_2-related health effects in people with COPD focused on measuring respiratory endpoints (Gong et al., 2005; Vagaggini et al., 1996; Morrow et al., 1992; Linn et al., 1985a). In comparisons of older adults with and without COPD, Morrow et al. (1992) reported larger NO_2-induced decrements in lung function in adults with COPD than never-smoker elderly subjects: 8.2% decrease versus 0.22% decrease in FVC and a 4.82% decrease versus a 1.25% increase in FEV_1. Similarly, Vagaggini et al. (1996) reported decreased (approximately 10%) FEV_1 in subjects with COPD following NO_2 exposure compared to air control exposures, while decrements were not observed in healthy adult subjects. However, other studies that examined only adults with COPD did

not report any changes in lung function or pulmonary inflammation following NO_2 exposure (Gong et al., 2005; Linn et al., 1985a).

In conclusion, controlled human exposure studies provide some evidence indicating that NO_2 exposure can result in larger pulmonary function decrements in individuals with COPD, relative to healthy controls. Epidemiologic evidence points to increased risk in adults with COPD, but in relation to cardiovascular effects, limiting the ability to assess coherence between disciplines. However, the cardiovascular and respiratory systems are linked in that inflammation and poor gas exchange associated with COPD can lead to cardiac tissue damage. Overall, the collective evidence, particularly from controlled human exposure studies, suggests that people with COPD are at increased risk of NO_2-related health effects relative to individuals without COPD.

6.3.3 Cardiovascular Disease (CVD)

Cardiovascular disease is the primary cause of death in the United States, and it is estimated that 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 (Schiller et al., 2012). Many studies investigating health effects associated with NO_2 have included individuals with pre-existing CVD, allowing for evaluation of whether pre-existing CVD modifies risk for NO_2-related health effects.

Associations between short-term increases in ambient NO_2 concentrations and cardiovascular hospital admissions or ED visits were not consistently greater among individuals with pre-existing cardiovascular disease. Individuals with hypertension were found to have larger NO_2-related risks of ED visits for arrhythmia (Peel et al., 2007) but not ED visits for ischemic heart disease (IHD) or congestive heart failure (CHF) (Peel et al., 2007) or hospital admissions for myocardial infarction (MI) (D'Ippoliti et al., 2003). The association between ambient NO_2 and hospital admissions for MI was larger among individuals with conduction disorders but not individuals with cardiac arrhythmia or heart failure (D'Ippoliti et al., 2003). A larger NO_2-associated increase in hospital admissions for IHD was found among individuals with a secondary diagnosis of CHF; however, this could have been attributable to the large percentage of IHD cases with a secondary CHF diagnosis (Mann et al., 2002).

Other studies found that pre-existing cardiovascular disease modified risk of NO_2-associated mortality. Chiusolo et al. (2011) found the strongest evidence for increased risk of NO_2-related mortality among individuals with diseases of the cardiovascular system, which was consistent with results from Berglind et al. (2009) that demonstrated increased NO_2-associated mortality among survivors of MI.

Some studies have found that individuals with pre-existing CVD are not at increased risk for NO_2-associated effects on cardiac function; however, another study found no difference in NO_2-associated ventricular tachyarrhythmia among individuals with and without ischemic heart disease or among categories of left ventricular ejection fraction (Ljungman et al., 2008). The latter finding is supported by results from a controlled human exposure study in which subjects with coronary heart disease or impaired left ventricular systolic function experienced no changes in heart rate or HRV with a 1-hour exposure to 400 ppb NO_2 (Scaife et al., 2012).

Toxicological studies using ApoE deficient ($ApoE^{-/-}$) mice as a model of hyperlipidemia and atherosclerosis have demonstrated effects following NO_2 exposure. Campen et al. (2010) and (Seilkop et al., 2012) reported changes in heme oxygenase-1, endothelin-1, and tissue inhibitor of metalloproteinase-2 as well as lipid peroxidation in the aorta following exposure to NO_2. However the connection between expression of these genes and cardiovascular outcomes is not well described, limiting the ability of these studies to provide biological plausibility for the potential increased risk of NO_2-related health effects observed in epidemiologic studies.

The evidence evaluating risk for NO_2-associated health effects in individuals with and without pre-existing CVD is inconsistent within and across various study designs and outcomes in epidemiologic and toxicological studies. The epidemiologic evidence indicates that risk for NO_2-associated mortality may be greater among individuals with pre-existing CVD, but evidence is inconsistent for cardiovascular hospital admissions, ED visits, and measures of cardiac function. Further, animal toxicological studies do not provide biological plausibility for epidemiologic results. Because of the inconsistencies across epidemiologic study designs and outcomes and lack of clear biological plausibility, the evidence is inadequate to determine whether pre-existing CVD increases risk for health effects associated with NO_2 exposure.

6.3.4 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). The causes of type 2 diabetes are not fully understood but chronic inflammation has been suggested as an important factor in the development of the disease. The available epidemiologic studies each examined a different outcome and produced inconsistent evidence of associations with NO_2 exposure. An Italian multicity study found stronger associations between NO_2 and

mortality among individuals with pre-existing diseases of the cardiovascular system, including diabetes (Chiusolo et al., 2011), whereas no effect modification by diabetes was observed in studies of respiratory hospital admissions or lung cancer mortality (Faustini et al., 2013; Yorifuji et al., 2010). Results also were inconsistent for cardiovascular effects. A stronger association between NO_2 and heart rate-corrected QT interval was found in individuals with diabetes (Baja et al., 2010), whereas a stronger association between decreased HRV and ambient NO_2 was found among individuals without a history of diabetes (Huang et al., 2012a). No effect modification was reported for ventricular tachyarrhythmia (Ljungman et al., 2008). Overall, the epidemiologic evidence across studies is inconsistent for cardiovascular effects as well as other outcomes, the collective evidence is inadequate to determine if diabetes increases risk NO_2-associated health effects.

6.4 Sociodemographic Factors

6.4.1 Lifestage

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) indicated that there was supporting evidence for age-related differences in health effects related to NO_2 exposure, particularly for children with asthma and older adults. Differential health effects of NO_2 across age groups may be due to several factors:

1) 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.
2) Older adults (typically considered those 65 years of age or greater) are generally at greater risk for ill health for a variety of reasons, including weakened immune function, impaired healing, decrements in pulmonary and cardiovascular function, and greater prevalence of pre-existing disease (Table 6-3).
3) Dose/exposure to oxides of nitrogen due to ventilation and time-activity patterns may vary across age groups.

More specifically, studies of exposure to oxides of nitrogen have identified time-activity patterns and spatial variability in NO_2 concentrations to be determinants of inter-individual variability in exposure [(Mölter et al., 2012; Kousa et al., 2001), and Section 2.6] and time-activity patterns have been shown to differ between children and adults. In comparisons of children (mostly less than 8 years of age), parents of young children (mostly under age 55), and older adults (mostly older than 54 years of age), children were more likely than adults or older adults to take part in vigorous activity or aerobic exercise

(indoors and outdoors). Children were also more likely to spend over 30 minutes performing vigorous outdoor physical activity compared with either group of adults (Wu et al., 2011b). Additionally, this study demonstrated small differences among age groups in time spent in various microenvironments. Differences in time-activity patterns across age groups could potentially result in different NO_2 exposure and may inform differential health effects associated with NO_2 in children and older adults. However, Meng et al. (2012a) suggested a weaker association between personal NO_2 exposure of children and ambient NO_2 concentrations in a meta-analysis.

6.4.1.1 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). Furthermore, it is generally recognized that children spend more time outdoors than adults in addition to having higher ventilation rates relative to lung volume, and thus, may have greater exposure to air pollutants, including NO_2.

The 2008 ISA for Oxides of Nitrogen reviewed several studies demonstrating larger increases in asthma-related hospital admissions in association with ambient NO_2 in children compared to adults and reported that children may be at greater risk for NO_2-associated health effects (U.S. EPA, 2008c). Consistent with these findings, several recent studies reported larger NO_2-related asthma hospital admissions and ED visits in children living in diverse locations including the U.S., Canada, Greece, and Hong Kong (Son et al., 2013; Ko et al., 2007b; Villeneuve et al., 2007). Son et al. (2013) found that children had a 3.4 fold increase in asthma hospitalizations relative to adults (15-64 years), similar to a study by Ko et al. (2007b) reporting a 2.2 fold increase in hospitalizations due to acute asthma exacerbation in children (0-14 years) compared to adults (15-65). In comparisons of children in different age groups, studies found larger NO_2-related risks of asthma hospital admissions or ED visits among younger children (e.g., age 0-4 years, 2-4 years) than children ages 5-14 years (Samoli et al., 2011; Villeneuve et al., 2007). These latter results may have weaker implications since diagnosis in children below the age of 5 years is less reliable. Evidence for other asthma outcomes did not clearly indicate increased risk for children. Sinclair et al. (2010) found increased risk for children for NO_2-related asthma outpatient visits, whereas Burra et al. (2009) did not find differences for asthma physician visits by age. NO_2-associated asthma medication sales also did not vary in children or adults (Laurent et al., 2009).

Because there are differences in lung development over the course of childhood, risk may vary among children according to the time window of exposure. Information on critical

time windows of exposure has been provided by studies of long-term NO_2 exposure and respiratory effects (Section 5.2.2.1), which found associations of asthma, lung function decrements, and allergic sensitization or conditions with NO_2 concentrations at birth or the first year of life and with exposure in the year of diagnosis, other periods in childhood, or lifetime exposure. Among studies that compared time periods of exposure, several found larger risks of respiratory effects in individuals ages 4-21 years (mostly children) associated with NO_2 exposure in the first year of life compared with NO_2 exposure in the first three years of life, year before diagnosis, or over a lifetime (Gruzieva et al., 2013; Nishimura et al., 2013a; Gruzieva et al., 2012; Schultz et al., 2012). These results suggest that higher NO_2 exposure of children early rather than later in life may increase their risk of developing respiratory effects.

Toxicological studies have evaluated differential effects of NO_2 among juvenile and mature animals on indicators of lung injury, inflammation, and lung host defense. These studies found effects of NO_2 exposure in mature rats and guinea pigs (exposures beginning at 5 or 8 weeks) and not juvenile animals (exposures beginning after birth or 5 days) with respect to increases in pulmonary inflammation and impaired alveolar macrophage function (Kumae and Arakawa, 2006) and lung damage (Azoulay-Dupuis et al., 1983). Although these results are in contrast to epidemiologic observations, the endpoints examined in experimental animals do not have direct coherence with asthma morbidity endpoints examined in the majority of epidemiologic studies.

In conclusion, recent epidemiologic studies generally demonstrate that NO_2-associated respiratory and asthma hospital admissions and ED visits are greater in children compared to adults, which is consistent with previous conclusions from the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c). There are some inconsistencies in the evidence as some studies did not find children to be at greater risk and age groups examined across studies varied. In children, results indicate that NO_2 exposure early in life may increase risk of respiratory effects. Limited toxicological evidence suggests greater NO_2-related respiratory effects in mature animals than juveniles, though the endpoints examined do not have direct coherence with the asthma morbidity found in the epidemiologic evidence and is not considered to be in conflict. In addition, time-activity patterns and ventilation rates have been shown to differ between children and adults, such that children are more likely to partake in vigorous outdoor activities and have higher ventilation. While these factors can influence exposure and/or dose, such information is not available for oxides of nitrogen. Overall, there is substantial, consistent evidence from epidemiologic studies to conclude that children are at greater risk of NO_2-associated health effects, particularly respiratory hospital admissions and ED visits.

6.4.1.2 Older Adults

Older adults may be at greater risk for NO_2-associated health effects due to a number of intrinsic factors as well as differences in time-activity patterns relative to other lifestages. In addition, time-activity patterns and ventilation rates are different among older adults than children and young to middle-aged adults, which likely contribute to different NO_2 exposure for this lifestage. According to the 2008 National Population Projections issued by the U.S. Census Bureau, approximately 12.9% of the U.S. population is 65 years or older and it is estimated that by 2030, this fraction will 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 associated with NO_2 exposure.

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) indicated that older adults were at increased risk for NO_2-associated morality and respiratory hospital admissions but not cardiovascular effects. Recent studies strengthen the evidence for hospital admissions and indicate elevated risk of older adults for admissions for asthma, respiratory disease, COPD, and lower respiratory tract infections (LRTI). Ko et al. (2007b) and Villeneuve et al. (2007) demonstrated larger associations between NO_2 and asthma hospital admissions in adults older than 65 years compared to individuals 15-64 years old or 45-64 years old, respectively. Adults aged 65 years and older were found to have <1 or 2.6 fold greater risk than younger adults in these studies. Villeneuve et al. (2007) also found the largest risk among adults ages 75 years and older, though larger associations were not observed for adults 45-64 years compared to individuals 15-44 years. Wong et al. (2009) found NO_2-related respiratory hospital admissions (i.e., respiratory disease, COPD, and LRTI in individuals with COPD) to be greater among older adults (>65 years) relative to all ages. Son et al. (2013) also evaluated risk for NO_2-related respiratory hospital admissions, but only found older adults (65-74 years and ≥ 75 years) to have larger associations for allergic disease relative to other adults (15-64 years). Arbex et al. (2009) specifically examined ED visit for COPD association with ambient NO_2 and found greater risk among those older than 65 compared to adults 40-64. Evidence for biological plausibility of differential effects of NO_2 by age is limited. Controlled human exposure studies of older adults found no significant or small decrements in lung function with NO_2 exposure (Gong et al., 2005; Morrow et al., 1992) and no changes in SpO_2 or sputum cell counts (Gong et al., 2005).

Increased risk in older adults is substantiated by evidence for mortality, particularly recent multicity studies finding NO_2-associated mortality to be greatest among individuals older than 65 years, and even greater among those older than 75 years (Chen et al., 2012b; Cakmak et al., 2011b). Chiusolo et al. (2011) also observed larger

associations between NO_2 and mortality among those greater than 85 years old compared to other adults; however, this study did not demonstrate increasing risk with increasing age as individuals between 35-64 years of age had larger associations compared to those 65-74 or 75-84 years of age. A few studies did not find elevated risk of NO_2-associated mortality among older adults. Cesaroni et al. (2013) reported that adults less than age 60 years old have higher NO_2-associated all-cause, cardiovascular, and ischemic heart disease mortality. For NO_2-associated lung cancer mortality, risk was increased among adults age 60 years and older (Cesaroni et al., 2013) but similar between those less than or older than age 75 years (Yorifuji et al., 2010). Although age comparisons differed among studies, adults ages 65-74 years were found to have 2 or 6 fold greater risk of NO_2-related mortality (Cakmak et al., 2011b; Chiusolo et al., 2011)

Consistent with previous studies, recent studies did not consistently show older adults to be at increased risk of NO_2-related cardiovascular effects. A larger NO_2-associated decrease in the LF component of HRV was found among adults older than age 50 years compared with younger adults or children (Min et al., 2008), whereas no association was found between NO_2 and ventricular tachyarrhythmias in any age group (Ljungman et al., 2008). Controlled human exposure studies have not evaluated effects of NO_2 on cardiovascular function in older adults.

There is substantial and consistent evidence demonstrating older adults are at increased risk for NO_2-associated respiratory hospitalizations and mortality. Furthermore, this recent evidence is consistent with that from the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) and strengthens previous conclusions. There is inconsistent evidence of effect modification by age for cardiovascular outcomes associated with NO_2 exposure. Additionally, the limited evidence from controlled human exposure studies in older, healthy adults is not coherent with the evidence from epidemiologic studies. Overall, there is consistent epidemiologic evidence for respiratory effects and mortality across various age ranges to conclude that there is adequate evidence indicating that older adults are at increased risk for NO_2-related health effects.

6.4.2 Socioeconomic Status

Socioeconomic status (SES) is a measure of an individual's income, education, and occupation that can indicate inequities in access to resources such as healthcare. According to U.S. Census data, 15.9% (approximately 48.5 million) of Americans were of poverty status in 2011 according to household income, which is one metric used to define SES (Bishaw, 2012). A number of other indicators are also used including: education level, employment status, insurance status, social deprivation, and access to

health care. Exposure to air pollution may vary according to poverty status and SES, and studies have further demonstrated that SES indicators can modify the association between air pollution and health effects, though the influence of SES on this relationship is not uniform. Deguen and Zmirou-Navier (2010) concluded that individuals having lower SES generally are exposed to higher levels of ambient air NO_2. However, O'Neill et al. (2003) noted that several factors might alter this relationship, including changing development, migration, and transportation patterns that could result in individuals of higher socioeconomic status having higher exposures. Yu and Stuart (2013) and Chaix et al. (2006) estimated the relationship between NO_X concentrations and income brackets in Tampa, FL and between NO_2 concentrations and income brackets in Malmö, Sweden, and observed that the ambient exposures declined with increasing income. Kruize et al. (2007) also estimated the relationship between residential NO_2 and income; at the top 50% of the distribution, little difference was observed among the income brackets, but across the lower 30% of the distribution, exposures were higher for the lower income groups. Several studies have shown that NO_2 exposures increase with factors reflecting lower SES, such as unemployment, overcrowding, lack of car ownership, lack of home ownership, low educational attainment, and not residing in country of birth (Mitchell, 2005; Stroh et al., 2005; Rotko et al., 2001). However, Mitchell (2005) did observe that discrepancy in NO_2 exposures for a unit increase in a deprivation index declined over the period 1993-2005. These studies support the possibility that individuals of low SES may be at increased risk for exposure to NO_2.

Studies presented in this ISA provide evidence for SES as a potential risk factor for NO_2 associated health effects, although it is important to note that some 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. It can be challenging to make comparisons among these studies given this variation, but there are a number of studies available across disciplines to evaluate SES as a risk factor for NO_2-related health effects.

Across respiratory outcomes, positive associations were generally reported with ambient NO_2 exposure (Section 4.2), but studies stratifying results by various SES indicators did not consistently show differences. Grineski et al. (2010) conducted a study in Phoenix, AZ and found that children without insurance were at greater risk of NO_2-associated asthma hospital admissions compared to children with private insurance or Medicaid. However, a study in Toronto, Canada that compared quintiles of household income did not find any evidence of differences in the risk of asthma physician visits by income (Burra et al., 2009). Studies that examined community-level SES indicators, which may not correspond with individual-level SES, produced inconsistent results. Larger NO_2-

related increases in asthma were found in children with higher maternal- or self-reported community violence (Clougherty et al., 2007); however, associations of asthma ED visits and asthma medication sales did not vary by income-based health insurance premiums in Korean communities (Kim et al., 2007), across census blocks or areas with varying average household income in Vancouver (Lin et al., 2004b), or across a composite index of SES indicators in Strasbourg, France (income, job category, education, and housing characteristics) (Laurent et al., 2009).

Results for effect modification by SES were more consistent for mortality. A number of studies that reported associations between short-term NO_2 exposure and mortality (Section 4.4.5), also demonstrated effect modification by SES. In a study of 10 Italian cities, Chiusolo et al. (2011) found inconsistent results when examining socioeconomic position and income by dividing the average of the census tract for both indicators into low (<20th percentile), middle (20th to 80th percentile) and high (>80th percentile) categories. Increased risk was observed for the low and high socioeconomic position groups while higher risk was identified for low and middle income groups. In contrast, a study of 7 Chilean cities using several indicators of SES (education attainment, community income level, and employment category) to measure effect modification found evidence of increased risk of NO_2-related mortality for individuals of low SES (i.e., were not white-collar workers, had a lower income and education level) (Cakmak et al., 2011b). These results are consistent with those of Wong et al. (2008b) and (Chen et al., 2012b); Wong et al. (2008a) found evidence that the most socially deprived areas of Hong Kong, as measured by a composite metric of socioeconomic status, had higher mortality risks, and Chen et al. (2012b) found that education level modified the relationship between NO_2 exposure and mortality in a study of 17 Chinese studies. More specifically, examining education as low, illiterate or completion of primary school; high, middle school education and above, Chen et al. (2012b) found those with less education to have increased risk of NO_2-related mortality. Overall, the trend across these studies indicates that those of low SES are at increased risk for mortality associated with short-term NO_2 exposure.

In addition to short-term exposure studies, a long-term study of mortality found evidence for low SES to increase risk of NO_2-associated mortality. In a cohort study conducted in Rome, Italy, Cesaroni et al. (2013) found positive associations between ambient NO_2 and mortality for all SES categories and education levels, but associations were more precise and generally larger for low SES and education. Other studies of long-term NO_2 exposure did not find SES to consistently modify the association between NO_2 and lung cancer incidence and mortality. Yorifuji et al. (2010) reported that financial capability did not modify the association between NO_2 and lung cancer mortality, while studies that

examined long-term NO_2 exposure and lung cancer incidence reported inconsistent findings when stratifying results by educational attainment (<8 years, 8-10 years, >8 years) (Raaschou-Nielsen et al., 2011; Raaschou-Nielsen et al., 2010a).

The potential modification of NO_2 associations by SES was also examined in studies of other health effects including birth and developmental outcomes. Results from these studies were generally null or inconsistent (Section 5.4), and stratified analyses did not clearly identify a particular level of SES within the population for which there is evidence of association (Becerra et al., 2013; Guxens et al., 2012; Pereira et al., 2012; Morello-Frosch et al., 2010). While evidence overall demonstrates no association between low birth weight and long-term NO_2 exposure (Section 5.4.3.3), one study demonstrated that decreases in birth weight associated with NO_2 were greatest for mothers living in communities with high rates of neighborhood-level poverty (Morello-Frosch et al., 2010). In contrast, a study in Australia found inconsistent results for modification by SES of the association between NO_2 and fetal growth measurements; those with low and high SES (community-level index) had positive associations for small for gestational age with NO_2 exposure in the 2nd trimester while those of middle SES had positive associations in the 3rd trimester (Pereira et al., 2012). Effect modification by SES was also inconsistent for associations between prenatal NO_2 exposure and neurodevelopmental effects (Becerra et al., 2013; Guxens et al., 2012) (Section 5.4.4.1). Guxens et al. (2012) did not find parental social class or education level to modify the relationship between NO_2 and mental development in infants, whereas Becerra et al. (2013) reported the strongest associations between NO_2 and autistic disorder in children born to mothers with the lowest education level.

Across epidemiologic studies, there is consistent evidence demonstrating risk for NO_2-related mortality among individuals with low SES, while there is limited and inconsistent evidence regarding cancer incidence and reproductive and developmental outcomes. Interpreting this body of evidence is challenging given the wide variety of SES indicators used across studies in addition to the breadth of countries where studies have been conducted. Educational attainment was the most commonly used indicator of SES across studies, and generally, lower levels of education were associated with NO_2-related health effects. Overall, the collective epidemiologic evidence suggests that low SES may increase risk for NO_2-related health effects.

6.4.3 Race/Ethnicity

Based on the 2010 U.S. Census, 63.7% of the U.S. population identified 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 risk factors that are often closely related to other risk factors including genetics, diet, and socioeconomic status, and both intrinsic and extrinsic mechanisms are likely to be involved in risk attributed to race and ethnicity. While it can be difficult to understand the complexities of these relationships, race/ethnicity is routinely examined as a risk factor for health outcomes and has been studied in the context of NO_2-related birth outcomes. Although NO_2 exposure during pregnancy was not consistently associated with birth weight across a multitude of studies (Section 5.4.3.3), some studies that identified associations examined effect modification by race (Darrow et al., 2011b; Madsen et al., 2010; Morello-Frosch et al., 2010; Bell et al., 2007). The results were varied as one study found NO_2-associated decreases in birth weight to be greatest for black mothers compared to white (Bell et al., 2007) while another study found NO_2-associated decreases in birth weight to be greatest for white (non-Hispanic) mothers, moderate for black (non-Hispanic), Asian (non-Hispanic), and Pacific Islander mothers, and smallest for Hispanic mothers (Morello-Frosch et al., 2010). Additionally, Darrow et al. (2011b) found decreases in birth weight associated with NO_2 exposure in the 3rd trimester to be similar among white (non-Hispanic), black (non-Hispanic), and Hispanic mothers. Rich et al. (2009) did find effect modification by race for associations between NO_2 and very small for gestational age; however, risk estimates were greatest for Hispanic mothers compared to those for white (non-Hispanic) and black (non-Hispanic). Further evidence is available from a study conducted outside of the U.S. that did not find differences in associations of NO_2 with birth weight, low birth weight, or small for gestational age (SGA) among mothers of non-Western or Western descent living in Oslo, Norway (Madsen et al., 2010).

Beyond birth outcomes, only one study examined effect modification by race. Grineski et al. (2010) examined whether race modified the association between NO_2 exposure and asthma hospital admission in children <14 years of age. Black children were at increased risk of NO_2-related asthma ED visits compared to Hispanic children, but no difference was observed between black and white children.

The results from some individual studies demonstrate effect modification by race, but the evidence across these studies is not consistent for any particular race/ethnicity Furthermore, there are studies that demonstrate no effect modification for race. Overall, this evidence is inadequate to determine whether race increases risk of NO_2-related health effects.

6.4.4 Sex

According to the 2010 U.S. Census, the distribution of males and females is approximately equal: 49.2% male and 50.8% female (Howden and Meyer, 2011). This distribution does vary by age with a greater prevalence of females above 65 years of age compared to males. Additionally, sex is a potential risk factor for a vast number of health conditions and diseases, including air pollutant-associated health effects. It is likely that there are complex biological phenomena that underlie these differences in addition to sex-based differences in exposure. A few studies have examined this more closely and report mixed results. Kan et al. (2007), Kan et al. (2008), and Sunyer et al. (2006) observed no difference in NO_2 exposure between men and women in the multi-country EXPOLIS study. Studies in children showed mixed results with respect to sex-related differences in both proximity to roads and NO_2 exposure, with some studies in Canada, Europe, and Mexico showing larger effects in girls (Rosenlund et al., 2009b; Oftedal et al., 2008; Rojas-Martinez et al., 2007a; Luginaah et al., 2005) and studies in Germany and Los Angeles showing larger effects in boys (Gehring et al., 2002; Peters et al., 1999).

The inconsistency in NO_2 exposure differences among men and women is mirrored in studies examining sex-specific respiratory effects associated with NO_2 exposure. Some studies did not clearly indicate differences in associations between males and females (Sarnat et al., 2012; Liu et al., 2009b; Lin et al., 2005). Stronger associations of short-term NO_2 exposure with outcomes such as wheeze and asthma hospital admissions were observed in boys with intermittent asthma or boys of low SES relative to girls (Mann et al., 2010; Lin et al., 2004b) and stronger associations for outcomes such as respiratory hospital admissions or ED visits for otitis media were observed in females (Zemek et al., 2010; Luginaah et al., 2005). Inconsistency was also reported for respiratory effects of long-term NO_2 exposure. The association with asthma diagnosis was greater in females (Kim et al., 2004), although no differences in lung function growth were reported between males and females (Rojas-Martinez et al., 2007a).

Other long-term and short-term exposure studies examined associations between NO_2 and mortality, and in general, indicate that females may be at greater risk. Some studies (Cesaroni et al., 2013; Katanoda et al., 2011; Raaschou-Nielsen et al., 2011; Raaschou-Nielsen et al., 2010a; Yorifuji et al., 2010) did not observe differences between males and females for NO_2 –associated lung cancer incidence or mortality, but other studies demonstrated that associations were significantly greater in females (Naess et al., 2007; Abbey et al., 1999). Associations between long-term NO_2 and cardiovascular or respiratory mortality were not different among men or women in a few studies (Cesaroni et al., 2013; Naess et al., 2007; Abbey et al., 1999), while Katanoda et al. (2011) reported respiratory mortality to be greater for females and Naess et al. (2007) reported COPD

mortality to be greater for males. In short-term studies, Chiusolo et al. (2011) found males to be at slightly greater risk for NO_2-related mortality, but more studies found associations to be greater for females (Chen et al., 2012b; Cakmak et al., 2011b; Kan et al., 2008).

For other health outcomes, results did not clearly indicate greater NO_2-associated risk in males or females. The association with small for gestation age was greater in baby girls than boys. The association between short-term NO_2 exposure and the HRV measure SDNN was stronger among females (Huang et al., 2012a).

Taken together, the results vary across studies, with recent evidence for increased risk for NO_2-related health effects present for males in some studies and females in others. The majority of studies examining a sex being at increased or decreased risk for NO_2-related health effects focused on respiratory outcomes and did not clearly indicate increased risk for either sex; however, a considerable number of epidemiologic studies examining NO_2-associated mortality demonstrate that females are at greater risk compared to males. Overall, the collective evidence suggests that women may be at increased risk for NO_2-related health effects.

6.5 Behavioral and Other Factors

6.5.1 Diet

Diet is a plausible risk factor for health effects of air pollutants as it is an important contributor to health status, but few epidemiologic studies have evaluated NO_2-associated health outcomes in the context of diet and the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) did not discuss diet as a risk factor; however, toxicological evidence is available from the 2008 ISA and previous AQCDs that has primarily focused on respiratory endpoints in animals deficient in or supplemented with Vitamin C and Vitamin E.

Vitamin C, or ascorbic acid, can act as an antioxidant in the airways and neutralize reactive oxygen species, and while epidemiologic studies are not available, a wealth of toxicological studies have demonstrated that a diet rich is Vitamin C can mitigate NO_2-related oxidant injury. Guinea pigs with a Vitamin C-deficient diet had increased BALF protein following NO_2 exposure relative to air controls or guinea pigs with a normal diet (Hatch et al., 1986; Selgrade et al., 1981). Rats with diets deficient in Vitamin E had increases in lipid peroxidation and protein content in lung homogenates following NO_2 exposure (Elsayed and Mustafa, 1982; Sevanian et al., 1982b). Additional support for an

influence of Vitamin E is provided by observations that NO_2-induced increases in BALF protein or decreases in glutathione peroxidase activity were attenuated in animals on Vitamin E-supplemented diets, relative to animals not supplemented with Vitamin E (Guth and Mavis, 1986; Ayaz and Csallany, 1978). These studies demonstrate that Vitamin C and E can modify the effects of NO_2 on pulmonary injury in animals.

Limited, recent epidemiologic evidence stratifying results according to dietary components includes two studies discussed in this ISA (Guxens et al., 2012; Raaschou-Nielsen et al., 2011). Guxens et al. (2012) found the association between prenatal NO_2 exposure during pregnancy and mental development in children during their second year of life was greater among children with low maternal fruit and vegetable intake during the first trimester of pregnancy. There was little evidence of an association among mothers with higher fruit and vegetable intake. In addition, children of mothers with low levels of circulating Vitamin D were also at greater risk for NO_2-associated decrements in mental development. Raaschou-Nielsen et al. (2011) found an association between the highest quartile of NO_X concentrations and lung cancer that was larger in individuals with the lowest fruit intake compared with those in the highest quartile of fruit intake.

Although the limited epidemiologic evidence examined different outcomes, both demonstrated response modification by dietary fruit or vegetable intake. The toxicological evidence consistently demonstrated that Vitamin C or E dietary levels affected pulmonary injury following NO_2 exposure, but no epidemiologic or controlled human exposure studies have examined these effects in humans. Coherence for the animal toxicological evidence is provided by the well-characterized effect of NO_2 in inducing ROS/RNS (Section 3.3.2.1). Overall, the toxicological evidence suggests that diet may modify response to NO_2 and contribute to increased or decreased risk for health effects associated with NO_2 exposure.

6.5.2 Obesity

Obesity is defined as having a body mass index (BMI) of 30 kg/m^2 or greater. It is an 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 BMI, may increase risk of NO_2-related health effects through multiple mechanisms including persistent, low-grade inflammation, increasing likelihood of comorbidities, and poor diet.

The 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c) did not evaluate obesity as a risk factor, but recent studies of NO_2-related health effects allow for evaluation of effect modification by obesity status. The majority of these studies have examined effect

modification of cardiovascular outcomes. There is biological plausibility for effect modification given that weight and cardiovascular disease are related; however, the evidence is inconsistent. In panel studies of individuals with pre-existing cardiovascular disease, NO_2 was associated with decreases in HRV but not ventricular tachyarrhythmias; however, BMI did not modify either association (Huang et al., 2012a; Ljungman et al., 2008). In contrast, Baja et al. (2010) found that the association between NO_2 and heart rate-corrected QT interval was greater among obese individuals compared to non-obese individuals.

Animal evidence regarding effect modification by obesity is limited. Long-term exposure to 160 ppb NO_2 resulted in increased triglycerides and decreased in HDL in obese rats compared to non-obese rats, though differences between strains were not observed at higher NO_2 exposures. This study suggests that obesity may contribute to NO_2-induced dyslipidemia, which is a known risk factor for cardiovascular disease.

Effect modification by obesity status has also been examined in the context of lung cancer (Yorifuji et al., 2010) and hypertensive disorders in pregnancy (Mobasher et al., 2013); associations between NO_2 and these outcomes were not modified by obesity status.

The epidemiologic and toxicological studies evaluating obesity as a risk factor are limited in quantity, and results are inconsistent across studies. Overall, the evidence is inadequate to determine whether obese individuals are at increased risk for NO_2-associated health effects.

6.5.3 Smoking

Smoking is a common behavior within the U.S. adult population as 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 a variety of diseases, but it is unclear if smoking exacerbates health effects associated with air pollutant exposures, including NO_2. Only a few studies included in this ISA examined effect modification by smoking status, and the limited amount of evidence is inconsistent.

In studies of lung cancer incidence, associations with ambient NO_2 were not stronger among smokers or nonsmokers (Raaschou-Nielsen et al., 2011; Raaschou-Nielsen et al., 2010a). Studies evaluating lung cancer mortality reported contrasting results as Yorifuji et al. (2010) found an increased hazard ratio among non-smokers and Katanoda et al. (2011) found greater associations among male smokers and female nonsmokers.

Although many controlled human exposure studies report smoking status, comparisons between smokers and non-smokers are infrequent due to small sample size. Despite this limitation, Morrow et al. (1992) did analyze smoking subgroups and demonstrated that among seven smoking subjects, the mean FEV_1 during a 4-hour exposure to 300 ppb NO_2 was significantly lower than the mean from 13 non-smoking subjects.

Taken together, evidence from both epidemiologic and controlled human exposure studies is limited and inconsistent. Further, the epidemiologic evidence is provided by studies of lung cancer, for which overall results are inconsistent. The limited and inconsistent evidence is inadequate to determine whether smoking is a risk modifying factor for NO_2-related health effects.

6.5.4 Residential Location

A few epidemiologic studies examined health effects related to exposure to oxides of nitrogen relative to residential location, specifically urban versus rural. Differences in NO_2 concentrations by residential location and building characteristics suggest that these factors may contribute to variation in NO_2 exposure. For example, Rotko et al. (2001) found higher NO_2 concentrations associated with downtown versus suburban location (14.9 ppb versus 11.7 ppb), high-rise building versus single family home (13.1 ppb versus 11.3 ppb), and older versus newer construction (before 1970: 14.5 ppb versus during or after 1970: 11.5 ppb). Additionally, location of residence with respect to a busy roadway can also affect exposure. 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). The influence of roadway proximity on personal NO_2 exposure was demonstrated in a study finding the lowest correlations among personal, indoor, and outdoor NO_2 and NO_X concentrations within a 500 meter buffer (r = 0.1-0.3) of a road compared to 100 meter (r = 0.2-0.3) and 250 meter (r = 0.3-0.4) buffers (Schembari et al., 2013). However, closest proximity to roadways was not the only determinant influencing personal exposure as correlations were strongest for NO_2 and the 250 meter buffer (r = 0.3-0.4), suggesting that NO_2 is higher after some of the NO reacts photochemically to become NO_2. The topography of urban communities also may contribute to higher NO_2 exposure 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 exposures to oxides of nitrogen for pedestrians, outdoor workers, and those living on lower levels.

Although the potential for higher exposure to NO_2 in urban areas is well characterized, epidemiologic comparisons of NO_2-related health effects between urban and nonurban

residence are mixed. Further, these studies encompassed diverse outcomes: lung function and respiratory symptoms, leukemia incidence, and autism. For respiratory effects and leukemia incidence in children, no associations were found with NO_2 in the entire study populations or in analyses stratified by urban or rural residence in Italy (Ranzi et al., 2004) or results stratified by urban, semi-urban or rural residence in France (Amigou et al., 2011). In a study of autism in children in California, in the highest quartile of NO_2 exposure in infancy, the association with autism was slightly lower among children living in an urban area relative to children residing in rural areas (Volk et al., 2013).

While it has been demonstrated that urban residence can result in higher exposure to oxides of nitrogen, the limited epidemiologic evidence does not consistently indicate residential location to be a risk modifying factor. Because there are so few studies, consistency within or across outcomes cannot be determined. Thus, the evidence is inadequate to determine whether residential location modifies NO_2-associated health risks.

6.6 Summary

In this section, epidemiologic, exposure assessment, controlled human exposure, and toxicological studies have been evaluated and indicate that various factors may lead to increased risk of NO_2-related health effects (Table 6-4)

The evaluation of evidence in this section demonstrates that there is adequate evidence to conclude that different lifestages may be at increased risk for health effects related to exposure to oxides of nitrogen. Moreover, both children and older adults are potentially at greater risk for NO_2-related health effects. Recent evidence demonstrates that children are at increased risk for NO_2-associated asthma hospitalizations and ED visits, which is consistent with conclusions from the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c). There is limited toxicological evidence to provide biological plausibility, but these observations are plausible based on studies demonstrating differences in NO_2 exposure among children in addition to higher ventilation rates. There is also consistent and substantial evidence showing larger associations in older adults between NO_2 exposure and hospitalization and mortality, though limited evidence of respiratory outcomes from controlled human exposures is not coherent with these observations.

Table 6-4 Summary of evidence for potential increased risk of health effects related to exposure to oxides of nitrogen.

Evidence Classification	Potential At Risk Factor
Adequate evidence	Lifestage (Children [Section 6.4.1.1] and Older adults [Section 6.4.1.2])
Suggestive evidence	Genetic background (Section 6.2) Asthma (Section 6.3.1) COPD (Section 6.3.2) SES (Section 6.4.2) Sex (Section 6.4.4) Diet (Section 6.5.1)
Inadequate evidence	Cardiovascular disease (Section 6.3.3) Diabetes (Section 6.3.4) Race/Ethnicity (Section 6.4.3) Obesity(Section 6.5.2) Smoking (Section 6.5.3) Residential location (Section 6.5.4)
Evidence of no effect	--

For several other factors, including genetic background, pre-existing asthma or COPD, SES, sex, and diet, it was determined that there is suggestive evidence of increased risk for respective populations as presented in Table 6-4. Studies have indicated increased risk of exposure to oxides of nitrogen with declining measures of SES. For all of these factors, there is a body of evidence for an outcome or scientific discipline indicating differential response to NO_2; however, there are inconsistencies within or across outcomes or scientific disciplines that present uncertainties in the interpretation. In contrast to conclusions from the 2008 ISA for Oxides of Nitrogen (U.S. EPA, 2008c), recent epidemiologic evidence provides inconsistent evidence that pre-existing asthma increases risk for hospitalizations or ED visits related to ambient NO_2; however, meta-analyses of controlled human exposure studies of AHR demonstrate that individuals with asthma are more sensitive to NO_2. Consistent with previous observations, individuals of low SES may be at greater risk for NO_2-related health effects, but limitations exist due to variations in definitions of SES and indicators of SES within and across different countries. Furthermore, epidemiologic evidence demonstrates that females appear to be at greater risk for NO_2-related mortality, though there are inconsistencies for other outcomes relative to NO_2. Animal studies provide a wealth of evidence that diets rich in Vitamin C or E may protect against respiratory effects of NO_2, while those deficient in these vitamins may be at greater risk for NO_2-induced pulmonary injury and inflammation.

In addition, there was inadequate evidence to determine increased risk for pre-existing cardiovascular disease, diabetes, race/ethnicity, obesity, smoking, and residential location (Table 6-4). For the majority of these factors, there was limited evidence to allow for evaluation of consistency or coherence.

Overall, the available evidence evaluated in this ISA in combination with previous evidence from the 2008 ISA for Oxides of Nitrogen demonstrates that lifestage is an important contributor to increased risk of NO_2-related health effects.

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