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Integrated Science Assessment for Oxides of Nitrogen – Health Criteria

Chapter 3: Respiratory Effects – Short-Term Exposure

External Review Draft

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U.S. Environmental Protection Agency

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Document Guide

This Document Guide is intended to orient readers to the organization of the Oxides of Nitrogen Integrated Science Assessment (ISA) in its entirety and to the sub-section of the ISA at hand (indicated in bold). The ISA consists of the Front Matter (list of authors, contributors, reviewers, and acronyms), Executive Summary, Integrated Synthesis, and 14 chapters, which can all be found at https://www.epa.gov/isa/integrated-science-assessment-isa-oxides-nitrogen-health-criteria.

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Acronyms and Abbreviation

µg	microgram
AQCD	Air Quality Criteria Document
AQS	air quality system
avg	average
BC	black carbon
BMI	body mass index
C_ANO	alveolar nitric oxide concentration
CHS	Children's Health Study
CI	confidence interval
CMAQ	community multiscale air quality
COPD	chronic obstructive pulmonary disease
C-R	concentration-response
d	day(s)
$D_{aw}NO$	diffusing capacity of the airway wall tissue for nitric oxide
df	degrees of freedom
DNA	deoxyribonucleic acid
DTH	delayed type hypersensitivity
e.g.	exempli gratia (for example)
EC	elemental carbon
ECP	eosinophilic cationic protein
ED	emergency department
EE	effect estimate
EEM	effect measure modification
ELF	epithelial lining fluid
eNO	exhaled nitric oxide
EOS	eosinophil
EPA	U.S. Environmental Protection Agency
etc.	et cetera
F	female(s)
FEF	forced expiratory flow
$FeNO_{50}$	exhaled nitric oxide fraction at a flow rate of 50 mL/s
FEV_1	forced expiratory volume in one second
FVC	forced vital capacity
g	gram(s)
GPS	Global Positioning System
H_2SO_4	sulfuric acid
hr	hour(s)
HR	hazard ratio
i.e.	id est (that is)
IDW	inverse distance weighted
IHD	ischemic heart disease
iNOS	inducible nitric oxide synthase
IQR	interquartile range
ISA	Integrated Science Assessment
ITGV	intrathoracic gas volume
$J'_{aw}NO$	maximum airway wall nitric oxide flux
LOAEL	lowest observed adverse effect level
LOESS	locally weighted scatterplot smoothing
LPS	lipopolysaccharide
LRTI	lower respiratory tract infection
LUR	land use regression
M	male
m	milligram
m	meter(s)
Max	maximum
MEC	Multiethnic Cohort Study
Min	minimum
min	minute(s)
mL	milliliter
mo	month(s)
n	sample size
N	population size
NA	not applicable
NK	natural killer
NO	nitric oxide
NO_2	nitrogen dioxide
NO_X	NO_2 + NO
NOAEL	no observable effect level
NR	not reported
NSES	neighborhood socioeconomic status
O_3	ozone
OC	Organic carbon
OR	odds ratio
OVA	ovalbumin
ρ	rho
P10	10^{th} percentile
P25	25^{th} percentile
P5	5^{th} percentile
P75	75^{th} percentile
P95	95^{th} percentile
PECOS	Population, Exposure, Comparison, Outcome, and Study Design
PEF	peak expiratory flow
PHA	phytohemagglutinin
PM	particulate matter
PM_{10}	particulate matter with a nominal mean aerodynamic diameter less than or equal to 10 µm
$PM_{2.5}$	particulate matter with a nominal mean aerodynamic diameter less than or equal to 2.5 µm
ppb	parts per billion
ppm	parts per million
RR	relative risk or risk ratio
RV	residual volume
SD	standard deviation
SDI	Socioeconomic Deprivation Index
SE	standard error
SES	socioeconomic status
SO_2	sulfur dioxide
sRaw/sReff	specific airway resistance
TDAR	T-dependent antibody response
TLC	Total lung capacity
UCLA	University of California, Los Angeles
U.K.	United Kingdom

U.S.	United States
UFP	ultrafine particles
URI	upper respiratory infection
VOC	volatile organic compounds
vs.	versus
WBC	white blood cells
WHI	Women's Health Initiative
wk	week(s)
YLL	year of life lost
yr	year(s)
ZCTA	ZIP code tabulation area
β	beta
$\mu g/m^3$	micrograms per cubic meter
μm	micrometer
ρ	rho

CHAPTER 3 Respiratory Effects – Short-Term Exposure

Summary of Causality Determinations for Short-Term Oxides of Nitrogen Exposure and Respiratory Effects

This chapter characterizes the scientific evidence that supports causality determinations for short-term exposure to oxides of nitrogen and respiratory effects. The types of studies evaluated within this chapter are consistent with the overall scope of the ISA as detailed in Chapter 14 (see Section 14.4.3.2). In assessing the overall evidence, the strengths and limitations of individual studies were evaluated based on scientific considerations detailed in Table 14-5 of Chapter 14 (Section 14.4.3.2). More details on the approach used to make causality determinations is included in Appendix A of Volume 2 of the Integrated Review Plan for the Primary National Ambient Air Quality Standards for Oxides of Nitrogen (U.S. EPA, 2024), which builds on the approach described in the 2015 Preamble to the Integrated Science Assessments (U.S. EPA, 2015a). The evidence presented throughout this chapter supports the following causality conclusion(s):

Outcome	Causality Determination
Respiratory Effects	Causal relationship

The Executive Summary, Integrated Synthesis, and all other chapters of this ISA can be found at https://www.epa.gov/isa/integrated-science-assessment-isa-oxides-nitrogen-health-criteria.

3.1 Introduction

3.1.1 Summary of Previous ISA

The U.S. Environmental Protection Agency's (EPA) 2016 Integrated Science Assessment for Oxides of Nitrogen – Health Criteria (hereafter referred to as the 2016 Oxides of Nitrogen ISA) concluded that the available scientific evidence supported a "causal relationship" between short-term nitrogen dioxide (NO_2) exposure and respiratory effects based on the coherence among multiple lines of evidence and biological plausibility for effects on asthma exacerbation (U.S. EPA, 2016). The evidence underpinning this causality determination is briefly summarized below.

The determination of a "causal relationship" was supported by consistent evidence from epidemiologic studies of associations between estimated short-term NO_2 exposures and increased

incidence of hospital admissions and emergency department (ED) visits for asthma. Biological plausibility for these findings was provided by controlled human exposure studies that consistently showed increased airway reactivity and allergic inflammation in adults with asthma exposed at rest to NO_2 at ambient-relevant concentrations. Integration of the multiple lines of evidence reduced uncertainties pertaining to potential copollutant confounding in the epidemiologic evidence. There was remaining uncertainty regarding the apparent lack of a dose-response relationship in controlled human exposure studies examining NO_2-induced airway reactivity, as well as a lack of clarity as to why increased airway reactivity is seen predominantly in humans exposed at rest and is absent when exposed during intermittent exercise. There was some support for NO_2-related exacerbation of respiratory allergy and chronic obstructive pulmonary disease (COPD), respiratory infection, respiratory mortality, and respiratory effects in healthy populations; however, evidence for these other respiratory health effects was less consistent and often lacked coherence between epidemiology, human, and animal toxicological data. Together, epidemiologic evidence for NO_2-associated asthma exacerbation and biological plausibility from NO_2-induced increases in airway responsiveness and allergic inflammation in adults with asthma were sufficient to conclude that there is a "causal relationship" between short-term NO_2 exposure and respiratory effects.

3.1.2 Chapter Organization and Conventions

This chapter evaluates recent evidence examining the relationship between short-term oxides of nitrogen exposure and respiratory effects. Its conclusions are drawn from integration of the full body of evidence available in the 2016 Oxides of Nitrogen ISA and the evidence that has become available since the 2016 Oxides of Nitrogen ISA. The following sections provide an overview of study inclusion criteria for this chapter (see Section 3.2), summaries of recent health effects evidence (see Sections 3.3 to 3.8), a discussion of biological plausibility (see Section 3.9), a summary of evidence that some life stages and populations may be at increased risk (see Section 3.10, Table 3-1), and a discussion of the causality determination for short-term oxides of nitrogen exposure and respiratory effects (see Section 3.11, Table 3-2). Evidence inventories include study-specific details for the epidemiologic and toxicological studies including, for example, study population characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), potential confounders, and select results are located in Appendix A – Appendix Tables. For outcomes supported by evidence from multiple disciplines, subsections titled "Synthesis Across Lines of Evidence" are included.

3.1.2.1 Epidemiologic Studies

In sections assessing the epidemiologic evidence, this chapter summarizes the evidence from previous ISAs and from recent studies that have become available since the 2016 Oxides of Nitrogen ISA. When relevant information is available from older or more recent epidemiologic studies, these sections

additionally include targeted discussion of available analyses examining concentration-response relationships, effects in particular lifestages and populations, and the potential for confounding by copollutants. For apical endpoints with no or limited evidence on these topics, the subsections addressing them are not included.

In the assessment of the recent epidemiologic evidence, the "Lifestages and Populations" sections, as well as "Summary of Lifestage and Populations" section, discuss analyses of effect measure modification. Effect measure modification occurs when the effect of a pollutant exposure on a health outcome of interest differs between subgroups or strata of risk factors (Rothman and Greenland, 1998). When a risk factor is an effect measure modifier, it changes the magnitude of the association between the pollutant exposure and the health outcome in stratified analyses. For risk factors that modify the association, effect estimates for each stratum will differ from one another and from the overall estimate, indicating there to be different quantitative relationships between exposure metric and outcome for populations represented by these variables.

In the assessment of recent epidemiologic evidence, the "Copollutant" section discussed examination of potential confounding by copollutants. Copollutant modeling, sometimes referred to as two-pollutant models or co-adjusted models, can reduce concern for confounding by copollutants when correlations between the copollutants are relatively low (e.g., correlation coefficient, rho (ρ) <0.4). However, when correlations are high (e.g., ρ>0.7), collinearity between pollutants makes copollutant modeling less informative, leading to greater uncertainty in the degree to which reported associations reflect health effects of exposure to oxides of nitrogen independently.

Throughout this chapter, short-term exposure to oxides of nitrogen is assumed to be approximately 30 days or less. Additionally, in the summaries of the recent epidemiologic evidence, the abbreviation "NO_X" is widely used in the epidemiologic literature and is assumed to be the sum of nitric oxide (NO) and NO_2, which are the dominant forms of oxides of nitrogen emitted from major sources (see Chapter 1, Section 1.3.1 and Chapter 2, Section 2.2).

To increase comparability of results across epidemiologic studies, this Oxides of Nitrogen ISA presents effect estimates for associations with health outcomes scaled to the same increment of oxide of nitrogen concentration.[1] The increments for standardization for short-term exposure vary by averaging time (e.g., 24-hour average, 8-hour max, 1-hour max) and oxide of nitrogen. For 24-hour average, effect estimates were scaled to a 20 ppb increase for NO_2 or NO and a 30 ppb increase for NO_X. For a 1-hour maximum, effect estimates were scaled to a 25 ppb increase for NO_2, a 60 ppb increase for NO, and an 80 ppb increase for NO_X. For an 8-hour maximum, the increments for standardization are a 25 ppb increase for NO_2, a 40 ppb increase for NO, and a 55 ppb increase for NO_X. These increments were derived by calculating the United States (U.S.)-wide percentile distributions for a given averaging time and then calculating the approximate difference between the median (a typical pollution day) and the 95th

[1]This is in contrast with reported effect estimates that are scaled to various changes in concentration, such as interquartile range for the study period or an arbitrary unit such as 10 parts per billion (ppb).

percentile (a more polluted day) for a given averaging time (U.S. EPA, 2015b). There were common exceptions to this standardization method. Averaging times other than 24-hour average or 1-hour maximum were examined, for example, 2- to 15-hour averages. Effect estimates based on these averaging times were not standardized but are presented in the ISA as reported in their respective studies.

Some studies reported effect estimates in terms of micrograms per cubic meter ($\mu g/m^3$) increases in oxides of nitrogen, which could be converted to ppb and standardized for NO_2 and NO but not NO_X. This conversion could not be made for NO_X because the proportions of NO_2 and NO are unknown for the various NO_X metrics. Also, data are not available to calculate the percentiles of NO_X concentrations in $\mu g/m^3$ at a national scale for the United States or other countries. Therefore, the ISA presents effect estimates based on $\mu g/m^3$ of NO_X as they are reported in their respective studies.

3.1.2.2 Animal Toxicological Studies

In sections assessing the animal toxicological evidence, this chapter summarizes the evidence from previous ISAs and from recent studies that have become available since the 2016 Oxides of Nitrogen ISA. When relevant information is available from recent animal toxicological studies, targeted discussions of analyses examining dose-response relationships, relevance of toxicological responses to human responses, and multipollutant exposures are included. For apical endpoints with no or limited evidence on these topics, the subsections addressing them are not included.

3.2 Scope

The evidence that has become available since the 2016 Oxides of Nitrogen ISA has been evaluated for relevance using scoping criteria defined by Population, Exposure, Comparison, Outcome, and Study Design (PECOS) statements. The PECOS statements help to define the scope of the assessment and establish study inclusion criteria, thereby facilitating the identification of the relevant literature for this Oxides of Nitrogen ISA.[2] In order to identify the most relevant literature, the body of evidence from the 2016 Oxides of Nitrogen ISA was considered in the development of the PECOS statements for this chapter. Specifically, well-established areas of research; gaps in the literature; and inherent uncertainties in specific populations, exposure metrics, comparison groups, and study designs identified in the 2016 Oxides of Nitrogen ISA inform the scope of this chapter. Studies that were included in the 2016 Oxides of

[2]The following types of publications are generally considered to fall outside the scope and are not included in the ISA: review (narrative, scoping, systematic, and meta-analyses of systematic reviews) articles (which typically present summaries or interpretations of existing studies rather than bringing forward recent information in the form of original research or analyses), risk or benefits analyses (e.g., that apply concentration-response functions or effect estimates to exposure estimates for differing cases), and health impact assessments (an assessment tool used to designed to investigate how a proposed program, project, policy, or plan may impact health and well-being and inform decision-makers of these potential outcomes before the decision is made).

Nitrogen ISA, including studies that do not meet the current PECOS criteria, are discussed in this chapter to establish the state of the evidence prior to this assessment. Except for supporting evidence used to examine the biological plausibility of oxides of nitrogen-associated respiratory effects, recent studies were only included if they met all components of the discipline-specific PECOS statements (see below) and satisfied discipline-specific study quality criteria (Chapter 14).

Epidemiologic Studies:

Population: Any human population, including populations or lifestages that might be at increased risk.

Exposure: Exposure to oxides of nitrogen concentrations relevant to ambient air in the United States.[3]

Comparison: Per unit increase (in ppb) for humans exposed to lower concentrations of oxides of nitrogen compared to higher concentrations (e.g., categorical comparisons between different exposure metric quantiles).

Outcome: Change or difference in risk (incidence/prevalence) of respiratory health effects.

Study Design: Epidemiologic studies, such as panel, case-crossover, time-series, case-control studies, cohort, cross-sectional studies, and quasi-experimental, with appropriate timing of exposure for the health outcome of interest.

Controlled Human Exposure Studies:

Population: Human volunteers enrolled in controlled exposure studies, including volunteers representing populations or lifestages that might be at increased risk of pollutant-related health effects.

Exposure: Controlled inhalation exposure to NO_2 or any other oxide of nitrogen-pollutant exposures must be controlled by the experimenters and not simply a measure of ambient or occupational exposure.

Comparison: Appropriate comparison group exposed to filtered air or room air.

Outcome: Respiratory health effects.

[3]Epidemiologic studies examining associations with NO_2 that report an overall average of NO_2 exposures (i.e., averaged across study populations/locations and over study periods) above 22 ppb were flagged for additional study quality evaluation. This concentration cutoff reflects the 98th percentile of annual average NO_2 concentrations measured at ambient monitors in the United States during the most recent 15 years of available data (i.e., 2008 to 2022) (U.S. EPA, 2023). Studies reporting average NO_2 exposures greater than 22 ppb were considered for inclusion in the ISA if they meet additional study quality criteria described in Chapter 14.

Study Design: Studies that perform controlled human exposures meeting the above criteria or that analyze data from previously conducted controlled human exposures (e.g., reanalysis, meta-analysis).

Animal Toxicological Studies:

Population: Laboratory nonhuman mammalian animal species (e.g., nonhuman primate, mouse, rat, guinea pig, minipig, rabbit, cat, dog) of any lifestage including models of increased susceptibility.

Exposure: Inhalation exposure to relevant oxides of nitrogen concentrations (i.e., 5 ppm or below).[4]

Comparison: An appropriate control group exposed to clean air (e.g., room air, filtered air).

Outcome: Respiratory health effects.

Study Design: Controlled exposure studies of animals in vivo meeting the above criteria.

3.3 Asthma Exacerbation and Related Respiratory Effects in Populations with Asthma

Asthma is a chronic inflammatory lung disease characterized by reversible airway obstruction and increased airway responsiveness. Exacerbation of disease is associated with symptoms such as wheeze, cough, chest tightness, and shortness of breath. Symptoms may be treated with asthma medication, and uncontrollable symptoms may result in individuals seeking medical treatment (e.g., visiting a hospital). Previous findings from studies evaluated in the 2016 Oxides of Nitrogen ISA provided several lines of evidence in support of a relationship between short-term NO_2 exposure and asthma exacerbation, represented as respiratory effects in populations with asthma. These findings provided the primary line of evidence informing the determination of a causal relationship with respiratory effects in the 2016 Oxides of Nitrogen ISA.

In characterizing the current state of the evidence, this section begins by considering effects of short-term NO_2 exposure on asthma-related hospital and ED visits (see Section 3.3.1) and increased respiratory symptoms and use of asthma-controlling medication (see Section 3.3.2). These are indicators of acute worsening of asthma symptoms. The evaluation follows with consideration of evidence for

[4]Five ppm is approximately two orders of magnitude higher than peak NO_2 concentrations in the ambient air in the United States. As discussed in Volume 2 of the Integrated Review Plan for Oxides of Nitrogen (U.S. EPA, 2024) and the 2015 Preamble to the ISAs (U.S. EPA, 2015a) (U.S. EPA, 2024), animal exposures within one to two orders of magnitude of recent ambient air concentrations are considered relevant to ambient air exposures. This concentration cutoff is also consistent with that used in the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016). Experimental studies investigating the effects of concentrations greater than 5 ppm may be considered for inclusion in the ISA if they provide insight into biological plausibility.

increases in respiratory effects that can underlie asthma exacerbations (see Section 3.3.3). This section is divided into evidence examining changes in airway responsiveness (see Section 3.3.3.1), lung function (see Section 3.3.3.2), and markers of pulmonary inflammation and oxidative stress (see Section 3.3.3.3) in asthmatics resulting from short-term exposure to NO_2. Finally, a summary of the recent evidence is provided by synthesizing and integrating the evidence across health endpoints and scientific disciplines (see Section 3.3.4).

3.3.1 Hospital Admissions and Emergency Department Visits

The evidence for NO_2-related effects on airway responsiveness, lung function, and respiratory symptoms detailed in the ensuing sections are all indicators of asthma exacerbation that may ultimately lead people with asthma to seek medical interventions (i.e., hospital admissions and ED visits). Epidemiologic studies that were evaluated in the 2016 Oxides of Nitrogen ISA provided consistent evidence of associations between short-term increases in NO_2 concentrations and increases in asthma hospital admissions and ED visits among children and adults. Robust evidence was observed across time-series and case-crossover studies conducted in diverse locations in the United States, Canada, and Asia, including several multicity studies. Epidemiologic studies of hospital admissions and ED visits also reported associations with other traffic-related pollutants, including particulate matter with a nominal mean aerodynamic diameter less than or equal to 2.5 μm ($PM_{2.5}$), carbon monoxide (CO), black carbon (BC) and elemental carbon (EC), ultrafine particles (UFP), other particulate matter (PM) constituents, and volatile organic compounds (VOCs). Among the studies that examined potential confounding by one or more of these copollutants, most indicated an independent association with NO_2. However, most of these studies utilized co-adjusted regression models, which have well-recognized limitations for distinguishing independent pollutant associations of highly correlated pollutants. Additionally, most of the studies evaluated in the 2016 Oxides of Nitrogen ISA assigned exposure using average concentrations from fixed-site monitors. In time-series and case-crossover studies, uncertainty associated with using central site measurements of NO_2 concentrations to represent personal exposure arises due to the difference between the temporal variability of the true exposure and the temporal variability of the central site exposure estimate. Most simulation studies demonstrated that these exposure errors may widen the confidence intervals of the health effect estimate and bias the estimate towards the null (see Chapter 2, Section 2.5.1).

In the following subsections, asthma hospital admissions and ED visit studies are evaluated separately because only a small percentage of asthma ED visits result in a hospital admission. As a result, asthma ED visits may represent a less severe outcome compared with asthma hospital admissions, which require extended care.

3.3.1.1 Epidemiologic Studies of Emergency Department Visits and Hospital Admissions

Emergency Department Visits

The 2016 Oxides of Nitrogen ISA evaluated several studies that examined the association between short-term NO_2 exposure and ED visits for asthma. The available studies reported generally consistent evidence of positive associations. Evidence consisted mostly of single-city studies conducted in the United States, Canada, and Australia. The only multicity study, an analysis of asthma ED visits in seven Canadian cities, reported a null association between asthma ED visits and NO_2 concentrations two days prior to presentation (Stieb et al., 2009). Across the single-city ED visit studies, there was evidence for effects of prolonged short-term exposures, with a number of studies demonstrating associations with multiday lags ranging from 0 to 2 and 0 to 4 days (Li et al., 2011; Strickland et al., 2010; Villeneuve et al., 2007; Peel et al., 2005). The evidence was consistent for study populations including people of all ages and those focused more specifically on children. Due to the limited number of studies and a lack of age-stratified analyses, it was difficult to discern any trends related to the strength of observed associations across different age groups. A limited number of studies evaluated potential copollutant confounding and reported that observed associations between NO_2 and asthma ED visits remained positive, though were sometimes attenuated, in copollutant models adjusting for particulate matter with a nominal mean aerodynamic diameter less than or equal to 10 μm (PM_{10}), $PM_{2.5}$, ozone (O_3), CO, or sulfur dioxide (SO_2). Evidence from recent studies is summarized below. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 3-1.

Several recent multicity studies conducted in the United States and Canada expand the existing evidence base and provide generally consistent support for an association between short-term oxides of nitrogen exposure and ED visits for asthma (see Figure 3-1). Most recent studies focused specifically on asthma ED visits among children or evaluated ED visits among people of all ages. Of the few studies that stratified by age, associations were typically stronger (i.e., greater magnitude) among children compared to adults. Associations were observed across diverse study locations, a range of mean NO_2 concentrations, and a variety of exposure assignment techniques, including population-weighted monitor averages, community multiscale air quality (CMAQ) modeling estimates, and land use regression (LUR) models. The evaluated studies utilized time-series or case-crossover study designs and used either 24-hour average or 1-hour daily max averaging times. A short, bulleted summary of the recent evidence is provided below, and study results are presented in Figure 3-1. Subsequent sections provide a more detailed synthesis of information from these studies on concentration-response relationships (see Section 3.3.1.1.1), exposure assignment (see Section 3.3.1.1.2), lag structure (see Section 3.3.1.1.3), seasonal differences (see Section 3.3.1.1.4), the lifestages and populations evaluated (see Section 3.3.1.1.5), and the potential impact of copollutants (see Section 3.3.1.1.6). Section 3.3.1.1.7 discusses conclusions and summarizes remaining uncertainties.

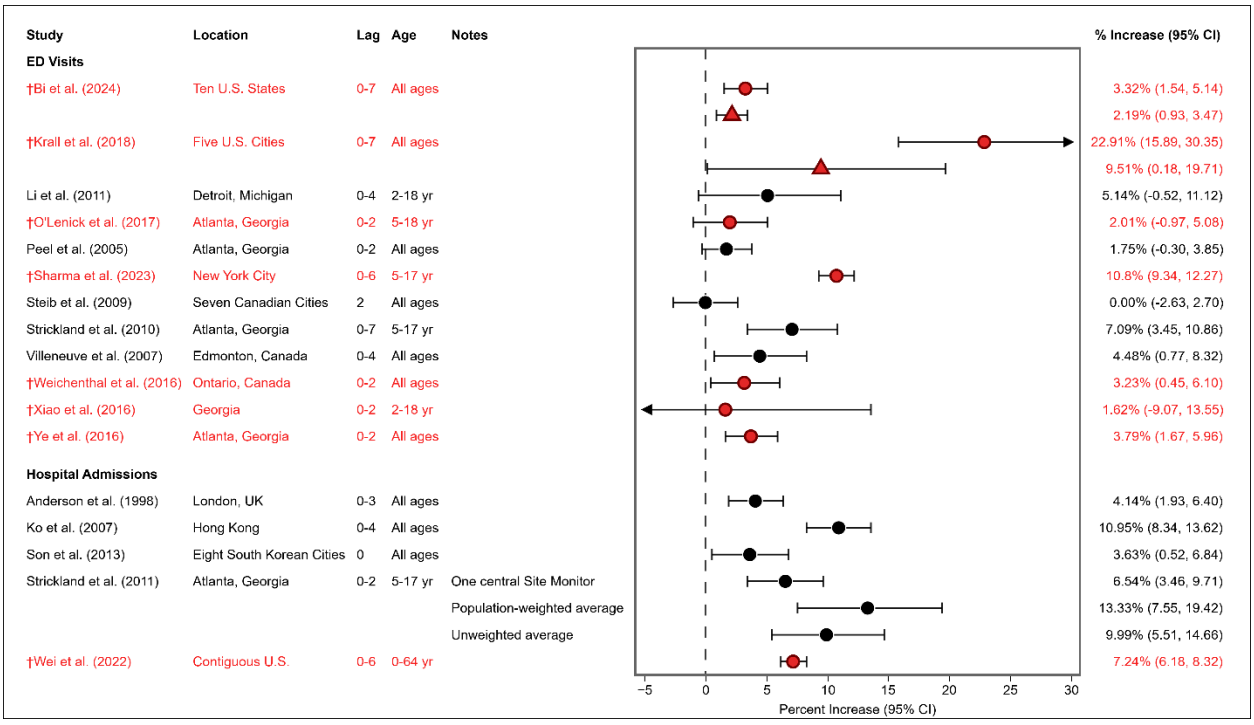
- The strongest evidence informing the relationship between NO_2 exposure and asthma ED visits comes from large (i.e., 100,000+ ED visits) multicity studies in the United States (Bi et al., 2023; Krall et al., 2018; Alhanti et al., 2016) and Canada (Szyszkowicz et al., 2018; Weichenthal et al., 2016). These studies, which include ED visits recorded over multi-year periods, reported consistent evidence of positive associations between NO_2 exposure and ED visits for asthma among people of all ages (Bi et al., 2023; Krall et al., 2018; Szyszkowicz et al., 2018; Alhanti et al., 2016; Weichenthal et al., 2016) and in an analysis restricted to children (Szyszkowicz et al., 2018). Bi et al. (2023) analyzed the most expansive dataset, including over 3 million ED visits for asthma across 10 U.S. states. Compared to the other multicity studies, a statewide study in Georgia, United States, observed an increase in the odds of asthma exacerbation that was much less precise, with confidence intervals that crossed the null, providing little evidence of an association (Xiao et al., 2016).
- There was notable overlap in study populations in many of the larger multicity studies. Krall et al. (2018) and Alhanti et al. (2016) both examined asthma ED visits in Atlanta, GA; Dallas, TX; and St. Louis, MO; though Krall et al. (2018) also included ED visits in Birmingham, AL and Pittsburgh, PA. The multicity studies in Canada each examined ED visits across Ontario during similar time periods (Szyszkowicz et al., 2018; Weichenthal et al., 2016).
- A smaller multi-city study in California (Cisneros et al., 2021), as well as single-city studies in Atlanta, GA (O'Lenick et al., 2017; Ye et al., 2016) and New York City, NY (Sharma et al., 2023) provide supporting evidence of an association between increases in NO_2 concentrations and increases in ED visits for asthma.
- In addition to studies examining ED visits for asthma, a recent study conducted in Houston, TX reported a 14.9% (95% CI: 7.4, 22.9) increase in ambulance-treated asthma attacks associated with same-day 24-hour avg NO_2 concentrations (Raun et al., 2019).

Hospital Admissions

The 2016 Oxides of Nitrogen ISA reported consistent evidence of positive associations between short-term exposure to NO_2 and hospital admissions for asthma. Except for one multicity study in South Korea, the majority of studies evaluated were single-city studies conducted in diverse locations, including the United States, Canada, Europe, and Asia. The available evidence included some studies with participants of all ages and others focused solely on children, with consistent positive associations observed in both cases. A few of these studies compared results in children ages 0−14 years and participants ages 15−64 years and observed NO2-associated increases in asthma hospital admissions that were 1.8 to 3.4-fold greater in children (Son et al., 2013; Ko et al., 2007; Anderson et al., 1998). The examination of potential copollutant confounding was not thoroughly considered by the studies evaluated in the 2016 Oxides of Nitrogen ISA, but some of the observed NO_2-asthma hospital admission associations were robust to the inclusion of O_3, $PM_{2.5}$, and UFPs in copollutant models. Across studies, associations were observed for same day (i.e., lag 0) and multiday average (i.e., lag 0−4) exposures (see Section 3.3.1.1.2). Evidence from a recent study is summarized below. Study specific details including study population characteristics, exposure assessment metrics, model covariates, and select results are in Appendix A – Appendix Table 3-1.

One recent study analyzed Medicaid data capturing over one and a half million hospitalizations in the contiguous United States from 2000 to 2012 (Wei et al., 2022). Wei et al. (2022) examined 1-hour

max NO_2 concentrations predicted from a hybrid ensemble exposure model in relation to hospital admissions for asthma in Medicaid patients less than 65 years old. In addition to adjusting for traditional potential confounders, such as temperature and humidity, the authors also used a negative exposure control (i.e., NO_2 exposure the day after hospital admission) as a proxy to detect the presence of residual or unmeasured time-varying confounders that would likely be correlated with exposure the day after admission (e.g., other pollutants, meteorological conditions, and behavioral factors; see Section 3.8.5 for background assumptions related to negative exposure controls). Consistent with findings of positive associations between oxides of nitrogen exposures and hospital admissions summarized in the 2016 Oxides of Nitrogen ISA, the authors reported a 7.24% (95% CI: 6.18, 8.32) increase in asthma hospital admissions per 25 ppb increase in lag 0−6 NO_2 concentrations. Wei et al. (2022) also analyzed effects at low concentrations, the lag structure of the observed associations, and effect modification by a range of demographic factors, which are discussed in detail in Sections 3.3.1.1.1, 3.3.1.1.3, and 3.3.1.1.5, respectively.



List of abbreviations in alphabetical order: ED = emergency department; yr = year.
Notes: Black = studies from the 2016 Oxides of Nitrogen ISA; Red = recent studies and those not included in 2016 Oxides of Nitrogen ISA. Circles = NO_2; triangles = NO_X. Results are standardized to a 20 ppb increase in 24-hr avg NO_2, a 25 ppb increase in 1-hr max NO_2, and an 80 ppb increase in 1-hour max NO_X.
†Studies published since the 2016 Oxides of Nitrogen ISA.

Figure 3-1 Percentage increase in asthma hospital admissions and emergency department visits in relation to short-term increases in oxides of nitrogen concentrations in ambient air.

3.3.1.1.1 Concentration-Response Relationships and Effects at Low Concentrations

As discussed in the 2016 Oxides of Nitrogen ISA, only a few studies examined the shape of the concentration-response relationship between short-term NO_2 exposure and hospital admissions or ED visits for asthma. Strickland et al. (2010) and Li et al. (2011) examined the shape of the NO_2-pediatric asthma ED visit relationship using different analytical approaches. Strickland et al. (2010) evaluated locally weighted scatterplot smoothing (LOESS) concentration-response (C-R) analyses across the 5th to 95th percentile NO_2 concentrations. Visual inspection of the C-R plot indicated a monotonically increasing relationship across the entire distribution, with some indication of a stronger relationship at lower NO_2 concentrations. In a study conducted in Detroit, MI, Li et al. (2011) used a change-point model to examine whether the relationship between short-term NO_2 exposure and asthma ED visits deviated from linearity across the exposure distribution. In models 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 of the concentration distribution where a change in linearity was likely to occur; ~80th percentile), the authors observed equivocal evidence of a threshold below which an association is no longer observed. When the authors utilized a time-series study design, there appeared to be evidence supporting a threshold at 23 ppb; however, using a case-crossover design, the C-R relationship was comparable above and below the threshold point and the relationship appeared linear across the range of the exposure distribution.

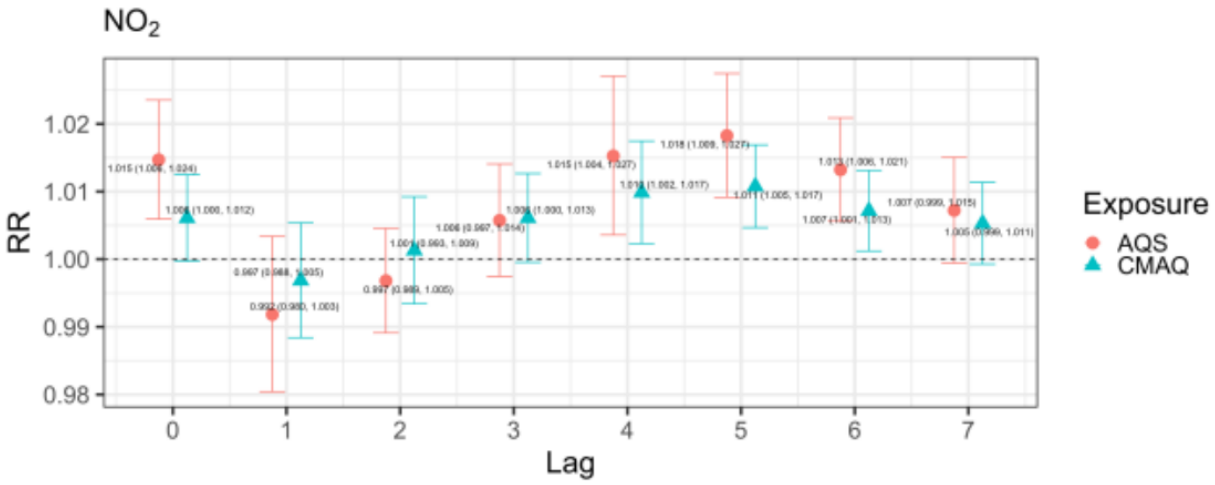
In a recent study analyzing asthma hospital admissions among Medicaid beneficiaries in the contiguous United States, Wei et al. (2022) employed restricted analyses to examine the C-R relationship between asthma hospital admissions and 7-day moving average (i.e., lag 0−7) of 1-hour max NO_2 concentrations below increasingly stringent concentration cut points. The magnitude of the observed association in the full population was comparable to the associations in each of the thresholds from 100 ppb down to 60 ppb, ranging from a 7.24% to 8.31% increase in hospital admissions per 25 ppb increase in 1-hour max NO_2 concentrations. Associations were notably larger at NO_2 concentrations below 40 ppb (13.28% [95% CI: 11.32, 15.27] increase) and 30 ppb (14.41% [95% CI: 11.46, 17.44] increase). These findings were consistent with results from Strickland et al. (2010) that indicated a stronger association at lower concentrations. The collective evidence does not indicate the presence of a threshold below which an association is not observed, and there continues to be support for an association at lower concentrations (e.g., <30 ppb).

3.3.1.1.2 Exposure Assignment

An important uncertainty in air pollution epidemiology is the potential for exposure measurement error resulting from the various methods used to assign exposure. Chapter 2 provides a thorough discussion of the bias resulting from particular exposure assessment methods common to the epidemiologic literature and their impact on the inferences that can be drawn about health effects. In the 2016 Oxides of Nitrogen ISA, epidemiologic studies examining the relationship between short-term NO_2

concentrations and hospital admissions and ED visits for asthma all used fixed or central-site monitors to assign exposure. These exposure surrogates included single monitors and unweighted or population-weighted averages of multiple monitors. One study examined the relative influence of different exposure assignment approaches (i.e., a single central-site monitor, unweighted averages across multiple monitors, and population-weighted averages) on the magnitude and direction of association between NO_2 and pediatric asthma hospital admission in Atlanta, GA (Strickland et al., 2011). Strickland et al. (2011) reported that based on a standardized increment, the magnitude of the association between NO_2 and pediatric asthma ED visits varied (central site monitor: 6.5% [95% CI: 3.5, 9.7] <unweighted average: 10.0% [95% CI: 5.5, 14.7] <population-weighted average: 13.3% [95% CI: 7.6, 19.4] for a 25 ppb increase in 1-hour max NO_2 concentrations at lag 0−2 days). The results suggest that exposure measurement error resulting from the different approaches used to assign exposure across the studies evaluated may alter the magnitude, but in this case not the direction, of the associations observed. This is consistent with exposure studies that have demonstrated that exposure measurement error in the temporal variability of the true short-term exposure concentration decreases the correlation between the exposure surrogate and the incidence of the effect, causing the true effect of exposure on incidence of the health outcome to be underestimated and imprecise.

Similar to studies from the 2016 Oxides of Nitrogen ISA, recent studies mostly use monitors to provide a surrogate for NO_2 exposure. This is common for time-series and case-crossover studies, which tend to rely on temporal variability in exposure, rather than spatial variability, to assess health effects. However, given the potential for finer scale spatiotemporal variation than is captured by air quality monitors, some recent studies have incorporated modeling data to assign exposure. In an analysis of over three million hospital admissions for asthma across 10 U.S. states, Bi et al. (2023) used a hybrid exposure model fusing ground observations from monitors with CMAQ estimates at a 12 km resolution. In general, positive associations were observed using both exposure assessment approaches. RRs derived from NO_2 concentrations estimated from the fused CMAQ model were slightly weaker in magnitude but more precise (e.g., smaller 95% CIs) than those derived from NO_2 concentrations estimated from AQS monitors (see Figure 3-2).



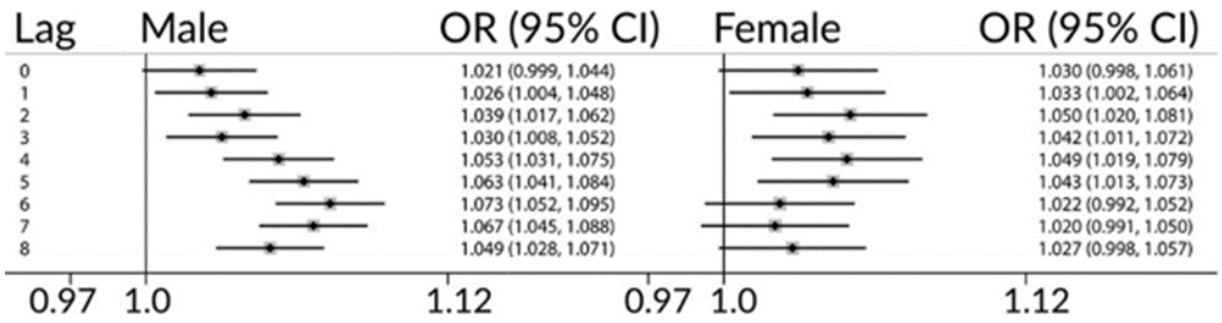
List of abbreviations in alphabetical order: AQS = air quality system; CMAQ = Community Multiscale Air Quality Model; NO_2 = nitrogen dioxide; RR = relative risk.
Note: RRs per 18.9 ppb increase 1-hr max NO_2.
Source: Adapted from Bi et al. (2023). Copyright permission pending.

Figure 3-2 Relative risks for asthma emergency department visits in relation to short-term increases in modeled and monitored ambient nitrogen dioxide concentrations estimated across several individual lag days in 10 states from the United States.

Other recent studies used modeled estimates, but did not compare results to effect estimates derived from monitored concentrations. Sharma et al. (2023) combined a LUR regression model with a mean time trend derived from AQS monitors in New York City to provide more refined spatiotemporal exposure estimates. In a recent study of asthma hospital admissions among Medicaid beneficiaries, Wei et al. (2022) used ensemble predictions of three machine learning models combining air monitoring data, satellite aerosol optical depth, meteorological conditions, chemical transport model simulations, and land-use variables to estimate the daily 1-hour max NO_2 concentrations at the centroids of 1 km × 1 km grid cells across the contiguous United States. Both studies reported positive associations with asthma hospital admissions or ED visits that are similar in magnitude to studies that used central site monitors.

3.3.1.1.3 Lag Structure of Associations

As discussed in the 2016 Oxides of Nitrogen ISA, there was some evidence of different lag structures for associations with asthma hospital admissions versus ED visits. More immediate effects (i.e., lag 0) were reported for asthma hospital admissions and evidence of prolonged effects of short-term exposures was reported for asthma ED visits, with a number of studies showing effects on ED visits at multiday lags ranging from 0−2 to 0−4 days. Most recent studies defined lags a priori, with many studies of asthma ED visits examining multiday lags, as informed by previous studies described in the 2016 Oxides of Nitrogen ISA. A few recent studies modeled several different lag periods. Bi et al. (2023) reported the largest relative risks (RR) for asthma ED visits associated with immediate (i.e., lag 0) and delayed exposure lags (i.e., individual lag days four through 7), with null or weak positive associations at intermediate lags (see Figure 3-2). In a multicity study in Ontario, Canada, Szyszkowicz et al. (2018) examined nine single day lags ranging from same-day exposure to exposure eight days prior to an asthma ED visit. Patterns of associations across lags varied by sex, but positive associations were observed for each individual lag day for both sexes (see Figure 3-3). Especially among males, where odds ratios (OR) were largest at later lags, these results indicate the potential for effects of prolonged short-term NO_2 exposure. Cisneros et al. (2021) reported similar findings with evidence of positive associations corresponding to exposures up to 30 days prior to ED visits for asthma in the cold season in a multi-city study in California.



List of abbreviations in alphabetical order: CI = confidence interval; OR = odds ratio.
Note: ORs per 9 ppb increase 24-hr avg NO_2.
Source: Adapted from Szyszkowicz et al. (2018). Copyright permission pending.

Figure 3-3 Odds ratios for asthma ED visits in children in relation to short-term increases in nitrogen dioxide concentrations in ambient air in the warm season across several individual lag days in Ontario, Canada.

In contrast to studies described in the 2016 Oxides of Nitrogen ISA that demonstrated mostly immediate effects of oxides of nitrogen exposure on asthma hospital admissions (i.e., lag 0), Wei et al. (2022) reported prolonged effects of short-term NO_2 exposure with hospital admissions for asthma (i.e., associations with exposure lags averaged from zero to five or more days prior to outcome). As noted previously, the authors reported associations with 7-day moving average NO_2 exposure (see Section 3.3.1.1) and noted positive associations at each of the seven individual lag days from same-day exposure to exposures six days prior to hospital admissions. The strongest associations were observed at lag days two through four.

3.3.1.1.4 Examination of Seasonal Differences

In addition to examining the association between short-term NO_2 exposure and asthma hospital admissions and ED visits in all-year analyses, several studies discussed in the 2016 Oxides of Nitrogen ISA also conducted seasonal analyses. Overall, these studies generally provided evidence of larger associations in the warm or summer season compared to cooler months. Notably, these studies did not examine potential copollutant confounding by season, and the correlation between personal and ambient air measurements often differs by season, both of which could influence the results of seasonal analyses.

Although a few recent studies reported results for a specific season, there was limited examination of seasonal differences in the association between short-term NO_2 concentrations and ED visits for asthma. Consistent with evidence from the 2016 Oxides of Nitrogen ISA, a recent study conducted in New York City, NY reported a larger NO_2-related increase in pediatric ED visits for asthma in the warm season (16.34% [95% CI: 13.77, 18.97] per 20 ppb increase in lag 0-6 NO_2) compared to the cold season (8.06% [95% CI: 6.29, 9.85]) (Sharma et al., 2023).

3.3.1.1.5 Lifestages and Population

3.3.1.1.5.1 Race/Ethnicity

In the 2016 Oxides of Nitrogen ISA, a single study of pediatric hospital admissions for asthma specifically aimed to analyze racial/ethnic differences in risk related to short-term NO_2 exposure (Grineski et al., 2010). The authors noted a larger risk of NO_2-related asthma ED visits for Black children compared to Hispanic children, but no difference between Black children and white children. Analyses from several recent studies are generally inconsistent. A recent nationwide study of hospital admissions among Medicaid beneficiaries in the United States (Wei et al., 2022) and a study of pediatric asthma ED visits in New York City, NY (Sharma et al., 2023) did not observe notable differences in NO_2-related risk of asthma exacerbation across racial/ethnic groups. Other recent studies in the United States provided contrasting evidence. In a multi-city study in California, Cisneros et al. (2021) noted stronger associations between same day NO_2 exposure and asthma ED visits in the cold season among white participants compared to Black, Hispanic, or Asian participants (quantitative results not provided). Conversely, a multi-city study across multiple states in the Southern United States reported that 3-day moving average (i.e., lag 0–2) NO_2 concentrations were more strongly associated with pediatric asthma ED visits among non-white children ages 5-18 years (RR: 1.20 [95% CI: 1.14, 1.26]) compared to white children of the same age (RR: 1.04 [95% CI: 1.00, 1.09]) (Alhanti et al., 2016). Alhanti et al. (2016) reported similar racial differences among adults.

3.3.1.1.5.2 Sex

A study evaluated in the 2016 Oxides of Nitrogen ISA observed increased risk of hospital admissions for females compared to males in a time-series analysis of low-income children in Vancouver, Canada (Lin et al., 2004). Results from recent studies are inconsistent. In a multi-city study conducted in Ontario, Canada, Szyszkowicz et al. (2018) observed that patterns of associations for short-term NO_2 exposure and asthma ED visits by lag day varied between sexes (see Figure 3-3), but the general strength of associations was comparable across males and females. Cisneros et al. (2021) similarly reported no sex-specific differences in risk of NO_2-related asthma ED visits in the cold season in a multi-city study in California (quantitative results not provided). In contrast, recent multi-city studies in the United States reported positive associations between short-term NO_2 exposure and hospital admissions (Wei et al., 2022) and ED visits (Alhanti et al., 2016) for asthma that were stronger among males compared to females.

3.3.1.1.5.3 Obesity

Although several epidemiologic studies in the 2016 Oxides of Nitrogen ISA examined obesity as a potential modifier of the effect of NO_2 exposure on cardiovascular health outcomes, no studies of respiratory outcomes considered the role of body mass index (BMI) or obesity as a mediator or moderator of the effect of NO_2 exposure. One recent study of asthma hospital admissions among Medicaid beneficiaries evaluated the relationship between short-term NO_2 exposure and asthma hospital admissions stratified by annual average BMI at the ZIP code tabulation area (ZCTA) level (Wei et al., 2022). The reported increase in hospital admissions for asthma for a 25 ppb increase in 1-hour max NO_2 concentrations was higher in high BMI ZCTAs (9.95% [95% CI: 6.71, 13.28]) compared to medium to low BMI ZCTAs (6.71% [95% CI: 5.38, 8.05]). Notably, at the population level, BMI is strongly correlated with several sociodemographic and behavioral factors, including diet, neighborhood resources, and neighborhood safety.

3.3.1.1.5.4 Socioeconomic Status and Social Stressors

Across a strong body of epidemiologic evidence from the 2016 Oxides of Nitrogen ISA that indicated a relationship between short-term NO_2 exposure and asthma exacerbation, relatively few studies examined potential effect modification by socioeconomic status (SES) or social stressors as an *a priori* objective. Of those studies that did, each used different indicators of SES or social stress, which limits the strength of inference across studies. U.S. studies observed higher risk of NO_2-related asthma exacerbation and incidence in groups of children with high exposure to community violence (Clougherty et al., 2007) or with no health insurance (Grineski et al., 2010). In another study in Canada, census block-level income did not modify associations of short-term NO_2 exposure with asthma hospital admissions (Lin et al., 2004).

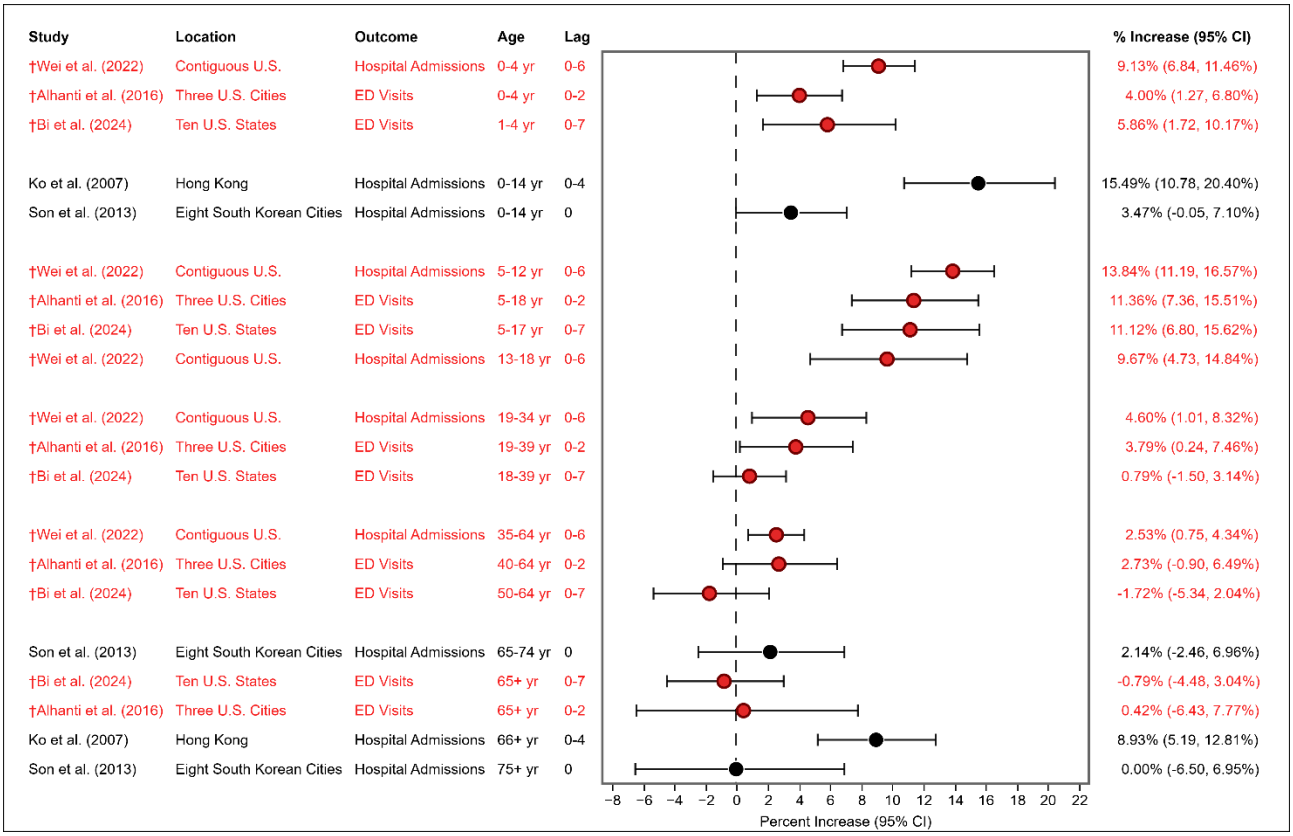
A limited number of recent studies also examined the influence of sociodemographic factors on the association between short-term exposure to NO_2 and asthma exacerbation. In a large case-crossover study in New York City, NY, Sharma et al. (2023) examined the impact of social stressors on the relationship between short-term NO_2 exposure and pediatric asthma ED visits. The authors considered both census tract-level spatial estimates of violent crime rate and Socioeconomic Deprivation Index (SDI) score, which comprises various components of SES, including income, education, employment, and housing. In contrast to a similar study described in the 2016 Oxides of Nitrogen ISA (Clougherty et al., 2007), Sharma et al. (2023) did not observe modification by neighborhood violence. The authors did report evidence of modification by SDI score, with a higher risk of NO_2-related pediatric ED visits in communities with lower SDI scores (i.e., less socioeconomic deprivation). In contrast, a recent nationwide study of Medicaid beneficiaries reported stronger associations between short-term NO_2 exposure and hospital admissions in ZCTAs with higher socioeconomic deprivation (Wei et al., 2022).

Notably, because Medicaid beneficiaries comprise a potentially vulnerable lower SES population, the results may not be directly comparable to Sharma et al. (2023).

3.3.1.1.5.5 Lifestage

Epidemiologic evidence in the 2016 Oxides of Nitrogen ISA consistently demonstrated that short-term increases in ambient NO_2 concentrations are associated with larger increases in asthma-related hospital admissions, ED visits, or outpatient visits among children compared to adults (Son et al., 2013; Sinclair et al., 2010; Ko et al., 2007; Hinwood et al., 2006; Peel et al., 2005; Atkinson et al., 1999; Anderson et al., 1998). The available evidence included single and multicity studies that were conducted in diverse locations (i.e., United States, Canada, Europe, Asia, Australia). Most comparisons were between children ages 0−14 years and people ages 15−64 years, which showed NO_2-associated increases in asthma hospital admissions that were 1.8- to 3.4-fold greater in children.

A few recent multi-city U.S. studies also conducted *a priori* stratified analyses to examine how age modifies the association between NO_2 exposure and hospital admissions or ED visits for asthma (Bi et al., 2023; Wei et al., 2022; Alhanti et al., 2016). Bi et al. (2023), Wei et al. (2022), and Alhanti et al. (2016) observed the largest increases in hospital admissions or ED visits for asthma among children ages 5−17 years (11.1% [95% CI: 6.8, 15.6] per 25 ppb increase in 1-hour max NO_2); 5−12 years (13.8% [95% CI: 11.2, 16.6] per 25 ppb increase in 1-hour max NO_2); and 5−18 years (11.4% [95% CI: 7.4, 15.5] per 25 ppb increase in 1-hour max NO_2), respectively (see Figure 3-4). The associations observed by Wei et al. (2022) and Alhanti et al. (2016) in these age groups were 2.5- to 5-fold larger than associations in pre-defined adult age groups. In both studies, there was a trend of decreasing magnitude of association with increasing age among adults. Bi et al. (2023) reported null associations for each of the adult age groups examined. Taken together, the evidence continues to demonstrate larger NO_2-associated increases in hospital admissions or ED visits for asthma among children compared to adults.



List of abbreviations in alphabetical order: CI = confidence interval; ED = emergency department; U.S. = United States; yr = year.
Notes: Black = studies from the 2016 Oxides of Nitrogen ISA; Red = recent studies and those not included in the 2016 Oxides of Nitrogen ISA. Circles = NO_2. Results are standardized to a 20 ppb increase in 24-hour avg NO_2 or a 25 ppb increase in 1-hr max NO_2.
†Studies published since the 2016 Oxides of Nitrogen ISA.

Figure 3-4 Age-stratified percentage increase in asthma hospital admissions and emergency department visits in relation to short-term increases in nitrogen dioxide concentrations in ambient air.

3.3.1.1.6 Copollutants

NO_2 is often highly correlated with other traffic-related copollutants (e.g., CO, BC, UFP), adding uncertainty to whether short-term NO_2 exposures are independently associated with asthma hospital admissions and ED visits. Studies included in the 2016 Oxides of Nitrogen ISA that examined potential confounding by $PM_{2.5}$, UFP, or CO provided evidence that associations between short-term NO_2 exposures and asthma hospital admissions and ED visits remained relatively unchanged (i.e., similar in magnitude or slightly attenuated, but still positive) in copollutant models (see Table 3-3). Due to high correlations of traffic-related pollutants with NO_2, recent studies include limited evaluation of potential copollutant confounding. Studies that do adjust for traffic-related pollutants and other pollutants provide further support for previous findings.

In a recent study in Atlanta, GA, Ye et al. (2016) observed an increase in ED visits for asthma associated with an increase in 3-day moving average (i.e., lag 0–2) 1-hour max NO_2 concentrations that was generally attenuated, but still positive following adjustment for indicator pollutants for 10 VOC groups classified by chemical structure (e.g., ethylene as an indicator for alkenes, toluene as an indicator for aromatics). Cisneros et al. (2021) similarly noted positive effect estimates that were attenuated compared to single pollutant NO_2 models, following adjustment for CO in a small multi-city case-crossover study of asthma ED visits in the cold season in California. The authors reported that the positive association between NO_2 and asthma ED visits was robust to adjustment for $PM_{2.5}$ and SO_2.

Table 3-3 Copollutant-adjusted associations between short-term exposure to nitrogen dioxide and hospital admissions or emergency department visits for asthma

Study and Location	Averaging Time	Lag	Correlations (*r*)	Effect Estimate (95% CI)
Hospital Admissions				
Samoli et al. (2011) Athens, Greece	1-hr max	0	PM_{10}: NR (<0.55) SO_2: 0.55 O_3: NR (<0.55)	% increase: 5.28% (-3.2, 14.5%) +PM_{10}: 2.6% (-6.1, 12.1%) +SO_2: -3.6% (-14.3, 8.4%) +O_3: 5.8% (-2.8, 15.1%)
Iskandar et al. (2012) Copenhagen, Denmark	24-hr avg	0	PM_{10}: 0.43 $PM_{2.5}$: 0.33 UFP: 0.51	OR: 1.3 (1.1, 1.6) +PM_{10}: 1.3 (1.0, 1.5) +$PM_{2.5}$: 1.4 (1.2, 1.7) +UFP: 1.5 (1.2, 1.8)
Ko et al. (2007) Hong Kong, China	24-hr avg	0–4	O_3: 0.41	% increase: 11.0% (8.1, 13.8%) +O_3: 2.3% (-0.8, 5.8%)
ED Visits				
Ito et al. (2007) New York, NY, United States	24-hr avg	0	NR	$PM_{2.5}$-, O_3-, CO-, and SO_2-adjusted NO_2 associations were relatively similar to single pollutant results (quantitative results not provided)
Strickland et al. (2010) Atlanta, GA, United States	1-hr max	0–7	NR	O_3-adjusted NO_2 associations were relatively similar to single pollutant results (quantitative results not provided)
Villeneuve et al. (2007) Edmonton, Canada	24-hr avg	0–4	CO: 0.74	CO-adjusted NO_2 associations were relatively similar to single pollutant results, except among 15–44-year-olds, where adjusted were attenuated but still positive (quantitative results not provided)
Jalaludin et al. (2008) Sydney, Australia	1-hr max	0	PM_{10}: 0.67 $PM_{2.5}$: 0.68 O_3: 0.21 CO: 0.71 SO_2: 0.52	% increase: 6.2% (3.7, 8.7%) +PM_{10}: 2.7% (1.1, 4.3%) +$PM_{2.5}$: 2.9% (1.6, 4.3%) +O_3: 3.2% (1.7, 4.7%) +CO: 4.8% (2.7, 7.0%)

Study and Location	Averaging Time	Lag	Correlations (*r*)	Effect Estimate (95% CI)
				+SO_2: 2.7% (0.3, 5.1%)
†Cisneros et al. (2021) 5 cities in California, United States	24-hr avg	0–30	NR	OR: 1.401 (1.307, 1.503) +$PM_{2.5}$: 1.396 (1.292, 1.508) +CO: 1.239 (1.118, 1.372) +SO_2: 1.376 (1.28, 1.478)
†Ye et al. (2016) Atlanta, GA, United States	1-hr max	0–2	VOC Group N-Alkane: 0.48 Iso-Alkane: 0.52 Other Alkane: 0.51 Cylcloalkane: 0.53 Alkene: 0.47 Alkyne: 0.5 Aromatic: 0.51 Aldehyde: 0.18 Acid: -0.03 Ketone: 0.05	% increase: 3.79% (1.67, 5.96%) +N-Alkane: 3.30% (1.06, 5.60%) +Iso-Alkane: 2.93% (0.57, 5.35%) +Other Alkane: 3.18% (0.82, 5.60%) +Cycloalkane: 2.81% (0.45, 5.23%) +Alkene: 3.55% (1.24, 5.91%) +Alkyne: 3.30% (1.00, 5.66%) +Aromatic: 2.93% (0.57, 5.35%) +Aldehyde: 3.92% (1.73, 6.15%) +Acid: 3.79% (1.61, 6.03%) +Ketone: 3.55% (1.36, 5.78%)

List of abbreviations in alphabetical order: Avg = average; CA = California; CI = confidence interval; CO = carbon monoxide; GA = Georgia; h = hour; IQR = interquartile range; max = maximum; NO_2 = nitrogen dioxide; NR = not reported; NY = New York; OR = odds ratio; $PM_{2.5}$ = particulate matter with a diameter less than 2.5 micrometers; PM_{10} = particulate matter with a diameter less than 10 micrometers; SO_2 = sulfur dioxide; UFP = ultrafine particles; VOC = volatile organic compounds.
†Studies published since the 2016 Oxides of Nitrogen ISA.

3.3.1.1.7 Conclusions and Remaining Uncertainties

Recent epidemiologic studies examining the association between short-term NO_2 exposure and asthma hospital admissions and ED visits support and extend findings from the 2016 Oxides of Nitrogen ISA. Relative to evidence evaluated in the 2016 Oxides of Nitrogen ISA, recent evidence includes an increased number of multicity or nationwide studies, including several in the United States and Canada with large case numbers, which consistently observed positive associations between short-term NO_2 exposures and asthma hospital admissions and ED visits. Across studies of asthma ED visits, there continues to be evidence of immediate and delayed effects of short-term exposures, with a few recent studies demonstrating associations with individual exposure lags of up to eight days. In contrast to studies described in the 2016 Oxides of Nitrogen ISA that demonstrated stronger evidence of more immediate effects (i.e., lag 0) for asthma hospital admissions, a recent study noted positive associations with each of seven individual exposure lag days ranging from same-day exposure to exposures six days prior to hospital admissions. On the whole, evidence suggests that short-term exposures to NO_2 may elicit immediate and delayed asthma exacerbation as indicated by asthma-related hospital admissions and ED visits.

A number of recent studies also evaluated whether there is evidence that the association between short-term NO_2 exposures and asthma hospital admissions and ED visits is modified by season or some

individual- or population-level factors. Consistent with evidence from the 2016 Oxides of Nitrogen ISA, a recent study provides further evidence that NO_2 associations with ED visits are larger in magnitude in the summer or warm season compared to the winter or cold season. There is also consistent evidence demonstrating stronger associations among children compared to adults. Across studies that further stratify into narrower age groups, the strongest associations are generally observed in children between the ages of five and 18 years. Evidence for other factors that may modify the association between short-term NO_2 exposure and asthma hospital admissions and ED visits, including race/ethnicity, sex, and SES factors, remains inconsistent.

Recent studies also include novel or expanded examination of various study designs and methodologies, including alternative methods for confounder control and additional exposure assignment approaches. A recent study of hospital admissions for asthma using a negative exposure control did not detect evidence that the observed positive association was a result of residual or unmeasured confounding. Additionally, recent studies, as well as those discussed in the 2016 Oxides of Nitrogen ISA, provide generally consistent evidence of positive associations across a variety of exposure assignment techniques, including population-weighted monitor averages, CMAQ modeling estimates, and LUR models. Studies that compared results across exposure assignment methods observed that exposure measurement error resulting from the different approaches impacted the magnitude, but not the direction, of the observed associations.

Notable uncertainties in the evidence base still remain. Oxides of nitrogen are often highly correlated with other traffic-related pollutants, making it a challenge to discern an independent effect of NO_2 in the epidemiologic literature. Of the limited number of studies that examined potential confounding by $PM_{2.5}$ or traffic-related pollutants (e.g., UFP, CO, or VOCs), associations between short-term NO_2 exposures and asthma hospital admissions and ED visits remained relatively unchanged in copollutant models (i.e., similar in magnitude or attenuated slightly, but still positive). Recent studies provide additional support for the robustness of these associations in copollutant models. However, relative to the overall body of evidence, evaluation of potential copollutant confounding by traffic-related pollutants remains limited, and high correlations between NO_2 and these pollutants complicate the interpretation of the available copollutant models. There has also been limited evaluation of the shape of the C-R relationship. In the few studies available that did account for potential non-linearities in the C-R relationship or estimate the relationship between short-term NO_2 exposures and asthma hospital admissions and ED visits in restricted low-exposure analyses, the evidence does not indicate the presence of a threshold, and there continues to be support for an association at lower concentrations (e.g., <30 ppb).

3.3.2 Respiratory Symptoms and Asthma Medication Use

Respiratory symptoms in children with asthma, including cough, wheeze, sputum production, shortness of breath, and chest tightness, may indicate an exacerbation of disease. Further, uncontrollable symptoms may lead people with asthma to treat symptoms with medication, such as short-acting beta agonists. Thus, studies examining the association between NO_2 and increases in asthma symptoms and medication use may provide insight into NO_2-induced acute respiratory effects and into the biological plausibility of NO_2-related increases in asthma hospital admissions and ED visits in children.

3.3.2.1 Epidemiologic Studies of Respiratory Symptoms and Asthma Medication Use

Epidemiologic studies that were evaluated in the 2016 Oxides of Nitrogen ISA consistently reported increased respiratory symptoms and asthma medication use among people with asthma in association with increases in indoor, personal, and ambient air NO_2 concentrations. Evidence was stronger for children with asthma compared to adults with asthma. Study populations were recruited from schools, asthma or allergy clinics, or doctors' offices. 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. None of these methodological choices appeared to significantly influence results. In a limited number of studies, larger NO_2-associated increases in respiratory symptoms were reported in children with asthma who also had allergies. Analysis of confounding by traffic-related copollutants was limited in both the number of studies and the array of copollutants considered. There was limited evidence that associations between short-term NO_2 exposure and respiratory symptoms in children with asthma were robust or attenuated but still positive in copollutant models adjusted for VOCs, SO_2, CO, $PM_{10-2.5}$, or O_3.

A few recent studies add to the existing evidence base and provide some additional evidence of an association between short-term NO_2 exposure and asthma medication use. Recent evidence is not entirely consistent, though the available studies that report null associations have notable limitations. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 3-2. A short, bulleted summary of the recent evidence is provided below, followed by additional discussion of the concentration-response relationship in a recent study (see Section 3.3.2.1.1). Section 3.3.2.1.2 discusses conclusions from this evidence and summarizes remaining uncertainties.

A few recent studies that used digital inhaler sensors and Global Positioning System (GPS) tracking provide strong evidence for an association between short-term oxides of nitrogen exposure and increased medication use among children and adults with asthma (Su et al., 2024; Hao et al., 2022; Su et al., 2022).

- Recent studies in California (Su et al., 2024) and Jefferson County, KY (Su et al., 2022) tracked rescue medication use in panels of volunteers with asthma. Participants were given digital sensors and a GPS app to track inhaler use and map participants' locations to either the nearest monitor (Su et al., 2022) or a hybrid-modeled 100m resolution air quality surface (Su et al., 2024) at the time of rescue medication use. Su et al. (2022) reported a 28.0% (95% CI: 24.6, 31.4) increase in daily rescue medication use over average (1.24 rescue uses/person/day) in relation to a 20 ppb increase in same day 24-hour avg NO_2. In the analysis of participants from California, Su et al. (2024) observed a smaller, 7.3% (95% CI: 5.3%, 9.4) increase in daily rescue medication use over average (0.86 rescue uses/person/day) in relation to a 20 ppb increase in same day 24-hour avg NO_2. The California study included 352 participants with COPD in addition to 3,034 volunteers with asthma, and the results do not distinguish between those with asthma and COPD.
- Hao et al. (2022) utilized a similar design with a small panel of 40 children with asthma who were recruited from the University of California, Los Angeles (UCLA) pediatric asthma clinics in Los Angeles and Santa Monica, CA. In addition to digital inhaler sensors and GPS tracking, Hao et al. (2022) assigned spatially resolved modeled exposure estimates using inverse-distance squared spatial interpolation from city-wide monitors. The authors reported imprecise (i.e., wide 95% CIs) increases in the rate of rescue inhaler use associated with increases in same day 24-hour avg NO_X (RR: 1.61 [95% CI: 1.23, 2.11] per 6.1 ppb increase), NO (RR: 1.80 [95% CI: 1.37, 2.35] per 2.4 ppb increase), and NO_2 (RR: 1.20 [95% CI: 0.85, 1.68] per 4.6 ppb increase).

Other recent evidence provides little support for an association between short-term NO_2 concentrations and asthma symptoms and medication use.

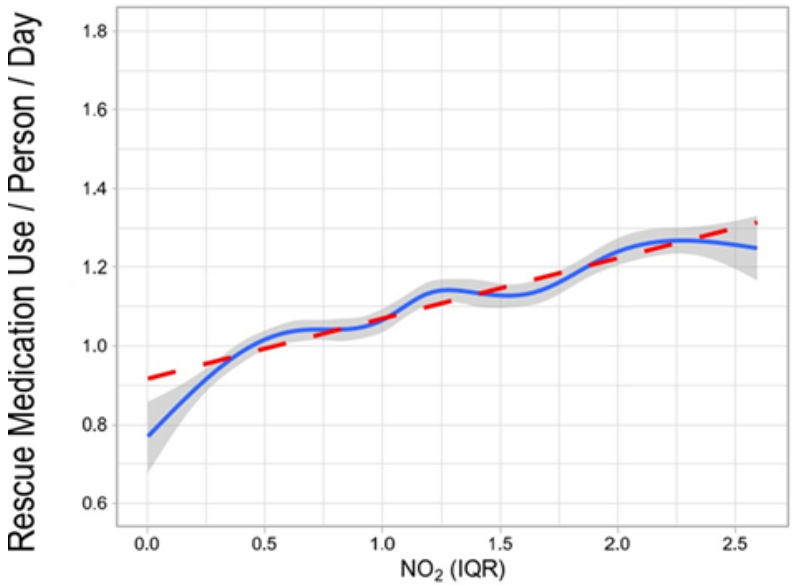
- A recent intervention study analyzed the impact of air cleaners on indoor NO_2 concentrations and asthma symptoms among children aged 5–11 years with persistent asthma living in homes with gas stoves (Gent et al., 2023). Although NO_2 scrubbers reduced indoor NO_2 concentrations by 4 ppb, the intervention did not reduce self-reported asthma symptoms compared to the control. Notably, the authors did not consider the participating children's ambient NO_2 exposures.
- Similarly, a small panel study of 36 inner-city New York, NY children conducted over two 2-week periods did not observe an association between same day 24-hour avg NO_2 concentrations measured at a single central site monitor and self-reported asthma symptoms (Schachter et al., 2016). Schachter et al. (2016) did observe a positive association between 24-hour avg NO_2 exposure and albuterol puffs during the summer observation period, but the reported OR was imprecise (OR: 1.36 [95% CI: 0.87, 2.12] per IQR increase in NO_2 - increment not reported).

Given the use of GPS data to aid exposure assignment, digital tracking of medication use, and a large number of person-days (~200,000), Su et al. (2022) provides the strongest recent evidence informing the relationship between short-term NO_2 exposure and asthma medication use. This study may be susceptible to potential selection bias due to self-selection, particularly as it relates to the generalizability of the study population; however, any factors associated with self-selection are unlikely to be associated with temporal variability in NO_2 exposure. Su et al. (2024) provides similarly strong evidence, but does not distinguish between participants with asthma and COPD. Hao et al. (2022) utilized similar design strengths as Su et al. (2022) and Su et al. (2024), but had a much smaller sample size and

consequently, less precise effect estimates. Other recent studies are limited for the purposes of this assessment either by limited statistical power to detect an association (Schachter et al., 2016) or a lack of consideration of NO_2 exposures from ambient air (Gent et al., 2023).

3.3.2.1.1 Concentration Response and Exposure Response

Although several epidemiologic studies in the 2016 Oxides of Nitrogen ISA examined the shape of the concentration-response relationship between short-term NO_2 exposure and hospital admissions or ED visits for asthma (see Section 3.3.1.1), no studies of respiratory symptoms or medication use evaluated potential non-linearities in the relationship with short-term NO_2 exposure. A recent panel study of over 1,000 volunteers with asthma analyzed daily rescue medication use in relation to NO_2 concentrations using penalized regression splines to account for potential non-linearities (Su et al., 2022). The authors modeled IQR increases truncated at the 95th percentile to avoid unstable estimates due to sparse data. The shape of the C-R relationship was approximately linear (see Figure 3-5). Precision was decreased at the tails of the concentration distribution, which appeared to have some deviation from linearity.



List of abbreviations in alphabetical order: IQR = interquartile range; NO_2 = nitrogen dioxide; ppb = parts per billion.\
Note: IQR = 9.44 ppb
Source: Adapted from Su et al. (2022). Copyright permission pending.

Figure 3-5 Penalized partial dependence plot of nitrogen dioxide with daily rescue medication use.

3.3.2.1.2 Conclusions and Remaining Uncertainties

A limited number of recent studies add to a large body of epidemiologic studies from the 2016 Oxides of Nitrogen ISA, which provided consistent evidence of increased respiratory symptoms and asthma medication use among people with asthma in association with increases in indoor, personal, and ambient NO_2 concentrations. Recent studies providing the strongest evidence utilize digital tracking of inhaler use and GPS data to geolocate participants for exposure assignment. In general, evidence is stronger for children with asthma compared to adults with asthma. Positive associations were observed across studies using a variety of recruitment approaches, methods for symptom evaluation, and exposure assignment techniques. Similar to studies of hospital admissions and ED visits, the analysis of confounding by traffic-related copollutants in studies of respiratory symptoms or asthma medication use was limited in both the number of studies and the array of copollutants considered. Associations between short-term NO_2 exposure and respiratory symptoms in children with asthma were robust or attenuated but still positive in copollutant models adjusted for VOCs, SO_2, CO, $PM_{10-2.5}$, or O_3. Additionally, there has been limited evaluation of the C-R response relationship and a lack of examination of how individual- or population-level factors may modify the observed associations.

3.3.2.2 Controlled Human Exposure Studies of Respiratory Symptoms and Asthma Medication Use

The controlled human exposure evidence assessed in the 2016 Oxides of Nitrogen ISA provided limited support for induction of respiratory symptoms in participants with asthma following short-term exposure to NO_2. No recent controlled human exposure studies have looked at respiratory symptoms or medication use, thus the evidence base is limited to that presented in 2016. A summary of the existing literature base is provided below. Study specific details, exposure conditions, and endpoints examined are highlighted in the text below and described in Table 5-9 of U.S. EPA (2016). Conclusions from this evidence and remaining uncertainties are discussed in Section 3.3.2.2.1.

- Participants with asthma exposed for 30-minutes to 2-hours of NO_2 in the range of 120 ppb to 350 ppb with exercise generally showed little effect on reported respiratory symptoms either immediately following or up to one week after exposure (Riedl et al., 2012; Vagaggini et al., 1996; Koenig et al., 1987; Kleinman et al., 1983).
- Small but significant increases in symptoms were seen in healthy subjects exposed to 300 ppb for 2-hours with intermediate exercise. This was not seen in subjects with asthma (Vagaggini et al., 1996).
- No change in subjective symptom scores was observed following 2-hour exposures to 100, 200, or 800 ppb NO_2 with 10 minutes of submaximal exercise midway through exposures in 20 healthy subjects or 20 subjects with asthma (Rasmussen et al., 1990).
- Subjects with asthma exposed to 350 ppb NO_2 for 2-hours with intermittent exercise had increased symptoms during exposure but not after (Riedl et al., 2012).

- NO_2 exposure (350 ppb for 2-hours) did not change symptom scores in response to an allergen challenge performed after the NO_2 exposure (Riedl et al., 2012).

Together, the small number of existing controlled human exposure studies provides limited support for an impact of short-term NO_2 exposure on respiratory symptoms in subjects with asthma.

3.3.2.2.1 Conclusions and Remaining Uncertainties

Controlled human exposure studies do not provide strong evidence for NO_2-induced increases in respiratory symptoms in adults or adolescents with asthma. Most studies of respiratory symptoms in asthmatics did not utilize a challenge agent but reported no change in symptom scores over controls. A single study that assessed symptoms after allergen challenge similarly showed null findings. It is important to note that controlled human exposure studies do not include those with the most serious underlying respiratory disease, potentially limiting the ability of these studies to capture populations at greatest risk of exacerbating asthma symptoms.

3.3.3 Respiratory Effects Underlying Asthma Exacerbation

3.3.3.1 Airway Responsiveness

Studies included in the 2016 Oxides of Nitrogen ISA show that exposure to NO_2 enhances the reactivity of airway smooth muscles to a variety of natural or pharmaceutical stimuli. These stimuli (i.e., challenge agents) can be classified as either "specific" or "nonspecific," with specific agents being those whose bronchoconstriction potential is related to individual sensitivity (i.e., an allergen) and nonspecific stimuli being more general activators of airway smooth muscle contraction (e.g., cold air, SO_2, histamine, carbachol). Nonspecific agents that act on smooth muscle receptors are considered "direct" acting (e.g., histamine) and those that utilize intermediate signaling to activate bronchial muscles are termed "indirect" acting (e.g., cold air). The broncho-constrictive response to indirect acting agents (especially specific allergens) can be more difficult to predict and control than the broncho-constrictive response to nonspecific agents that act directly on airway smooth muscle receptors (O'Byrne et al., 2009).

Airway responsiveness can be measured using spirometry or plethysmography coupled with increasing doses of allergen or chemical or physiological stimuli. Studies have shown that the provocative dose of challenge agent to induce bronchoconstriction and airway obstruction can be an order of magnitude less in people with asthma than in healthy age-matched controls (Morrow and Utell, 1989a; Hazucha et al., 1983). Baseline airway responsiveness is log-normally distributed in the general population of people with asthma commonly among those considered to have airway hyperresponsiveness (Postma and Boezen, 2004; Cockcroft et al., 1983). Airway hyperresponsiveness (synonymous with hyperreactivity) is a primary feature in the clinical definition and characterization of asthma severity

(Reddel et al., 2009). Airway hyperreactivity is also a risk factor for the development/diagnosis of asthma (Brutsche et al., 2006). This propensity for increased airway reactivity and subsequent reductions in lung function potentially put people with asthma at higher risk than the general population for experiencing respiratory complications in response to inhaled pollutants like NO_2. The following sections describe the evidence from studies investigating the impacts of NO_2 or other oxides of nitrogen exposure on airway reactivity.

3.3.3.1.1 Controlled Human Exposure Studies of Airway Responsiveness

The 2016 Oxides of Nitrogen ISA described several controlled human exposure studies of asthmatics that investigated airway reactivity changes in response to short term NO_2 exposure [see Tables 3-6 and 3-7 in U.S. EPA (2016)].[5] As discussed in the 2016 Oxides of Nitrogen ISA and in the above section, airway challenge agents can be classified as specific (i.e., an allergen) or nonspecific. The changes in airway responsiveness to nonspecific stimuli are more predictable than to allergen challenge, likely because allergen responses depend on an individual's level of sensitivity, which may vary considerably. Additionally, use of specific stimuli can pose greater risk to people with asthma, in part due to a second, more delayed (3–12 hours after allergen exposure) onset of symptoms that are usually more severe than the early response (which resolves within one to two hours) and are less responsive to bronchodilators. As a result, studies often evaluate responsiveness to nonspecific stimuli. While the results from individual studies are mixed, with some studies showing statistically significant increases in group mean airway reactivity following NO_2 exposure and others showing no significant changes [see Table 3-6 in U.S. EPA (2016)] broader patterns have emerged when the individual-level data from these studies has been combined and evaluated in several meta-analyses. The 2008 Oxides of Nitrogen ISA and 2016 Oxides of Nitrogen ISA described four meta-analyses that aimed to further understand the potential impacts of NO_2 exposure on airway responsiveness by looking at the fraction of individuals experiencing increased airway reactivity (measured as a decrease in the provocative dose to achieve a standardized decrease in lung function or increase in airway resistance) (Brown, 2015; Goodman et al., 2009; Kjaergaard and Rasmussen, 1996; Folinsbee, 1992). Findings from these analyses are summarized below. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Table 3-4 and Table 3-5. There have been no recent published studies of controlled human exposures that include people with asthma.

- In studies where participants with asthma were at rest, NO_2 exposures at 100 ppb, 100 to less than 200 ppb, 200 to less than 300 ppb, and greater than 300 ppb resulted in statistically significant proportions of study participants (i.e., 66% to 78%) experiencing increased airway responsiveness to nonspecific stimuli (Brown, 2015; Goodman et al., 2009; U.S. EPA, 2008b; Kjaergaard and Rasmussen, 1996; Folinsbee, 1992) (see Table 3-6). As noted in the 2016

[5]Studies discussed in this section examine effect of NO_2 exposure on airway responsiveness by measuring changes in lung function in response to bronchoconstricting agents. The direct effects of NO_2 exposure on lung function in the absence of bronchoconstricting agents is discussed in Section 3.3.3.1 .

Oxides of Nitrogen ISA, the analyses of (Folinsbee, 1992) and Kjaergaard and Rasmussen (1996) were effectively analyses of nonspecific responsiveness since few studies were available at the time evaluated allergen responses.

- A decrease by 50% in the provocative dose required to elicit airway constriction (measured by increased specific airway resistance (sRaw) or decreased forced expiratory volume in one second (FEV_1) is recognized as a potential indicator of clinically relevant changes in airway responsiveness (Reddel et al., 2009). Alternatively, a doubling of the provocative dose (100% increase) is considered a potential indicator of a meaningful decreased airway reactivity. One meta-analysis showed that a greater proportion of study participants experienced a reduction of the provocative dose by 50% (indicating increased airway reactivity) following NO_2 exposure compared to those that experienced a doubling of provocative dose (indicating decreased airway reactivity) (Brown, 2015).
- Asthmatics exposed at rest to NO_2 from 100 to 300 ppb did not result in increased airway responsiveness following allergen challenge. Resting exposure to 400 ppb NO_2 did increase airway responsiveness to house dust mite allergen in a single study of eight participants (Tunnicliffe et al., 1994) (see Figure 3-6).
- NO_2 exposure during exercise did not result in changes in airway reactivity in response to a nonspecific or specific stimuli in several meta-analyses (Brown, 2015; Goodman et al., 2009; U.S. EPA, 2008b; Folinsbee, 1992) (see Table 3-6 and Figure 3-6).
- There was no clear dose-response relationship between NO_2 exposure at rest and the change in airway reactivity (i.e., higher NO_2 concentrations or exposure durations do not lead to greater increases in airway responsiveness) (Brown, 2015; Goodman et al., 2009).
- People with asthma experience statistically significant increases in airway reactivity following NO_2 exposures as low as 100 ppb NO_2, whereas healthy volunteers without respiratory disease do not have significant increases in airway responses except following NO_2 exposures of greater than 1,000 ppb (Folinsbee, 1992).

Table 3-4 Resting exposures to nitrogen dioxide and airway responsiveness in individuals with asthma

Reference	N	NO_2 ppb	Exp. (min)	Chall-enge Type	End Point	Time Post-exp min	Change in AR[a]		Average PD±SE[b]		*p*-value[c]
							+	–	Air	NO_2	
Specific Challenge Agents											
Ahmed et al. (1983b)	20	100	60	RAG	sGaw	IM	10	8	9.0±5.7	11.7±7.6	n.s.
Tunnicliffe et al. (1994)	8	100	60	HDM	FEV_1	IM	3	5	-14.62 ΔFEV_1	-14.41 ΔFEV_1	n.s.
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.

Reference	N	NO_2 ppb	Exp. (min)	Chall-enge Type	End Point	Time Post-exp min	Change in AR[a]		Average PD±SE[b]		*p*-value[c]
							+	–	Air	NO_2	
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
Tunnicliffe et al. (1994)	8	400	60	HDM	FEV_1	IM	8	0	-14.62 ΔFEV_1	-18.64 ΔFEV_1	0.009
Nonspecific Challenge Agents											
Ahmed et al. (1983a)	20	100	60	CARB	sGaw	NA	13	7	6.0±2.4	2.7±0.8	NA
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]
Bylin et al. (1988)	20	140	30	HIST	sRaw	25	14	6	0.39±0.07	0.28±0.05	n.s.
Orehek et al. (1976)	4	200	60	CARB	sRaw	IM	3	0	0.60±0.10	0.32±0.02	n.s.
Jörres and Magnussen (1990)	14	250	30	SO_2	sRaw	27	11	2	46.5±5.1	37.7±3.5	<0.01
Bylin et al. (1988)	20	270	30	HIST	sRaw	25	14	6	0.39±0.07	0.24±0.04	<0.01
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.

List of abbreviations in alphabetical order: AR = airway responsiveness; BIR = birch; CARB = carbachol; Exp. = exposure, FEV_1 = forced expiratory volume in one second; HDM = house dust mite allergen; HIST = histamine; IM = immediately after exposure; METH = methacholine; NA = not available; NO_2 = nitrogen dioxide; n.s. = less than marginal statistical significance, p>0.10; pEF = partial expiratory flow at 40% vital capacity; RAG = ragweed; SO_2 = sulfur dioxide; sGaw = specific airway conductance; sRaw = specific airway resistance; TIM = timothy pollen.
[a]Change in AR: number of individuals showing increased (+) or decreased (−) airway responsiveness after NO_2 exposure compared to air.
[b]PD±SE: arithmetic or geometric mean provocative dose (PD)±standard error (SE). See individual papers for PD calculation and dosage units. ΔFEV_1 indicates the change in FEV_1 response at a constant challenge dose.
[c]Statistical significance of increase in AR to bronchial challenge following NO_2 exposure compared to filtered air as reported in the original study unless otherwise specified. Statistical tests varied between studies (e.g., sign test, *t*-test, analysis of variance).[d]Statistical significance for all individuals with asthma from analysis by Dawson and Schenker (1979). Orehek et al. (1976) only tested for differences in subsets of individuals classified as "responders" and "nonresponders."

Table 3-5 Exercising exposures to nitrogen dioxide and airway responsiveness in individuals with asthma

Reference	n	NO_2 ppb	Exp. min	Challenge Type	End Point	Time Post-exp min	Change in AR[a]		Average PD±SE[b]		*p*-value[c]
							+	−	Air	NO_2	
Specific Challenge Agents											
Jenkins et al. (1999)	11	200	360	HDM	FEV_1	IM	6	5	2.94	2.77	n.s.
Riedl et al. (2012)	15	350	120	CA	FEV_1	90	4	11	-6.9±1.7 ΔFEV_1	-0.5±1.7 ΔFEV_1	<0.05[f]
Jenkins et al. (1999)	10	400	180	HDM	FEV_1	IM	7	3	3.0	2.78	0.018
Witten et al. (2005)	15	400	180	HDM	FEV_1	IM	8	7	550±240	160±60	n.s.
Nonspecific Challenge Agents											
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
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.
Avol et al. (1988)	37	600	120	COLD	FEV_1	60	13[e]	16[e]	-8.4±1.8 ΔFEV_1	-10.4±2.2 ΔFEV_1	n.s.
Roger et al. (1990)	19	600	80	METH	sRaw	120	11[d]	8[d]	3.3±0.7	3.7±1.1	n.s.

List of abbreviations in alphabetical order: AR = airway responsiveness; CARB = carbachol; CA = cat allergen; COLD = cold-dry air; Exp. = exposure; FEV1 = forced expiratory volume in one second; HDM = house dust mite allergen; HIST = histamine; IM = immediately after exposure; METH = methacholine; NO_2 = nitrogen dioxide; n.s. = less than marginal statistical significance, $p>0.10$; SO_2 = sulfur dioxide; sRaw = specific airway resistance.

[a]Change in AR: number of individuals showing increased (+) or decreased (−) airway responsiveness after NO_2 exposure compared to air.
[b]PD±SE: arithmetic or geometric mean provocative dose (PD)±standard error (SE). See individual papers for PD calculation and dosage units. ΔFEV1 indicates the change in FEV1 response at a constant challenge dose.
[c]Statistical significance of increase in AR to bronchial challenge following NO_2 exposure compared to filtered air as reported in the original study. Statistical tests varied between studies (e.g., sign test, t-test, analysis of variance).
[d]Number of individuals having an increase or decrease in airway responsiveness is from Folinsbee (1992).
[e]Data for 0.25% carbachol challenge from Appendix H of Morrow and Utell (1989b).
[f]Significantly greater ΔFEV1 in response to a constant challenge dose following exposure to filtered air than NO_2 (i.e., a protective effect of NO_2 exposure).
Source: Adapted from Brown (2015). Copyright permission pending.

Table 3-6 Fraction of individuals with asthma having NO_2-induced increases in airway reactivity in response to a non-specific challenge

NO_2 Concentration (ppb)	All Exposures[a,b]	Exposure with Exercise[a,b]	Exposure at Rest[a,b]
$[NO_2] = 100$	0.66 (50; p = 0.033)	-	0.66 (50; p = 0.033)[c]
$100 \leq [NO_2] < 200$	0.66 (87; p = 0.005)	0.59 (17; p = 0.63)[d]	0.67 (70; p = 0.006)[e]
$200 \leq [NO_2] \leq 300$	0.59 (199; p = 0.011)	0.55 (163; p = 0.21)[f]	0.78 (36; p = 0.001)[g]
$[NO_2] > 300$	0.57 (94; p = 0.18)	0.49 (61; p = 1.0)[h]	0.73 (33; p = 0.014)[i]
All $[NO_2]$	0.60 (380; p<0.001)	0.54 (241; p = 0.25)	0.71 (139; p<0.001)

List of abbreviations in alphabetical order:; NO_2 = nitrogen dioxide; ppb = parts per billion.
[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 3- and Table 3- having a change (±) in nonspecific airway responsiveness.
[c]Thirty-three increases, 17 decreases; 100 ppb data from Ahmed et al. (1983a), Hazucha et al. (1983), and Orehek et al. (1976).
[d]Ten increases, 7 decreases; 150 ppb data from Roger et al. (1990).
[e]Forty-seven increases, 23 decreases; 100 ppb data from Ahmed et al. (1983a), Hazucha et al. (1983), and Orehek et al. (1976); 140 ppb data from.Bylin et al. (1988).
[f]Ninety increases, 73 decreases; 200 ppb data from Kleinman et al. (1983); 250 ppb data from Jörres and Magnussen (1991); 260 ppb data from Strand et al. (1996); 300 ppb data from Avol et al. (1988), Avol et al. (1989), Bauer et al. (1986), Morrow and Utell (1989a), Roger et al. (1990), and Rubinstein et al. (1990).
[g]Twenty-eight increases, 8 decreases; 200 ppb data from Orehek et al. (1976); 250 ppb data from Jörres and Magnussen (1990); 270 ppb data from Bylin et al. (1988).
[h]Thirty increases, 31 decreases; 350 ppb data from Riedl et al. (2012); 600 ppb data from Avol et al. (1988) and Roger et al. (1990).
[I]Twenty-four increases, 9 decreases; 480 ppb data from Bylin et al. (1985); 500 ppb data from Mohsenin (1987a); 530 ppb data from Bylin et al. (1988).
Source: Adapted from Brown (2015). Copyright permission pending.

NO_2 Concentration, ppb	All exposures[a,b]	Exposure with exercise[a,b]	Exposure at rest[a,b]
$[NO_2] = 100$	0.50 (26; n.s.)	–	0.50 (26; n.s.)[c]
$100 \leq [NO_2] < 200$	0.50 (26; n.s.)	–	0.50 (26; n.s.)[c]
$200 \leq [NO_2] \leq 300$	0.55 (56; n.s.)	0.55 (11; n.s.)[d]	0.56 (45; n.s.)[e]
$[NO_2] > 300$	0.56 (48; n.s.)	0.48 (40; n.s.)[f]	1.00 (8; $p = 0.008$)[g]
All $[NO_2]$	0.55 (130; n.s.)	0.49 (51; n.s.)	0.58 (79; n.s.)

n.s., less than marginal statistical significance ($p > 0.10$).
[a]See Footnote ''a'' of Table 3.
[b]Analysis is for the 130 subjects with asthma in Tables 1 and 2 having a change (+/−) in specific allergen airway responsiveness.
[c]13 increases, 13 decreases; 100 ppb data from Ahmed et al. (1983b) and (Tunnicliffe et al., 1994).
[d]6 increases, 5 decreases; 200 ppb data from Jenkins et al. (1999).
[e]25 increases, 20 decreases; 260 ppb data from Barck et al. (2002), Strand et al. (1997) and Strand et al. (1998).
[f]19 increases, 21 decreases; 350 ppb data from Riedl et al. (2012); 400 ppb data from Jenkins et al. (1999) and Witten et al. (2005).
[g]8 increases, 0 decreases; 400 ppb data from Tunnicliffe et al. (1994).

Source: Adapted from Brown (2015). Copyright permission pending.

Figure 3-6 Fraction of individuals with asthma having nitrogen dioxide-induced increases in airway reactivity in response to an allergen challenge.

3.3.3.1.1.1 Conclusions and Remaining Uncertainties

Available meta-analyses of individual-level data from controlled human exposure studies indicate that exposure to NO_2 at and above 100 ppb can enhance the reactivity of airway smooth muscles in people with asthma under some circumstances (i.e., NO_2 exposures at rest, non-specific challenge agents). Uncertainties in NO_2 controlled human exposure studies of airway reactivity contribute to limitations observed in meta-analyses of individual-level data from these studies. For example, meta-analyses did not detect a discernible dose-response relationship with airway reactivity in resting study participants (i.e., higher concentrations do not lead to greater effects) (Brown, 2015; Goodman et al., 2009). This could be due to inconsistencies in study design and methods across individual studies included in the meta-analyses. Consistent with the broad literature concerning airway responsiveness and its measurement, Brown (2015) suggested that a variety of factors including exercise, bronchial challenge agents, and their method of delivery may affect or modulate airway responsiveness following NO_2 exposures. Goodman et al. (2017) assessed the statistical significance of these modulating factors in studies of airway responsiveness following NO_2 exposure. Within the limited body of NO_2 studies, they concluded that the evidence does not indicate that these modulating factors explained a lack of change in airway responsiveness in studies involving exercise or using allergens as airway challenge agents. Another important uncertainty relates to why increased airway responsiveness following NO_2 exposures in people with asthma is seen following exposures at rest but not following exposures with concurrent exercise. The 2016 Oxides of Nitrogen ISA suggested several possible explanations for such findings. A recent analysis by Goodman et al. (2017) hypothesized that these potential explanations were not adequately supported based on postulation and limited meta-analysis of NO_2 controlled human exposure literature.

3.3.3.1.2 Animal Toxicological Studies of Airway Responsiveness

Animal studies investigating the impact of short-term NO_2 exposures on airway responsiveness in response to allergen sensitization and subsequent challenge are limited in number likely due to the extended time it takes to sensitize animals to an allergen. The process of sensitizing rodents often takes repeated exposure or injection of the challenge agent over the course of several days to weeks followed by challenge with the allergen through aerosolized exposure over several days. To maximize the impact of NO_2 exposure, experiments often expose animals prior to and during sensitization. The details of animal studies of long-term NO_2 exposure on airway reactivity in asthmatic model systems are described in Chapter 4, Section 4.4.1.2. The animal studies of short-term NO_2 exposure in the 2016 Oxides of Nitrogen ISA showed mixed results. A summary of the results is below. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 3-3.

- Mice sensitized and then challenged with ovalbumin (OVA) and then exposed to 5 ppm or 25 ppm NO_2 six hours/day for three days showed no change in airway reactivity compared to (ovalbumin) OVA sensitization and challenge in air exposed mice (Poynter et al., 2006). After exposure to 25 ppm NO_2 for five days and a 20-day recovery period, the airway responsiveness was greater, although not statistically significant, than in sensitized mice exposed to air.
- Short-term exposure to 0.7 or 5 ppb NO_2 (two to three hours/day for one to four days) resulted in reduced airway reactivity measures to OVA challenge in OVA sensitized mice (Hubbard et al., 2002; Proust et al., 2002). In contrast, exposure to 20 ppm NO_2 for three hours/day for four days resulted in increased airway reactivity to OVA in sensitized mice (Proust et al., 2002).

Recent animal studies show that exposure to 2.5 ppm NO_2 for five hours/day during gestation (~21 days) results in increased airway reactivity to methacholine in the offspring following challenge with OVA at seven or nine weeks of age (Yue et al., 2018; Yue et al., 2017). These studies expand evidence that NO_2 exposure during gestation can affect the airway responsiveness of offspring. Similar to epidemiology studies, recent animal studies support more pronounced effects of NO_2 in children compared to adults.

3.3.3.1.2.1 Relevance of Animal Responses to Human Responses

Given the extensive resources and knowledge, rodent models are the most common animal type used in animal toxicological studies and recapitulate many of the inflammatory phenotype seen in human asthma. However, species differences in the lung factors like airway autonomic innervation and responsiveness to bronchoconstricting stimuli may impact the observation of lung reactivity. For example, mice and rats are also obligate nose breathers and lack a cough reflex. Additionally, the smooth muscles of mouse and rat airways are not responsive to many bronchoconstrictors that are implicated in human asthma and are responsive to serotonin, which is not a potent constricting agent in human airways

(Canning, 2003). Guinea pig smooth muscle more closely mimic those of humans and may provide a more accurate model of human airway reactivity (Canning, 2003). It should be noted that both human and rodent smooth muscle respond to methacholine which is the provocative agent used in most animal studies of NO_2 and airway reactivity.

3.3.3.1.2.2 Conclusions and Remaining Uncertainties

The available animal studies examining the effects of short-term exposures to NO_2 on airway reactivity in rodents is relatively limited, and the results are mixed at ambient-relevant concentrations, though recent studies provide some support for effects of gestational exposures. As noted above, the sensitization and challenge paradigm for rodent models often requires several weeks to complete, and additional studies examining NO_2-induced effects on airway responsiveness using repeated exposures are discussed in the chapter on long-term exposures and respiratory effects (see Chapter 4, Section 4.3.2.2).

3.3.3.2 Lung Function Changes in Populations with Asthma

This section evaluates the evidence for changes in lung function due to short-term NO_2 exposure. Section 3.3.3.2.1 discusses epidemiologic studies, and Section 3.3.3.2.2 discusses controlled human exposure studies.

3.3.3.2.1 Epidemiologic Studies of Lung Function Changes in Populations with Asthma

Collectively, epidemiologic studies that were evaluated in the 2016 Oxides of Nitrogen ISA reported consistent associations between increases in ambient NO_2 concentrations and decrements in supervised spirometry measures (primarily FEV1) in children with asthma. Key evidence for NO_2-associated decrements in lung function measured under supervised conditions is provided by studies that assessed exposure in subjects' locations, including total personal NO_2, personal outdoor NO_2 estimated from measurements at school and other locations using time-activity information, or outdoor NO_2 at schools (Greenwald et al., 2013; Martins et al., 2012; Delfino et al., 2008; Holguin et al., 2007). These studies consistently reported associations between multiday averages (i.e., lag 0–1 avg, 0–4 avg) of 24-hour avg NO_2 and decrements in lung function. Results were less consistent for exposures assigned using NO_2 concentrations measured at central sites. Across the various populations examined, results were also inconsistent for lung function measured under unsupervised conditions, primarily peak expiratory flow (PEF) at home. Although unsupervised home spirometry offers some advantages related to increased frequency and ease of lung function testing, a recent meta-analysis reported higher variability and underestimation of lung function measured using unsupervised compared to supervised spirometry (Anand et al., 2023).

Fewer studies reviewed in the 2016 Oxides of Nitrogen ISA examined lung function changes in adults with asthma. While associations were inconsistent overall, the only study with supervised spirometry measures and personal ambient NO_2 measurements reported decrements FEV_1 and forced expiratory flow (FEF) from 25 to 75% of vital capacity ($FEF_{25–75\%}$) two to 22 hours after NO_2 exposures (McCreanor et al., 2007). Observed associations with $FEF_{25–75\%}$ were attenuated but still positive in copollutant models adjusted for UFP, EC, or $PM_{2.5}$.

A limited number of recent studies add inconsistent findings to the existing evidence base. A short summary of the recent evidence is provided below. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 3-4.

- Recent panel studies conducted among children with asthma examined supervised (Ierodiakonou et al., 2015) or unsupervised (Hao et al., 2022; Schachter et al., 2016) spirometry measures, including FEV_1, forced vital capacity (FVC), and peak expiratory flow (PEF). No PECOS-relevant recent studies examined lung function in adults with asthma. 24-hour avg NO_2 concentrations were not associated with supervised spirometry measures (FEV_1 or PEF) in an analysis of 1,003 5- to 12-year-old children enrolled in the 1993 to 1999 multicity Childhood Asthma Management Program (CAMP) study (Ierodiakonou et al., 2015). Although the Ierodiakonou et al. (2015) study had several strengths, including a large sample size and supervised spirometry measures, one limitation is the potential for exposure measurement error due to the use of central site monitors up to 50-km away from participants' residences, which is most likely to bias effect estimates toward the null and decrease precision.

In contrast, a few recent studies provided some limited evidence of associations between short-term NO_2 exposure and lung function in children with asthma.

- A recent panel study of 40 children with asthma recruited from UCLA pediatric asthma clinics in Los Angeles and Santa Monica, CA, observed decreases in % predicted FEV1 (-7.32% [95% CI: -17.32, 2.68] per 20 ppb increase) and PEF (-7.18% [95% CI: -16.39, 2.03] per 7.1 ppb increase) associated with increases in two-day lag 24-hr average traffic-related NO_2 concentrations estimated using the RLINE line source dispersion model (Hao et al., 2022). Associations were comparable for % predicted FEV_1 and ambient NO_2 concentrations assigned using spatially resolved modeled exposure estimated from inverse-distance squared spatial interpolation of city-wide monitors, though the NO_2-PEF association based on spatial interpolation of monitors was positive and imprecise.
- In a panel study of 36 inner-city New York, NY children with asthma, Schachter et al. (2016) observed an imprecise association between 2-week average NO_2 exposure and higher odds of decreased FEV_1 during the summer observation period (OR: 1.23 [95% CI: 0.84, 1.82] per IQR increase; increment not reported), but not in the winter. The smaller sample size and number of observations may have resulted in limited statistical power to detect an association, as reflected in the imprecise effect estimates reported by the authors.

3.3.3.2.1.1 Conclusions and Remaining Uncertainties

Although recent studies examining the association between short-term NO_2 exposure and lung function in children with asthma are limited in number and the results are inconsistent, a large number of studies evaluated in the 2016 Oxides of Nitrogen ISA provide a strong body of evidence for NO_2-related decrements in lung function in children with asthma. The strongest evidence for NO_2-associated decrements in lung function comes from studies that measured lung function under supervised conditions and assessed exposure in subjects' locations. There is notable remaining uncertainty regarding potential copollutant confounding, which has not been thoroughly evaluated. Additionally, there has been limited evaluation of lung function in adults with asthma.

3.3.3.2.2 Controlled Human Exposure Studies of Lung Function Changes in Populations with Asthma

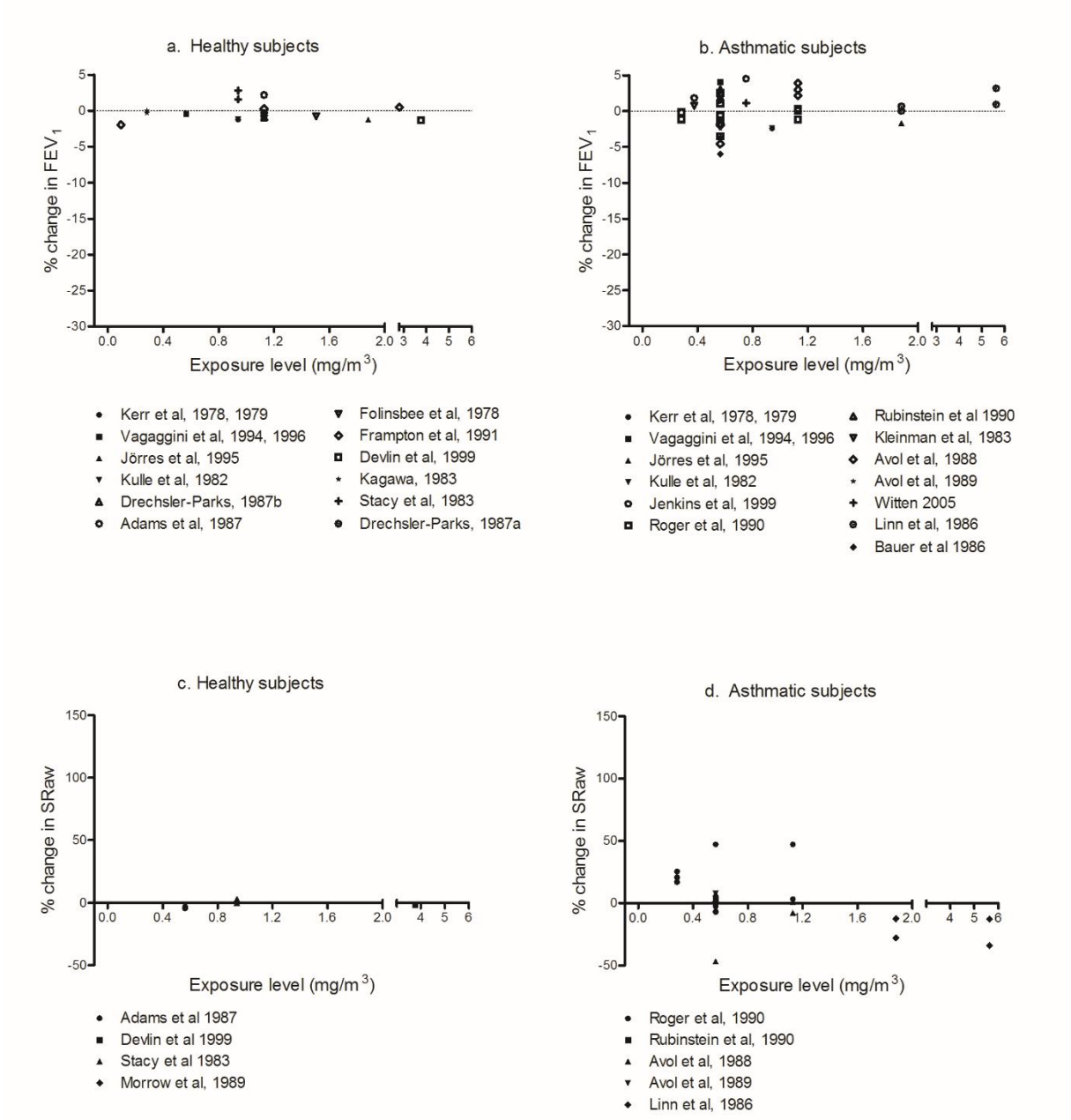
No recent controlled human exposure studies have investigated lung function changes following NO_2 exposures in people with asthma.[6] Older studies of controlled human exposures to NO_2 and lung function changes have been described previously (U.S. EPA, 2016, 2008b) and have reported minimal effects of NO_2 exposures on lung function (i.e., spirometry or airway resistance). Study specific details, exposure conditions, and endpoints examined are highlighted in the text below and described in more detail in Table 5-6 of U.S. EPA (2016).

- Exposures in the range of 100 to 400 ppb NO_2 did not result in changes in lung function in adolescents with asthma (Koenig et al., 1987) or adults with asthma (Riedl et al., 2012; Jenkins et al., 1999; Vagaggini et al., 1996; Jörres and Magnussen, 1991; Kleinman et al., 1983).
- No change in FEV_1 or airway resistance was observed following 2-hour exposures to 100, 200, or 800 ppb NO_2 with 10 minutes of submaximal exercise midway through exposures in 20 healthy subjects or 20 subjects with asthma (Rasmussen et al., 1990).
- One study observed NO_2-induced decreases in FEV_1 and partial expiratory flow rate after exposure to 300 ppb NO_2 for 30 minutes with exercise in subjects with asthma but not follow exposures during rest (Bauer et al., 1986).
- Exposure to higher NO_2 concentrations did not consistently show impacts on lung function with small, but statistically significant, reductions in FEV_1 seen after 1,000 ppb NO_2 (Jörres et al., 1995) but no impacts on airway resistance at 4,000 ppb (Linn et al., 1985b).

One recent meta-analysis reviewed controlled human exposure studies to determine if there was a difference in the lung function response to NO_2 (and other pollutants) between healthy individuals and individuals with asthma (Johansson et al., 2015). One analysis derived an estimated differential response factor defined as the ratio of the lowest observed adverse effect level (LOAEL), and in some cases the no

[6]It is important to note that studies discussed in this section examine the direct effects of NO_2 exposure on lung function, while the studies discussed in Section 3.3.3.1 use lung function to assess the effect of NO_2 exposure on airway responsiveness to bronchoconstricting agents.

observable effect level (NOAEL), for lung function responses reported in controlled human exposures of healthy individuals and individuals with asthma subjects. An estimated differential response factor of one would suggest no difference in the LOEL or NOEL between healthy and subjects with asthma. An estimated differential response factor greater than one would suggest that those with asthma are more sensitive to NO_2 than healthy subjects. While this is a crude estimate that is highly dependent on the exposure concentrations used in the studies analyzed, the estimated differential response for NO_2 was >1 suggesting that those with asthma were more sensitive to NO_2 effects on lung function than healthy subjects. In a second analysis, the authors reported that subjects with asthma had a greater FEV_1 decrement and larger increase in sRaw in response to NO_2 at all concentrations reported. However, subjects with asthma showed a similar FEV_1 decrement and increase in sRaw following exposure to filtered air suggesting that a factor outside of NO_2 exposure was responsible for this difference. It is important to note that all of the studies evaluated in this analysis involved NO_2 exposure with exercise regimens, and the authors suggest that the general lack of NO_2 effects could reflect the increased airway responsiveness in subjects with asthma in response to exercise. In support of this hypothesis, there was no discernible dose response observed between NO_2 exposure concentration and lung function measures when the data were corrected for the impact of exercise as determined from the change in lung function in the filter air group (see Figure 3-7).



List of abbreviations in alphabetical order: FEV_1 = forced expiratory volume in one second; mg/m^3 = milligrams per cubic meter; sRaw = specific airway resistance.
Notes: 0.4, 0.8, 1.2, 1.6, 2.0, 4, 5, and 6 mg/m^3 are approximately equivalent to 210, 430, 640, 850, 1060, 2130, 2660, and 3190 ppb, respectively.
Source: Adapted from Johansson et al. (2015). Copyright permission pending.

Figure 3-7 Percentage change in forced expiratory volume in one second and specific airway resistance in healthy and asthmatic subjects following exposure to nitrogen dioxide with exercise.

3.3.3.2.2.1 Conclusions and Remaining Uncertainties

The body of controlled human exposure studies is limited. Overall, most studies investigating the direct effects of NO_2 exposure on lung function, whether in adolescents or adults with asthma, have reported null findings. Uncertainties include the exposure pattern evaluated in studies (i.e., constant concentration compared to variable concentration patterns that are more real-world relevant), the degree to which study populations are representative of those with more severe or not well controlled asthma who would be excluded from controlled human exposure studies, and the relevance of exposure to NO_2 alone compared to more ambient-relevant mixtures of oxides of nitrogen species.

3.3.3.3 Pulmonary Inflammation and Oxidative Stress in Populations with Asthma

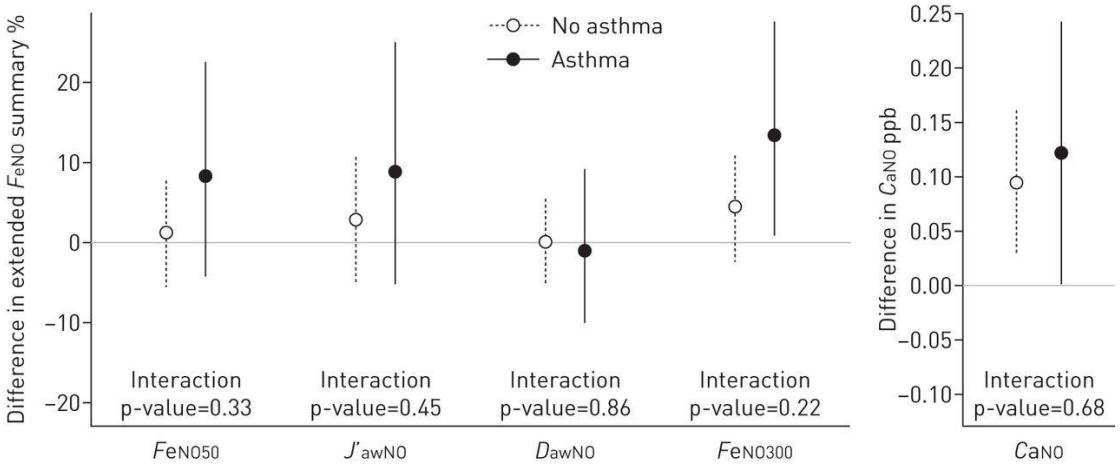
Pulmonary inflammation is a key early event in asthma exacerbation that can mediate increases in airway responsiveness, and increased oxidative stress can contribute to that inflammation. Studies examining NO_2 exposure-related pulmonary inflammation or oxidative stress can provide insight into a potential mode of action by which NO_2 exposures could result in respiratory effects serious enough that, in some cases, they result in an ED visit or hospital admission (see Section 3.9). This section discusses epidemiologic (see Section 3.3.3.3.1), controlled human exposure (see Section 3.3.3.3.2), and animal toxicological (see Section 3.3.3.3.3) studies that evaluate short-term NO_2 exposures and pulmonary inflammation and oxidative stress.

3.3.3.3.1 Epidemiologic Studies of Pulmonary Inflammation

A large number of studies that were evaluated in the 2016 Oxides of Nitrogen ISA provided consistent evidence of an association between short-term NO_2 exposures and pulmonary inflammation. The most studied biomarker was exhaled nitric oxide (eNO), a common indicator of pulmonary inflammation. A link between eNO and asthma exacerbation is well supported in the literature (Soto-Ramos et al., 2013; Carraro et al., 2007; Jones et al., 2001; Kharitonov and Barnes, 2000). Studies examining eNO often utilized exposure and biomarker measurements taken on site (e.g., homes, schools) and included examination of potential confounding by traffic-related copollutants. The 2016 Oxides of Nitrogen ISA described generally consistent epidemiologic evidence of an association between short-term NO_2 exposure and increased eNO in children with asthma. Associations were observed with both personal exposure measurements and central site NO_2, and reported effect estimates were larger for short, multiday average concentrations (i.e., lag 0–1) compared with single day lag exposures (e.g., lag 0 or 1). A panel study of children with asthma in southern California reported positive NO_2-eNO associations that were robust to copollutant adjustment for personal $PM_{2.5}$, EC, or organic carbon (OC) [(Delfino et al., 2006); quantitative results not provided by authors]. Epidemiologic studies of pulmonary inflammation in adults with asthma were limited in number and reported inconsistent evidence of associations with ambient NO_2.

Evidence from a recent study is summarized below. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 3-4.

One recent cross-sectional analysis of the Children's Health Study (CHS) cohort examined the relationship between indoor NO concentrations and eNO in 1,635 school children (aged 12–15 years) with and without asthma in southern California (Eckel et al., 2016). The authors measured eNO from proximal [exhaled nitric oxide fraction at a flow rate of 50 mL/s ($FeNO_{50}$), maximum airway wall nitric oxide flux ($J'_{aw}NO$), and diffusing capacity of the airway wall tissue for nitric oxide ($D_{aw}NO$)] or distal [$FeNO_{300}$ and alveolar nitric oxide concentration (C_ANO)] airways, and indoor NO measured at schools was used as a surrogate for traffic-related air pollutant exposure in the indoor microenvironment. Eckel et al. (2016) observed higher average eNO from distal airways associated with a 10 ppb increase in indoor NO concentrations (C_ANO: 7.1% increase [95% CI: 2.9, 11.4]; $FeNO_{300}$: 6.5% increase [95% CI: 0.3, 13.1]). Associations with eNO from proximal airways were less robust, as smaller increases were reported for $J'_{aw}NO$ (4.0% [95% CI: -2.8, 11.3]) and $FeNO_{50}$ (2.8% [95% CI: -3.3, 9.3]), but not $D_{aw}NO$ (0.2% [95% CI: -4.8, 4.6]). In models with interaction terms for asthma status and indoor NO, the previously described positive associations were larger among children with asthma (see Figure 3-8).



List of abbreviations in alphabetical order: C_{ANO} = alveolar nitric oxide concentration; D_{awNO} = diffusing capacity of the airway wall tissue for nitric oxide; F_{eNO} = exhaled nitric oxide fraction; F_{eNOx} = F_{eNO} measured at an exhalation flow rate of x mL·s^{-1}; J'_{awNO} = maximum airway wall nitric oxide flux; NO = nitric oxide; ppb = parts per billion.
Note: 95% confidence interval.
Source: Adapted from Eckel et al. (2016). Copyright permission pending.

Figure 3-8 Estimated differences in each extended exhaled nitric oxide fraction summary associated with a 10 ppb increase in indoor nitric oxide from models with an interaction between asthma and indoor nitric oxide.

3.3.3.3.1.1 Conclusions and Remaining Uncertainties

Recent epidemiologic evidence evaluating the association between short-term oxides of nitrogen exposures and pulmonary inflammation is limited to one recent cross-sectional study that observed a positive association between short-term NO exposure and biomarkers of pulmonary inflammation in children with asthma. This study was consistent with a much larger body of evidence from the 2016 Oxides of Nitrogen ISA that supported a positive association between short-term NO_2 exposure and increased eNO in children with asthma. These studies include longitudinal panel studies utilizing either personal exposure measurements or central site NO_2 concentrations. The available evidence supports a stronger association with short, multiday average concentrations (i.e., lag 0–1) compared with single day lag exposures (e.g., lag 0 or 1), suggesting a potential cumulative effect of short-term NO_2 exposure. While the observed NO_2-eNO associations were robust to copollutant adjustment for personal $PM_{2.5}$, EC, or OC, copollutant evaluations were limited in number. There is also remaining uncertainty regarding pulmonary inflammation in adults with asthma, which has been less frequently examined in relation to short-term NO_2 exposures.

3.3.3.3.2 Controlled Human Exposure Studies of Pulmonary Inflammation

In the 2016 Oxides of Nitrogen ISA, studies in adults with asthma provided generally consistent evidence of exacerbation of allergic inflammation responses that can underlie increased airway reactivity and asthma exacerbations. No recent controlled human exposure studies are available. Study specific details, exposure conditions, and endpoints examined in previously evaluated studies are highlighted in the text below and are described in more detail in Table 5-13 of U.S. EPA (2016).

- Exposure to 260 ppb NO_2 for 30 minutes followed by allergen (timothy or birch pollen) challenge four hours post exposure resulted in increased neutrophil recruitment into bronchial and bronchoalveolar lavage fluid (BAL) as well as increased concentrations of eosinophilic cationic protein (ECP) (Barck et al., 2002). Levels of other inflammatory markers including IL-5, eotaxin, IL-8, myeloperoxidase, and soluble ICAM were either not detectable or not significantly changed between the air/allergen exposed group. Also, the level of albumin in the lung lavage fluid was not different after allergen treatment between the air- and NO_2-exposed groups.
- A study by the same group exposed participants with asthma to 260 ppb NO_2 or filtered air for 15 minutes on day 1, followed four hours later by allergen challenge. Day two of the experiment consisted of two 15-minute exposures to NO_2 or air with another allergen challenge three hours after the final exposure. The authors reported increases in ECP in the blood and sputum (day 1–3 difference) but no increase in myeloperoxidase and no statistically significant change in differential cell counts in the sputum at any timepoint (Barck et al., 2005a).
- Study participants with asthma and rhinitis exposed at rest to 30 minutes of 260 ppb NO_2 followed four hours later with allergen challenge did not impact the cell counts or ECP or myeloperoxidase levels seen in nasal lavage (Barck et al., 2005b).

- In two separate studies from the same group, exposure to 400 ppb NO_2 for six hours followed by allergen challenge increased ECP in nasal lavage fluid, compared to the air/allergen-exposed control group, but did not result in changes to nasal airway resistance or levels of other inflammatory mediators (Wang et al., 1999; Wang et al., 1995a). This group showed that 400 ppb NO_2 exposure increased both ECP levels and eosinophil numbers in nasal lavage samples from subjects with allergic rhinitis (Wang et al., 1995b). These NO_2-induced increases could be partly reversed with treatment with a corticosteroid spray to suppress inflammation.
- People with asthma exposed to 400 ppb NO_2 for three hours with intermittent exercise followed by allergen challenge caused reduced eosinophils in the sputum six hours after allergen challenge but otherwise did not change markers of inflammation in the sputum (Witten et al., 2005). Sputum ECP levels were increased although this change did not reach statistical significance.

A few studies examined the direct effects of NO_2 exposure on markers of airway inflammation without allergen challenge. These studies did not report changes in inflammatory markers following NO_2 exposures.

- People with mild asthma exposed to 350 ppb NO_2 for two hours with intermittent exercise showed increased sputum IgG4 but no changes in sputum cell counts or other airway inflammatory responses (Riedl et al., 2012). Sputum measures were taken roughly 23 hours after the end of NO_2 exposure.
- No change in inflammatory cells in sputum or nasal lavage was seen two hours following a 1-hour exposure to 300 ppb NO_2 in people with asthma (Vagaggini et al., 1996).
- People with mild asthma exposed to 1 ppm NO_2 for three hours with exercise showed no changes in lavage inflammatory cell influx or levels of histamine leukotrienes, PGE2, or PGf2α but did show decreases in thromboxane B_2, prostaglandin D_2, and 6-keto-prostaglandin$_{1\alpha}$ (Jörres et al., 1995). People without asthma with the same NO_2 exposure protocol only experienced a small increase in thromboxane B2 after NO_2.

3.3.3.3.2.1 Conclusions and Remaining Uncertainties

Existing studies of NO_2 exposure followed by allergen challenge in resting individuals with asthma or allergic rhinitis generally showed consistent increases in ECP protein, a marker of asthma-like eosinophilic inflammation, in respiratory washes and sputum. Corresponding increases in inflammatory cells was seen in some, but not all, studies. It is important to note the different respiratory sample (e.g. sputum, BAL, or nasal lavage) may impact the ability to observe cell inflammatory changes and differences in results may be due to the sample type analyzed. For example (Rutgers et al., 2000) reported that cell differential counts from sputum did not correlate with the cell counts in bronchial wash, bronchoalveolar lavage, or from airway biopsies Changes to the production of other inflammatory mediators did not show consistent trends. Markers of inflammation were not changed in the single study that looked at NO_2 exposure in those with asthma with intermittent exercise followed by allergen challenge. Studies of NO_2 exposure in asthmatics in the absence of allergen challenge did not show direct

effects of NO_2 exposure on airway inflammation. Timing of biological sample collection post-NO_2 exposure or allergen challenge varied among studies and could impact the ability to detect small or transient changes in inflammatory markers.

3.3.3.3.3 Animal Toxicological Studies of Pulmonary Inflammation

As discussed for airway reactivity, the timeline for inducing allergen sensitization and challenge in mice can take multiple weeks and as a result, exposures to NO_2 in these models tend to be longer than those that do not sensitize to allergen. The 2016 Oxides of Nitrogen ISA described animal studies that looked at short-term exposure to NO_2 in the context of allergen-induced inflammation. A summary of the results of these studies is highlighted in the text below and study details are described in more detail in Appendix A – Appendix Table 3-5.

- Exposure of mice to 25 ppm, but not 5 ppm, NO_2 for three days following OVA sensitization and challenge resulted in heightened numbers of neutrophils and eosinophils in the BAL (Poynter et al., 2006). Despite the increased inflammatory cells, there was no difference in the expression of several mucus and inflammatory genes in the lung tissue. Residual inflammation was still visible in the lung after five days of NO_2 exposure to 25 ppm and a 20-day recovery period whereas the inflammation had resolved in air and OVA challenged mice.
- Exposure of mice to two hours of 0.7 or 5 ppm NO_2 following OVA sensitization on each of three consecutive days resulted in decreased eosinophil numbers in the BAL (Hubbard et al., 2002). A similar protocol of OVA and NO_2 exposure for 10 days resulted in complete suppression of OVA-induced eosinophilic response.
- Proust et al. (2002) challenged OVA-sensitized mice to OVA immediately before a 5-hour exposure to either 5 or 20 ppm NO_2. The study reported lower eosinophil counts in the BAL of 5 ppm exposed mice 24 and 72 hours after exposure which corresponded to reduced IL-5 compared to air controls. Interestingly, there was no change in eosinophil response in mice exposed to 20 ppb NO_2 compared to air exposed and the IL-5 levels in the BALF were increased over air controls. NO_2 exposure did not affect BAL levels of IgG1, IL-4, or OVA-specific IgE.
- Rats sensitized to house dust mite allergen and exposed to three hours of 5 ppm NO_2 had increased inflammatory cell influx into the BAL and enhanced specific immune responses (Gilmour et al., 1996).

Recently identified studies expand these findings and provide evidence for heightened inflammation in the offspring of NO_2-exposed dams. A summary of the recently evaluated literature is as follows:

- Exposure of pregnant mice to 2.5 ppm NO_2 for five hours/day throughout gestation (approximately 21 days) heightened the number of macrophages, eosinophils, and T helper 2 (Th2) cells in the bronchoalveolar lavage [BAL; (Yue et al., 2018; Yue et al., 2017)]. These studies also reported increased inflammatory mediator production (TNFα and IL-1β) and worsened histologic inflammation in the lungs.

- Gestational exposure of mice to NO_2 with subsequent allergen sensitization and challenge in the offspring resulted in signs of a shift toward a pro-asthmatic, Th2 inflammatory signature which included increases in lung IL-4 and IL-13 with subsequent suppression of Th1 associated mediators like interferon gamma (IFNγ) (Yue et al., 2018; Yue et al., 2017). This shift was also seen as increases in the Th2 transcription factor GATA-3 and a decrease in the Th-1 transcription marker T-bet.
- Levels of total IgE and allergen specific IgE were increased in gestationally exposed mice following OVA challenge (Yue et al., 2018; Yue et al., 2017).
- Rats sensitized to OVA and exposed to 2 ppm NO_2 for six hours/day, five days/week, over four weeks showed heightened levels of 8-isoprostane in the exhaled breath condensate over the course of the exposures (de Broucker et al., 2015). This increase was not seen in unsensitized or sensitized rats exposed to clean air. Lavage samples at the end of the 4-week exposure showed no difference in total cells or percentage of neutrophils in the BAL.

3.3.3.3.3.1 Conclusions and Remaining Uncertainties

Animal studies of inflammation in allergen-sensitized animals often involves repeated exposures over several weeks or months. The evidence of NO_2-induced inflammation for shorter periods of time or in already sensitized animals is more limited. Emerging evidence from a small number of recent studies provides consistent support for increased allergen sensitization following gestational exposures to NO_2. In adult animals without gestational exposures, the effects of NO_2 exposure on markers of inflammation are more mixed. These studies in adult animals provide some support for heightened inflammation following NO_2 exposures, though results across studies are not consistent and several studies found no effect with ambient-relevant exposure concentrations (i.e., 5 ppm or below). The array of inflammatory and immune related cytokines between studies makes comparison of results difficult. There also remains some uncertainty in the dose of exposure that can lead to effects on inflammation as inflammatory responses in adult animals do not always show dose dependence and, in some instances, increases in inflammation are only seen after exposures to NO_2 that greatly exceed ambient concentrations (>10 ppm). Uncertainty remains regarding the relevance of mechanisms underlying allergic inflammation phenotypes in rodents compared to humans.

3.3.4 Synthesis Across Lines of Evidence

Consistent with conclusions from the 2016 Oxides of Nitrogen ISA, evidence integrated across health outcomes and scientific disciplines strongly supports a relationship between short-term NO_2 exposure and asthma exacerbation. In some cases, this asthma exacerbation is serious enough to result in a visit to the emergency room and/or admission to the hospital. There is evidence for a continuum of effects that can lead to serious asthma exacerbations. These effects range from subclinical changes, including allergic inflammation in the respiratory system and increased airway responsiveness, to clinical events indicative of asthma exacerbation, such as respiratory symptoms in populations with asthma.

Epidemiologic studies provide strong support for positive associations between short-term NO_2 exposures and asthma exacerbations severe enough to result in ED visits or hospital admissions (see Section 3.3.1). Recent evidence includes several multicity or nationwide studies, including several in the United States and Canada, that reported positive associations across various study designs, methodologies, and exposure assignment approaches. While associations with hospital admissions and ED visits were observed among subjects of all ages, there is consistent evidence demonstrating stronger associations among children compared to adults. The largest increases in risk of NO_2-related hospital admissions or ED visits are generally observed in children between the ages of 5- and 18-years. Additional support for asthma exacerbation comes from epidemiologic studies that consistently demonstrate that increases in ambient NO_2 concentrations are associated with increases in asthma symptoms and medication use in children (see Section 3.3.2.1). Particularly strong evidence comes from recent studies of asthma medication use that utilize digital tracking of inhaler use and GPS data to geolocate participants for exposure assignment.

Though recent epidemiologic studies support and extend findings from previous reviews, there remains some uncertainty as to whether the observed associations are independent from other traffic-related pollutants. Of the limited number of studies that examined potential confounding by $PM_{2.5}$ or traffic-related pollutants (e.g., UFP, CO, or VOCs), associations between short-term NO_2 exposures and asthma hospital admissions and ED visits remained relatively unchanged in copollutant models (i.e., similar in magnitude or attenuated slightly, but still positive). Although copollutant models have well-recognized limitations for distinguishing independent pollutant associations, coherence with results from experimental studies provide strong support for an independent effect of short-term NO_2 exposures on the physiological processes that underly asthma exacerbation. Specifically, controlled human exposure studies of adults with asthma provide evidence of NO_2-enhanced markers of allergic inflammation, including eosinophil activation, pro-allergic cytokine production, and increased infiltration of neutrophils at relevant concentrations (e.g., following exposures of 260 ppb NO_2 for 15−30 minutes or 400 ppb NO_2 for six hours). As discussed in Section 3.9, increased allergic inflammation may promote asthma exacerbation through increases in airway reactivity, reductions in lung function, and/or increased airway resistance.

Additional support for an independent effect of NO_2 exposure on asthma exacerbation comes from several meta-analyses of controlled human exposure studies demonstrating increases in airway responsiveness in adults with asthma exposed to NO_2 at rest (see Section 3.3.3.1). Specifically, exposures as low as 100 ppb in resting subjects with asthma resulted in approximately 70% of study participants experiencing increased airway responsiveness to nonspecific stimuli (Brown, 2015; Goodman et al., 2009; Kjaergaard and Rasmussen, 1996; Folinsbee, 1992). Increased airway reactivity can be indicative of poor asthma control and thus the evidence for clinically relevant increases in airway responsiveness in adults with asthma following relatively low NO_2 exposures provides key support that ambient concentrations of NO_2 can exacerbate asthma. Although controlled human exposure studies do not provide strong evidence for NO_2-induced increases in respiratory symptoms in adults or adolescents with

asthma, ethical considerations in the design of controlled human exposure studies, especially in populations with underlying respiratory disease, may preclude these studies from capturing populations that may be most at risk for asthma exacerbation. Controlled human exposure studies of subjects without asthma or those with asthma that are exposed during exercise does not support NO_2-induced changes to airway responsiveness or lung function.

There was limited coherence with animal toxicological studies of airway responsiveness. However, a small but consistent body of recent evidence demonstrating increased markers of allergic inflammation in animal models supports controlled human exposure findings for allergic inflammation (see Section 3.3.3.3.3). This expanded evidence suggests that in utero exposure to NO_2 can heighten allergic responses later in life. While the epidemiologic evidence for inflammatory cell counts in populations with asthma is limited and inconsistent, there continues to be strong support for ambient or personal NO_2 exposure-related increases in eNO (see Section 3.3.3.3.1). These findings are coherent with experimental evidence for allergic inflammation because increases in eosinophils and neutrophils are linked with NO production during an inflammatory response. Further, the epidemiologic evidence provides support for NO_2-related decrements in lung function in children with asthma (see Section 3.3.3.2.1). The most consistent evidence comes from well-designed, well-conducted studies that measured lung function under supervised conditions and assessed exposure in subjects' locations.

In summary, epidemiologic evidence provides strong support for an association between short-term NO_2 exposure and asthma exacerbation, including hospital admissions, ED visits, and respiratory symptoms and medication use in children and adults with asthma. Epidemiologic studies are coherent with experimental evidence demonstrating effects of ambient NO_2 exposure on allergic inflammation and airway responsiveness in adults with asthma. Together, the evidence supports an effect of short-term ambient NO_2 exposure on asthma exacerbation independent of other traffic-related pollutants.

3.4 Chronic Obstructive Pulmonary Disease Exacerbation

COPD is a chronic lung disorder characterized by destruction of alveolar tissue, airway remodeling, and minimally reversible airflow limitation (Vestbo et al., 2013). Reduced airflow is associated with decreased lung function, and clinical symptoms demonstrating exacerbation of COPD include cough, sputum production, and shortness of breath (Vestbo et al., 2013). Severe exacerbation can lead to ED visits or hospital admissions.

3.4.1 Hospital Admissions and ED Visits for COPD

Severe COPD exacerbations may lead people with COPD to seek medical interventions (i.e., reflected in data for hospital admissions and ED visits). A limited number of epidemiologic studies that

were evaluated in the 2016 Oxides of Nitrogen ISA provided support for a positive association between short-term NO_2 exposure and hospital admissions for COPD. Evidence for ED visits were less consistent.

3.4.1.1 Epidemiologic Studies of Hospital Admissions and ED Visits for COPD

A small body of evidence evaluated in the 2016 Oxides of Nitrogen ISA supported a positive association between NO_2 exposure and hospital admissions for COPD. The strongest evidence came from a multi-city time-series analysis in Italy in which increases in COPD hospital admissions were associated with increases in same-day and lag 0–1 24-hour avg NO_2 concentrations (Faustini et al., 2013). This evidence was supported by several single-city studies conducted in North America and Asia reporting comparable associations. Evidence was more limited and inconsistent in studies of ED visits for COPD, with a null association observed in a multicity Canadian study (Stieb et al., 2009) and positive associations reported in a few single-city studies, including one in the United States (Peel et al., 2005). Evidence from recent studies is summarized below. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 3-6.

Although there are no recent studies examining hospital admissions for COPD, a few recent multicity studies in the United States (Krall et al., 2018) and Canada (Szyszkowicz et al., 2018; Weichenthal et al., 2016) reported positive associations between short-term NO_2 exposure estimated from central site monitors and ED visits for COPD (see Table 3-7).

- In a time-series study conducted in five U.S. cities (Atlanta, GA; Dallas, TX; St. Louis, MO; Birmingham, AL; and Pittsburgh, PA), Krall et al. (2018) observed a 9.8% increase in ED visits for COPD (95% PI: 1.5, 18.9%) per 25 ppb increase in 8-day moving average (i.e., lag 0–7) 1-hour max NO_2 concentrations. The authors used Bayesian multicity multi-outcome (MCM) models to improve precision of the pooled city-specific estimates by incorporating information from multiple outcomes to estimate shared intercity variation. The estimates from the Bayesian MCM models were comparable to estimates derived from traditional Bayesian hierarchical pooling, but slightly more precise (i.e., narrower 95% CIs).

Cases-crossover analyses of ED visit data collected across Ontario, Canada observed similarly positive, but weaker associations with 24-hour avg NO_2 concentrations.

- Weichenthal et al. (2016) analyzed over 280,000 ED visits for COPD across 15 cities in Ontario from 2004–2011. Same-day NO_2 concentrations were associated with increased risk of ED visits for COPD (0.69% [95% CI: -0.1, 1.5] per 10.6 ppb increase). The association was null at later lags (i.e., lag 1 and lag 2), and positive, but imprecise in relation to multiday average NO_2 concentrations (lag 0–2: 0.52% [95% CI: -0.80, 1.9]).
- Szyszkowicz et al. (2018) examined sex-specific associations between short-term NO_2 concentrations and ED visits for COPD in a smaller subset of cities in Ontario across the same time frame. The authors reported positive associations among males, but not females. Notably, in contrast to the lag structure of associations observed by Weichenthal et al. (2016),

Szyszkowicz et al. (2018) reported null associations with same day NO_2 concentrations among males, and positive associations with delayed exposures up to seven days.

- Each of the recent studies of ED visits for COPD assigned NO_2 exposure as concentrations measured at central site monitors, either from the nearest monitor to each patient's residential postal code (Weichenthal et al., 2016), the average of all monitors within 35 km of each patient's residential postal code (Szyszkowicz et al., 2018), or the population-weighted average of all monitors in a given city (Krall et al., 2018). As noted previously, the use of central site monitors in time-series and case-crossover studies is likely to bias effect estimates toward the null and reduce precision.
- In addition to uncertainties regarding exposure assessment, none of the recent studies evaluated potential confounding by copollutant exposures. In an analysis of the effect of the oxidant capacity of NO_2 and O_3 on ED visits for asthma, Weichenthal et al. (2016) observed that the weighted average of NO_2 and O_3 using redox potentials was associated with a much larger increased risk of ED visits for COPD than NO_2 alone. However, the association with redox-weighted oxidant capacity, which accounts for O_3 being a stronger oxidant than NO_2, was comparable in magnitude and precision to the association observed for a single pollutant O_3 model. This suggests some level of confounding is present between the pollutants.

Table 3-7 Percentage increase in hospital admissions or emergency department visits for chronic obstructive pulmonary disease in relation to short-term increases in ambient nitrogen dioxide concentrations

Study	Location	Ages	Lag	Averaging Time	Effect Estimate (95% CI)
Hospital Admissions					
Faustini et al. (2013)	Seven Italian cities	35+ yrs	0–1 d	24-hr avg	OR (NO_2) 3.7% (-0.6, 8.3%)
ED Visits					
†Krall et al. (2018)	Five U.S. cities	All ages	0–7 d	1-hr max	RRs NO_2: 9.8% (95% PI: 1.5, 8.9%) NO_X: 3.5% (95% PI: -6.1, 14.1%)
Peel et al. (2005)	Atlanta, GA, U.S.	All ages	0–2 d	1-hr max	RR (NO_2) 4.4% (0.7, 8.2%)
Stieb et al. (2009)	Seven Canadian Cities	All ages	0 d	24-hr avg	RR (NO_2) 0.1% (-5.5, 6.1%)
†Szyszkowicz et al. (2018)	Ontario, Canada	All ages	2 d	24-hr avg	ORs (for NO_2 in the warm season) Males: 2.7 (-0.5, 6.0%) Females: -0.2% (-3.3, 2.9%)
†Weichenthal et al. (2016)	Ontario, Canada	All ages	0 d	24-hr avg	OR (NO_2) 1.3% (-0.2%, 2.8%)

List of abbreviations in alphabetical order: avg = average; CI = confidence interval; d = day; ED = emergency department; HDI = highest density interval; h = hour; max = maximum; NO_2 = nitrogen dioxide; NO_X = NO + NO_2; NR = not reported; OR = odds ratio; PI = posterior interval; ppb = parts per billion; RR = relative risk; yr = year.
Notes: Results are standardized to a 20 ppb increase in 24-hr avg NO_2, a 25 ppb increase in 1-hr max NO_2, and an 80 ppb increase in 1-hr max NO_X.
†Studies published since the 2016 Oxides of Nitrogen ISA.

3.4.1.1.1 Conclusions and Remaining Uncertainties

Recent studies provide more consistent evidence of NO_2-associated increased risk of COPD ED visits relative to the limited evidence evaluated in the 2016 Oxides of Nitrogen ISA. While the evidence base remains small, several recent multicity studies in the United States and Canada provide consistent evidence of a positive association between COPD ED visits and short-term NO_2 concentrations measured at central site monitors. No recent PECOS-relevant studies have examined hospital admissions for COPD, but a small number of studies in the 2016 Oxides of Nitrogen ISA provided evidence of positive associations with short-term NO_2 exposure. The collective evidence for ED visits and hospital admissions includes a lack of evaluation of potential copollutant confounding, which remains a key uncertainty. Additionally, there has been limited consideration of the lag structure of associations, the impact of different exposure assignment methods, the shape of the C-R relationship, or how individual- or population-level factors may modify the observed associations.

3.4.2 Respiratory Symptoms and Medication Use

Respiratory symptoms in people with COPD, including cough, wheeze, sputum production, shortness of breath, and chest tightness, may indicate an exacerbation of disease. Further, uncontrollable symptoms may lead people with COPD to treat symptoms with medication. Thus, studies examining the association between NO_2 and increases in respiratory symptoms and medication use may provide biological plausibility for NO_2-induced acute respiratory effects, such as evidence of NO_2-related increases in hospital admissions and ED visits for COPD.

3.4.2.1 Epidemiologic Studies of Respiratory Symptoms and Medication Use

The 2016 Oxides of Nitrogen ISA included a limited number of epidemiologic panel studies that examined respiratory symptoms and medication use among adults with COPD. Associations between ambient NO_2 and respiratory symptoms were either null or inconsistent across the range of outcomes and exposure lags examined. Results were equally inconsistent for individual symptoms of cough, wheeze, dyspnea, total symptoms, and medication use. Several recent studies have examined oxides of nitrogen exposures in relation to respiratory symptoms and medication use among COPD patients. Recent evidence is not entirely consistent, but generally indicates positive associations between short-term NO_2 exposure and increased respiratory symptoms and/or medication use. Recent studies reported NO_2-related COPD exacerbations with exposures assigned using both personal monitors and central-site monitors. Additionally, positive associations were observed in both case-crossover and panel studies. Evidence

from recent studies is summarized below. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 3-7.

A few recent panel studies evaluated COPD exacerbations measured as a sustained worsening of symptoms and/or increased medication use (Evangelopoulos et al., 2021; Magzamen et al., 2018). These studies provide better inference than studies examining individual symptoms over a short period, which may be transient and not indicative of an exacerbation.

- In a panel of 115 COPD patients followed for 128 days, Evangelopoulos et al. (2021) reported that increases in same-day 24-hour avg NO and NO_2 concentrations measured using personal monitors were associated with an increase in COPD exacerbations (OR: 1.41 per 20 ppb increase in NO [95% CI: 1.22, 1.63] and 1.53 per 20 ppb increase in NO_2 [95% CI: 1.26, 1.85]). A pulmonologist reviewed each patient's self-reported symptoms and defined exacerbations based on a worsening of symptoms that lasted at least two days longer than normal symptom variation. In comparison to personal monitors, associations with fixed-site monitors were slightly attenuated but still positive. That is consistent with findings from exposure studies that demonstrate attenuation of effect estimates attributable to short-term exposures assigned via fixed site monitors. Additionally, Evangelopoulos et al. (2021) examined copollutant models and observed that NO_2 associations were robust to adjustment for PM_{10}, $PM_{2.5}$, and O_3, and slightly attenuated but still positive after adjustment for NO and CO.
- Similar results were observed in a smaller panel of 35 COPD patients followed for three months in the Seattle metropolitan area (Magzamen et al., 2018). Magzamen et al. (2018) reported a 9.8% increase in daily SABA inhaler puffs (95% CI: 1.5, 18.7) per 20 ppb increase in same-day NO_2 concentrations. Inhaler use was tracked by a portable electronic sensor and exposure was assigned using a single central-site monitor.

Pfeffer et al. (2019) and Ross et al. (2023) also examined COPD exacerbations measured as a sustained worsening of symptoms using time-series and case-crossover study designs, respectively. These studies had somewhat larger sample sizes than the panel studies but analyzed a comparable number of exacerbations.

- Pfeffer et al. (2019) focused on classifying exacerbations as "viral-type" (e.g., nasal congestion/discharge) and "bacterial-type" (e.g., increased sputum). Similar to the positive associations noted in the panel studies, the authors reported that short-term NO_X concentrations were associated with increased viral-type exacerbations. The association was present across single day lags from one to six days, with the strongest association at cumulative lags 2–4, indicating a delayed effect. In contrast, the authors observed null associations with bacterial-type exacerbations and with both types of exacerbations combined.
- A multicity case-crossover study conducted in Canada provides evidence of a small but imprecise positive association between short-term NO_2 exposure in the cold season and symptom-based exacerbation (lag 0–6 OR: 1.33 [95% CI: 0.71, 2.49] per 20 ppb). A negative association of similar magnitude was observed in relation to warm season NO_2 exposure, which indicates that the two observed associations may be the result of random variation due to low precision (Ross et al., 2023).

Other recent studies support more limited inference than the aforementioned studies and provide inconsistent evidence of associations between short-term NO_2 exposure and respiratory symptoms or medication use.

- A large panel study tracked rescue medication use among 3,386 volunteers in California with self-reported diagnosis of asthma or COPD (Su et al., 2024). Participants were given digital sensors and a GPS app to track inhaler use and map participants' locations to a hybrid-modeled 100m resolution air quality surface at the time of rescue medication use. The authors reported a 7.3% increase (95% CI: 5.3, 9.4) in daily rescue medication use over average (0.86 rescue uses/person/day) in relation to a 20 ppb increase in same day 24-hour average NO_2. However, the results do not distinguish between participants with asthma and COPD, and the large majority of participants in the study had asthma (n = 3,034).
- Aglan et al. (2023) reported a positive association between NO_2 measured at central site monitors and self-reported worsening of bronchitic symptoms in a panel of 30 former smokers with moderate to severe COPD. Notably, NO_2 exposure estimated from personal monitors was not associated with worsening of bronchitic symptoms. A limitation of this study is the case definition of worsening symptoms as increases in self-reported symptoms over a 24-hour period rather than more sustained increases that would better indicate a departure from normal day-to-day variation in COPD-related symptoms. This may have reduced specificity of the outcome, which would potentially bias effect estimates toward the null.
- In a case-crossover study of 168 patients, Devries et al. (2016) reported an increase in the odds of medication use associated with an increase in 7-day moving average (i.e., lag 1–7) NO_2 concentrations (OR: 1.06 [95% CI: 0.98, 1.16] per 1 ppb). The association was stronger after adjustment for $PM_{2.5}$ (OR: 1.17 [95% CI: 1.05, 1.30]) and null after adjustment for SO_2 (OR: 0.96 [95% CI: 0.88, 1.06]) in copollutant models. The authors implemented a unidirectional case-crossover design, which may have introduced seasonal confounding, particularly given that control periods were identified via random selection that may have significantly predated the exacerbation.

3.4.2.1.1 Conclusions and Remaining Uncertainties

A few recent panel studies add to a previously inconsistent evidence base and provide some additional evidence supporting a positive association between short-term NO_2 exposure and COPD symptom exacerbation or medication use, particularly from studies providing stronger inference due to a focus on sustained worsening of symptoms and/or increased medication use. Consistent with evidence from the 2016 Oxides of Nitrogen ISA, recent studies remain limited in number and are subject to key uncertainties due to the small number of cases, inconsistent results, and limited adjustment for potential copollutant confounding, particularly for traffic pollutants.

3.4.2.2 Controlled Human Exposure Studies of Respiratory Symptoms and Medication Use

No recent controlled human exposure studies evaluated the impact of NO_2 exposure in subjects with COPD. The results of the four controlled human exposure studies evaluated in the 2016 Oxides of Nitrogen ISA were mixed regarding symptom changes in study participants with COPD following NO_2 exposure. Exposure of participants with COPD to 400 ppb NO_2 for two hours (Gong et al., 2005) or 300 ppb for four hours (Morrow et al., 1992) did not result in changes in symptom scores. In other studies, small but statistically significant increases in symptoms were seen in individuals with COPD exposed to NO_2 ranging from 300–2000 ppb NO_2 for one hour with intermittent exercise (Vagaggini et al., 1996; Linn et al., 1985a). In one study, three of the participants with COPD reported increased medication usage in response to excessive symptoms in the week following NO_2 exposure at 1 or 2 ppm (Linn et al., 1985a).

3.4.2.2.1 Conclusions and Remaining Uncertainties

The evidence from controlled human exposure studies for NO_2-induced effects on respiratory symptoms in people with COPD is limited to a small number of studies. NO_2-induced changes in symptoms are inconsistent across studies, and the variation in exposure and exercise paradigms make synthesis of the data challenging. Other uncertainties that are inherent to controlled human exposure studies persist. This includes the factors like exposure pattern (constant concentration compared to variable concentration patterns that are more real-world relevant), relatability of results to effects in populations with more severe COPD than was reflected by the study participants, and the relevance of exposure to NO_2 alone compared to more ambient-relevant mixtures of oxides of nitrogen species.

3.4.3 Respiratory Effects Underlying COPD Exacerbation

3.4.3.1 Lung Function Changes in Adults with COPD

Exacerbation of COPD is typified by increased inflammation that accompanies acute worsening of lung function. The available epidemiologic and controlled human exposure evidence on NO_2-related changes in lung function in adults with COPD is discussed in Sections 3.4.3.1.1 and 3.4.3.1.2, respectively.

3.4.3.1.1 Epidemiologic Studies of Lung Function Changes in Adults with COPD

A limited number of epidemiologic studies evaluated in the 2016 Oxides of Nitrogen ISA examined lung function measures in adults with COPD. Most studies of adults with COPD assessed lung

function with unsupervised home measurements, and associations with ambient NO_2 concentrations measured at central site monitors were inconsistent among the various lung function parameters (e.g., FEV_1, PEF) and the NO_2 exposure lags (0-, 1-, 2-, or 2- to 7-day average) examined. A few recent panel studies of older adults with COPD strengthen the evidence base by measuring lung function with supervised spirometry (McHugh et al., 2023; Sinharay et al., 2017) and estimating exposure using personal monitors (Chen et al., 2023; Nurhussien et al., 2022; Evangelopoulos et al., 2021; Sinharay et al., 2017). Recent studies provide evidence of NO_2-related decrements in FEV1, FVC, and PEF in older adults with COPD (Nurhussien et al., 2022; Evangelopoulos et al., 2021; Sinharay et al., 2017). A summary of the recent evidence is provided below. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 3-8.

- Sinharay et al. (2017) conducted a randomized crossover study in which each participant alternated walking for two hours on two different days alongside a high-traffic road and a traffic-free park in London. Participants consisted of 40 volunteers with stable COPD, 39 volunteers with ischemic heart disease (IHD), and 40 age-matched healthy volunteers. In the participants with COPD, higher NO_2 concentrations at the time of the walk were associated with decrements in % predicted FEV1 (-4.85% [95% CI: -9.03, -0.67]) and FVC (-4.99% [95% CI: -8.27, -1.70]) 1 hour after the walk. Notably, the authors reported null associations in healthy participants and participants with stable IHD.
- Similar results were observed in a panel of 30 former smokers in Boston, MA with moderate to severe COPD who were given portable monitors and followed for up to four months (Chen et al., 2023; Nurhussien et al., 2022). Nurhussien et al. (2022) reported that higher previous-day 24-hour average NO_2 exposure was associated with lower FEV1 (-42.6 ml [95% CI: -70.4, -14.9]) and FVC (-67.9 ml [95% CI: -120.4, -15.5]). In the same population, Chen et al. (2023) observed that the inverse NO_2-FEV_1 associations were strongest in magnitude for participants with lowest levels of physical activity (<1,988 steps on the previous day; -105.6 mL [95% CI: -152.0, -59.1]) compared to those with moderate physical activity (1,988–4,168 steps; -49.3 mL [95% CI: -94.6, -3.9]), and nonexistent among participants with the highest level of physical activity (≥4,169 steps; -5.9 mL [95% CI: -68.2, 56.3]). Similar patterns were observed for FVC (quantitative results not provided).
- Nurhussien et al. (2022) also compared exposure assignment methods and evaluated potential effect modification by blood eosinophil (EOS) levels. While each of the recent studies estimated exposure using personal exposure monitors, Nurhussien et al. (2022) compared results based on exposure estimated from personal and community-based fixed site monitors. Similar to the results from personal monitors, inverse associations were also observed in analyses using community monitors, but the associations were smaller in magnitude, which is consistent with exposure studies that have demonstrated attenuation of effect estimates attributable to short-term exposures assigned via fixed site monitors. The authors also reported strong effect modification by EOS levels, with NO_2-related FEV1 decrements observed in the high EOS group (-9.5 mL [95% CI: -17.1, -1.9]) compared to small increases in the low EOS group (4.5 mL [95% CI: -4.1, 13.1]). Higher EOS levels are an indicator that COPD may be responsive to corticosteroids and may also be associated with higher rates of COPD exacerbation (Kerkhof et al., 2017).
- Other recent studies provide some additional support for an association between short-term NO_2 exposure and changes in lung function in adults with COPD, however the associations

are conditional on other factors, such as season and disease severity. Evangelopoulos et al. (2021) observed inverse associations between NO_2 and PEF in the spring and fall among a panel of COPD patients in London. The relationship was null in year-round analyses, as well as analyses focused on NO_2 concentrations in the summer and winter. In a panel of former smokers in Boston, MA, McHugh et al. (2023) did not observe strong evidence for an association between indoor NO_2 concentrations and pre- or post-bronchodilator FEV1 or FVC levels in the total study populations, but did observe imprecise NO_2-associated decrements in prebronchodilator FEV_1 (-17.36 mL [95% CI: -58.35, 23.60]) and FVC (-28.22 mL [95% CI: -91.49, 35.07]) levels in participants with more severe COPD (i.e., FEV_1 <50% predicted).

- Recent studies include limited evaluation of potential copollutant confounding. Evangelopoulos et al. (2021) examined copollutant models adjusting for CO, $PM_{2.5}$, PM_{10}, or O_3 in the year-round models, but not the seasonal models in which NO_2 was associated with PEF. However, year-round NO_2 concentrations were not strongly correlated with any of the other pollutants that were measured (r <0.25; seasonal correlations not reported). The crossover study conducted by Sinharay et al. (2017) had inherently highly correlated exposure concentrations due to the design of the study (i.e., participants walking alongside high-traffic roads), resulting in significant uncertainty regarding potential copollutant confounding by other traffic-related pollutants.

3.4.3.1.1.1 Conclusions and Remaining Uncertainties

Recent epidemiologic studies of lung function changes in adults with COPD have addressed some of the key uncertainties identified in the design of studies evaluated in the 2016 Oxides of Nitrogen ISA. In particular, a few recent studies used supervised spirometry, rather than unsupervised home spirometry, to measure lung function, and recent panel studies utilized personal monitors to better estimate exposure. In one recent study that compared results from analyses using personal monitors and a fixed-site community monitor, associations with lung function were stronger (i.e., larger in magnitude) with NO_2 estimated from personal monitors compared to the fixed-site monitor. Although the overall body of evidence is small, studies that provide stronger inference indicate an association between short-term NO_2 exposure and lung function in adults with COPD. In some cases, these associations were only present in stratified analyses examining seasonal impacts or lung function changes across participants with varying levels of COPD severity. Additionally, recent studies do not fully address some key uncertainties. The evidence consists of smaller panel studies with a limited number of repeated lung function measures, reducing statistical power to detect an association. This is reflected in the imprecise effect estimates observed in many of these studies. Recent studies also do not adequately address the potential for copollutant confounding, particularly by traffic-related pollutants (e.g., EC or UFPs). This uncertainty is particularly notable in studies that measured lung function in participants exercising near a busy roadway (Chen et al., 2023; Sinharay et al., 2017).

3.4.3.1.2 Controlled Human Exposure Studies of Lung Function Changes in Adults with COPD

Only four controlled human studies investigating the effect of lung function testing in subjects with COPD were evaluated in the 2016 Oxides of Nitrogen ISA [see Figure 5-19 of U.S. EPA (2016)]. The studies have shown mixed effects of NO_2 exposure on lung function. FEV_1 and FVC measures were increased in one study that exposed subjects with COPD to 300 ppb NO_2 for one hour with exercise (Vagaggini et al., 1996). Another study found decreased FEV_1 and FVC following 4-hour exposure to 300 ppb NO_2 with intermittent exercise that was not seen with air exposure (Morrow et al., 1992). In contrast, other studies showed no statistically significant changes to FVC or FEV_1 with exposure to 400–2000 ppb NO_2 (Gong et al., 2005; Linn et al., 1985a). In an exposure of NO_2 with concentrated ambient particulates, NO_2 exposure did not impact the PM-induced lung function decrements (Gong et al., 2005). No recent studies were identified that investigated the impact of NO_2 exposure on lung function in subjects with COPD.

3.4.3.1.2.1 Conclusions and Remaining Uncertainties

The body of controlled human exposure data is limited in number and report inconsistent results. Uncertainty remains regarding the exposure concentration required to see effects of lung function as the two studies with statistically significant decreases in lung function involved exposures to 300 ppb whereas studies that used higher exposure concentrations did not. Of note, all the reported studies of subjects with COPD involved an exercise paradigm. Implicitly, these studies would exclude those with COPD who cannot tolerate intermittent exercise and may thus miss a population potentially sensitive to NO_2-induced effects on lung function.

3.4.3.2 Pulmonary Inflammation in Populations with COPD

Increased markers of inflammation are often used as surrogates for COPD exacerbation. The evidence from epidemiologic and experimental studies is discussed in the ensuing sections.

3.4.3.2.1 Epidemiologic Studies of Pulmonary Inflammation in Adults with COPD

A single epidemiologic study in the 2016 Oxides of Nitrogen ISA reported that neither ambient NO_2 nor NO was associated with indicators of inflammation, such as increases in the numbers of blood eosinophils or lymphocytes, in adults with COPD. One recent panel study similarly provides limited evidence of an association between short-term NO_2 exposure and respiratory tract inflammation in populations with COPD [Wu et al. (2016); Appendix A – Appendix Table 3-8]. In a small panel of 23 stable COPD patients repeatedly measured for markers of airway inflammation in Beijing, China, Wu et al. (2016) reported null associations between 24-hr avg NO_2 concentrations measured at central site

monitors and eNO levels. The authors also examined exhaled hydrogen sulfide (FeH_2S), which has been shown to predict airway inflammation in COPD. While NO_2 concentrations were positively associated with FeH_2S at later exposure lags, indicating delayed NO_2-related airway inflammation, there was an inverse association after adjustment for SO_2, PM_{10}, and $PM_{2.5}$ in copollutant models. Additionally, median 24-hour avg NO_2 concentrations (~42 ppb) were higher than most recent studies in the United States and Canada.

3.4.3.2.1.1 Conclusions and Remaining Uncertainties

Few studies have evaluated the relationship between short-term NO_2 exposure and pulmonary inflammation in adults with COPD. The available studies do not provide evidence of an association. In addition to the limited number of studies, the evaluated studies include small sample sizes and a small number of repeated exhaled breath samples collected from the participants.

3.4.3.2.2 Controlled Human Exposure Studies of Pulmonary Inflammation in Adults with COPD

Given the small number of controlled human exposure studies, there is limited information with regards to subclinical signaling events that relate to inflammation in subjects with COPD. As mentioned in Section 3.3.3.3.2 and Section 3.6.4.2, some controlled human exposure studies in healthy and asthmatic populations have shown increased markers of inflammation. However, one study that exposed subjects with COPD to 300 ppb NO_2 for one hour with intermittent exercise showed no changes in inflammatory cells in the sputum following exposure (Vagaggini et al., 1996). Another study of a 2-hour exposure to 400 ppb NO_2 reported no change in white blood cell counts in sputum from subjects with COPD (Gong et al., 2005). The limited controlled human exposure data does not provide support for NO_2-increased inflammation in subjects with COPD that could underly exacerbation of disease.

3.4.3.2.2.1 Conclusions and Remaining Uncertainties

The body of controlled human exposure studies examining inflammatory effects in those with COPD is extremely limited in number and show null results. Given the role of chronic inflammation in the development of COPD, controlled human studies likely did not include the most severely affected individuals.

3.4.3.2.3 Animal Toxicological Studies of Pulmonary Inflammation

While animal models do not develop COPD, evidence of NO_2-induced increases in inflammation, a key feature of COPD, have been studied in naive and allergen sensitized animals and is discussed in Sections 3.3.3.3.3 and 3.6.4.3, respectively.

3.4.4 Synthesis Across Lines of Evidence

Evidence for the effects of short-term NO_2 exposure on COPD exacerbation lacks coherence across disciplines. There is some epidemiologic support for an association between short-term NO_2 exposure and COPD exacerbation, but the experimental evidence is extremely limited and mostly inconsistent. The strongest evidence comes from epidemiologic studies that reported generally consistent NO_2-associated increases in risk of COPD ED visits and hospital admissions, including several recent multicity studies in the United States and Canada (see Section 3.4.1). Epidemiologic evidence for respiratory symptoms and medication use among adults with COPD is limited but is consistent with the evidence for more severe exacerbations (see Section 3.4.2.1). A limited number of epidemiologic studies have examined lung function in adults with COPD, and those that provide stronger inference indicate an association between short-term NO_2 exposure and reduced lung function (see Section 3.4.3.1.1).

Although the epidemiologic evidence is generally consistent, the limited number of controlled human exposure studies do not clearly indicate respiratory symptoms, lung function changes, or pulmonary inflammation following NO_2 exposure in adults with COPD. A major uncertainty in the controlled human exposure studies is the generalizability of results to effects in populations with more severe COPD that would not qualify for enrollment based on ethical and safety concerns. The lack of coherence from experimental lines of evidence does not reduce uncertainty in the degree to which the epidemiologic evidence is indicative of an association with NO_2 exposure independent of traffic-related pollutants. This is a particularly important consideration given the limited evaluation of potential copollutant confounding in epidemiologic studies. In summary, the lack of coherent evidence across disciplines, along with limited evaluation of potential copollutant confounding in the epidemiologic evidence base, contributes to overall uncertainty regarding the independent relationship between short-term NO_2 exposure and COPD exacerbation.

3.5 Respiratory Infection

The respiratory tract is protected from exogenous pathogens by lung host defenses that include mucociliary clearance and clearance by alveolar macrophages, as well as innate and adaptive immunity. Impairment of these defense mechanisms can increase the risk of respiratory infection. The 2016 Oxides of Nitrogen ISA included evidence from experimental animals demonstrating increased susceptibility to

bacterial infection following NO_2 exposures of two to 48 hours in the range of 1,000 to 5,000 ppb. Animal toxicological evidence did not indicate infection-induced mortality or changes in bacterial clearance in response to lower NO_2 exposures (i.e., 500 to 600 ppb). Many of the epidemiologic studies reported null or imprecise associations with hospital admissions and ED visits for respiratory infection, as well as for parental reports of infection. There was some epidemiologic evidence for NO_2-related increases in hospital admissions or ED visits for bronchiolitis or ear infection in study populations with pre-existing respiratory disease (i.e., children with asthma and adults with COPD). However, most epidemiologic studies did not examine copollutant confounding, and respiratory infections in these populations were also associated with other traffic-related pollutants, as well as other pollutants that were highly correlated with NO_2.

3.5.1 Hospital Admissions and ED Visits for Respiratory Infection

This section evaluates the epidemiologic evidence for hospital admissions and ED visits for respiratory infection. The available evidence from the 2016 ISA and from recent studies is summarized, followed by additional discussion of the lifestages and populations evaluated in recent studies (see Section 3.5.1.1) and the exposure assessment approaches used (see Section 3.5.1.2). Section 3.5.1.3 discusses conclusions from these studies and summarizes remaining uncertainties.

Hospital Admissions for Respiratory Infection

A limited number of epidemiologic studies evaluated in the 2016 Oxides of Nitrogen ISA examined associations between short-term exposure to NO_2 and hospital admissions for respiratory infection. Target populations and respiratory infection types varied considerably, limiting inference across these studies. Evidence for NO_2-related hospital admissions since the 2016 Oxides of Nitrogen ISA is limited to a few studies. A short summary of the recent evidence is provided below. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 3-9.

A few recent studies have examined short-term NO_2 exposure in relation to hospital admissions for respiratory infection.

- A nationwide time-series study conducted in Canada examined aggregated influenza and pneumonia hospital admissions and did not observe strong evidence of an association with same-day NO_2 concentrations (Shin et al., 2022). In models stratified by sex, the authors reported an increase in hospital admissions among women (2.4% increase [95% PI: -1.0, 5.9] per 20-pbb increase in 24-hour avg NO_2); however, in seasonal analyses, the association was slightly negative or null in the warm and cold seasons, respectively. These attenuated effect estimates in season-specific analyses suggest the presence of residual confounding, despite a natural cubic spline fitted to account for the long-term temporal trends. In contrast, a positive association was observed among males in the warm season, but not in the cold season or in year-round analyses.

Other recent studies examined acute respiratory distress syndrome (ARDS), an endpoint that was not evaluated in the 2016 Oxides of Nitrogen ISA. ARDS is an often-fatal condition characterized by pulmonary edema and low blood oxygen levels resulting from infection or traumatic injury.

- Recent studies evaluated ARDS incidence in patients hospitalized with sepsis (Reilly et al., 2023) or acute traumatic injury (Reilly et al., 2019) during two overlapping 10-year periods in Philadelphia, PA. In both cases, 3-day average (i.e., lag 1–3) NO_2 concentrations were associated with large but imprecise increases in the odds of ARDS (OR: 1.37 [95% CI: 1.10, 1.71] per 8.1 ppb increase and 1.42 [95% CI: 0.99, 2.04] per 4 ppb increase, respectively).

ED Visits for Respiratory Infection

In the 2016 Oxides of Nitrogen ISA, epidemiologic studies of ED visits for respiratory infection were limited in number and examined disparate infection types. Results from a small number of studies analyzing ED visits for aggregated respiratory infections were equivocal. Separate studies provided evidence of positive associations between NO_2 exposure and ED visits for bronchiolitis and otitis media in young children, but not for pneumonia among people all ages. A few recent studies provide evidence of positive associations between short-term NO_2 exposure and ED visits for pneumonia, upper respiratory infection (URI), and lower respiratory tract infection (LRTI). However, outside of the small number of studies that examined ED visits for upper respiratory tract infection, specific respiratory infection endpoints (e.g., pneumonia, LRTI) have not been evaluated across multiple studies. A short, bulleted summary of the recent evidence is provided below, followed by more detailed discussion of the lifestages and populations evaluated in these studies (see Section 3.5.1.1) and the exposure assignment methods used in recent studies (see Section 3.5.1.2). Section 3.5.1.3 discusses conclusions from these studies and summarizes remaining uncertainties. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 3-9.

- A few large multi-city studies in the United States and Canada reported increased risk of ED visits for pneumonia (Krall et al., 2018), URI (Krall et al., 2018; Szyszkowicz et al., 2018; Xiao et al., 2016), and LRTI (Szyszkowicz et al., 2018) in association with short-term increases in NO_2 (see Table 3-8). Each study included multiple years of data on ED visits among people of all ages (Krall et al., 2018; Szyszkowicz et al., 2018) or in analyses restricted to children (Xiao et al., 2016). Compared to the results from Krall et al. (2018), Xiao et al. (2016) observed an odds ratio for NO_2-associated ED visits for pneumonia that was much closer to the null and had confidence intervals that crossed the null, providing little evidence of an association. Given the different age ranges of the populations examined, the contrasting results observed by Krall et al. (2018) and Xiao et al. (2016) may reflect differences in risk across age groups (see Section 3.5.1.1).
- A much smaller regional study in Utah reported inconsistent evidence for increased risk of ED visits for pneumonia among adults across different lags (Pirozzi et al., 2017). Positive associations were observed at individual lag days four through 7, but not for shorter lags.

- Similar to evidence presented in the 2016 Oxides of Nitrogen ISA, the recent studies discussed above report strong correlations between NO_2 concentrations and traffic-related pollutants and do not evaluate copollutant models.
- Evidence examining the effects of short-term exposure to NO_2 and COVID-19 was limited, but a large study in Canada reported a positive association between exposure to NO_2 and ED visits for COVID-19 over lags 0–3 days (Lavigne et al., 2023).

Table 3-8 Associations between short-term exposure to oxides of nitrogen and emergency department visits for respiratory infection

Study	Location	Ages	Lag	Averaging Time	Effect Estimate (95% CI)
†Pirozzi et al. (2017)	Wasatch Front, UT, U.S.	Adults (18+)	Individual lags 1 through 7 d	24-hr avg	Pneumonia ED Visits (NO_2) OR: Lag 1: 0.98 (0.90, 1.06) Lag 4: 1.04 (1.01, 1.07) Complete results for other lags provided in Appendix A – Appendix Table 3-9
† Xiao et al. (2016)	Georgia, U.S.	2–18	0–2 d	1-hr max	ORs for ED Visits (NO_2) Pneumonia: 1.016 (0.976, 1.058) URI: 1.023 (1.017, 1.028)
†Krall et al. (2018)	5 U.S. cities	All ages	0–7 d	1-hr max	RRs for ED Visits *Pneumonia* NO_2: 1.076 (95% PI: 1.013, 1.143) NO_X: 1.040 (95% PI: 0.953, 1.135) *URI* NO_2: 1.115 (95% PI: 1.06, 1.173) NO_X: 1.056 (95% PI: 0.973, 1.147)
†Szyszkowicz et al. (2018)	Ontario, Canada	All ages	8 d	24-hr avg	ORs for ED Visits (NO_2) *URI* Male: 1.04 (1.015, 1.067) Female: 1.027 (1.001, 1.053) *Acute Lower Respiratory Infection* Cold: 1.018 (0.993, 1.043) Warm: 1.124 (1.073, 1.177)

List of abbreviations in alphabetical order: avg = average; CI = confidence interval; d = day; ED = emergency department; HDI = highest density interval; h = hour; max = maximum; NO_2 = nitrogen dioxide; NO_X = NO + NO_2; NR = not reported; OR = odds ratio; ppb = parts per billion; RR = relative risk; URI = upper respiratory infection; yr = year.
Notes: Results are standardized to a 20 ppb increase in 24-hr avg NO_2, a 25 ppb increase in 1-hr max NO_2, and an 80 ppb increase in 1-hr max NO_X.
†Studies published since the 2016 Oxides of Nitrogen ISA.

3.5.1.1 Lifestages and Population Differences

3.5.1.1.1 Lifestage

In the 2016 Oxides of Nitrogen ISA, the strongest evidence of an association between short-term NO_2 concentrations and respiratory infection was provided by studies that focused on children, specifically those less than five years of age. Most recent studies examine hospital admissions or ED visits for respiratory infections among people of all ages and thus do not inform the relationship in specific lifestages. In a statewide study of ED visits for URI and pneumonia among 0- to 18-year-old children living in Georgia, a 20 ppb increase in 3-day moving average (i.e., lag 0–2) NO_2 concentrations was associated with a 6.3% (95% CI: 4.8, 7.8) increase in ED visits for URI (Xiao et al., 2016). The authors observed a smaller, imprecise increase for pneumonia with confidence intervals that crossed the null (1.6% [95% CI: -2.4, 5.8]), providing little evidence of an association.

3.5.1.2 Exposure Assessment

The majority of respiratory infection hospital admission and ED visit studies evaluated in the 2016 Oxides of Nitrogen ISA assigned NO_2 exposure using average concentrations across multiple monitors in a given study area. Recent studies utilize similar methods, assigning average or inverse distance weighted (IDW) average NO_2 concentrations from multiple monitors, in some cases using a maximum distance buffer of 35 to 50 km. For case-crossover and time-series epidemiologic studies of short-term exposure, such as those examining hospital admissions or ED visits for respiratory infection, the temporal variability in concentrations is of primary importance to relate to variability in the outcome. Spatial variability in NO_2 concentrations across the study area that is not captured by central-site monitors could attenuate an epidemiologic study's effect estimate if the exposures are not correlated in time with monitored ambient concentration.

3.5.1.3 Conclusions and Remaining Uncertainties

The 2016 Oxides of Nitrogen ISA included a limited number of epidemiologic studies examining ED visits of hospital admissions for respiratory infection. The available studies examined disparate infection types and reported inconsistent results. Several recent large multicity time-series and case-crossover studies in the United States and Canada provide generally consistent evidence of an association between short-term NO_2 exposure and ED visits for respiratory infection. Most recent studies include populations of all ages, though one focuses specifically on respiratory infection ED visits in children. Notably, there are still a limited number of studies examining the same endpoints (e.g., pneumonia, LRTI). The most consistent evidence for any given endpoint is from multiple studies that reported an

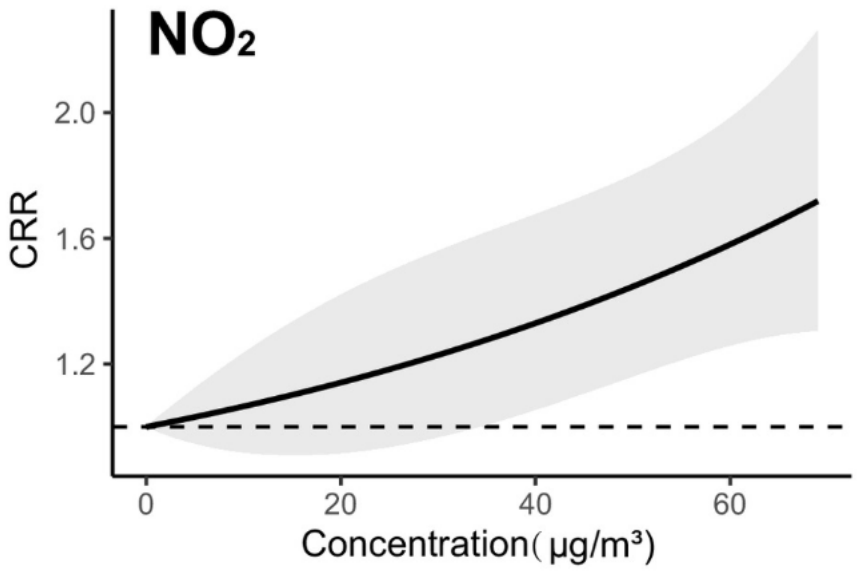
association between short-term NO_2 exposure and ED visits for upper respiratory tract infection. Consistent with the evidence presented in the 2016 Oxides of Nitrogen ISA, recent studies reported strong correlations between NO_2 concentrations and traffic-related pollutants and did not evaluate potential copollutant confounding. Studies of hospital admissions for respiratory infection remain limited in number and results are equivocal.

3.5.2 Self-Reported or Diagnosed Respiratory Infection

3.5.2.1 Epidemiologic Studies of Self-Reported or Laboratory Confirmed Infection

Epidemiologic evidence discussed in the 2016 Oxides of Nitrogen ISA did not clearly indicate a relationship between short-term NO_2 exposure and self- or parental-reported respiratory infection in children or more objectively ascertained as laboratory-confirmed or physician-diagnosed cases. Several studies examined these outcomes and provided inconsistent evidence of an association. Those studies that did report positive associations were limited by small sample sizes, lack of temporal resolution of outcomes (i.e., "ever diagnosed"), and limitations in exposure assignment, precluding strong inferences from the results. A limited number of recent studies examine self-reported or laboratory-confirmed respiratory infection, providing some evidence of an association. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 3-10.

- A large study examining nearly four million clinical cases of influenza across 82 cities in China reported a 39.7% increase in influenza incidence (95% CI: 27.8, 52.8) associated with a 5.32 ppb increase in NO_2 concentrations averaged over the week preceding case identification (Yu et al., 2023). In a stratified analysis, the NO_2 association with influenza was stronger in magnitude for 0- to 14-year-olds (RR: 1.506 [95% CI: 1.357, 1.672]) compared to those aged 15 years and older (1.206 [95% CI: 1.117, 1.302]). The authors also modeled the relationship using a cubic polynomial function to assess potential nonlinearity and observed an approximately linear relationship between NO_2 concentrations and influenza risk (see Figure 3-9).
- Relative to Yu et al. (2023), Zhang et al. (2023) observed a stronger association across a longer distributed lag (RR: 3.03 [95% CI: 2.22, 4.12] per 10 ppb increase in 0–14-day lag NO_2 concentrations) using similar data sources but focusing on a smaller number of cities in China (i.e., eight capital cities and two municipalities; 1+ million clinical cases).



List of abbreviations in alphabetical order: CRR = cumulative risk ratio; NO_2 = nitrogen dioxide; $\mu g/m^3$ = micrograms per cubic meter.
Source: Adapted from Yu et al. (2023). Copyright permission pending.

Figure 3-9 Concentration-response curve between nitrogen dioxide concentrations (lag 1-7) and influenza incidence in 82 cities across China.

- In an analysis of the MESA Air study comprising a much smaller study population (n = 6,536), Kirwa et al. (2021) reported an increased risk of self-reported respiratory infection, including influenza, febrile illness, sinus infection, bronchitis, or pneumonia, associated with an increase in 14-day and 28-day average NO_2 and NO_X concentrations. The authors used a high-resolution spatiotemporal model to assign NO_2 exposures to participants' residences. Kirwa et al. (2021) considered potential effect modification across a range of demographic variables, reporting slightly higher risk of respiratory infection associated with short-term NO_2 concentrations among older adults (65+ years), Chinese Americans, males, smokers, and non-diabetics.

3.5.2.1.1 Conclusions and Remaining Uncertainties

Overall, the evidence for self-reported, laboratory confirmed, or physician-diagnosed respiratory infection is limited. The strongest evidence informing the relationship between NO_2 exposure and laboratory confirmed respiratory ED visits comes from recent multicity studies in China that reported an increase in influenza cases associated with short-term NO_2 exposure. However, the studies in China evaluate overlapping study populations and therefore do not provide independent evidence of associations. In an analysis of nearly four million clinical cases of influenza across 82 cities in China, a flexible spline model revealed an approximately linear relationship between NO_2 concentrations and influenza incidence. Self-reported respiratory infection is more prone to misclassification of outcome than laboratory confirmed diagnoses.

3.5.3 Experimental Support for Respiratory Infection

This section evaluates evidence from controlled human exposure studies (see Section 3.5.3.1) and animal toxicology studies (see Sections 3.5.3.2 to 3.5.3.4) that examine effects related to respiratory infection following short-term NO_2 exposures. No recent PECOS-relevant experimental studies examining respiratory infections were identified. Thus, the studies discussed in this section are those that were available in the 2016 Oxides of Nitrogen ISA.

3.5.3.1 Controlled Human Exposure Studies of Subclinical Effects Underlying Exacerbation of Respiratory Infection

A small number of controlled human exposure studies have examined the effects of NO_2 exposure on susceptibility to viral infections and alveolar macrophage functions in healthy adults (U.S. EPA, 2016, 2008b, 1993a). Study specific details, exposure conditions, and endpoints examined are highlighted in the text below and are described in more detail in Tables 5-22 and 5-26 of U.S. EPA (2016).

Overall, studies provide little evidence supporting compromised host resistance to viral infection following NO_2 exposure. Frampton et al. (1989) reported that alveolar macrophages collected from a subset of subjects exposed to 0.6 ppm NO_2 for three hours were less effective at inactivating influenza virus ex vivo than air-exposed subjects. In the same study, however, inactivation of influenza virus was unaffected in alveolar macrophages collected from subjects continuously exposed to 0.05 ppm NO_2 plus 2 ppm intermittent NO_2 pulses and exercise (Frampton et al., 1989). Frampton et al. (2002) found that inactivation of influenza virus and respiratory syncytial virus was unaffected in alveolar macrophages collected from subjects continuously exposed to 0.6 ppm NO_2 or 1.5 ppm NO_2 with intermittent exercise. Similarly, other investigators found no effect of NO_2 exposure (6 hours to 2 ppm with intermittent exercise) on inactivation of influenza virus ex vivo by alveolar macrophages (Azadniv et al., 1998) or on the frequency of infection, virus shedding, or overall antibody responses in subjects exposed to NO_2 (2 ppm, two hours/day for three consecutive days) and inoculated with influenza virus after the second exposure (Goings et al., 1989).

A few studies investigated other immune endpoints. Phagocytosis of *Candida albicans* particles and superoxide anion production were reduced in alveolar macrophages collected from subjects exposed for four hours to 2 ppm NO_2 (Devlin et al., 1999). However, Azadniv et al. (1998) reported that exposure to NO_2 (6 hours to 2 ppm with intermittent exercise) had no effect on phorbol ester-stimulated superoxide anion production or on antibody-dependent cell-mediated cytotoxicity as determined by measuring the lysis of opsonized red blood cells. Frampton et al. (1989) reported that production of the inflammatory mediator, IL-1, was related to the viral inactivation capacity of the alveolar macrophages. In cells collected from NO_2 exposed subjects (see above), IL-1 secretion increased when measured in the subset of alveolar macrophages with impaired viral neutralization capability and decreased in unaffected alveolar

macrophages (Frampton et al., 1989). These results may indicate the presence of differing susceptibility of individuals to NO_2-induced suppression of macrophage function

There are no recent controlled human exposure studies investigating the effects of short-term NO_2 exposure on susceptibility to viral infection or other related immune endpoints. Overall, the older controlled human exposure evidence of subclinical effects underlying exacerbation of respiratory infection does not consistently indicate impaired immune function.

3.5.3.1.1 Conclusions and Remaining Uncertainties

Controlled human exposure studies of subclinical effects underlying exacerbation of respiratory infection do not consistently indicate impaired immune function under the conditions studied. Importantly, however, the evidence base is limited. For example, there are no controlled human exposure studies investigating the effects of NO_2 exposure on susceptibility to bacterial infections or other important immunological endpoints (e.g., antibody responses). Furthermore, the available controlled human exposure studies examined healthy adults and did not include populations potentially at increased risk. Consequently, there is uncertainty in interpreting the available controlled human exposure studies within the context of the broader populations evaluated in epidemiologic studies, including children.

3.5.3.2 Animal Toxicological Studies of Effects Underlying Exacerbation of Respiratory Infection – Susceptibility to Bacterial Infection

No recent studies investigated the impact of NO_2 exposure on bacterial infection in mammalian models. Available animal toxicological studies evaluated in the 2008 and 2016 Oxides of Nitrogen ISAs provide clear evidence that host resistance to bacterial infection is compromised following short-term exposures to PECOS-relevant NO_2 concentrations in mice (U.S. EPA, 2016, 2008b). These studies used a variety of experimental approaches but in most cases included an infectivity model where mice were exposed to NO_2 or filtered air and then challenged by exposure to an aerosol containing an infectious organism. Some of these studies measured mortality following challenge, while others examined bacterial clearance and bactericidal activity. Experimental infectivity models are well-accepted as indicators of impaired or weakened host defense (i.e., immunosuppression). Study specific details, exposure conditions, and endpoints examined are highlighted in the text below, followed by additional discussion of the dose-response relationships evaluated in these studies (see Section 3.5.3.2.1), lifestage and population differences (see Section 3.5.3.2.2), and the relevance of the animal responses to humans (see Section 3.5.3.2.3). Section 3.5.3.2.4 discusses conclusions from these studies and summarizes remaining uncertainties. Further study details are described in Appendix A – Appendix Table 3-11.

3.5.3.2.1.1 Mortality from Bacterial Infection

The effects of NO_2 exposure on mortality resulting from infection with five bacterial strains, including three gram-positive (i.e., *Streptococcus pyogenes*, *Streptococcus zooepidemicus*, and *Staphylococcus aureus*) and two gram-negative strains (i.e., *Klebsiella pneumoniae* and *Pasteurella pneumotropica*), were investigated in nine studies conducted in seven different strains of mice, as well as hamsters and squirrel monkeys. Mortality associated with bacterial infection was unaffected when mice were exposed to NO_2 concentrations of 0.5 ppm (Ehrlich, 1980; Gardner et al., 1979), 1 ppm (Sherwood et al., 1981; Illing et al., 1980), or 1.5 ppm (Ehrlich, 1980) for up to 48 hours. Effects on mortality were evident, however, when higher concentrations of NO_2 were evaluated. For example, mortality was increased in mice exposed to 2 or 3 ppm NO_2 for three hours (Illing et al., 1980; Ehrlich et al., 1977). Other investigations evaluating PECOS-relevant concentrations (0.5–5 ppm) of NO_2 in mice also reported increased mortality (Mcgrath and Oyervides, 1985; Ehrlich, 1980; Ehrlich et al., 1977; Ehrlich and Henry, 1968; Ehrlich, 1966; Purvis and Ehrlich, 1963). Henry et al. (1970) reported that exposure to 5 ppm NO_2 for two months increased bacterial infection-related mortality in squirrel monkeys. Ehrlich (1980) reported that NO_2 exposure increased bacterial infection-related mortality in hamsters and squirrel monkeys, but only when animals were exposed to very high concentrations (i.e., greater than 35 ppm and 50 ppm, respectively). Considering findings across published studies, the investigators suggested that the effects of NO_2 exposure on mortality from infection may differ among animal species.

In addition to investigating the effects of different NO_2 exposure concentrations on mortality from bacterial infection in different species and mouse strains, the impact of other experimental variables has been assessed. When examining the relationship between NO_2 concentration and exposure duration, concentration was found to be more of a factor driving mortality due to bacterial infection than exposure duration (Ehrlich et al., 1979; Gardner et al., 1979). The sequence and interval between exposure to NO_2 and the challenge with an infectious agent is also important. Ehrlich (1980) provided evidence that increasing the interval between short-term NO_2 exposure and challenge reduced mortality. Differences in experimental designs (e.g., mouse strain, NO_2 concentration, exposure duration) make it difficult to make comparisons across studies evaluating the same infectious organism. However, when the same infectious organism was used in more than one study, increased mortality was observed in all of those studies (Mcgrath and Oyervides, 1985; Ehrlich, 1980; Illing et al., 1980; Gardner et al., 1979; Ehrlich et al., 1977; Ehrlich, 1966; Purvis and Ehrlich, 1963). There is also some evidence that the NO_2 concentration associated with increased mortality is influenced by the challenge organism used in the study. For example, compared to a study investigating *Klebsiella pneumoniae* (Ehrlich, 1966), increased mortality was observed when mice were infected with *S. pyogenes* (Ehrlich et al., 1977) suggesting differential sensitivity to the challenge organism. Finally, based on a single study conducted in mice, there is no indication that intermittent peak exposures superimposed on a lower continuous background exposure to NO_2 alter susceptibility to infection (Graham et al., 1987). In that study, there was no difference in mortality when comparing 4.5 ppm NO_2 spikes alone with NO_2 spikes superimposed on a 1.5 ppm NO_2 continuous background exposure when infection occurred immediately after NO_2 exposure. Mortality

increased in proportion to duration of the 4.5 ppm spike from one to three and a half to seven hours. In mice challenged for 18 hours after NO_2 exposure, increases in mortality were statistically significant only with 3.5- and 7-hour exposures to 4.5 ppm NO_2 (combining groups with spikes alone and with 1.5 ppm background NO_2 exposure).

Overall, animal toxicological studies examining exacerbation of respiratory infection consistently indicate increased mortality in multiple strains of NO_2-exposed mice infected with gram-positive and gram-negative bacteria.

3.5.3.2.1.2 Bacterial Clearance from the Lung

Studies investigating the effect of NO_2 exposure on bacterial clearance from the lung can be divided into two categories – physical removal and bactericidal activity. The effects of NO_2 exposure on bacterial clearance from the lung was investigated in seven studies involving infection with six bacterial strains, including three gram-positive (i.e., group C *Streptococci*, *Listeria monocytogenes*, and *Staphylococcus aureus*), four gram-negative strains (i.e., *Klebsiella pneumoniae*, *Pasteurella pneumotropica*, *Proteus mirabilis*, and *Mycoplasma pulmonis*) in five different strains of mice.

NO_2 exposures of mice in the range of 1 to 5 ppm reduced bacterial clearance and/or bactericidal activity from the lungs (Davis et al., 1992; Davis et al., 1991; Parker et al., 1989; Jakab, 1988; Sherwood et al., 1981; Goldstein et al., 1973). In these studies, mice were challenged with radiolabeled bacteria either immediately before or after NO_2 exposures of four to 48 hours. For studies of 4-hour exposures, bacterial clearance was not affected with 0.5 ppm NO_2 (Davis et al., 1991) but decreased dose-dependently with NO_2 concentrations of 2.3 ppm and higher (Davis et al., 1992; Parker et al., 1989; Jakab, 1988; Goldstein et al., 1973). Studies involving NO_2 exposure for four hours both before and after bacterial challenge with *S. aureus* demonstrate that the lung is more susceptible to adverse effects of NO_2 when the lung contains an infectious organism (Jakab, 1988). Statistically significant decreases in bactericidal activity to *S. aureus* was seen in mice exposed to 2.3 ppm or 6.6 ppm NO_2 for 17 hours but not following exposure to 1 ppm for the same duration (Goldstein et al., 1973). Exposure to 1 ppm NO_2 for 24 or 48 hours did not impact initial clearance of pathogenic *C Streptococci* but proliferation of bacteria in the lungs was greater in mice exposed to 1 ppm NO_2 for 48 hours (Sherwood et al., 1981).

Consistent with mortality, there is some evidence that the effects of NO_2 on bacterial clearance and bactericidal activity is influenced by the challenge organism used in the study. Increased bacterial proliferation was observed in NO_2-exposed (1 ppm, 24 or 48 hours) mice after infection with the virulent group *C Streptococci* but not *S. aureus* (Sherwood et al., 1981). A 4-hour exposure to 5 ppm NO_2 resulted in a decrease in bactericidal activity after challenge with the gram-positive bacteria *S. aureus*. However, NO_2 exposure at concentrations less than 20 ppm did not affect killing of two gram-negative strains (i.e., *P. mirabilis* or *P. pneumotropica*) (Jakab, 1988).

Overall, animal toxicological evidence of subclinical effects underlying exacerbation of respiratory infection consistently indicates decreased removal of gram-positive and gram-negative bacteria from the lungs of mice exposed to NO_2. There are no recent animal toxicological studies investigating the effects of short-term NO_2 exposure on susceptibility to bactericidal clearance from the lung.

3.5.3.2.1.3 Resistance to Bacterial Infection in a Predisposed Host

Recognizing that influenza virus infections are known to predispose the host to bacterial infections, mice were infected with murine influenza A/PR8/34 eight days before challenge with *S. aureus*. Challenged mice were subsequently exposed to 5 or 10 ppm NO_2 for four hours. As demonstrated in other experiments, 5 and 10 ppm NO_2 exposure dose-dependently impaired bactericidal activity, but influenza infection only impacted bactericidal activity in mice exposed to 10 ppm NO_2 (Jakab, 1988).

In another experiment, the impact of NO_2 exposure on bactericidal activity in immunosuppressed mice was investigated. For this experiment, mice were injected with methylprednisolone acetate, a corticosteroid, four days before challenge with *S. aureus*. Challenged mice were immediately exposed to 1, 2.5, 5, or 10 ppm NO_2 for four hours. As demonstrated in other experiments, 5 and 10 ppm NO_2 exposure dose-dependently impaired bactericidal activity. The combination of corticosteroid treatment and NO_2 exposure dose-dependently reduced bactericidal activity in mice exposed 2.5, 5, and 10 ppm NO_2 (Jakab, 1988).

Overall, animal toxicological evidence of subclinical effects underlying exacerbation of respiratory infection indicates increased susceptibility to bacterial lung infection in NO_2-exposed mice pre-disposed to infection. There are no recent animal toxicological studies investigating the effects of short-term NO_2 exposure on resistance to bacterial infection in a predisposed host.

3.5.3.2.1 Dose Response

Dose-response relationships in experimental studies can strongly support conclusions on causality (See Appendix A of U.S. EPA (2024)). Dose-response relationships were reported for several lines of evidence related to susceptibility to infection, including mortality from bacterial infection ($\geq$2 ppm) (Ehrlich, 1980), bacterial clearance ($\geq$5 ppm) (Parker et al., 1989; Jakab, 1988) and bactericidal activity ($\geq$2.3 ppm) (Jakab, 1988; Goldstein et al., 1973). In all instances, PECOS-relevant concentrations of NO_2 promoted enhanced susceptibility to bacterial infection in animal models.

3.5.3.2.2 Lifestages and Population Differences

The body of animal evidence suggests that individuals with bacterial infections might be at higher risk of complications from NO_2 exposures. In addition, one short-term exposure study demonstrated that exposure to NO_2 (5 and 10 ppm) dose-dependently impaired bactericidal activity in immunosuppressed mice (Jakab, 1988). Together, these results suggest that immunosuppressed individuals may be at increased risk of developing bacterial infections in lung following NO_2 exposure.

3.5.3.2.3 Relevance of Animal Responses to Human Responses

Several animal models consistently showed increased susceptibility to bacterial infection following exposure to NO_2. Host resistance assays are considered the gold standard of immunotoxicity testing because clearance of a self-replicating infectious agent requires the integration of immune system responses to protect the host, and disruption of this integrated response at any point can be detected as a reduction in host resistance. Well-designed studies of this nature are judged sufficient for the determination of immunotoxicity in humans (IPCS, 2012).

3.5.3.2.4 Conclusions and Remaining Uncertainties

Animal toxicological evidence of post-infection mortality and subclinical effects underlying exacerbation of respiratory infection suggest that host resistance to bacterial infection is compromised following short-term exposures to PECOS-relevant NO_2 concentrations. These data are supported by other studies reporting decreased antibody responses (see Section 3.5.3.4.1.1, Antibody Responses) and impairment of many ex vivo WBC functions (see Section 3.5.3.4.1.2, Ex vivo WBC Function), all of which suggest that NO_2 exposure may lead to immunosuppression. Additional studies are needed to better understand the effects of NO_2 on host defense to bacterial pathogens.

In addition to the uncertainties associated with the existing evidence base, assessing the impact of NO_2 exposure on immune system-related endpoints is generally hindered by the lack of studies investigating other mechanisms of immunosuppression. For example, there are no published animal toxicological studies investigating natural killer (NK) cell function and delayed type hypersensitivity responses. Studies utilizing these endpoints and others are needed to better understand the potential for and the mechanisms underlying immunosuppression observed in some NO_2 investigations.

3.5.3.3 Animal Toxicological Studies of Effects Underlying Exacerbation of Respiratory Infection – Resistance to Viral Infection

Available animal toxicological evidence evaluated in the 2008 and 2016 Oxides of Nitrogen ISAs provide evidence that host resistance to viral infection is compromised following short-term exposures to PECOS-relevant in NO_2 concentrations in mice (U.S. EPA, 2016, 2008b) Study specific details, exposure conditions, and endpoints examined are highlighted in the text below, followed by additional discussion of the relevance of the animal responses to humans (see Section 3.5.3.3.1). Section 3.5.3.3.2 discusses conclusions from these studies and summarizes remaining uncertainties. Further study details are provided in Appendix A – Appendix Table 3-11.

The effect of NO_2 exposure on the severity of viral infection was investigated in a few studies conducted in mice. Notably, exposure to 5 ppm NO_2 at the time of inoculation reduced the amount of murine cytomegalovirus required to infect mice 100-fold compared to air-exposed controls (Rose et al., 1988). In a second study, mice previously inoculated with a cytomegalovirus while being exposed to 5 ppm NO_2 were susceptible to reinfection 30 days later while animals exposed to 2.5 ppm NO_2 during the initial infection were not (Rose et al., 1989). Exposure to NO_2 (5, 10, or 20 ppm) for four hours had no effect on proliferation and elimination of parainfluenza virus type 1 (murine Sendai virus) in mice exposed to the virus one hour before pollutant exposure (Jakab, 1988).

Overall, the small body of animal toxicological evidence of subclinical effects underlying exacerbation of respiratory infection indicates decreased resistance to viral lung infection in NO_2-exposed animals. Importantly, however, the evidence base from previous ISAs is highly limited, and there are no recent animal toxicological studies investigating the effects of short-term NO_2 exposure on resistance to viral infection.

3.5.3.3.1 Relevance of Animal Responses to Human Responses

Animal toxicological studies found evidence for increased susceptibility to viral infection following NO_2 exposure. Host resistance assays are considered the gold standard of immunotoxicity testing because clearance of a self-replicating infectious agent requires the integration of immune system responses to protect the host, and disruption of this integrated response at any point can be detected as a reduction in host resistance. Well-designed studies of this nature are judged sufficient for the determination of immunotoxicity in humans (IPCS, 2012). However, uncertainty in interpreting these animal results stems from the lack of effect of NO_2 exposure on susceptibility to viral infection in controlled human exposure studies (see Section 3.5.3.1). Recognizing that the number of studies is limited and that experimental designs vary, it is challenging to directly compare the outcomes of these studies. Consequently, additional studies are needed to better understand the effect of NO_2 exposure on susceptibility to viral infection.

3.5.3.3.2 Conclusions and Remaining Uncertainties

The limited available animal toxicological evidence of subclinical effects underlying exacerbation of respiratory infection suggests that host resistance to viral infection is compromised following short-term exposures to PECOS-relevant NO_2 concentrations. These data are supported by other studies reporting decreased antibody responses (see Section 3.5.3.4.1.1, Antibody Responses) and impaired ex vivo WBC function (see Section 3.5.3.4.1.2, Ex vivo WBC Function), all of which suggest that NO_2 exposure may lead to immunosuppression. While that may be the case, it is important to recognize limitations in the evidence. For example, most available animal toxicological studies investigating host resistance are focused on bacterial infections. Only three animal toxicological studies investigated the effects of NO_2 exposure on viral infections. In two of those studies, host resistance to viral infection was reduced by exposure to NO_2. In contrast, the available controlled human exposure studies did not report decrements in host resistance to viral infection (see Section 3.5.3.1). Recognizing that the available evidence is limited and that viral infections can predispose the host to bacterial infections, for which there is an abundance of animal toxicological evidence, additional studies are needed to better understand the effects of NO_2 on host defense to viruses and bacterial pathogens.

In addition to the uncertainties associated with the existing evidence base, assessing the impact of NO_2 exposure on the immune system-related endpoints is generally hindered by the lack of studies investigating other mechanisms of immunosuppression. For example, there are no published studies utilizing the NK cell function, which is crucial to defense against viral infection. Given the conflicting results of animal models and controlled human exposure studies investigating viral infection, application of this well-accepted test is important for understanding whether NO_2 exposure alters host susceptibility to viral infections. Furthermore, there are no studies evaluating the potential for NO_2 exposure to alter the delayed type hypersensitivity (DTH) response. Studies like these are needed to better understand the potential for and the mechanisms underlying immunosuppression observed in some NO_2 investigations.

3.5.3.4 Animal Toxicological Studies of Effects Underlying Exacerbation of Respiratory Infection – Other Measures of Immunosuppression

Immunosuppression is a condition where the immune system is weakened, making it more difficult to fight infections and disease. As discussed in Sections 3.5.3.2 and 3.5.3.3, short-term exposure to PECOS-relevant NO_2 concentrations weakened host resistance to bacterial and viral infection in laboratory animals. Several other lines of evidence evaluated in the 2008 and 2016 reviews provide additional evidence of immunosuppression following short-term exposures to PECOS-relevant NO_2 concentrations in mice (U.S. EPA, 2016, 2008b). These lines of evidence were organized into two broad categories: (1) antibody responses and (2) ex vivo white blood cell function (e.g., mitogen response, phagocytosis). Study specific details, exposure conditions, and endpoints examined are highlighted in the text below, followed by additional discussion of the relevance of these animal models for humans (see

Section 3.5.3.4.1). Section 3.5.3.4.2 discusses conclusions from these studies and summarizes remaining uncertainties. Further detail on these studies is provided in Appendix A – Appendix Table 3-11.

3.5.3.4.1.1 Antibody Responses

The production of antigen-specific antibodies is a major defense mechanism of humoral immune responses. A study describing effects of NO_2 exposure on the T cell dependent antibody response (TDAR) was reviewed in the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016). The TDAR is a comprehensive immune function assay that integrates several aspects of immune responses. Thus, xenobiotic-induced alterations in antigen processing and presentation, B and T cell interactions, antibody production, and isotype class switching and modification have the potential to modify this defense mechanism (IPCS, 2012). The TDAR was reduced in mice exposed to 5 ppm NO_2 for seven days (Lefkowitz et al., 1986). In another study, Fujimaki et al. (1982) reported decreased primary antibody response to sheep red blood cells measured ex vivo in lymphocytes collected from mice exposed to 0.4 and 1.6 ppm NO_2 for four weeks.

3.5.3.4.1.2 Ex vivo WBC Function

White blood cells (WBCs) are cells of the immune system involved in protecting the body from infectious disease. These cells can be organized into two lineages—myeloid cells and lymphoid cells. Myeloid cells (i.e., myelocytes) include neutrophils, eosinophils, mast cells, basophils, and monocytes. Lymphoid cells (i.e., lymphocytes) include T cells, B cells, and NK cells. Xenobiotic-induced impairment of ex vivo WBC function is considered clear evidence of immunosuppression (IPCS, 2012). Ex vivo WBC function assays are performed outside the body using immune cells collected from exposed individuals.

NO_2 exposure (0.25, 0.1, and 1 ppm) suppressed splenic T and B cell proliferative responses to the mitogens phytohemagglutinin (PHA) and lipopolysaccharide (LPS), respectively. However, the extent of the decrease did not appear to be related to the concentration of NO_2 or the duration exposure, except for PHA which was related to the duration of exposure (Maigetter et al., 1978).

Results of investigations of phagocytic activity have been mixed. Phagocytosis is a crucial innate immune response where cells (i.e., phagocytes) nonspecifically bind to and ingest particles (Rosales and Uribe-Querol, 2017). Exposure to 5 ppm NO_2 either reduced (Rose et al., 1989) or had no effect (Lefkowitz et al., 1986) on phagocytosis by alveolar macrophages. In another study, the number of rabbit alveolar macrophages capable of phagocytosing latex particles was unaffected, but the number of particles internalized by phagocytosing cells either decreased or increased depending on the NO_2 exposure dose (i.e., 0.3 ppm or 1 ppm, respectively) (Schlesinger, 1987).

A single study investigated the ability of mouse pulmonary macrophages to phagocytose ^{198}Au-colloid particles in vivo (Rose et al., 1989). Exposure to 1 or 5 ppm NO_2 decreased phagocytosis while phagocytosis returned to baseline levels in mice exposed to 15 ppm NO_2. The investigators hypothesized that the "apparent improvement" in phagocyte capacity may be due to an inflammatory response promoting an influx of additional phagocytes.

Respiratory burst is the rapid production of reactive oxygen species that occurs during phagocytosis of microbes (Thomas, 2017). Phorbol ester-stimulated superoxide production was significantly reduced in macrophages from NO_2-exposed rats (Robison et al., 1993; Amoruso et al., 1981). Superoxide production was dose-dependently decreased in alveolar macrophages isolated from weanling rats that were exposed to 0.5 and 2.0 ppm NO_2 for eight weeks (Kumae and Arakawa, 2006). While that is the case, exposure to the same concentrations of NO_2 dose-dependently increased superoxide production following 12 weeks of exposure.

Movement of cells along a concentration gradient of chemoattractant is known as chemotaxis. Chemotaxis is an essential function that enables, for example, phagocytes to migrate to sites of infection (Mañes et al., 2005). Chemotaxis, stimulated by macrophage products, was unaffected in neutrophils isolated from rats exposed to 5 or 20 ppm NO_2 for one hour (Robison et al., 1990). Acton and Myrvik (1972) reported that spontaneous and zymosan-induced release of neutrophil chemotactic factor by rabbit alveolar macrophages was not affected exposure to 5–50 ppm NO_2. Random cell movement (i.e., chemokinesis) was reduced in alveolar macrophages harvested from rabbits exposed to 0.3 ppm, but not 1 ppm NO_2 (Schlesinger, 1987). Although not conducted using animal cells, a single study investigated the effects of NO_2 exposure on release of neutrophil chemotactic factor by alveolar macrophages collected from healthy volunteers and were subsequently exposed to 5, 10, or 15 ppm NO_2 ex vivo for three hours (Pinkston et al., 1988). In that study, neither spontaneous nor zymosan-stimulated release of neutrophil chemotactic factor was affected by exposure to NO_2.

Pinkston et al. (1988) reported that IL-1 release was unaffected in alveolar macrophages collected from healthy volunteers exposed to 5, 10, or 15 ppm NO_2 for three hours and challenged ex vivo with influenza virus.

Overall, animal toxicological evidence of subclinical effects underlying exacerbation of respiratory infection indicates that NO_2 exposure decreased TDAR and impaired some ex vivo WBC functions (i.e., decreased mitogen response and decreased superoxide anion production). However, release of neutrophilic chemotactic factor following NO_2 exposures were inconsistent when measured in mouse and human alveolar macrophages. There are no recent animal toxicological studies investigating the effects of short-term NO_2 exposure on antibody response or ex vivo white blood cell function.

3.5.3.4.1 Relevance of Animal Responses to Human Responses

The TDAR and ex vivo primary antibody response were reduced in mice exposed to NO_2. The TDAR is a comprehensive immune function assay that integrates several aspects of immune responses. Thus, xenobiotic-induced alterations in antigen processing and presentation, B and T cell interactions, antibody production, and isotype class switching and modification have the potential to modify this defense mechanism (IPCS, 2012). Positive results obtained using the TDAR assay are considered clear evidence of adverse immunosuppression with applicability to humans.

3.5.3.4.2 Conclusions and Remaining Uncertainties

Animal toxicological evidence of subclinical effects underlying exacerbation of respiratory infection suggest that host resistance to bacterial and viral infection is compromised following short-term exposures to PECOS-relevant NO_2 concentrations. These data are supported by other studies reporting decreased antibody responses and impaired ex vivo WBC function, all of which suggest that NO_2 exposure may lead to immunosuppression. Additional studies are needed to better understand the effects of NO_2 on host defense to viruses and bacterial pathogens.

An area of uncertainty relates to the impact of NO_2 exposure on antibody responses. The TDAR assay is the gold standard animal model for assessing antibody responses. However, suppression of the antibody response by NO_2 was only investigated in a single study by Lefkowitz et al. (1986). Additional confirmatory animal toxicological studies, including other antigens, are needed as are evaluations of the vaccine response in humans to better understand the potential effects of NO_2 on antibody responses.

3.5.4 Synthesis Across Lines of Evidence

The strongest evidence for an effect of short-term NO_2 exposure on respiratory infection continues to come from animal toxicological studies evaluated in the 2016 Oxides of Nitrogen ISA. Animal toxicological evidence of mortality and subclinical effects underlying exacerbation of respiratory infection suggests that host resistance to bacterial and viral infection is compromised following short-term exposures to PECOS-relevant NO_2 concentrations (see Sections 3.5.3.2 and 3.5.3.3). Additionally, studies report decreased antibody responses and impaired ex vivo WBC function (see Section 3.5.3.4). These well-designed animal studies utilizing host resistance assays, TDAR assays, and measures of ex vivo primary antibody response provide strong support for immunotoxicity in humans (IPCS, 2012). In contrast to animal models, controlled human exposure studies provide limited support for NO_2 effects on antibody response or susceptibility to bacterial or viral infection. Specifically, the controlled human exposure evidence is limited in terms of the number of studies and the range of approaches that have been used to evaluate immune function.

Expanded epidemiologic evidence provides some support for a positive association between short-term NO_2 exposure and ED visits for respiratory infection (see Section 3.5.1.1). However, other than a few studies that reported an NO_2-related increase in risk of ED visits for upper respiratory tract infection, there are still a limited number of studies providing consistent evidence for the same respiratory infection endpoints (e.g., pneumonia, LRTI). The epidemiologic evidence is also limited and inconsistent for hospital admissions for respiratory infection (see Section 3.5.1.1) and self-reported, laboratory-confirmed or physician-confirmed respiratory infection (see Section 3.5.2.1). Additionally, consistent with the evidence presented in the 2016 Oxides of Nitrogen ISA, recent epidemiologic studies reported strong correlations between NO_2 concentrations and traffic-related pollutants and did not evaluate potential copollutant confounding.

In summary, animal toxicological evidence provides strong support for increased susceptibility to infection following short-term exposure to NO_2. However, there is a lack of coherence with controlled human exposure studies, which have not examined important immunological endpoints. Additionally, although there is some epidemiologic support for NO_2-related increases in ED visits for respiratory infections, there is limited evidence of consistent associations across specific infection types, and evidence is inconsistent for other outcomes, including hospital admissions and self-reported, laboratory-confirmed, or physician-diagnosed respiratory infection. Together, while experimental studies in animals supports immunotoxicity of short-term NO_2 exposure, there is limited coherence from other lines of evidence.

3.6 Respiratory Effects in Populations Without Pre-Existing Respiratory Disease

While those with underlying lung disease may be more likely to experience respiratory effects, the study of respiratory effects in healthy populations after short-term exposure to oxides of nitrogen is important for understanding risk in populations with generally uncompromised respiratory symptoms and for understanding endpoints related to development of disease. The following sections summarize previous findings and describe recent evidence of respiratory health effects in the general population, undifferentiated by disease status, and in populations without pre-existing respiratory disease. These populations are evaluated for respiratory symptoms (see Section 3.6.1), airway responsiveness (see Section 3.6.2), lung function (see Section 3.6.3), and respiratory tract inflammation, injury, and oxidative stress (see Section 3.6.4).

3.6.1 Respiratory Symptoms

Impacts to lung function and activation of respiratory sensory nerves can result in respiratory symptoms. Most commonly, this is measured by symptom questionnaires that report the presence or

severity of respiratory symptoms such as cough and wheeze. The evidence for respiratory symptoms in populations with asthma and COPD is discussed in Sections 3.3.3 and 3.4.2, respectively. This section discusses evidence for respiratory symptoms in healthy adults or in the general population (i.e., studies that do not differentiate the population by disease status).

3.6.1.1 Epidemiologic Studies of Respiratory Symptoms in the General Population

As detailed in the 2016 Oxides of Nitrogen ISA, NO_2-related respiratory symptoms have not been examined in epidemiologic studies of healthy adults; however, associations are indicated in studies among school-aged children. In studies of school-aged children, there was a consistent pattern of positive associations between short-term NO_2 exposure and respiratory symptoms. Children were recruited primarily from schools but also from a birth cohort, suggesting study populations were representative of the general population. A wide range of participation rates were reported, but no study reported differential participation by a particular group. An important limitation of the evidence was the limited specification of health status of study populations. It is not clear whether the NO_2-associated increases in respiratory symptoms reflect associations among all children or those with a pre-existing respiratory condition. Studies of respiratory symptoms in children also did not adequately consider potential confounding by $PM_{2.5}$ and other traffic-related copollutants. Respiratory symptoms were consistently associated with $PM_{2.5}$, CO, BC, and UFP, which were also highly correlated with NO_2 ($\rho = 0.61-0.75$).

A single recent study of college students in Shanghai, China examined respiratory symptoms in 56 healthy children and young adults following scripted exposures to traffic-related air pollution (Zhu et al., 2023). Participants alternated walking sessions in high- and low-traffic-related-pollutant environments, completing a validated respiratory tract symptom survey prior to and immediately after the walking sessions. The authors observed that the difference between pre- and post-walk symptom scores increased 3.360 (95% CI: 1.252, 5.467) points per 31 ppb increase in 4-hour avg NO_2 concentrations measured via personal monitors. Each one-point increase in symptom scores represents a change in self-perceived severity of any one of 14 respiratory tract symptoms on a 0-to-5-point scale. Notably, due to strong correlations between the traffic-related pollutants, the authors did not evaluate copollutant models, limiting inference of independent effects that could be attributed to individual pollutants.

3.6.1.1.1 Conclusions and Remaining Uncertainties

Epidemiologic evidence for respiratory symptoms in populations without pre-existing disease remains largely the same as in the 2016 Oxides of Nitrogen ISA. One recent randomized crossover analysis adds to the existing evidence of an association between short-term NO_2 exposure and respiratory symptoms in children and young adults without pre-existing disease. This study addresses previous uncertainty regarding disease status by excluding participants with pre-existing respiratory disease as

compared to other studies that recruited participants from the general population without specification of health status. A key remaining uncertainty in the evidence is the potential for copollutant confounding, particularly by traffic-related pollutants. The available studies discussed in this section report strong correlations between NO_2 concentrations and traffic-related pollutants.

3.6.1.2 Controlled Human Exposure Studies of Respiratory Symptoms

The 2016 Oxides of Nitrogen ISA found that controlled human studies of exposures to NO_2 in healthy volunteers did not result in marked changes in respiratory symptoms. No recent controlled human studies have investigated the onset of respiratory symptoms in healthy volunteers. Thus, the results of previously reviewed studies are summarized below. Study specific details, exposure conditions, and endpoints examined are highlighted in the text below and described in more detail in Table 5-31 of U.S. EPA (2016).

- Studies of healthy adult volunteers exposed to NO_2 in the range of 300–600 ppb for 1–3 hours showed no change in symptom scores following exposure (Gong et al., 2005; Hazucha et al., 1994; Morrow et al., 1992; Frampton et al., 1991; Adams et al., 1987).
- NO_2 exposures of 400 or 600 ppb in adults also did not affect respiratory symptoms with co-exposure or subsequent exposure to ozone of concentrated ambient $PM_{2.5}$ (Gong et al., 2005; Hazucha et al., 1994; Adams et al., 1987).
- Higher exposure concentrations of 1000 or 4000 ppb also had minimal effects on respiratory symptoms in healthy adults (Jörres et al., 1995; Rasmussen et al., 1992; Frampton et al., 1991; Linn et al., 1985b; Hackney et al., 1978).

3.6.1.2.1 Conclusions and Remaining Uncertainties

Together, the controlled human exposure evidence does not indicate a relationship between exposure to NO_2 in healthy adults and the onset of respiratory symptoms. A key uncertainty in interpreting these studies stems from their evaluation of healthy adults, while the strongest evidence for NO_2-induced respiratory effects in humans comes from studies conducted in people with pre-existing disease, particularly children. Additional uncertainty results from the exposure paradigms that focus on single short exposures to NO_2, while some animal studies and epidemiologic studies indicate more serious effects following repeated exposures.

3.6.2 Airway Responsiveness

As discussed in Section 3.3.3.1, the bronchi can respond to external challenges resulting in airway constriction and airway narrowing. Increased sensitivity of the airways to these bronchial challenges can cause a predisposition to reduced lung function and respiratory symptoms. The evidence for airway

responsiveness in asthmatics is discussed in Section 3.3.3.1. Unlike asthmatics, healthy populations generally have lower sensitization toward many common airway allergens making measurement of airway responsiveness changes to specific agents unfeasible. Consequently, the available studies in healthy individuals measure airway responses to bronchial challenge with "nonspecific" broncho-constricting agents (e.g., methacholine, SO_2, cold air).

3.6.2.1 Controlled Human Exposure Studies of Airway Responsiveness

The 2016 Oxides of Nitrogen ISA evaluated several controlled human exposure studies of short-term NO_2 exposure and airway responsiveness to a bronchoconstricting agent in healthy adult volunteers. There are no recent controlled human exposure studies identified that investigate airway responsiveness in healthy populations, thus, the evaluation is limited to the existing literature base. Key findings from existing controlled human exposure studies are highlighted below.

- Increases in nonspecific airway responsiveness were observed in healthy adults following exposure to NO_2 in the range of 1500–2,000 ppb (Frampton et al., 1991; Mohsenin, 1988). In addition, Kulle and Clements (1988) observed statistically non-significant trends toward larger FEV1 decrements following 2-hour exposure to 2,000 or 3,000 ppb NO_2 on multiple days.
- Exposure to 100 ppm NO_2 did not affect nonspecific airway reactivity measurements in healthy volunteers (Ahmed et al., 1983a; Hazucha et al., 1983)
- Two meta-analyses show statistically significant increases in airway reactivity in healthy individuals after NO_2 exposures above 1000 ppb but not below (Kjaergaard and Rasmussen, 1996; Folinsbee, 1992).

3.6.2.1.1 Conclusions and Remaining Uncertainties

The controlled human exposure data provide evidence that increases in airway reactivity may be seen in people without respiratory disease, although only at exposure concentrations higher than are likely to be seen in ambient air. A key uncertainty in interpreting these studies stems from their evaluation of healthy adults, while the strongest evidence for NO_2-induced respiratory effects in humans comes from studies conducted in people with pre-existing disease, particularly children. Additional uncertainty in these studies results from the use of exposure concentrations and exposure paradigms that may not fully replicate or recapitulate responses with exposure to the complex mixture of oxides of nitrogen that can be present in ambient air.

3.6.2.2 Animal Toxicological Studies of Airway Responsiveness

The animal evidence of NO_2-induced effects on baseline airway reactivity is limited. One previously reviewed study showed that three days of exposure of guinea pigs to 4 ppm NO_2 resulted in increased airway reactivity to histamine compared to controls however there were no significant changes at one or seven days (Kobayashi and Shinozaki, 1990). Three recent studies have looked at the effect of NO_2 exposure on airway reactivity in naive mice. While these studies were designed to investigate the impact of NO_2 exposures on airway responsiveness after subsequent sensitization to an allergen, the data provided gives insight into the impacts of NO_2 exposure on baseline airway responsiveness. (Lu et al., 2023) found that exposure of mice to 5 ppm for 30 days increased both the inspiratory and expiratory resistance in the lung with no statistically significant change to the dynamic compliance in response to methacholine. However, exposure of pregnant BALB/c mice to 2.5 ppm NO_2 five hours/day during gestation (approximately 21 days) resulted in no changes in airway resistance, expiratory resistance, or dynamic compliance in response to methacholine in the offspring (Yue et al., 2018; Yue et al., 2017). Study specific details, exposure conditions, and endpoints examined are described in more detail in Appendix A – Appendix Table 3-12.

3.6.2.2.1 Conclusions and Remaining Uncertainties

Animal toxicological evidence for NO_2-induced changes in airway responsiveness in otherwise healthy animals is limited. Results from existing studies provide some evidence of increased airway reactivity in response to nonspecific bronchoconstricting agents at PECOS-relevant concentrations (≤5 ppm) and responses do not necessarily show dose dependence. Studies of gestational NO_2 exposure show null results however the airway reactivity tests in these studies occur in the offspring several weeks after the end of NO_2 exposure.

3.6.3 Lung Function

The primary function of the lungs is to take in oxygen and remove waste products (namely CO_2) from the blood. Correspondingly, lung function testing seeks to measure the ability of the lungs to transport air and perform gas exchange. Spirometry is a common means of measuring lung function by measuring the ability to breath air in and out of the lungs and can be used to calculate a number of measures related to lung function, including forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), and other measures of expiratory flow rate like peak expiratory flow (PEF). Other tests, like plethysmography and gas diffusion testing, can be used to measure airway resistance, total lung capacity, residual volume, vital capacity, lung airway resistance, and the diffusion capacity of inhaled gases into the lung. The evidence for the lung function effects of NO_2 exposure in populations with asthma is discussed in Section 3.3.3. This section discusses the evidence for lung function effects in

healthy populations and in the general population, undifferentiated by disease status. The 2016 Oxides of Nitrogen ISA concluded that the evidence base for short-term exposure to oxides of nitrogen and lung function impairment in populations without respiratory disease was generally inconsistent.

3.6.3.1 Epidemiologic Studies of Lung Function in the General Population

3.6.3.1.1.1 Studies in Children

The 2016 Oxides of Nitrogen ISA discusses an extensive body of epidemiologic literature analyzing the relationship between short-term NO_2 exposure and lung function in children. The evidence did not strongly support NO_2-associated changes in lung function measured in children without asthma. The most informative lung function studies in children included those with supervised spirometry and NO_2 concentrations measured outside at schools or at a central site monitor adjacent to schools. Studies that satisfied these criteria reported inconsistent associations between short-term NO_2 exposure and lung function in children (U.S. EPA, 2016). While ambient NO_2 concentrations were more consistently associated with lung function decrements measured by supervised spirometry than at-home PEF, many studies of supervised spirometry did not observe associations with ambient NO_2 concentrations (U.S. EPA, 2016). The potential for confounding by other traffic pollutants and time-varying factors like weather were examined in a few studies, and both remain areas of uncertainty.

A recent case-cross over study of college students in Shanghai, China examined lung function parameters measured in 56 healthy children and young adults (mean age of 23 years) following scripted exposures to traffic-related air pollution (Zhu et al., 2023). Participants alternated walking sessions alongside a high-traffic road or through a park. Zhu et al. (2023) reported that a 31 ppb increase in NO_2 concentrations measured via personal monitors was associated with decreased FEV_1 (-0.071 L [95% CI: -0.135, -0.007]), FEV_1/FVC (-0.878 [95% CI: -1.630, -0.125]), and MMEF (-0.203 L/s [95% CI: -0.39, -0.036]) relative to pre-walk measures. Notably, despite strong correlations between the traffic-related pollutants, the authors did not evaluate copollutant models, limiting inference of independent effects that could be attributed to individual pollutants. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 3-13. No additional studies in children ages <18 years were available for review.

3.6.3.1.1.2 Studies in Adults

In studies of adults in the general population reviewed in the 2016 Oxides of Nitrogen ISA, there was some evidence that increases in ambient NO_2 concentrations were associated with decrements in lung function as measured by supervised spirometry. Studies evaluated in the 2016 Oxides of Nitrogen ISA examined a wide range of ages (i.e., 18 to 79 years) and analyzed populations restricted to healthy

participants as well as those including adults with asthma or allergies. While the evidence was inconsistent overall, a smaller number of studies with exposure assessments that more closely approximate personal exposure indicated ambient NO_2-associated decreases in lung function in healthy adults (Dales et al., 2013; Strak et al., 2012). These studies measured NO_2 concentrations at the site of outdoor activity, which aligned measurements with subjects in both time and space. Both studies observed high correlations with other traffic-related pollutants, though Strak et al. (2012) noted that NO_2 associations were robust to adjustment for particle number concentration (PNC), elemental carbon (EC), or $PM_{2.5}$ in copollutant models. Age and restrictions on disease status did not appear to influence results.

Recent panel studies do not provide robust evidence of an association between short-term NO_2 exposure and lung function measures in adults without pre-existing respiratory disease (see Appendix A – Appendix Table 3-13). Recent evidence comes from panel studies and a case-crossover study using exposure assessment methods that included central site monitors and fixed site monitors that measure NO_2 concentrations at the site of outdoor activity. Similar to the studies in children, the evidence in adults includes clinically measured spirometry and biomarkers. Recent studies were primarily focused on participants 55 years and older, except for one study that included younger and older adults (25 to 55 years old). Evidence from recent studies is summarized below. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 3-13.

- Collectively, a few recent panel studies and a case-crossover study of older adults exercising outdoors did not provide strong evidence of an association between NO_2 exposures and lung function measures, including FEV_1, FVC, and PEF, in healthy adults (Stieb et al., 2019; Stieb et al., 2018; Sinharay et al., 2017). A single study reported potential modification of the association by diet (Chen et al., 2021). In each study, NO_2 measurements were taken at a central site or near the place of exercise, and lung function was measured by supervised spirometry.
- A case-crossover study in the United Kingdom examined 119 adults aged 60 years and older. Participants included healthy volunteers and individuals with COPD or ischemic heart disease (Sinharay et al., 2017). In healthy individuals, no association was reported between short-term NO_2 exposure and lung function measures. Associations with decreased lung function, including FEV_1 and FVC, were only reported in individuals with COPD (see Section 3.4.3.1).
- A few studies in Canada that examined adults 55 years and older provide inconsistent evidence of associations between short-term NO_2 and lung function (Stieb et al., 2019; Stieb et al., 2018). In both studies, participants were encouraged to engage in light activity outdoors daily in a consistent location. In adults advised to avoid exercising outdoors on days with an air quality advisory, Stieb et al. (2018) reported inconsistent findings across exposure lags 0 to 2 days. A decrease in FEV_1 was reported following an increase in previous day NO_2 concentrations, while no association was observed on other individual days or with NO_2 concentrations averaged over a longer period. In a similar study, Stieb et al. (2019) reported contrasting findings. Participants were divided into a control group that received no guidance about avoiding outdoor activity on days with an air quality advisory and an intervention group that was instructed to do activities indoors on days with an air quality advisory. Among adults in the control group (exercising outdoors on high air quality days), 1-week average NO_2 concentrations were associated with lower FEV_1 prior to exercise compared to the

intervention group (advised to exercise indoors on high air quality days). No associations were observed between single-day lag NO_2 concentrations (lags 0–2) and FEV_1.

- Another recent panel study of younger adults in the United States. (25 to 55 years old) evaluated potential effect modification by dietary omega-3 (Chen et al., 2021). Short-term increases in NO_2 were associated with increased FEV_1 and FVC in individuals with a higher omega-3 diet, but not in individuals with a low omega-3 diet (Chen et al., 2021).

3.6.3.1.1 Conclusions and Remaining Uncertainties

Studies evaluated in the 2016 Oxides of Nitrogen ISA reported inconsistent evidence for an association between short-term NO_2 exposure and lung function deficits in the general population. Prior studies did report evidence of this association in children with preexisting respiratory health conditions (i.e. asthma), as discussed in Section 3.3.3.2.1. No recent studies in young children were identified.

In adults, studies evaluated in the 2016 Oxides of Nitrogen ISA provided some evidence of decreased lung function, as measured by supervised spirometry, in association with short-term ambient NO_2. Prior studies reported stronger evidence for this association in young adults. A recent case-crossover study in young adults provided additional evidence of NO_2-associated decreases in spirometry (i.e. FEV_1, FEV_1/FVC, and MMEF). This study adjusted for potential confounding by time-varying changes in weather, but did not examine potential confounding by copollutants. Potential confounding by copollutants remains an area of uncertainty. In older adults, a recent study provides some evidence of an inverse association between short-term NO_2 and lung function in individuals with COPD, but the results in healthy adults are inconsistent. Several studies accounted for potential confounding by time-varying meteorological variables and adjusted models with temperature. A few studies considered the relationship to be potentially confounded by temperature and relative humidity and adjusted models for both variables. As with examining the relationship in children, potential confounding by copollutants remains an area of uncertainty.

3.6.3.2 Controlled Human Exposure Studies of Lung Function

Conclusions on the impact of NO_2 exposure on lung function in the 2016 Oxides of Nitrogen ISA were based on older studies that had been previously reviewed in the 2008 Oxides of Nitrogen ISA and/or in earlier air quality criteria documents. The 2016 Oxides of Nitrogen ISA concluded that the evidence base provided limited support for the impact of NO_2 exposure on lung function in healthy populations. In general, studies of exposures to NO_2 concentrations ranging from 120−600 ppb (40 minutes to four hours) did not affect the lung function of healthy adolescents, young adults, or older adults. In a study of 4-hour exposure to 2,000 ppb NO_2 for four days, a significant reduction in FEV_1 and FVC was only seen after the first day of exposure and was ameliorated on subsequent exposure days (Blomberg et al., 1999). Another study found that 2-hour exposure to 1,000 ppb NO_2 induced a 1.5% drop in FVC only on the second day

of a 2-day exposure (Hackney et al., 1978). Similarly, studies of multipollutant exposures suggest that NO_2 exposure does not impact the lung function changes that result from simultaneous particulate matter exposure (Huang et al., 2012; Gong et al., 2005) or simultaneous (Adams et al., 1987) or sequential ozone exposure (Hazucha et al., 1994). One recent controlled human exposure study investigated the effect of NO_2 exposure on lung function in healthy populations (Brand et al., 2016) and the findings are summarized below. Study specific details, exposure conditions, and endpoints examined are highlighted in the text below and described in more detail in Appendix A – Appendix Table 3-14.

- 25 healthy subjects were exposed for three hours in a crossover design to 0.0, 0.1, 0.5, and 1.5 ppm NO_2 with moderate exercise (10-minute exercise at a workload of 80 watts).
- 12 lung function variables were measured before and after each exposure [vital capacity (VC), FEV_1, peak expiratory flow (PEF), Maximal expiratory flow (MEF) at 75% (MEF_{75}), 50% (MEF_{50}), and 25% (MEF_{25}) FVC, effective airway resistance (Reff), specific airway resistance (sReff), intrathoracic gas volume (ITGV), total lung capacity (TLC), residual volume (RV)].
- Only Intrathoracic gas volume (ITGV) was significantly dependent on NO_2 exposure concentration ($p = 0.048$). Post hoc analysis showed that the change in ITGV was increased following exposure to 0.5 ppm NO_2 compared to 0.1 ppm NO_2 but was not significantly different than control or 1.5 ppm NO_2.
- Vital capacity showed a borderline significant dependency on exposure concentration ($p = 0.09$ by Kruskil-Wallace test and $p = 0.08$ for analysis of variance).
- Authors hypothesized that the statistical significance found for ITGV was likely a spurious finding resulting from the measurement of multiple lung function parameters.
- The study also measured changes in lung diffusion capacity for NO and CO before and after exposures and found no changes in diffusion capacity at any NO_2 exposure concentration.

3.6.3.2.1 Conclusions and Remaining Uncertainties

The majority of the evidence from controlled human exposures suggests that NO_2 does not impact measures of lung function in healthy adults, particularly at exposure concentrations closer to those in ambient air. The existing literature investigating lung function in healthy populations covers multiple exposure concentrations, exposure patterns, and age ranges. However, all the studies reported exposure to NO_2 in subjects concurrent with intermittent exercise. As seen with airway reactivity responses in subjects with asthma (see Section 3.3.1.1), it is possible that exertion from exercise can also impact lung function and alter NO_2-driven effects. Other uncertainties that are inherent to controlled human exposures also exist. This includes the use of healthy adult volunteers which may not accurately represent potential effects in populations associated with greater epidemiologic associations with NO_2-induced respiratory effects, particularly children. Also, the relevance of studies of NO_2 in informing potential impacts from other oxides of nitrogen or ambient-relevant mixtures is unclear. In addition, the exposure patterns used in controlled humans are designed for experimental feasibility which are often constant exposures or

intermittent high exposure peaks superimposed on a low baseline exposure. Translation of the resulting data to the more variable exposures seen in real-world exposures remains an area of uncertainty.

3.6.3.3 Animal Toxicological Studies of Lung Function

This section evaluates available toxicological evidence for NO_2-induced lung function effects in healthy animals. Section 3.6.3.3.1 discusses the relevance of the various animal models for human responses, and Section 3.6.3.3.2 provides additional discussion of the impacts of multipollutant exposures on lung function in these models. Section 3.6.3.3.3 discusses conclusions from available studies and summarizes remaining uncertainties.

In the 2016 Oxides of Nitrogen ISA, toxicological studies evaluating lung function in healthy animals showed changes in lung function parameters following NO_2 exposure concentrations ≥5 ppm [see Table AX4.8 of U.S. EPA (2008a) and Table 13-8 of U.S. EPA (1993b)]. The evidence for effects of short term NO_2 exposure on lung function in naïve animals at concentrations under 5 ppm was limited. While no recent studies are available that look at the effect of NO_2 exposure on the lung function in otherwise healthy animals, two recent studies describe the effects of nitrous acid (HONO) exposure in an animal model. Study specific details, exposure conditions, and endpoints examined are highlighted in the text below and described in more detail in Appendix A – Appendix Table 3-15.

- One study exposed male Hartley guinea pigs to air or nitrous acid (HONO) at an average concertation of 0.659 ppm or 3.425 ppm continuously for seven weeks (Ohyama et al., 2022). The authors report that specific airway resistance for the guinea pigs was increased in both HONO exposed groups starting during the fourth week of exposure. The 0.659 ppm group had increased specific airway resistance during the first week compared to controls. Measured respiratory waveforms were qualitatively altered more frequently in the treated groups compared to controls. Tidal volumes and peak expiratory flows were unchanged in all treatment groups throughout the experiment.
- In a study by the same group, exposure of rats to 5.8 ppm HONO continuously for six weeks reported increased baseline airway resistance that was not seen with exposure to 4 ppm HONO (Ohyama et al., 2018). No change in dynamic compliance was seen in any exposure group tested.

It is difficult to determine if the respiratory effects were due to HONO as the exposures also generated significant NO_2 and NO concentrations. Older studies from the same group found that exposure of guinea pigs to 3.6 ppm HONO resulted in a borderline significant increase in sRaw ($p = 0.06$) that accompanied emphysema-like airspace enlargement (Ohyama et al., 2010). Increased airspace enlargement was not reported in mice exposed to 8.4 ppm HONO for three weeks (Ohyama et al., 2011). These studies suggest that exposure to high concentrations of HONO, potentially in combination with the resulting lower concentrations of NO_2 or NO, may impact lung function.

3.6.3.3.1 Relevance of Animal Responses to Human Responses

Most studies of NO_2-induced lung function are done in either rats or mice. However, physiological differences in the lung may impact the observation of lung function effects. For example, the smooth muscle of the rat and mouse airways has differing reactivity to many of the bronchoconstricting stimuli that are relevant to the pathogenesis of asthma in humans (Canning, 2003). In contrast, guinea pigs show more similarity in bronchoconstrictor responsiveness, airway autonomic innervation, and cough reflex to humans than either rats or mice (Zosky and Sly, 2007; Canning, 2003). Studies show that HONO exposure of guinea pigs results in emphysema-like changes that can be seen in lung function changes (Ohyama et al., 2010). These emphysematous changes are not seen in mice (Ohyama et al., 2011), suggesting that structural changes underlying lung function effects may not be recapitulated equally well in all species.

3.6.3.3.2 Multipollutant Exposure

While the majority of animal studies examine exposure to NO_2 alone, the recent study by Ohyama et al. (2022) gives insight into the effects of HONO exposure in combination with secondarily formed NO and NO_2. While HONO concentrations were measurably higher than NO or NO_2 (mean NO concentration 61 ppb, 877 ppb, and 3,318 ppb and mean NO_2 concentrations 18 ppb, 87 ppb, and 880 ppb for control, medium and high exposure groups respectively), the resulting respiratory effects may represent the impact of simultaneous exposure to several oxides of nitrogen species, potentially mimicking real-world exposures more closely than exposures to NO_2 alone.

3.6.3.3.3 Conclusions and Remaining Uncertainties

Animal toxicology studies generally show limited impact of NO_2 exposures on lung function measurements at ambient-relevant concentrations. Recent work with HONO exposure does suggest impact on lung function variables, but given that HONO concentrations are often much lower than NO_2 concentrations in the ambient air, there remains some question as to the human relevance of the observed health effects. Also, factors inherent to the use of animal models, such as physiological and biological differences between human and rodent animals and the impact of these differences on study results remain a source of uncertainty.

3.6.4 Respiratory Tract Inflammation, Injury, and Oxidative Stress

Changes in airway reactivity, lung function, and onset of respiratory symptoms can be mediated by pulmonary damage, inflammation, and oxidative stress. These subclinical markers can be observed in experimental and epidemiologic studies. Increases in the level of protein, such as albumin, in lung lavage

fluid can indicate a compromised pulmonary epithelial barrier in response to either damage to the epithelium or in response to inflammatory signals. Inflammation can also be gauged by differential cell counts from lavage fluid or induced sputum as well as by measurement of cytokine and chemokine mediators involved with coordinating immune cell responses. Measurement of cytokines is aided by the ability to use noninvasive sampling methods such as exhaled breath condensate and nasal fluids. Oxidative stress can be measured through the presence of markers of byproducts of reactions with proteins, lipids, or DNA. Such markers include 8-hydroxydeoxyguanosine (8-OHdG), malondialdehyde (MDA), and levels of carbonylated proteins. In a similar manner, decreases in antioxidant defenses can also indicate oxidative stress and are often measured as changes in the ratios of oxidized and unoxidized glutathione (GSH) and changes to the activity of the enzymes responsible for replenishing glutathione such as glutathione S-transferases (GST). This section discusses available evidence from epidemiologic studies (see Section 3.6.4.1), controlled human exposure studies (see Section 3.6.4.2), and animal toxicology studies (see Section 3.6.4.3) examining short-term oxides of nitrogen exposure and respiratory tract inflammation, injury, and oxidative stress in the general population, undifferentiated by disease status, and in healthy populations without pre-existing disease.

3.6.4.1 Epidemiologic Studies of Respiratory Tract Inflammation, Injury, and Oxidative Stress in the General Population

3.6.4.1.1.1 Studies in Children

The evidence evaluated in the 2016 Oxides of Nitrogen ISA indicated consistent associations between increases in ambient NO_2 and increases in pulmonary inflammation or oxidative stress among children in the general population. Studies demonstrated associations in populations without pre-existing respiratory disease and in general populations including children with and without asthma or allergy, with no consistent difference in magnitude of association between children with and without respiratory disease (Liu et al., 2014; Berhane et al., 2011; Lin et al., 2011). These findings suggest associations between NO_2 exposure and pulmonary inflammation in healthy children, as measured by eNO (Berhane et al., 2011; Lin et al., 2011; Steerenberg et al., 2001). In a study providing strong inference, (Lin et al., 2011) reported evidence of a positive association between NO_2 exposure and eNO in a panel of healthy children examined as a part of a natural experiment conducted in Beijing, China before and after the 2008 Olympics. The reported effect estimate was robust to adjustment for $PM_{2.5}$, and attenuated, but still positive after adjustment for BC. Associations were not reported with other markers of pulmonary inflammation and oxidative stress, including polymorphonuclear neutrophils (PMNs) (Chen et al., 2012a), eosinophils (Chen et al., 2012a), exhaled breath condensate pH (Patel et al., 2013), or methylation of inducible nitric oxide synthase (iNOS) (Salam et al., 2012).

One recent cross-sectional analysis of the CHS cohort examined the relationship between indoor NO concentrations and eNO in 1,635 school children (aged 12–15 years) with and without asthma in

southern California (Eckel et al., 2016). The authors measured eNO from proximal ($FeNO_{50}$, $J'_{aw}NO$ and $D_{aw}NO$) or distal ($FeNO_{300}$ and C_ANO) airways, and indoor NO measured at schools was used as a surrogate for traffic-related air pollutant exposure in the indoor microenvironment. While the authors observed higher average eNO associated with higher indoor NO concentrations in the full study population, in many cases the associations appeared to be driven by a stronger relationship among children with asthma (see Figure 3-8). The most robust associations among healthy children were reported for NO and pulmonary inflammation in the distal airways (i.e., $FeNO_{300}$ and C_ANO).

3.6.4.1.1.2 Studies in Adults

Among studies of older adults and adults performing outdoor exercise that were reviewed in the 2016 Oxides of Nitrogen ISA, several studies observed increases in pulmonary inflammation associated with increases in ambient NO_2 concentrations. Pulmonary inflammation was indicated as increases in eNO, nasal lavage IL-6, and indicators of pulmonary injury and lung permeability, such as club cell protein (CC16) and nasal lavage protein levels. In populations of mostly healthy adults performing outdoor exercise for less than one to five hours, increases in pulmonary inflammation were associated with increases in NO_2 measured at the locations of outdoor exposures (Steenhof et al., 2013; Strak et al., 2012) but not at central sites. Compared with studies that do not account for time-activity patterns, examination of subjects during time spent outdoors may better reflect effects related to ambient exposures, particularly when pollutants are measured in subjects' outdoor locations. Increases in pulmonary inflammation were, however, associated with 24-hr avg NO or NO_2 measured at central monitoring sites among older adults [ages: 53–90 years; (Madsen et al., 2008; Adamkiewicz et al., 2004)]. In a limited number of studies that included copollutant models, observed associations remained after adjustment for $PM_{2.5}$ (Adamkiewicz et al., 2004) but were attenuated after adjustment for EC, Abs, or PNC (Steenhof et al., 2013; Strak et al., 2012). Evidence from recent studies is summarized below.

A few recent panel studies examined the relationship between NO, NO_2, or NO_X exposures and repeated measures of eNO in adults without respiratory illness. Consistent with findings from the 2016 Oxides of Nitrogen ISA, these studies provide evidence of pulmonary inflammation related to exposure to oxides of nitrogen. A short, bulleted summary of the recent evidence is provided below, followed by additional discussion of copollutant confounding in these studies (see Section 3.6.4.1.1). Section 3.6.4.1.2 discusses conclusions from these studies and summarizes remaining uncertainties.

- Recent panel studies of adults without respiratory illness provide consistent evidence of positive associations between eNO and exposure to NO (Cakmak et al., 2020), NO_2 (Cakmak et al., 2020) and NO_X (Peng et al., 2016; Zhang et al., 2016).
- Associations were observed across a range of populations, including a panel of 69 Boston, MA residents (45–85 years) with Type II diabetes (Peng et al., 2016); a panel study of 97 older adults (65+ years) in Los Angeles, CA (Zhang et al., 2016); and a panel of 59 healthy young adults that were assigned to spend five consecutive days in one of two Ontario, Canada

locations of varying distances from a steel plant in a randomized cross-over design (Cakmak et al., 2020).

- While Zhang et al. (2016) reported that increased 24-hour average NO_X concentrations measured at central site monitors were associated with increased eNO (5.5% [95% CI: 2.0, 9.0] per 30 ppb increase), the authors observed a null association with personal monitors (0.1% [95% CI: -3.8, 4.1] per 21.3 ppb increase). Peng et al. (2016) and Cakmak et al. (2020) utilized central site and on-location monitors, respectively.

3.6.4.1.1 Copollutants

In a limited number of copollutant analyses evaluated in the 2016 Oxides of Nitrogen ISA, associations of eNO with oxides of nitrogen exposures were consistently attenuated with adjustment for other pollutants. In a panel study of exercising young adults, positive associations of eNO with NO_X and NO_2 concentrations were attenuated with adjustment for EC or $PM_{2.5}$ absorbance, and became negative with adjustment for PNC (Strak et al., 2012). Among older adults in Steubenville, OH, the association between NO concentrations and eNO was attenuated, but remained positive with adjustment for $PM_{2.5}$ (Adamkiewicz et al., 2004).

A limited number of recent studies of pulmonary inflammation examined copollutant models. In a panel of 59 healthy young adults in Ontario, Canada, Cakmak et al. (2020) observed a 2.34% (95% CI: -0.56, 5.31%) increase in eNO associated with 4.03 ppb increase in NO_2 concentrations. In models adjusting for ozone, the observed association was stronger (3.08% [95% CI: 1.26, 4.91]). The authors did not present quantitative results from other copollutant models, only noting that results were not statistically significant. Peng et al. (2016) reported NO_X-FeNO associations that were robust to adjustment for $PM_{2.5}$ in a small panel study of adults with Type II diabetes.

3.6.4.1.2 Conclusions and Remaining Uncertainties

The 2016 Oxides of Nitrogen ISA highlighted consistent evidence of increased pulmonary inflammation associated with increased NO_2 exposure among children and adults in the general population (i.e., study populations that are selected without consideration of pre-existing respiratory disease). Recent evidence for children is limited to a single cross-sectional study that reported some evidence of pulmonary inflammation in the distal airways associated with indoor NO exposure. However, as an indicator of nearby traffic-related pollutant, the association with indoor NO could be confounded by other pollutants. A few recent panel studies provide evidence of pulmonary inflammation related to exposure to oxides of nitrogen among adults without respiratory illness. A strength of these studies is repeated measurements of eNO. However, there continues to be limited evaluation of potential copollutant confounding in the evidence base, which remains a key uncertainty in understanding the relationship between short-term oxides of nitrogen exposure and pulmonary inflammation in populations without pre-existing respiratory disease.

3.6.4.2 Controlled Human Exposure Studies of Respiratory Tract Inflammation, Injury, and Oxidative Stress

Several controlled human exposure studies reviewed in previous ISAs have investigated the impact of short-term NO_2 exposure on subclinical markers of pulmonary inflammation, lung damage, and oxidative stress (U.S. EPA, 2016, 2008b). The evidence reviewed previously showed mixed effects of NO_2 exposure on these markers, and drawing broad conclusions was challenging due to the variety of endpoints examined. The following sections discuss the older evidence and a small number of more recent controlled human exposure studies that examine NO_2 exposures and markers of pulmonary inflammation, lung damage, and oxidative stress. Study-specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Table 5-36 of U.S. EPA (2016).

3.6.4.2.1.1 Inflammation

Previously reviewed controlled human exposure studies have looked at the effect of NO_2 exposure on markers of inflammation including differential inflammatory cell counts, eicosanoids, and cytokines. These studies typically exposed healthy volunteers to NO_2 concentrations ranging from 500 ppb to 2000 ppb for several hours (2–6 hours). All studies previously reviewed utilized intermittent exercise regimes.

In the 2016 Oxides of Nitrogen ISA, the strongest support for effects of NO_2 exposure on inflammation in healthy adults came from increases in PMNs. Previous studies have shown generally consistent increases in the percentage or number of PMNs in pulmonary washings following NO_2 exposures (Frampton et al., 2002; Blomberg et al., 1999; Devlin et al., 1999; Azadniv et al., 1998; Helleday et al., 1994). Studies that use NO_2 exposure concentrations at or in excess of 1,000 ppb generally see increased PMN levels however, the few studies that look at lower exposure concentrations (300–600 ppb) have not reported changes in PMN levels (Huang et al., 2012; Frampton et al., 2002; Vagaggini et al., 1996). The timing of lavage after cessation of NO_2 exposure could affect the ability to detect changes in inflammatory cell populations. (Azadniv et al., 1998) showed that the percentage of neutrophils in the BAL was increased immediately following exposure (within one hour) to 2,000 ppb NO_2 for six hours but was statistically significant only when the lavage procedure was performed 18 hours post exposure. Other studies that performed lavage immediately (≤1 hour post exposure) after a single NO_2 exposure did not see significant changes in PMNs (Jörres et al., 1995; Mohsenin, 1991). Studies that looked at lavage samples 1.5–24 hours after exposure cessation saw significant increases in percentage of PMNs (Frampton et al., 2002; Blomberg et al., 1999 Devlin, 1999, 40851; Helleday et al., 1994). In contrast, (Huang et al., 2012) saw no difference in PMNs 18 hours after exposure to 500 ppb NO_2 for two hours, and (Solomon et al., 2000) found that bronchial wash immediately after a 3-day exposure to 2,000 ppb NO_2 exposure resulted in increased PMNs in the bronchial lavage.

The lung lavage procedure involves instillation and recovery of several aliquots of saline that can be pooled to represent the bronchoalveolar lavage fluid. Many studies separate the first portion of instilled and recovered fluid as more representative of the bronchial space and characterized the subsequent washings as more indicative of more distal alveolar regions. In studies that separated the washings this way, the changes in PMNs and PMN activation markers were larger in the bronchial fractions (Frampton et al., 2002; Solomon et al., 2000; Blomberg et al., 1999 Devlin, 1999, 40851; Helleday et al., 1994). The increased percentage of PMNs was larger in the more distal sampling in smokers (Helleday et al., 1994).

The pattern of exposure may impact development of lung inflammation. Exposure for three hours to a constant 600 ppb NO_2 resulted in an increased, albeit not statistically significant, percentage of BAL PMNs whereas exposure to a constant concentration of 50 ppb NO_2 with intermittent 2000 ppb peaks (resulting in a time weighted average concentration of 600 ppb) resulted in PMN percentages that were nearly identical to clean air controls (Frampton et al., 1989).

Apart from cellular infiltration, production of inflammatory mediators can be indicators of lung inflammation. In general, the changes in inflammatory mediators measured in controlled human studies is inconsistent. Exposure to 1,000 or 2,000 ppb NO_2 for three or four hours increased thromboxane B_2 levels but not an array of other prostaglandins (Devlin et al., 1999; Jörres et al., 1995). Levels of IL-6 and IL-8 were not affected by NO_2 exposure in most studies (Huang et al., 2012 Pathmanathan, 2003, 89549; Blomberg et al., 1999), though (Devlin et al., 1999) showed increased IL-6 and IL-8 in bronchial washings following 2000 ppb NO_2 exposure for 4 hour. Cellular mediators related to a pro-asthmatic Th2 inflammatory response, including IL-13 and IL-5, were increased in participants exposed to 2 ppm NO_2 for four hours a day for four consecutive days (Pathmanathan et al., 2003).

A more recent controlled human exposure study investigated the impact of NO_2 exposure to inflammation in healthy volunteers. Brand et al. (2016) found no change in TNFα, IL-6, IL-8, and a number of inflammatory mediator proteins in sputum or nasal secretions from volunteers exposed to 100–1,500 ppb NO_2 for three hours with intermittent exercise. Similarly, no changes in eNO were observed. Though not significant in this study, the percentage of macrophages in the sputum tended to decrease with NO_2 exposure whereas neutrophil percentage tended to increase. In whole, the evidence of NO_2-induced increases in inflammation is strongest and most consistent with regards to increases in PMN cells in BAL following NO_2 exposures, however, evidence for increases in chemotactic or cytokine mediators that are expected to underlie cellular infiltration is inconsistent.

3.6.4.2.1.2 Lung damage

No recent controlled human studies have investigated the impact of NO_2 exposure on markers of lung damage, thus, the literature base available in the 2016 Oxides of Nitrogen ISA remains the foundation for understanding the impacts of NO_2 on lung damage. The following points summarize the findings:

- Null effects were observed for protein or albumin levels in lavage fluid in response to a wide range of NO_2 exposures (600−4,000 ppb) given for 20 minutes to six hours on a single day or repeated over three or four days (Frampton et al., 2002; Devlin et al., 1999; Azadniv et al., 1998; Helleday et al., 1994; Mohsenin, 1991).
- Pulmonary injury in healthy adults was indicated in two studies as an increase in lung lavage LDH following a 2-hour 500 ppb NO_2 exposure (Huang et al., 2012) and an increase, although not statistically significant, in lavage albumin and total protein levels following a 4-hour 2,000 ppb NO_2 exposure repeated over four days (Blomberg et al., 1999).
- In some instances, NO_2 exposure resulted in statistically significant decreases in total protein or albumin recovered in the bronchial portion of the lavage but not LDH (Solomon et al., 2000; Blomberg et al., 1999).

Together, there is limited evidence that acute exposure to NO_2 in controlled human exposures results in increased lung damage.

3.6.4.2.1.3 Oxidative stress

Controlled human exposure studies investigating NO_2-induced changes in antioxidant properties in the lung show mixed results. Indicators of lipid peroxidation increased in the BAL fluid of healthy adults exposed to 4,000 ppb NO_2 for three hours (Mohsenin, 1991), but not with exposure to 2,000 ppb for four hours (Kelly et al., 1996). NO_2 exposure impacts on antioxidants were similarly variable. A study of the kinetics of antioxidant response in the respiratory tract indicates that NO_2 exposure may have a transient effect. A single exposure to 2,000 ppb NO_2 reduced levels of uric acid and ascorbic acid in bronchial wash and BAL fluid one and a half hours post-exposure that either returned to baseline or increased 6-24 hours after exposure (Kelly et al., 1996). Glutathione increased in the proximal (bronchial) lavage fluid one and a half and six hours after exposure, but no changes in glutathione were found in the distal (bronchoalveolar) lavage fluid or for reduced glutathione at any time after exposure. Repeat exposure to 2,000 ppb NO_2 on four consecutive days did not alter levels of glutathione, ascorbic acid, or uric acid levels one and a half hours after the final exposure (Blomberg et al., 1999).

One recent controlled human exposure study looked at the levels of nitrate and nitrite in the exhaled breath condensate (EBC) of healthy volunteers after exposure to NO_2. Nitrate and nitrite levels were used as a surrogate of the inflammation-induced enzyme, induced nitric oxide synthase (iNOS), and are indicative of oxidative and nitrosative stress. Brand et al. (2016) found no change in the levels of either nitrate or nitrite in the EBC of volunteers exposed to 100–1,500 ppb NO_2 for three hours.

The evidence from controlled human exposures regarding NO_2 exposure and oxidative stress is mixed. Some studies report increased markers of oxidative damage and alterations in oxidative defenses although the findings are not always consistent and there is considerable variability in the endpoints evaluated in different studies which makes comparison difficult. The current controlled human exposure data for lung injury does not characterize the timing of changes in markers of lung injury in comparison to other endpoints such as lung inflammation. It is possible that lung injury precedes lung inflammation

which is not captured with lavages preformed several hours after the end of NO_2 exposure. In addition, inconsistencies in the range of inflammatory mediators, oxidative stress markers, and lung damage indicators that are measured in different studies makes comparison of results among studies difficult as related markers may be controlled by different cellular and physiological processes.

3.6.4.2.1 Conclusions and Remaining Uncertainties

Controlled human exposure evidence of lung injury, inflammation, and oxidative stress is generally inconsistent. Evidence of NO_2-induced increases in inflammation is strongest and most consistent with regards to increases in PMN cells in BAL following NO_2 exposures; however, evidence for increases in chemotactic or cytokine mediators that are expected to underlie cellular infiltration is less consistent. Increases in PMN cells were seen with exposure concentrations at or above 1,000 ppb but were generally not seen at lower concentrations. The inconsistency in findings may be due, in part, to the inconsistency in timing of sampling compared to NO_2 exposure and the wide array of markers that are evaluated to measure injury, inflammation, and oxidative stress. Uncertainties that are inherent to controlled human exposure studies as a whole still persist. This includes factors like exposure pattern (constant concentration compared to variable concentration patterns that are more real-world relevant), generalizability of results to susceptible populations that would not qualify for controlled human exposures, and the relevance of exposure to NO_2 alone compared to more ambient-relevant mixtures of oxides of nitrogen species.

3.6.4.3 Animal Toxicological Studies of Respiratory Tract Inflammation, Injury, and Oxidative Stress

This section evaluates animal toxicological studies examining short-term oxides of nitrogen exposure and markers of respiratory tract inflammation, injury, and oxidative stress. Studies are summarized below, followed by additional discussion of dose-response relationships in these studies (see Section 3.6.4.3.1) and multipollutant exposures (see Section 3.6.4.3.2). Section 3.6.4.3.3 discusses conclusions from these studies and summarizes remaining uncertainties.

3.6.4.3.1.1 Inflammation

The 2016 Oxides of Nitrogen ISA found inconsistent evidence from animal studies to support NO_2-induced inflammation. NO_2 exposures in the range of 300 to 5,000 ppb did not affect PMN levels in mice, rats, or rabbits whether exposure occurred on a single day or was repeated over two to 13 days (Poynter et al., 2006; Pagani et al., 1994; Schlesinger et al., 1990). However, other studies found increased inflammatory mediator production at NO_2 exposure concentrations in excess of 5,000 ppb (Müller et al., 1994; Robison and Forman, 1993; Schlesinger et al., 1990). Macrophages isolated from rats

exposed to 500–2,000 ppb NO_2 throughout gestation and the first eight weeks of life had increased baseline production of TNFα and IFNγ, but not IL-1β; inflammatory markers returned to control levels after continued exposure to 12 weeks of age (Kumae and Arakawa, 2006). When the exposure to NO_2 started at five weeks old, only baseline TNFα production was increased at eight weeks of age (3 weeks of NO_2 exposure) while production of TNFα and IFNγ were elevated at 12 weeks of age (7 weeks of exposure) for the 500 ppb and 2,000 ppb groups (Kumae and Arakawa, 2006).

Recent studies have added information regarding inflammation following NO_2 exposures. Study specific details, exposure conditions, and endpoints examined are highlighted in the text below and described in more detail in Appendix A – Appendix Table 3-16.

- Gestational exposure to 2.5 ppm NO_2 led to increased counts of inflammatory cells and Th2-related cytokines in the offspring early in life (PND 1–PND 14) but were similar to the offspring of air exposed animals by PND 21 (Yue et al., 2017). Studies that subsequently looked at OVA sensitization in offspring of NO_2 exposed dams did not see differences in the inflammatory cells or inflammatory mediators between the air exposed and NO_2 treated controls. This may reflect the longer time lag after NO_2 exposure (Yue et al., 2018; Yue et al., 2017).
- Prolonged exposure (27–30 days) of 2–5 ppm NO_2 exposure of adult mice or rats resulted in Th2 skewing and increased markers of inflammation (IL-6, IL-1β, and TNF-α) in the lung (Lu et al., 2023; Han et al., 2017) which corresponded to worsened airway histology (Han et al., 2017).

3.6.4.3.1.2 Lung damage

Animal data evaluated in the 2016 Oxides of Nitrogen ISA found inconsistent effects of NO_2 exposures from 100–5,000 ppb on markers of lung damage such as bronchial wash fluid levels of protein, albumin, or LDH (U.S. EPA, 2016).

- A wide range of NO_2 exposures (100−5,000 ppb for one hour to 20 days) showed no effect on protein levels, LDH, albumin, lipid, or surfactant levels in the BAL of rodents (Müller et al., 1994; Pagani et al., 1994; Robison and Forman, 1993; Rose et al., 1989; Last and Warren, 1987; Selgrade et al., 1981).
- Several rodent studies of NO_2 exposure showed increases in BAL fluid protein, LDH, or albumin following NO_2 exposures of 5,000 ppb for a few hours on a single day or for 2−5 days (Poynter et al., 2006; Müller et al., 1994; Rose et al., 1989; Last and Warren, 1987; Hatch et al., 1986; Gregory et al., 1983).
- NO_2 exposures of 400–3,000 ppb for 72 hours to one week increased indicators of pulmonary injury in rodents deficient in dietary antioxidant vitamin intake (Selgrade et al., 1981; Sherwin and Carlson, 1973) or with longer duration exposures of 1−3 weeks (Gregory et al., 1983; Elsayed and Mustafa, 1982; Sherwin et al., 1972).
- Exposures to 400–3,000 ppb NO_2 were found to affect lung injury markers in rodents that were deficient in vitamin E (Selgrade et al., 1981; Sherwin and Carlson, 1973) or with

exposures from a week to several weeks (Gregory et al., 1983; Elsayed and Mustafa, 1982; Sherwin et al., 1972).

No recent studies have looked at markers of lung injury in animals, however, one study looked at the impact of high NO exposure (5–20 ppm for seven days) on inflammation in rat lungs.

- Early life exposure to high concentrations of NO did not affect wet to dry lung weights but did increase vascular volume density, VEGF production, and an activator of MMP2 (Duong-Quy et al., 2014).

3.6.4.3.1.3 Oxidative stress

Animal toxicological studies that have previously been evaluated have shown mixed results on markers of oxidative stress. The observed results of previously reviewed studies are below:

- Exposure of rodents to low concentrations of NO_2 (≤500 ppb) for exposure times two weeks or less did not impact lipid peroxidation (Ichinose and Sagai, 1989), Vitamin C or E levels (Ichinose and Sagai, 1989), or activity of antioxidant enzymes (Ichinose et al., 1988).
- Exposures of rodents to higher concentrations of NO_2 (1,000–5,000 ppb) had varying effects with some studies showing no changes in antioxidant enzyme activity (Mustafa et al., 1984a) while others showed increased levels of antioxidant enzymes (de Burbure et al., 2007) and increased GSSH concentrations in the BAL fluid (Pagani et al., 1994).
- Vitamin E deficiency resulted in increased susceptibility to increased signs of oxidative damage and decreased antioxidant response in response to NO_2 exposure (1–3 ppm) that were predominantly absent with normal or supplemented dietary levels of vitamin E (Sevanian et al., 1982b; Ayaz and Csallany, 1978).
- Combined exposure to NO_2 (400 ppb or 4,800 ppb) and ozone (400–450 ppb) increased glutathione peroxidase activity synergistically, indicating that the effect of NO_2 in a photochemical oxidant mixture may differ from exposure to NO_2 alone (Ichinose and Sagai, 1989; Mustafa et al., 1984a).

3.6.4.3.1 Dose Response

Given the variability in endpoints measured and markers used to measure inflammation, damage, and oxidative stress, the dose response relationship is difficult to determine. Despite this, for many of the endpoints examined, the effects were more apparent with exposure to higher concentrations of NO_2. For example, inflammatory cell infiltration in the lungs were seen in several studies at NO_2 exposure concentrations ≥10 ppm but were generally not significant for short term exposures ≤5 ppm (Poynter et al., 2006; Müller et al., 1994; Pagani et al., 1994). In a more recent study, Han et al. (2017) showed that histologic signs of inflammation, pulmonary inflammatory mediator transcription, signs of Th2 mediated immune response, and mucus production were worsened in a dose dependent fashion in rats exposed to 2 or 5 ppm NO_2 for 28 days.

3.6.4.3.2 Multipollutant Exposure

The investigation of NO_2 in a multipollutant environment is limited but there are some animal studies that suggest that NO_2 exposure in conjunction or in series with other air pollutants (e.g., ozone) can result in changes in oxidative stress and inflammation that are greater than either pollutant alone. For example, exposure of rats and guinea pigs to 400 ppb NO_2 concurrently with 400 ppb O_3 resulted in increased levels of lipid peroxides that was not seen with exposure to either NO_2 or O_3 alone (Ichinose and Sagai, 1989). Similarly, in mice exposed eight hours a day for seven days to 4,800 ppb NO_2 concurrently with 450 ppb O_3 had significantly greater increase on markers of oxidant lung injury than either exposure alone (Mustafa et al., 1984b).

3.6.4.3.3 Conclusions and Remaining Uncertainties

Together, the toxicological data suggests that NO_2 exposure has the potential to result in lung inflammation, damage, and oxidative stress although there remain considerable uncertainties related to the timing and dose required to elicit these responses. Vitamin deficiency (specifically Vitamin C and D) has also been shown to augment oxidative stress and lung damage in response to NO_2 exposure, suggesting an interplay between nutritional status and susceptibility to respiratory responses to NO_2. Many of the animal studies on healthy populations are embedded in studies of allergen sensitization. Given the length of time needed to sensitize and challenge animals, the measurement of endpoints occurs significantly after the end of NO_2 exposure (30+ days post exposure). This would allow for recovery and reversal of transient effects, and null findings for some endpoints may not be surprising. However, some of the recent studies utilize separate animal groups that were sacrificed shortly after exposure and had more noticeable impacts on inflammation and damage markers. It is important to consider timing of endpoint measurement in interpreting the animal evidence and understanding the potential discrepancies among studies. More uniformity in the endpoints that are used to evaluate oxidative stress, inflammation, and lung damage would ease comparison of study results.

3.6.5 Synthesis Across Lines of Evidence

Epidemiologic studies evaluated in the 2016 Oxides of Nitrogen ISA reported consistent evidence of a positive association between NO_2 and increased respiratory symptoms and pulmonary inflammation in children and in the general population. Evidence from recent epidemiologic studies is limited but continues to support an association between short-term NO_2 exposure and increased respiratory symptoms in children and young adults without pre-existing disease, but not strong evidence of an association in older adults. In a recent randomized cross-over study in young-adults, short-term NO_2 exposure during scripted exposures to traffic-related air pollution, measured via personal monitors, was associated with an increase in self-reported respiratory symptoms and decreases in multiple lung function measurements. A

key uncertainty in this study was the independent nature of the NO_2 associations given the high correlations between NO_2 and other traffic-related pollutants and limited adjustment for potential copollutant confounding. A recent cross-sectional study in school children observed indoor NO-associated pulmonary inflammation in healthy children, which is consistent with findings from the 2016 Oxides of Nitrogen ISA and provides mechanistic support for respiratory impacts. Studies of pulmonary inflammation in healthy adults are limited and have reported inconsistent results. Additionally, panel studies do not provide robust evidence of an association between short-term NO_2 exposure and lung function measures in children or adults. Prior studies provided inconsistent support, though a few studies with stronger exposure assessment (e.g., personal or on-site measurements) reported a negative association between short-term NO_2 and lung function in adults. Across the epidemiologic evidence for respiratory effects in healthy populations, potential confounding by copollutants, particularly other traffic-related pollutants, remains an area of uncertainty.

Controlled human exposure studies provide limited and inconsistent evidence supporting an effect of short-term NO_2 exposure on respiratory effects in populations without pre-existing respiratory disease. Studies evaluated in the 2016 Oxides of Nitrogen ISA provide limited support for the impact of NO_2 exposure on lung function, respiratory symptoms, or subclinical markers of pulmonary injury, inflammation, or oxidative stress in healthy populations at exposure concentrations close to those in ambient air. Prior studies did indicate increases in nonspecific airway responsiveness at high concentrations, but there is uncertainty regarding effects at exposure concentrations closer to those present in ambient air. Additionally, some studies indicated elevated markers of oxidative damage and changes in oxidative defense mechanisms, but results were inconsistent. Recent studies are limited in number and do not generally support respiratory effects in adults without pre-existing respiratory disease. A small number of recent controlled human exposure studies reported minimal impacts on lung function endpoints and no change in multiple inflammatory mediators or oxidative stress measurements in healthy volunteers exposed to NO_2. No recent controlled human studies have investigated the impact of short-term NO_2 exposure on the onset of respiratory symptoms or airway responsiveness in healthy volunteers. Across the evidence base, differences in the specific endpoints assessed complicates direct comparisons of results. Also, while controlled human exposure studies control exposure duration and concentration, there is uncertainty as to the extent that study exposure concentrations and patterns simulate ambient air exposures.

Overall, animal studies are limited in number, but recent studies provide some evidence supporting increases in airway reactivity in response to nonspecific broncho constricting agents. Exposure was examined over several weeks in these studies, and the implications for impacts at shorter durations are not well understood. A recent study suggests HONO exposures, in combination with residual NO and NO_2, can impair lung function; however, in prior studies, NO_2-related effects on lung function at lower does have not been reported consistently. Increased markers of inflammation and oxidative stress are seen more consistently in studies of animals deficient in antioxidants and effects can be reversed with antioxidant supplementation (see Section 3.10.3). Overall, toxicological data from animal studies report

the potential for increases in inflammation, damage, and oxidative stress related to NO_2 exposure, particularly for longer exposure times and higher NO_2 exposures.

Epidemiologic studies provide consistent evidence an association between short-term NO_2 exposure and respiratory symptoms and increased inflammation in children without pre-existing respiratory disease. In adults, there is limited evidence of NO_2-associated increases in pulmonary inflammation and inconsistent evidence for changes in lung function. The role of copollutants as potential confounders is still unclear in epidemiologic studies, raising some uncertainty about the independent effect of NO_2. Controlled human exposure and animal studies provide limited and inconsistent results, further contributing to the uncertainty. Overall, the lack of coherence between epidemiologic, controlled human exposure, and experimental findings raises uncertainty about the plausibility of epidemiologic associations in populations without pre-existing disease and whether the observed associations can be specifically attributed to NO_2.

3.7 Hospital Admissions and Emergency Department (ED) Visits for Aggregated Conditions

Some epidemiologic studies examine respiratory diseases as an aggregate of multiple respiratory conditions. In the 2016 Oxides of Nitrogen ISA, several studies examined short-term NO_2 exposure and all respiratory-related diseases in the context of hospital admissions and ED visits. The evidence included both multicity and single-city studies and reported consistent, positive associations when examining effects in children, people of all ages, adults, and older adults (i.e., ≥65 years of age) at single day lags of zero or 2 days and multiday lags up to 0–5 days. The evidence base was notably larger for hospital admissions than for ED visits. Studies of aggregated respiratory effects did not thoroughly examine potential confounding by traffic-related copollutants, which in many studies showed high ($r = 0.61−0.76$) correlations with NO_2. When interpreting these results, it is often difficult to determine whether the observed associations indicate that NO_2 may affect the spectrum of respiratory diseases or reflects the evidence supporting associations with specific respiratory diseases, such as asthma.

Studies published since the completion of the 2016 Oxides of Nitrogen ISA continue to support associations between short-term exposure to NO_2 and hospital admissions and ED visits for aggregated respiratory conditions. As in previous sections (see Section 3.3), studies of hospital admissions and ED for aggregated respiratory conditions are evaluated separately (see Section 3.7.1). The summary of evidence for hospital admissions and ED visits is followed by additional discussion of seasonal differences in associations (see Section 3.7.1.1), the lifestages and populations evaluated (see Section 3.7.1.2), exposure assignment approaches (see Section 3.7.1.3), the potential impact of copollutants (see Section 3.7.1.4), and the implications of model specification (see Section 3.7.1.5).

3.7.1 Epidemiologic Studies of Hospital Admissions and ED Visits for Aggregated Conditions

3.7.1.1.1.1 Hospital Admissions for Aggregated Conditions

Epidemiologic studies evaluated in the 2016 Oxides of Nitrogen ISA consistently indicated associations between short-term increases in ambient NO_2 concentrations and increases in respiratory hospital admissions aggregated across specific conditions such as asthma, COPD, and respiratory infections. Associations of NO_2 with respiratory disease hospital admissions were observed to be larger for children and older adults. Evidence also indicated that the largest increases in total respiratory hospital admissions occurred in the first few days after NO_2 exposure, specifically lags of zero to two days. An examination of model specification indicated the NO_2-respiratory hospital admission relationship was robust to alternative lags and *df* for weather covariates.

Results from several recent large multicity studies conducted in Canada are consistent with findings from the 2016 Oxides of Nitrogen ISA (see Table 3-9). A remaining uncertainty is the limited evaluation of potential copollutant confounding, often because the strong correlations between NO_2 and other traffic-related pollutants limit the utility of copollutant models due to multicollinearity. A short, bulleted summary of the recent evidence is provided below. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 3-17.

- Collectively, several recent multicity Canadian studies provide strong evidence of a positive association between short-term NO_2 exposure and hospital admissions for respiratory disease among people of all ages (Huang et al., 2023; Huang et al., 2022; Shin et al., 2022; Parajuli et al., 2021; Shin et al., 2021).
- A few studies examined the same spatial domain of 24 urban areas representing ~50% of the Canadian population (Shin et al., 2022; Parajuli et al., 2021; Shin et al., 2021). These studies analyzed data from overlapping years [1996-2012 (Shin et al., 2022; Shin et al., 2021) and 2001-2012 (Parajuli et al., 2021)], such that the results do not provide independent evidence of an association. Huang et al. (2023) analyzed data representing a similarly large proportion of the Canadian population from 47 cities.
- Each of the recent studies used NO_2 concentrations from central site monitors to assign exposures. In most cases, concentrations were averaged across multiple monitors in each city. Huang et al. (2022) used a Bayesian framework to impute missing hourly pollutant data. Whereas most recent studies analyzed 24-hour avg NO_2 concentrations, (Huang et al., 2023) used a 3-hr max averaging time.
- Similar to studies evaluated in the 2016 Oxides of Nitrogen ISA, recent studies report associations with NO_2 concentrations between lags of zero to two days. Most studies selected this range of lag days *a priori*, though one study that evaluated additional lags reported null associations with exposure lags ranging from 3 to six days (Parajuli et al., 2021).

Table 3-9 Associations between short-term exposure to oxides of nitrogen and hospital admissions for aggregated respiratory conditions

Study	Location	Ages	Lag	Averaging Time	Effect Estimate (95% CI)
†Huang et al. (2023)	9 provinces in Canada	All ages	0–1 d	3-hr max	% change in risk: 1.15% (95% HDI: 0.58, 1.54) per 12.8 ppb increase in NO_2
†Huang et al. (2022)	4 cities in Canada	All ages	0–1 d	24-hr avg	% change in risk (NO_2): 1.97% (95% PI: -1.31, 5.36)
†Shin et al. (2021)	24 cities in Canada	All ages	0 d	24-hr avg	% change risk (NO_2) Respiratory hospitalizations Lag 0 Warm: 3.63% (95% PI: 0.52, 6.84) Lag 0 Cold: 0.60% (95% PI: -0.50, 1.71) Lag 0 YR: 1.40% (95% PI: 0.10, 2.72)
†Shin et al. (2022)	24 census divisions in Canada	All ages	0 d	24-hr avg	% change risk (NO_2) Warm season Female: 4.86% (95% PI: 1.33, 8.51) Male: 2.41% (95% PI: -0.97, 5.91) Cold season Female: 0.80% (95% PI: -1.09, 2.73) Male: 0.20% (95% PI: -1.19, 1.61) Year-round Female: 1.81% (95% PI: 0.01, 3.64) Male: 1.20% (95% PI: -0.49, 2.93)
†Parajuli et al. (2021)	24 cities in Canada	All ages	0–6 d	24-hr avg	% change in risk (NO_2): 1.2% (95% PI: -0.2, 2.62)
†Krall et al. (2018)	5 cities in United States	All ages	0–7 d	1-hr max	% change in risk: NO_2: 13.2% (95% PI: 6.6, 20.3) NO_X: 6.1% (95% PI: -4.9, 18.4)

List of abbreviations in alphabetical order: avg = average; CI = confidence interval; d = day; F = female; HDI = highest density interval; hr = hour; M = male; max = maximum; NO_2 = nitrogen dioxide; NO_X = NO + NO_2; NR = not reported; PI = posterior interval; ppb = parts per billion; RR = relative risk; yr = year; YR = year-round.
Notes: Unless otherwise noted, results are standardized to a 20 ppb increase in 24-hr avg NO_2, a 25 ppb increase in 1-hr max NO_2, and an 80 ppb increase in 1-hr max NO_X.
†Studies published since the 2016 Oxides of Nitrogen ISA.

3.7.1.1.1.2 ED Visits for Aggregated Conditions

Despite more limited evidence base than for hospital admissions, epidemiologic studies in the 2016 Oxides of Nitrogen ISA also provided consistent evidence for positive associations between NO_2 exposure and ED visits for respiratory outcomes aggregated across specific conditions. The NO_2-related

increases in ED visits were observed in study populations including people of all ages, and were noted in relation to shorter exposure lags, suggesting more immediate effects. Notably, the evidence for ED visits lacked geographic diversity, as all the studies were single-city analyses conducted in Atlanta, Georgia.

Several recent studies expand the geographic representation of the evidence base, including a multicity study that spans multiple U.S. states (Krall et al., 2018). Recent studies provide generally consistent evidence of associations between short-term NO_2 exposure and ED visits for respiratory disease (see Table 3-10). Uncertainties remain regarding potential copollutant confounding. A short, bulleted summary of the recent evidence is provided below. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 3-18.

- A few recent large multicity studies conducted in the United States (Cromar et al., 2020; Krall et al., 2018) and Canada (Weichenthal et al., 2016) examined the relationship between short-term NO_2 exposures and ED visits for respiratory disease among people of all ages. Positive associations were reported in some of these studies, including a time-series analysis of ED visits in Atlanta, GA; Dallas, TX; St. Louis, MO; Birmingham, AL; and Pittsburgh, PA (Krall et al., 2018) and a time-series analysis of ED visits in 3 California regions comprising 87% of the state's population (Cromar et al., 2020). In contrast, although Weichenthal et al. (2016) reported positive associations between 24-hour avg NO_2 concentrations and ED visits for asthma and COPD across 15 cities in Ontario, Canada (see Sections 3.3.1.1 and 3.4.1.1), the authors reported slightly inverse associations with aggregated respiratory conditions across all lags.
- Results from large single-city studies in Atlanta, GA (Ebelt et al., 2022) and New York City, NY (Perlmutt and Cromar, 2019) provide supporting evidence of an positive association between short-term NO_2 exposure and ED visits for respiratory disease. Notably, Ebelt et al. (2022) reported a null association with exposure assigned from a single central site monitor, but positive associations when exposures were assigned using spatially refined exposure estimates from IDW or kriging of all monitors within the 200 km study area (see Section 3.7.1.3 for more detail).
- In addition to recent studies of ED visits for respiratory disease, one recent case-crossover study conducted across 21 Japanese cities reported a 3.98% increase (95% CI: 3.05, 4.91) in respiratory outpatient visits associated with a 10-pbb increase in 4-day moving average (i.e., lag 0-3) 24-hour avg NO_2 concentrations (Seposo et al., 2020). The association was robust to adjustment for $PM_{2.5}$ and O_3 (see Section 3.7.1.4).
- Most recent studies defined pollutant lag structures *a priori* based on previous research. Associations were observed with cumulative lags ranging from 0–3 days (Cromar et al., 2020; Seposo et al., 2020; Perlmutt and Cromar, 2019) to 0–7 days (Ebelt et al., 2022; Krall et al., 2018). Ebelt et al. (2022) also tested alternative lag structures, including same-day NO_2 concentrations (i.e., lag 0) and 3- and 6-day moving average NO_2 concentrations (i.e., lag 0–2 and lag 0–5, respectively). The authors consistently noted stronger associations with lag 0–7 NO_2 concentrations, indicating an effect of prolonged short-term NO_2 exposure on respiratory ED visits.

Table 3-10 Association between short-term exposure to oxides of nitrogen and emergency department visits for aggregated respiratory conditions

Study	Location	Ages	Lag	Averaging Time	Effect Estimate (95% CI)
†Ebelt et al. (2022)	Atlanta, GA, U.S.	All ages	0–7 d	1-hr max	RRs (NO_2) *Exposure estimated at the central monitor location* Central monitor: 0.990 (0.974, 1.006) Site average: 0.990 (0.969, 1.011) IDW: 0.988 (0.972, 1.004) Kriging: 0.99 (0.974, 1.006) CMAQ: 1.025 (1.009, 1.042) CMAQ + kriging: 0.990 (0.974, 1.006) CMAQ fusion: 1.030 (1.013, 1.048) LUR: 1.058 (1.025, 1.093) *Exposure estimated using full spatial model* Site average: 0.990 (0.969, 1.011) IDW: 1.046 (1.025, 1.066) Kriging: 1.066 (1.046, 1.087) CMAQ: 1.03 (1.019, 1.042) CMAQ + kriging: 1.008 (0.993, 1.023) CMAQ fusion: 1.035 (1.020, 1.051) LUR: 1.064 (1.039, 1.089)
†Cromar et al. (2020)	25 counties in California	18+	0–3 d	1-hr max	Quantitative results presented in figures. Forest plots depict some variation across regions and seasons, but evidence of positive associations in general.
†Perlmutt and Cromar (2019)	New York City, NY, U.S.	All ages	0–3 d	1-hr max	RR (NO_2): 1.027 (1.007, 1.048)
†Weichenthal et al. (2016)	Ontario, Canada	All ages	0–2 d	24-hr avg	% change (NO_2): -0.94% (-2.89, 1.05)

List of abbreviations in alphabetical order: avg = average; CI = confidence interval; CMAQ = the community multiscale air quality model; d = day; h = hour; IDW = inverse distance weighting; LUR = land use regression model; max = maximum; NO_2 = nitrogen dioxide; NO_X = NO + NO_2; ppb = parts per billion; RR = relative risk; yr = year.
Notes: Unless otherwise noted, results are standardized to a 20 ppb increase in 24-hr avg NO_2, a 25 ppb increase in 1-hr max NO_2, and an 80 ppb increase in 1-hr max NO_X.
†Studies published since the 2016 Oxides of Nitrogen ISA.

3.7.1.1 Examination of Seasonal Differences

Respiratory hospital admission and ED visit studies discussed in the 2016 Oxides of Nitrogen ISA included limited analysis of potential seasonal differences. Results from a multicity study conducted in South Korea suggest that NO_2 associations with all respiratory disease hospital admissions are stronger during the summer (Son et al., 2013). A recent analysis of NO_2-related hospital admissions in 24

Canadian cities observed similar results (Shin et al., 2022; Shin et al., 2021). The authors reported increased risk of NO_2-related respiratory hospital admissions in the warm season (females: 4.9% [95% PI: 1.3, 8.5] per 10 ppb increase in same day NO_2; males: 2.4% [95% PI: -1.0, 5.9]), and null associations in the cold season.

3.7.1.2 Lifestages and Population Differences

3.7.1.2.1.1 Sex

One study discussed in the 2016 Oxides of Nitrogen ISA examined sex differences in the association between short-term NO_2 exposure and hospital admissions for respiratory disease (Luginaah et al., 2005). The authors examined respiratory hospital admissions in Windsor, Canada and observed a positive association with previous-day NO_2 concentrations for women and a null association for men. The sex differences were consistent across longer lags and study design methods (i.e., time-series and case-crossover). A recent study conducted in 24 Canadian cities observed similar differences in NO_2-related risk of warm season respiratory hospital admissions across sexes (Shin et al., 2022). Shin et al. (2022) reported positive associations for both sexes, but associations were stronger among females (4.9% [95% PI: 1.3, 8.5] per 20 ppb increase in same day NO_2) compared to males (2.4% [95% PI: -1.0, 5.9]).

3.7.1.2.1.2 Lifestages

Epidemiologic studies of respiratory hospital admissions and ED visits in the 2016 Oxides of Nitrogen ISA mostly included study populations of all ages. Those studies that did examine specific age groups reported associations of NO_2 with respiratory disease hospital admissions that were larger in magnitude among children and older adults compared to the population at-large. Notably, these comparisons were made across studies, and the differences could be the result of non-age-related factors that differed between the studies. One recent study compared the association between short-term NO_2 concentrations and hospital admissions for respiratory disease across age groups. In a case-crossover study conducted in 47 Canadian cities, Huang et al. (2023) observed a smaller increase in 3-day moving average NO_2-related risk among older adults (66+ years: 0.81% [95% HDI: 0.11, 1.42]) compared to the rest of the study population (1–65 years: 1.15% [95% CI: 0.58, 1.54]). Overall, the limited evaluation of age-modified risk of respiratory hospital admissions and ED visits limits the conclusions that can be drawn. However, results from studies of specific respiratory diseases clearly demonstrate differences in risk of hospitalization across lifestages, such as children with asthma (see Section 3.3) and older adults with COPD (see Section 3.4).

3.7.1.3 Exposure Assignment

As discussed previously, exposure measurement error in epidemiologic studies can bias effect estimates. Chapter 2 provides a thorough discussion of the bias resulting from particular exposure assessment methods common to the epidemiologic literature and its impact on the inferences that can be drawn about health effects. Most studies of short-term NO_2 exposure and hospital admissions and ED visits for respiratory disease assign exposure from central site monitors. Notably, one recent study compared monitoring and modeled NO_2 concentrations in a time-series study of respiratory ED visits in Atlanta, GA (Ebelt et al., 2022). The authors compared eight different exposure estimation approaches, including a single central-site monitor, an average of five central-site monitors across Atlanta, GA, an IDW average of 12 monitors, kriging of 12 monitors, CMAQ modeled estimates, kriging-adjusted CMAQ estimates, fused CMAQ estimates to correct for modeling biases, and LUR. In order to compare the alternative modeling approaches to the single central site monitor, each approach was used to assign NO_2 exposure based on the estimated concentration at the centroid of the ZIP code that housed the central site monitor. Additionally, the analyses were conducted by assigning exposures at the centroid of residential ZIP codes corresponding to each patient, allowing for utilization of the additional spatial information provided by some of the more spatially refined exposure estimation approaches. Ebelt et al. (2022) reported a null association corresponding to exposures estimated using the central site monitor, which was replicated in analyses using IDW, kriging, and CMAQ kriging estimations at the central site monitor location. Each of the other exposure assignment approaches used to estimate exposure at the central site monitor location (i.e., CMAQ, CMAQ fusion, and LUR) yielded positive associations despite monitor concentrations at the point of reference not being associated with the outcome, indicating that exposure measurement errors may have led to effect estimates that were biased away from the null for those approaches. In the full spatial analyses that estimated exposures at patients' residences, effect estimates derived from the IDW, kriging, and CMAQ kriging approaches were each positive. This suggests that the use of more spatially refined exposure estimates may be important to capture the effects of more spatially heterogeneous pollutants such as NO_2.

3.7.1.4 Copollutants

Studies examined in the 2016 Oxides of Nitrogen ISA included limited evaluation of potential copollutant confounding, often due to high correlations between other traffic-related pollutants and NO_2. A single study that examined copollutant models corresponding to traffic-related pollutants reported that NO_2 associations with respiratory ED visits persisted with adjustment for CO or $PM_{2.5}$ [quantitative results not provided by authors; (Tolbert et al., 2007)]. A few recent studies evaluated copollutant models and similarly reported NO_2 associations that were robust to adjustment for $PM_{2.5}$. In a case-crossover analysis of outpatient respiratory visits in 21 Japanese cities, increases in risk of outpatient visits associated with 20 ppb increases in 4-day moving average (i.e., lag 0–3) NO_2 (3.98% [95% CI: 3.05, 4.91]) were larger after adjustment for $PM_{2.5}$ (7.29% [95% CI: 6.20, 8.39]) and relatively unchanged after

adjustment for O_3 (4.14% [95% CI: 3.19, 5.11]) (Seposo et al., 2020). Parajuli et al. (2021) similarly reported an increase in hospital admissions for respiratory disease associated with short-term NO_2 concentrations (1.2% [95% CI: -0.2, 2.6] per 20 ppb increase) that was robust to adjustment for $PM_{2.5}$ (1.2% [95% CI: -0.2, 2.6]) and attenuated close to the null after adjustment for O_3 (0.4% [95% CI: -1.5, 2.3]). As a whole, the limited number of studies examining potential copollutant confounding provide some evidence that the observed NO_2 associations are independent of $PM_{2.5}$, O_3, and CO. Notably, potential confounding by other pollutants—traffic-related pollutants, in particular—remains an uncertainty.

3.7.1.5 Model Specification

The results of time-series and case-crossover studies described in this section rely on the assumption that the statistical models adequately control for the potential confounding effects of temporal trends and meteorological conditions. Each study of respiratory hospital admissions and ED visits included in this section, as well as those discussed in the 2016 Oxides of Nitrogen ISA, includes models that adjust for temporal trends and some combination of meteorology covariates, including temperature, humidity, and barometric pressure. Often, these covariates are modeled using splines and lags and can be sensitive to the specification of the *df* and lag structures used to account for their relationship with the outcome, which often differ across studies. In a study of eight South Korean cities evaluated in the 2016 Oxides of Nitrogen ISA, Son et al. (2013) conducted a sensitivity analysis to identify whether risk estimates changed based on the specification of temporal and meteorological covariates. The authors reported that the association between short-term NO_2 exposures and all respiratory disease hospital admissions was sensitive to using less than 6 *df* per year to control for temporal trends but was stable when using 6−10 *df* per year. This finding suggests potential residual confounding by temporal trends when using a smaller number of *df* that may not accurately capture temporal trends in hospital admissions, though the ideal *df* is likely study dependent. Regarding meteorology covariates, the authors observed that the NO_2 association remained robust (i.e., relatively unchanged) to different specifications of *df* (between 3 to 6 *df*) and lag structure (i.e., lag 0 and lag 0−3 days).

A recent study of respiratory ED visits in Atlanta, GA also conducted sensitivity analyses to assess model specification (Ebelt et al., 2022). In addition to a long list of covariates included in the primary model, the authors used parametric cubic splines with 2 knots per month to control for temporal trends, an indicator variable to account for same-day maximum temperature, and cubic terms for the moving averages of minimum temperature and mean dew point temperature. In sensitivity analyses, the authors considered one knot per month in the temporal spline, controlled for temperature with cubic terms of the moving average of mean temperature; and included product terms for season and temperature. Similar to findings from Son et al. (2013), Ebelt et al. (2022) noted that the results were most sensitive to less stringent control for temporal trends. The magnitudes of the observed associations were generally comparable across the alternate temperature covariate specifications.

3.7.1.6 Conclusions and Remaining Uncertainties

Epidemiologic studies evaluated in the 2016 Oxides of Nitrogen ISA consistently indicated associations between short-term increases in ambient NO_2 concentrations and increases in respiratory hospital admissions and ED visits aggregated across specific conditions. Several recent multicity studies in the United States and Canada expand the geographic representation of the evidence base and support the conclusions from the 2016 Oxides of Nitrogen ISA. Associations of NO_2 with respiratory disease hospital admissions and ED visits were mostly observed in study populations including people of all ages. Though some studies utilized stratified models and reported stronger associations of NO_2 with respiratory disease hospital admissions among children and older adults, the limited evaluation of age-modified risk limits the conclusions that can be drawn. However, these limited results are consistent with studies of specific respiratory diseases that clearly demonstrate differences in risk of hospitalization across lifestages, such as children with asthma (see Section 3.3) and older adults with COPD (see Section 3.4).

Although one recent study reported a positive association of respiratory ED visits with one week moving average NO_2 concentrations, the collective evidence supports larger increases in respiratory hospital admissions and ED visits relative to more recent NO_2 exposures (i.e., shorter exposure lags). One recent study examined model specification in a time-series analysis and reported that associations were robust to specifications of temporal splines and alternative lags and *df* for meteorological covariates, which is consistent with findings summarized in the 2016 Oxides of Nitrogen ISA. Potential copollutant confounding remains a key uncertainty in the epidemiologic evidence. Studies examined in the 2016 Oxides of Nitrogen ISA included limited evaluation of potential copollutant confounding, often because of concerns with multicollinearity due to high correlations between other traffic-related pollutants and NO_2. While a limited number of studies examining potential copollutant confounding provide some evidence that the observed NO_2 associations are robust to adjustment for $PM_{2.5}$, O_3, and CO, there are no PECOS-relevant studies that examined other traffic-related pollutants (e.g., EC and UFPs).

3.8 Respiratory Mortality

Studies that examine the association between short-term NO_2 exposure and cause-specific mortality outcomes, such as respiratory mortality, provide additional evidence for NO_2-related respiratory effects, specifically, whether there is evidence of an overall continuum of effects. Several multicity epidemiologic studies evaluated in the 2016 Oxides of Nitrogen ISA reported consistent positive associations between short-term NO_2 exposure and respiratory mortality. Positive associations were observed in multicity studies conducted in Asia (Chen et al., 2012b; Wong et al., 2008) and Italy (Faustini et al., 2013; Chiusolo et al., 2011; Bellini et al., 2007), as well as a meta-analysis of studies conducted in Asian cities (Atkinson et al., 2012). As with the interpretation of NO_2 associations with respiratory morbidity, it is challenging to determine whether NO_2 is independently associated with respiratory mortality because NO_2 is often highly correlated with other traffic-related pollutants. A limited number of

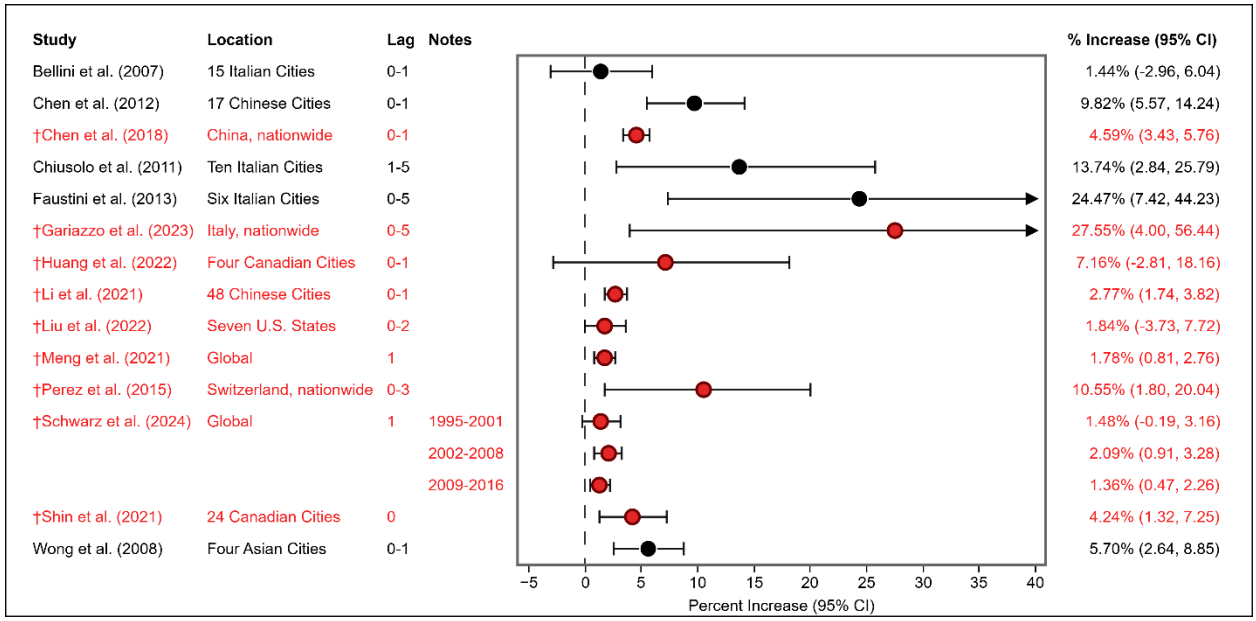
studies examined the potential confounding effects of copollutants on the NO_2-respiratory mortality relationship and indicated that associations remain relatively unchanged after adjustment for SO_2, O_3, and PM_{10}. Key traffic-related pollutants (e.g., EC) were not considered. Evidence from recent studies is summarized below. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 3-19.

A large number of recent studies expand the existing evidence and provide generally consistent support for an association between short-term NO_2 exposure and respiratory mortality (see Figure 3-10). Recent studies expand the geographic coverage of the evidence base, including a global analysis of respiratory mortalities recorded in 362 cities across 16 countries, as well as multicity studies conducted in North America and Europe. Recent studies also provide a limited evaluation of potential copollutant confounding by traffic-related pollutants, such as $PM_{2.5}$ and CO. A short, bulleted summary of the recent evidence is provided below, followed by additional discussion of concentration-response relationships in these studies (see Section 3.8.1), potential impacts of copollutants (see Section 3.8.2), the lifestages and populations evaluated (see Section 3.8.3), exposure assignment approaches (see Section 3.8.4), alternative methods for confounder control (see Section 3.8.5), implications of model specification (see Section 3.8.6), lag structure of associations (see Section 3.8.7), and potential seasonal differences in associations (see Section 3.8.8). Section 3.8.9 discusses conclusions from these studies and summarizes remaining uncertainties.

- Recent multicity studies in the United States (Liu et al., 2022), Canada (Huang et al., 2023; Huang et al., 2022; Shin et al., 2022; Parajuli et al., 2021; Shin et al., 2021), Europe (Gariazzo et al., 2023; Perez et al., 2015), and China (Li et al., 2021; Chen et al., 2018), as well global analyses (Schwarz et al., 2024; Meng et al., 2021), provide generally consistent evidence of an association between short-term NO_2 exposure and mortality due to respiratory disease.
- Studies providing the strongest inference include a U.S. study with state-wide mortality data from seven states (Liu et al., 2022) and global analyses of mortality and air pollution data from over 360 cities (Schwarz et al., 2024; Meng et al., 2021). In addition to including a large representative sample of the U.S. population, Liu et al. (2022) assigned NO_2 exposure using a high resolution (i.e., 1 km x 1 km) hybrid prediction model with a high out-of-sample predicted R^2 (0.79) and used negative exposure controls to assess the potential for unmeasured confounders (see Section 3.8.6). Meng et al. (2021) and Schwarz et al. (2024) used central site monitors to estimate the NO_2-respiratory mortality relationship using data from over 360 cities in the Multi-Country Multi-City Collaborative Research Network database, pooling effect estimates across cities to the country and global level. Meng et al. (2021) reported an overall increase in respiratory mortality of 1.78% (95% CI: 0.81, 2.76) for every 20 ppb increase in NO_2 concentrations, and further noted an increase in NO_2-related mortality risk in 15 of the 16 countries examined. In order to assess potential long-term temporal variation in the relationship between short-term NO_2 exposure and respiratory mortality, Schwarz et al. (2024) used longitudinal meta-regression models to pool effect estimates using either a linear or non-linear term for time. The authors observed a non-linear temporal trend in the NO_2-respiratory mortality relationship, as further demonstrated by effect estimates pooled across three sequential time periods that indicated a slightly stronger

association in the middle time period (1995–2001: 1.48% [95% CI: -0.19, 3.16]; 2002–2008: 2.09% [95% CI: 0.91, 3.28]; 2009–2016: 1.36% [95% CI: 0.47, 2.26]).

- While most multi-city studies provide consistent evidence of an association, the results from a few of the studies conducted in Canada were more heterogeneous. In multiple related studies conducted across 24 Canadian cities in overlapping years [1984–2012 (Shin et al., 2022; Shin et al., 2021) and 2001–2012 (Parajuli et al., 2021)], positive associations were observed inconsistently across seasons, sexes, and exposure lags (see Section 3.8.1 for more detail). Additionally, in a large study of 47 Canadian cities, positive associations were only observed in Bayesian pooled models that used weakly informative priors that specified a non-negative distribution with a 95th percentile of the effect estimate equivalent to a 2% increase in mortality rate per 10 ppb increase in NO_2 (Huang et al., 2023). The authors reported a null association between short-term NO_2 exposure and respiratory mortality when uninformative priors were used to pool effect estimates across cities.
- A few recent studies also examined mortality due to specific respiratory diseases. Large multicity studies conducted in China reported increases in COPD-related mortality associated with 2-day moving average (i.e., lag 0–1) increases in NO_2 that were comparable in magnitude to observed increases in respiratory mortality in the same populations (Huang et al., 2021; Chen et al., 2018). A much smaller study in Hubei Province, China observed increased odds of asthma mortality associated with short-term NO_2 exposure (Liu et al., 2019).



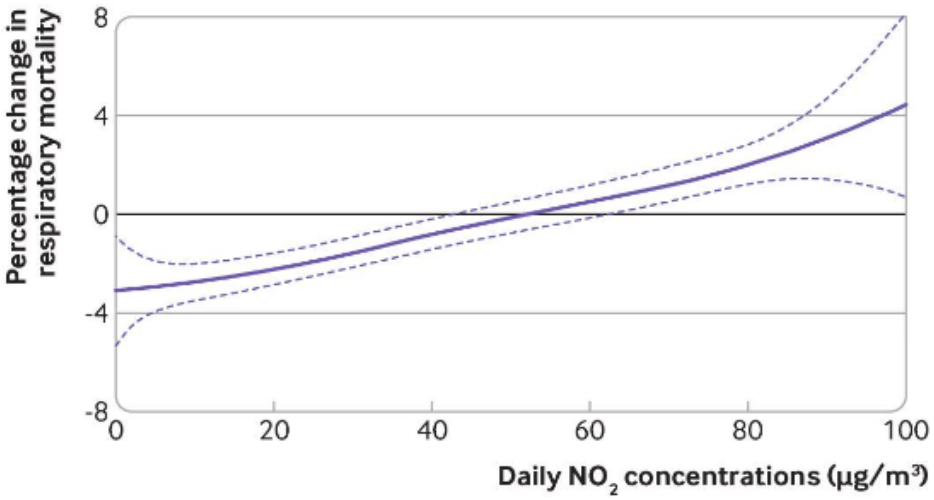
List of abbreviations in alphabetical order: CI = confidence interval; U.S. = United States.
Notes: Black = studies from the 2016 Integrated Science Assessment for Oxides of Nitrogen; Red = recent studies and those not included in 2016 Oxides of Nitrogen ISA. Results are standardized to a 20 ppb increase in 24-hr avg NO_2 and a 25 ppb increase in 1-hr max NO_2.
†Studies published since the 2016 Oxides of Nitrogen ISA.

Figure 3-10 Percentage increase in respiratory mortality in relation to short-term increases in ambient nitrogen dioxide concentrations.

3.8.1 Concentration-Response

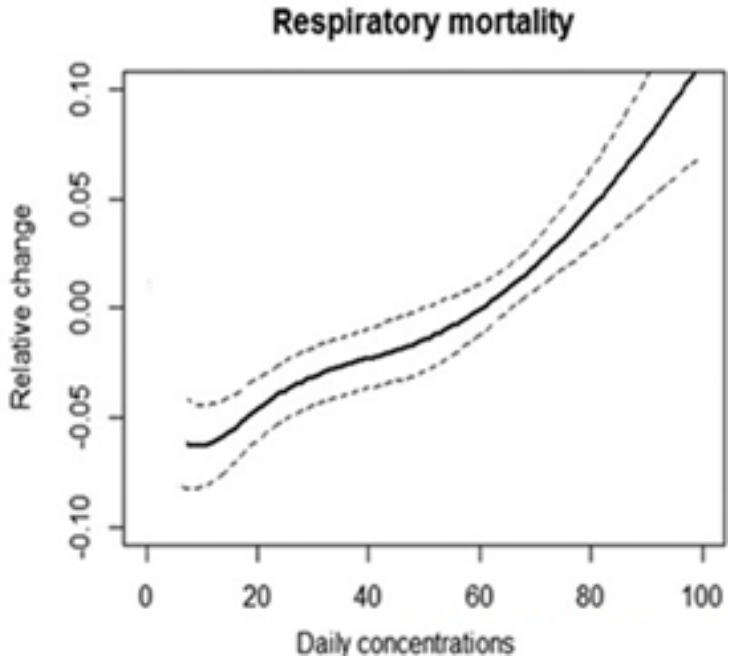
There was limited evaluation of the shape of the C-R relationship between short-term NO_2 exposure and respiratory mortality in the 2016 Oxides of Nitrogen ISA. A multicity study conducted in China fit both a linear and spline model to the city-specific NO_2-COPD mortality relationship and then computed the deviance between the two models to determine if there was evidence of nonlinearity (Meng et al., 2013). Visual inspection of the city-specific spline models indicated curves that ranged from linear to sublinear, but the examination of deviance did not indicate that the spline model improved the overall fit of the NO_2-COPD mortality relationship across the cities examined.

Several recent studies also used spline models to assess the shape of the NO_2-respiratory mortality C-R relationship (Li et al., 2021; Meng et al., 2021; Chen et al., 2018). In each study, authors used either cubic or natural splines with two knots to explore potential nonlinearities in the observed associations. Spline knots ranged from 20 to 30 μg/m^3 (10.64 to 15.96 ppb) on the lower end of the concentration distribution and 40 to 50 μg/m^3 (21.28 to 26.6 ppb) on the higher end. Visual inspection of each C-R curve indicated approximately linear relationships between short-term NO_2 exposure and respiratory mortality (see Figure 3-11, Figure 3-12, and Figure 3-13). The C-R curve presented in Li et al. (2021) appears to plateau slightly in the middle of the curve, but the 95% CIs are much wider than in the other spline models (see Figure 3-13). In a largely overlapping study population, Huang et al. (2021) examined COPD mortality and observed a C-R curve comparable to the respiratory mortality curve presented in Li et al. (2021) and Figure 3-14. The collective evidence does not indicate the presence of a threshold, and there continues to be support for an association across the full distribution of concentrations evaluated, including at lower concentrations.



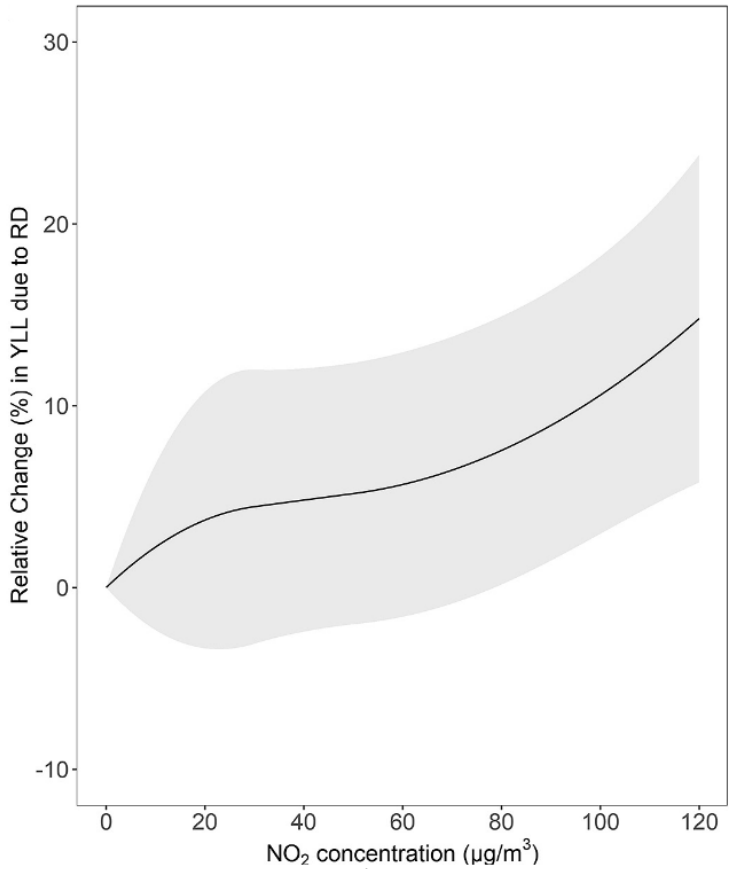
List of abbreviations in alphabetical order: NO_2 = nitrogen dioxide; $\mu g/m^3$ = micrograms per cubic meter.
Source: Adapted from Meng et al. (2021). Copyright permission pending.

Figure 3-11 Concentration-response curve between nitrogen dioxide concentrations (lag 1) and respiratory mortality in 383 cities across 16 countries; estimated from a natural spline model with knots at 20 and 40 $\mu g/m^3$.



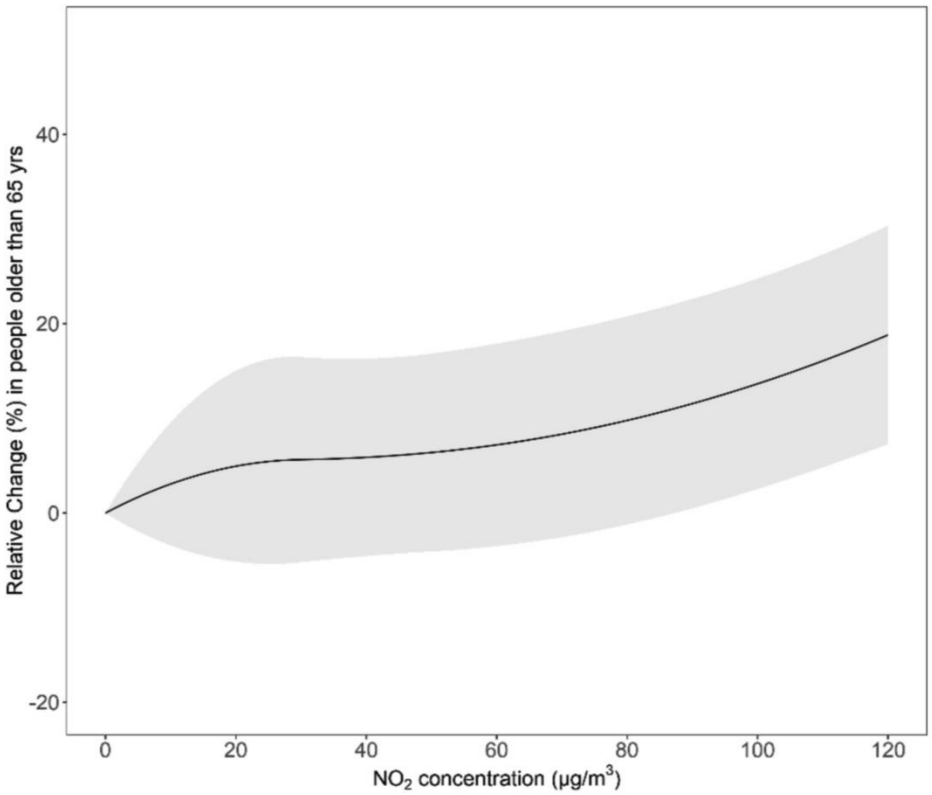
List of abbreviations in alphabetical order: $\mu g/m^3$ = micrograms per cubic meter.
Source: Adapted from Chen et al. (2018). Copyright permission pending.

Figure 3-12 Concentration-response curve between nitrogen dioxide concentrations (lag 0–1) and respiratory mortality in 272 Chinese cities; estimated from a natural spline model with knots at 20 and 50 $\mu g/m^3$.



List of abbreviations in alphabetical order: NO_2 = nitrogen dioxide; $\mu g/m^3$ = micrograms per cubic meter.
Source: Adapted from Li et al. (2021). Copyright permission pending.

Figure 3-13 Concentration-response curve between nitrogen dioxide concentrations (lag 0–1) and total years of life lost due to respiratory mortality in 48 Chinese cities; estimated from a natural spline model with knots at 30 and 50 µg/m³.



List of abbreviations in alphabetical order: COPD = Chronic Obstructive Pulmonary Disease; NO_2 = nitrogen dioxide; yr = years; $\mu g/m^3$ = micrograms per cubic meter.
Source: Adapted from Huang et al. (2021). Copyright permission pending.

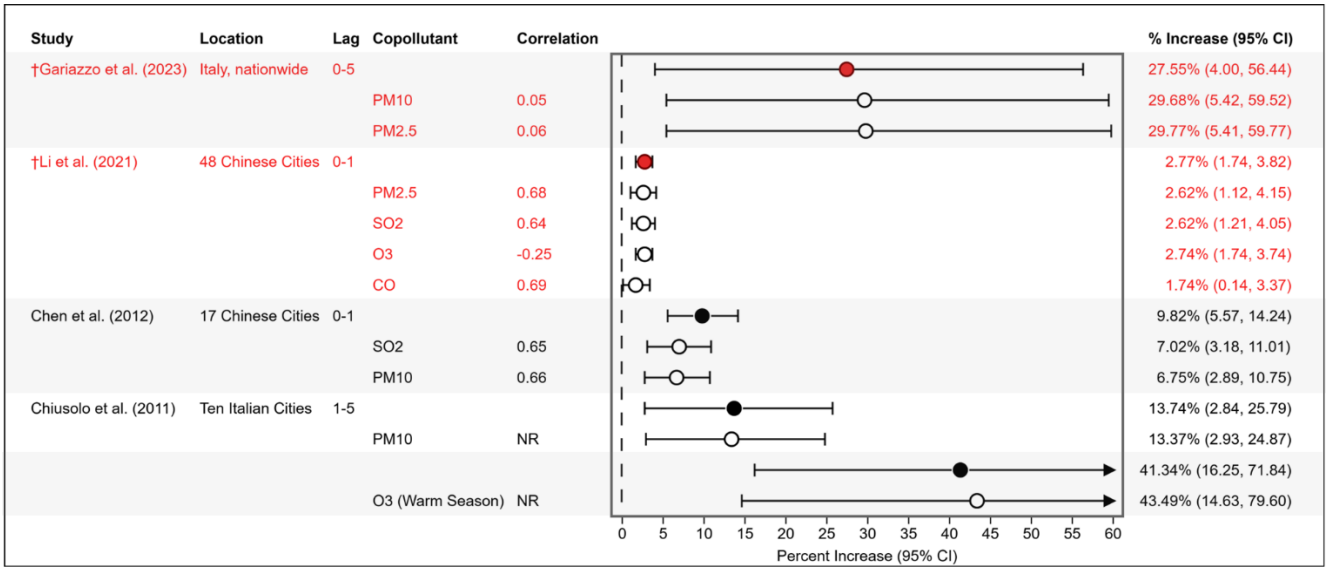
Figure 3-14 Concentration-response curve between nitrogen dioxide concentrations (lag 0–1) and total years of life lost due to COPD mortality in 37 Chinese cities; estimated from a natural spline model with knots at 30 and 50 $\mu g/m^3$.

3.8.2 Copollutants

NO_2 is often highly correlated with other traffic-related copollutants; therefore, the ability to determine whether short-term NO_2 exposures are independently associated with respiratory disease mortality is limited. In the 2016 Oxides of Nitrogen ISA, a small portion of the included studies of short-term exposure and respiratory mortality conducted copollutant analyses. Across multicity studies in China (Meng et al., 2013; Chen et al., 2012b) and Italy (Faustini et al., 2013; Chiusolo et al., 2011), associations between NO_2 and respiratory disease mortality were generally attenuated, but still positive after adjustment for PM_{10} (Faustini et al., 2013; Meng et al., 2013; Chen et al., 2012b; Chiusolo et al., 2011), SO_2 (Meng et al., 2013; Chen et al., 2012b), and O_3 (Chiusolo et al., 2011).

A few recent multicity and nationwide studies, also conducted in China (Huang et al., 2021; Li et al., 2021; Chen et al., 2018) and Italy (Gariazzo et al., 2023), examined copollutant models adjusting for

an expanded group of pollutants. Across a small number of studies, authors consistently observed positive associations that were relatively unchanged in models adjusting for PM_{10}, $PM_{2.5}$, SO_2, and O_3 (see Figure 3-15). In two analyses of similar study populations across larger Chinese cities, associations between short-term NO_2 exposure and respiratory (Li et al., 2021) or COPD mortality (Huang et al., 2021) were attenuated but still positive in copollutant models adjusted for CO. In contrast, a nationwide study in China reported comparable associations between two-day moving average (i.e., lag 0-1) NO_2 concentrations and respiratory mortality in single pollutant models and models adjusted for CO (Chen et al., 2018). While NO_2 concentrations are often highly correlated with $PM_{2.5}$ and traffic-related pollutants, such as CO, Gariazzo et al. (2023) reported weak correlations with $PM_{2.5}$ ($\rho = 0.06$) in a nationwide analysis of respiratory mortality in Italy. The weak relationship between NO_2 and $PM_{2.5}$ increases confidence that the NO_2-respiratory mortality association observed in this study was independent of $PM_{2.5}$.



List of abbreviations in alphabetical order: CI = confidence interval; CO = carbon monoxide; hr = hour; NO_2 = nitrogen dioxide; NR = not reported; O3 = ozone; PM_{10} = particulate matter with a nominal mean aerodynamic diameter less than or equal to 10 µm; $PM_{2.5}$ = particulate matter with a nominal mean aerodynamic diameter less than or equal to 2.5 µm; ppb = parts per billion; SO_2 = sulfur dioxide.
Notes: Black = studies from the 2016 Integrated Science Assessment for Oxides of Nitrogen; Red = recent studies and those not included in 2016 Integrated Science Assessment. Closed circles = effect of NO_2 in single pollutant models; open circles = effect of NO_2 in single pollutant models. Results are standardized to a 20 ppb increase in 24-hr avg NO_2, a 25 ppb increase in 1-hr max NO_2.
†Studies published since the 2016 Oxides of Nitrogen ISA.

Figure 3-15 Associations between short-term exposure to nitrogen dioxide and respiratory mortality with and without adjustment for copollutants.

3.8.3 Lifestages and Populations

3.8.3.1.1.1 Sex

Several recent studies examined effect modification by sex and reported generally consistent evidence of stronger NO_2-respiratory mortality associations among females [(Shin et al., 2022; Huang et al., 2021; Li et al., 2021; Chen et al., 2018); see Table 3-11]. Sex-differences were observed in analyses of respiratory mortality in large multi-city time-series analyses in Canada (Shin et al., 2022) and China (Li et al., 2021; Chen et al., 2018), as well as for NO_2-related COPD-related mortality in a nationwide time-series study in China (Huang et al., 2021). Although most studies observed stronger positive associations in females, a nationwide study in Italy reported comparable positive associations between short-term NO_2 exposure and respiratory mortality in analyses stratified by sex (Gariazzo et al., 2023).

3.8.3.1.1.2 Lifestage

Respiratory disease mortality rates increase with increasing age, and therefore the association between short-term NO_2 exposure and respiratory mortality disproportionately affects older adults. Results were inconsistent for several recent studies that conducted stratified analyses to examine potential effect measure modification by age (see Table 3-11). In a large time-series study in Switzerland, Perez et al. (2015) reported positive associations between short-term NO_2 and respiratory mortality that were of comparable magnitude in adults 65+ years old and 75+ years old. Similarly, Huang et al. (2021) did not observe notable difference in NO_2-related percent change in year of life lost (YLL) due to COPD mortality across age-stratified analyses of older adults living in China (65–4 years: 4.55% [95% CI: 1.85, 7.33]; 75–84 years: 3.31% [95% CI: 1.67, 4.98]; 85+ years: 4.13% [95% CI: 2.09, 6.2]). Other recent studies compared associations in older adults (65+ years) to all other ages (i.e., 0–64 years) and reported that associations increased in magnitude with increasing age (Gariazzo et al., 2023; Li et al., 2021; Chen et al., 2018). Notably, given the lower rate of respiratory disease-related mortality in the younger populations, effect estimates in those groups were much less precise.

Table 3-11 Epidemiologic studies evaluating effect-measure modification of the relationship between short-term NO_2 exposure and respiratory mortality by sociodemographic factors

Study	Location (Years)	EMM Reference Factor	EMM Comparison Factor	Direction of EMM[a]	Hazard Ratio (95% CI)	Spatial Scale of Effect Modifier
Age						
†Gariazzo et al. (2023)	Italy, nationwide (2013–2015)	0–64 yr	65–74 yr	–	EMM Reference Factor: 1.003 (0.23, 4.35) EMM Comparison Factor: 1.042 (0.49, 2.20)	Individual level
			75–84 yr	↑	1.25 (0.85, 1.84)	
			≥85 yr	↑	1.65 (1.10, 2.46)	
†Li et al. (2021)	48 Chinese Cities (2013–2017)	65–74 yr	75–84 yr	↑	EMM Reference Factor: 1.67% (-1.56, 5.00%)[b] EMM Comparison Factor: 2.85% (0.84, 4.91%)[b]	Individual level
			≥85 yr	↑	2.93% (1.65, 4.22%)[b]	
†Chen et al. (2018)	272 Chinese Cities (2013–2015)	5–64 yr	65–74 yr	↑	EMM Reference Factor: 1.02 (0.99, 1.04) EMM Comparison Factor: 1.04 (1.01, 1.07)	Individual level
			≥75 yr	↑	1.05 (1.04, 1.07)	
†Huang et al. (2023)	47 Canadian Cities (2001–2015)	<66 yr	≥66 yr	↓	EMM Reference Factor: 1.03 (0.97, 1.07)[c] EMM Comparison Factor: 1.00 (0.99, 1.01)[c]	Individual level
†Perez et al. (2015)	Switzerland, nationwide (2001–2010)	<75 yr	≥75 yr	↓	EMM Reference Factor: 1.11 (1.03, 1.20) EMM Comparison Factor: 1.10 (1.02, 1.19)	Individual level
Sex						
†Li et al. (2021)	48 Chinese Cities (2013–2017)	Female	Male	↓	EMM Reference Factor: 3.62% (1.88, 5.39%)[b] EMM Comparison Factor: 2.05% (0.47, 3.64%)[b]	Individual level
†Chen et al. (2018)	272 Chinese Cities (2013–2015)	Female	Male	↓	EMM Reference Factor: 1.056 (1.037, 1.075)	Individual level

Study	Location (Years)	EMM Reference Factor	EMM Comparison Factor	Direction of EMM[a]	Hazard Ratio (95% CI)	Spatial Scale of Effect Modifier
					EMM Comparison Factor: 1.039 (1.023, 1.056)	
†Shin et al. (2022)	47 Canadian Cities (1984–2012)	Female	Male	↓	EMM Reference Factor: 1.044 (1.008, 1.082) EMM Comparison Factor: 1.028 (0.982, 1.077)	Individual level
†Gariazzo et al. (2023)	Italy, nationwide (2013–2015)	Female	Male	–	EMM Reference Factor: 1.36 (1.01, 1.84) EMM Comparison Factor: 1.34 (0.97, 1.84)	Individual level

List of abbreviations in alphabetical order: avg = average; CI = confidence interval; hr = hour; NO_2 = nitrogen dioxide; ppb = parts per billion; yr = year.
Results are standardized to a 20 ppb increase in 24-hr avg NO_2 and a 25 ppb increase in 1-hr max NO_2.

[a]Up-facing arrow and yellow shading indicate that the effect of NO_2 is greater (e.g., larger risk of mortality) in the group with the factor evaluated than in the reference group. Down-facing arrow and blue shading indicate that the effect of NO_2 is smaller in the group with the factor evaluated than in the reference group. A dash and grey shading indicate that the effect of NO_2 is comparable between groups.

[b]Percent change in years of life lost.

[c]HR not standardized – per 12.8 ppb increase.

†Studies published since the 2016 Oxides of Nitrogen ISA.

3.8.4 Exposure Assignment

As discussed previously, exposure measurement error in epidemiologic studies can bias effect estimates. Chapter 2 provides a thorough discussion of the bias resulting from particular exposure assessment methods common to the epidemiologic literature and their impact on the inferences that can be drawn about health effects. To date, most studies of short-term NO_2 exposure and respiratory mortality assign exposure from central site monitors. A few recent studies have implemented hybrid prediction models to estimate NO_2 exposure at 1 km x 1 km grids (Gariazzo et al., 2023; Liu et al., 2022). Liu et al. (2022) modeled exposure across seven U.S. states using prediction models that incorporated combinations of monitoring data, chemical transport modeling, meteorological variables, and land use terms. In a nationwide study in Italy, Gariazzo et al. (2023) downscaled 5 km x 5 km chemical transport model estimates using a machine learning integrating similar spatial and spatiotemporal data as Liu et al. (2022). The exposure model used by Liu et al. (2022) performed better in out-of-sample cross-validation ($r^2 = 0.79$) than the model used by Gariazzo et al. (2023) ($r^2 = 0.60$). Each study reported positive associations between NO_2 exposure and respiratory mortality. The magnitude of the observed associations was similar to studies using central site monitors, though direct comparisons are complicated by differences in study populations and other methodological approaches.

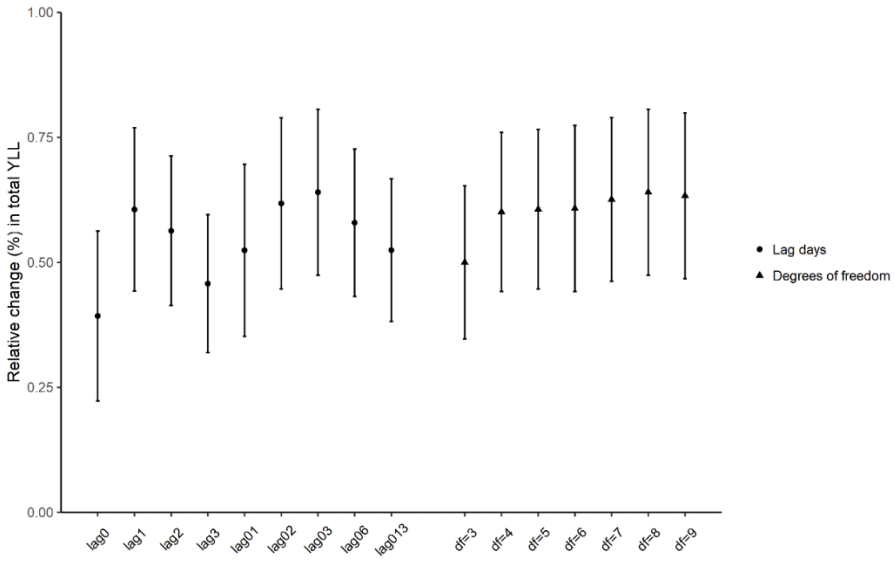
3.8.5 Alternative Methods for Confounder Control

Many traditional air pollution epidemiologic studies employ study designs and statistical methods to control for variables that could potentially confound the relationship between the exposure and outcome of interest. Some epidemiologic studies additionally attempt to account for potential unmeasured confounders. In a recent U.S. study with state-wide mortality data from seven states, Liu et al. (2022) employed a negative exposure control to adjust for potential unmeasured confounders. As a negative exposure control, the authors included a model term for NO_2 exposure on the day after death (lag -1). The assumption is that any omitted time-varying confounders that are associated with exposure on the same day as mortality are also likely to be associated with exposure on the subsequent day. Under the assumptions that lag -1 exposure is truly a negative control and that it is strong enough to correct for bias, the model could yield an effect estimate that is at least partially adjusted for unmeasured confounding, represented as the difference between the effect coefficient corresponding to exposure prior to mortality and the effect coefficient corresponding to exposure on the subsequent day. Liu et al. (2022) reported a larger increase in NO_2-related mortality after adjusting for the negative exposure control (4% [95% CI: NR]) compared to the uncorrected model (1.8% [95% CI: 0.0, 3.7]).

3.8.6 Model Specification

As discussed in Section 3.7.1.5, short-term epidemiologic studies of air pollution exposure often assess the sensitivity of results to different specifications of temporal and meteorology covariates. There was no discussion of model specification in studies of short-term NO_2 exposure and respiratory mortality in the 2016 Oxides of Nitrogen ISA. A few recent studies conducted sensitivity analyses to evaluate the impact of specifications of the spline *df* and lag structures of time and temperature covariates used to account for their relationship with the outcome (Gariazzo et al., 2023; Huang et al., 2021; Li et al., 2021). Across studies, authors noted some variation in the magnitude, but not the direction of effect estimates derived using different model specifications for time and temperature.

In a time-series analysis of respiratory mortality across 48 major Chinese cities, Li et al. (2021) observed positive associations between NO_2 concentrations and YLL from respiratory disease across models using various lag structures and *df* for temperature (see Figure 3-16). There was variation in the estimates across the temperature lag structures examined, and the results were most sensitive to less flexible control for temperature (i.e., $df = 3$). In another multicity study in China examining YLL due to COPD in a similar study population, Huang et al. (2021) noted sensitivity across model specifications of time covariates, though results were relatively unchanged for various *df* specified for temperature splines. The authors reported that the results were most sensitive to less stringent control for temporal trends, which is consistent with findings related to NO_2 associations with hospital admissions for aggregated respiratory conditions (see Section 3.7.1.5). Gariazzo et al. (2023) reported some minor variation in the magnitude of positive associations between NO_2 exposure and respiratory mortality in Italy corresponding to alternative knot placement and indicators in the modeling of temperature (e.g., apparent temperature). Together, these results suggest that while the magnitude of associations may be sensitive to the different model specifications of time and temperature covariates, the direction of observed associations remain relatively robust.



List of abbreviations in alphabetical order: df = degrees of freedom; ppb = parts per billion; YLL = years of life lost.
Source: Adapted from Li et al. (2021). Copyright permission pending.

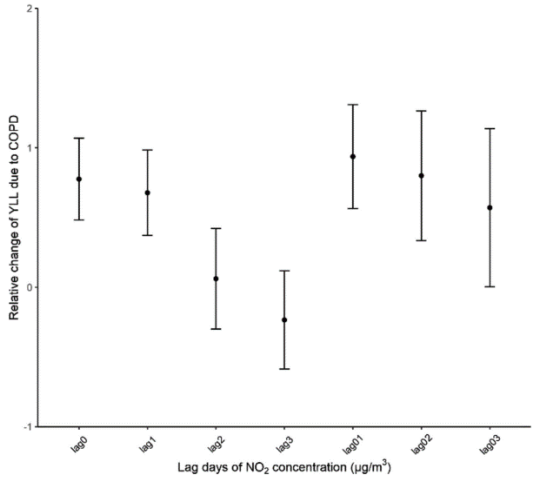
Figure 3-16 Estimates and corresponding 95% confidence intervals of total years of life lost due to respiratory disease per 5.32 ppb increase in nitrogen dioxide concentration (lag01 day) using different lag structures or degrees of freedom of temperature in 48 major cities, China, 2013–2017.

3.8.7 Lag Structure of Associations

As discussed in the 2016 Oxides of Nitrogen ISA, many studies reported that respiratory mortality was associated with shorter lags of NO_2 exposure. Most typically, respiratory mortality associations were reported with lags of 0−1 or 0−2 days, which are indicative of more immediate effects. Studies examining alternate lag structures provided equivocal results. Multicity studies in China provided some evidence of an effect of more prolonged short-term NO_2 exposures on respiratory mortality (Meng et al., 2013; Chen et al., 2012b). When examining single- and multi-day lags from 0 to 7 days, Chen et al. (2012b) and Meng et al. (2013) noted that the largest magnitude of an association was observed for lag 0−4 days. In contrast, multicity studies conducted in Italy reported the strongest evidence for an effect of NO_2 on respiratory mortality at later lags indicative of a delayed effect [i.e., lag 2−5 days (Faustini et al., 2013) or lag 1–5 days (Chiusolo et al., 2011)].

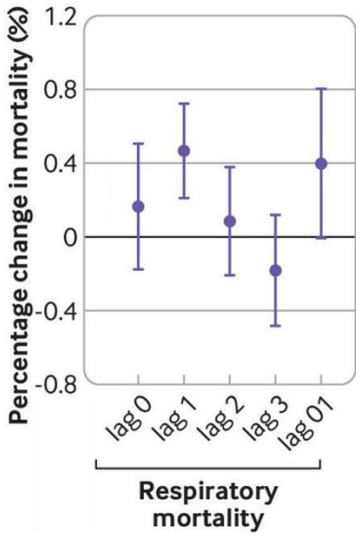
Many recent studies of respiratory mortality defined lags *a priori* as multiday averages of 0-1 or 0-2 days, as informed by previous studies described in the 2016 Oxides of Nitrogen ISA. A few recent studies examined the impact of different lag structures on the association between short-term NO_2 exposure and respiratory mortality. Some recent studies that considered lag structure of associations focused on a small number of lags ranging from 0 to 3 days. These studies included a global analysis of respiratory mortality and air pollution data from 362 cities (Meng et al., 2021) and large multicity time-

series analyses of COPD mortality (Huang et al., 2021) and respiratory mortality (Li et al., 2021; Chen et al., 2018) in China. Across these studies, there was a consistent pattern of stronger associations at earlier lags (e.g., 0 and 1 days) with decreasing magnitudes of associations at later lags (see Figure 3-17 and Figure 3-18 for examples from Huang et al. (2021) and Meng et al. (2021), respectively).



List of abbreviations in alphabetical order: COPD = chronic obstructive pulmonary disease; NO_2 = nitrogen dioxide; ppb = parts per billion; YLL = years of life lost; $\mu g/m^3$ = micrograms per cubic meter.
Source: Adapted from Huang et al. (2021). Copyright permission pending.

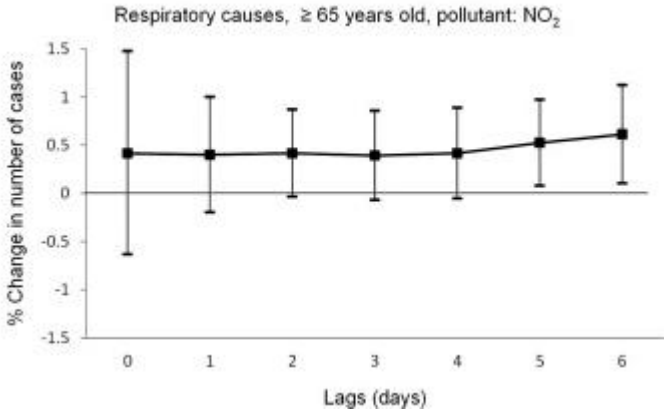
Figure 3-17 Relative change of years of life loss due to chronic obstructive pulmonary disease mortality associated with a 5.32 ppb increase in nitrogen dioxide on different lag days for NO_2 in 37 major cities, China, 2013–2017.



Source: Adapted from Meng et al. (2021). Copyright permission pending.

Figure 3-18 Relative risks of mortality associated with a 5.32 ppb increase in nitrogen dioxide on different lag days for NO_2 in 362 cities across 16 countries.

Other recent studies examined longer lags as far as six days before death and reported inconsistent results. Similar to the previously discussed studies that examined shorter lags, a recent analysis of NO_2-related respiratory mortality in 24 Canadian cities provides evidence of stronger associations with earlier single day lags (i.e., lags 0 through 2), with null associations reported for single day lags ranging from three to six days (Parajuli et al., 2021). Notably, the patterns of associations across lag structures are less clear in an analysis of the same population examining associations stratified by sex and season (Shin et al., 2022). In contrast, some recent studies provide evidence for effects of prolonged short-term NO_2 exposure on respiratory mortality. Perez et al. (2015) observed associations that were similar in magnitude across single day exposure lags ranging from zero to six days in a large time-series study in Switzerland (see Figure 3-19). Similarly, Gariazzo et al. (2023) conducted a nationwide analysis of over 100,000 respiratory mortalities in Italy and observed larger increases in risk of mortality corresponding to 6-day moving average NO_2 concentrations (lag 0–5: 27.6% [95% CI: 4.0, 56.4] per 20 ppb increase) relative to 2-day moving average (lag 0–1: 4.8% [95% CI: -5.6, 16.5]).



List of abbreviations in alphabetical order: NO_2= nitrogen dioxide; ppb = parts per billion.
Source: Adapted from Perez et al. (2015). Copyright permission pending.

Figure 3-19 Percent change in respiratory mortality associated with a 5.32 ppb increase in nitrogen dioxide on different lag days for NO_2 across 13 geographical regions of Switzerland.

3.8.8 Examination of Seasonal Differences

A limited number of studies evaluated in the 2016 Oxides of Nitrogen ISA indicated seasonal differences in the NO_2-respiratory mortality relationship with evidence of larger associations in the warm season in Italy (Chiusolo et al., 2011; Bellini et al., 2007). Several recent multicity studies conducted in Canada (Shin et al., 2022; Parajuli et al., 2021; Shin et al., 2021) and China (Huang et al., 2021; Li et al., 2021) report similar seasonal differences to those described in the 2016 Oxides of Nitrogen ISA.

Recent analyses of NO_2-related respiratory mortality in 24 Canadian cities provide evidence of stronger associations with previous-day NO_2 concentrations in the warm season (4.65% [95% CI: -0.34,

9.9] per 20 ppb) relative to the cold season (1.61% [95% CI: -0.88, 4.16]) (Parajuli et al., 2021; Shin et al., 2021). Recent multicity studies conducted in China provided additional evidence of seasonal or temperature-related differences in mortality risk associated with NO_2 exposure. In an analysis of COPD-related mortality in 37 Chinese cities, Huang et al. (2021) reported a larger NO_2-associated increase in YLL due to COPD mortality in the warm season (5.14% [95% CI: 2.47, 7.87] per 20 ppb increase in lag 0–1 NO_2) compared to the cool season (1.55% [95% CI: 0.13, 2.99]). Li et al. (2021) examined respiratory mortality in an expanded analysis including 48 Chinese cities over the same period and reported stronger associations on days with higher temperatures.

3.8.9 Conclusions and Remaining Uncertainties

A large number of recent epidemiologic studies support and extend conclusions from the 2016 Oxides of Nitrogen ISA that indicated an association between short-term NO_2 exposure and respiratory mortality. Recent evidence is provided by studies with greater geographic diversity, including several multicity studies in North America and analyses of a large dataset collected from over 360 cities in 16 different countries across the world. The recent evidence base also includes expanded evaluation of the C-R relationship that suggests an approximately linear relationship between short-term NO_2 exposure and respiratory mortality with no evidence of a threshold. Additionally, whereas studies evaluated in the 2016 Oxides of Nitrogen ISA utilized central site monitors, a few recent studies reported positive associations between respiratory mortality and short-term NO_2 concentrations estimated using more spatially refined hybrid prediction models. Most of the evaluated studies of respiratory mortality defined lags a priori as multiday averages of 0–1 or 0–2 days. In contrast to studies described in the 2016 Oxides of Nitrogen ISA that examined alternative lag structures and reported equivocal results, recent studies revealed a consistent pattern of stronger associations at earlier lags (e.g., zero and one days) with decreasing magnitudes of associations at later lags.

Several recent studies evaluated potential effect modification by season or individual-level biological factors, such as sex or lifestage. Consistent with evidence from the 2016 Oxides of Nitrogen ISA, several recent studies in Canada and China provide further evidence that NO_2 associations with respiratory mortality are larger in magnitude in the summer or warm season compared to the winter or cold season. This seasonal pattern of associations is consistent with evidence for other respiratory endpoints, including asthma exacerbation (see Section 3.3.1.1.4) and respiratory hospital admissions and ED visits aggregated across specific conditions (see Section 3.7.1.1). There is also consistent recent evidence demonstrating stronger associations among females compared to males. Results were equivocal across studies that stratified by age.

Recent studies also include novel or expanded examination of various study methodologies, including alternative methods for confounder control and examination of model specification. A recent study of respiratory mortality using a negative exposure control provides evidence of an association

independent of potential unmeasured time-varying confounders. Additionally, recent studies observed variance in the magnitude of associations across different model specifications for temporal splines and meteorological covariates. Despite the observed variance in magnitude of associations, authors reported consistent positive associations across different specifications of the potential confounders. These analyses reduce uncertainty regarding potential unmeasured or residual confounding.

A notable uncertainty in the epidemiologic evidence base is the potential for copollutant confounding. While a limited number of studies reported that associations between short-term NO_2 exposure and respiratory mortality remained relatively unchanged in copollutant models adjusting for PM_{10}, $PM_{2.5}$, SO_2, CO, or O_3 (i.e., similar in magnitude or attenuated slightly, but still positive), evaluation of potential copollutant confounding by traffic-related pollutants remains limited, and high correlations between NO_2 and these pollutants complicate the interpretation of the available copollutant models.

3.9 Biological Plausibility

As discussed in the 2016 Oxides of Nitrogen ISA, NO_2 and NO have distinct chemical properties that impact initiating events related to downstream health effects [see Section 4.3 of U.S. EPA (2016)]. Given the lack of recent mechanistic evidence related to the impacts of short-term NO exposure on the cardiovascular system, the following discussion will focus on plausible pathways linking NO_2 exposure to respiratory effects. As detailed in Chapter 2, NO_2 is a highly reactive gas that occurs as a radical that, once inhaled, encounters the epithelial lining fluid (ELF) of the respiratory tract. In the ELF, NO_2 chemically interacts with antioxidants, unsaturated lipids, and other compounds generating reactive oxygen and nitrogen species and provoking inflammation. Experimental evidence suggests several plausible pathways by which short-term exposure to NO_2 can result in respiratory effects. The structure of the biological plausibility figures is described in the Integrated Synthesis, Section IS.4.1.1.

Epidemiologic evidence showed consistently positive associations between short-term NO_2 levels and exacerbation of asthma. One plausible pathway for NO_2-induced exacerbation of asthma involves the activation of inflammation and oxidative stress signaling. Given the interrelated nature of oxidative stress and inflammation, these processes are grouped together in Figure 3-20. Reaction of NO_2 with antioxidant molecules can lead to inflammation and oxidative stress and, as discussed in Section 3.6.4, experimental studies in humans and in animal models provide evidence for the activation of cellular inflammatory signaling pathways and evidence of increased oxidative damage following inhalation of NO_2. For example, a controlled human exposure to 2,000 ppb NO_2 for four hours resulted in a decrease in the antioxidants urate and ascorbate in the BAL one and a half hours after exposure (Kelly et al., 1996). The importance of oxidative stress following NO_2 exposure is supported by another study showing that supplementation with the antioxidants ascorbate or α-tocopherol decreased the levels of oxidized lipids in BAL of human subjects following a 3-hour exposure to NO_2 at 4,000 ppb (Mohsenin, 1991). Antioxidant

(α-tocopherol) deficency resulted in reduced glutathione peroxidase activity in animals exposed to NO_2 (Ayaz and Csallany, 1978). Supplementation with α-tocopherol protected against this impairment.

Inflammation is a key feature of many respiratory diseases including asthma and asthma exacerbation. Controlled human exposure studies have consistently shown increases in ECP, a marker of epsinophilic inflammation, although evidence of increased inflammatory cell infitration is not consistently seen. Animal studies of short-term NO_2 exposure in the absence of allergen sensitization show inconsistent effects on inflammatory mediators production and recruited immune cell numbers although recent studies support the potential of NO_2 exposure to elicit lung inflammation (Lu et al., 2023; Yue et al., 2018; Han et al., 2017; Yue et al., 2017). Recent animal studies of gestational exposure show increased inflammation in offspring animals (Yue et al., 2018; Yue et al., 2017) which provides plausibility for the positive epidemiologic associations between short-term NO_2 concentrations and asthma exacerbation in children. Animal models of asthma provide mixed results regarding inflammation with some studies finding increased inflammatory markers with short term exposure to relatively high NO_2 exposures (>5 ppm). Consistent increases in inflammation in animal models is reported at lower NO_2 concentrations with longer exposures to NO_2 (see Chapter 4, Section 4.3.2.2). Inflammation is a hallmark of other lung diseases including COPD so it is plausible that inflammation resulting from NO_2 exposure could exacerbate COPD symptoms, potentially requiring clinical intervention.

While inflammation is a necessary part of the body's immune response, excessive or ectopic inflammation can result in superfluous oxidative and tissue damage and impairment of lung barrier permeability. Indeed, biomolecular markers of lung damage or increased epithelial barrier permeability, such as increased LDH or albumin in the BAL, have been reported in some, but not all, human studies of NO_2 exposure. Animal studies have shown histologic signs of lung repair, including lung epithelial cell hyperplasia, which is thought to be indicative of lung repair processes to replace injured or dead lung cells following NO_2 exposure. Ather et al. (2010) showed that NO_2 exposure in wild-type mice increased markers of airway inflammation, while mice deficent in a key inflammatory pathway had reduced lung inflammatory cell infitration and reduced histologic lung injury following NO_2 exposure. Similarly, deficencies in antioxidant molecules in animal models resulted in increased oxidative damage and BAL protein levels following NO_2 exposure (Hatch et al., 1986; Sevanian et al., 1982a). Because of this, a solid arrow is used in Figure 3-20 between inflammation and oxidative stress and lung damage. It has also been posited that increased permeability of the lung epithelium can allow for greater penetration of allergens and promote allergic sensitization that could also play a role in asthma development (Hellings and Steelant, 2020).

Excessive or repeated lung damage from oxidative stress and inflammation can result in upregulation of repair processes like extracellular matrix deposition that over time can lead to remodeling of the airway. Airway remodeling is a commonly seen in asthmatic airways. Animal studies have shown histologic changes including extracellular matrix deposition around the airways following NO_2 exposure

(Han et al., 2017) . This airway remodeling results in stiffening of the airway which can, in part, underlie increased airway resistance and lung obstruction seen in asthma.

A second pathway for NO_2-mediated increases in asthma exacerbation is through the augmentation of allergic responses. As discussed in Section 3.3.3.3, allergic asthma responses are typified by production of IgE and a Th2 cytokine response (namely IL-4, IL-5, and IL-13). Many animal studies have shown a shift toward a Th2 response following NO_2 exposure even in the absence of allergen sensitization (see Section 3.3.3.3). Studies of animals sensitized with allergen support these findings and show heightened allergic-related cytokines and IgE in response to NO_2 exposure. An animal study of mice deficent in inflammatory signalinag thorugh TLR 2 and MyD66 showed protection against NO_2-induced BAL inflammatory cell iniltration, excess mucus proditciont and antigen-specific IgE and IgG suggesting that there is a link between NO_2-induced inflammation and heightened allergic responses (Bevelander et al., 2007). Although these heightened allergic responses are impacted by inflammatory signaling, heightened inflammation is also a byproduct of allergic sensitization and it is unclear if inflammation is always necessary to drive the heightened allergic responses or is secondary to increased activation of allergic signaling pathways. In either case this augmented allergic phenotype following NO_2 exposure is seen concurrently with increases in airway reactivity which as mentioned above can underlie the reduction in lung function and increased airway resistance seen in those with asthma during an asthma attack.

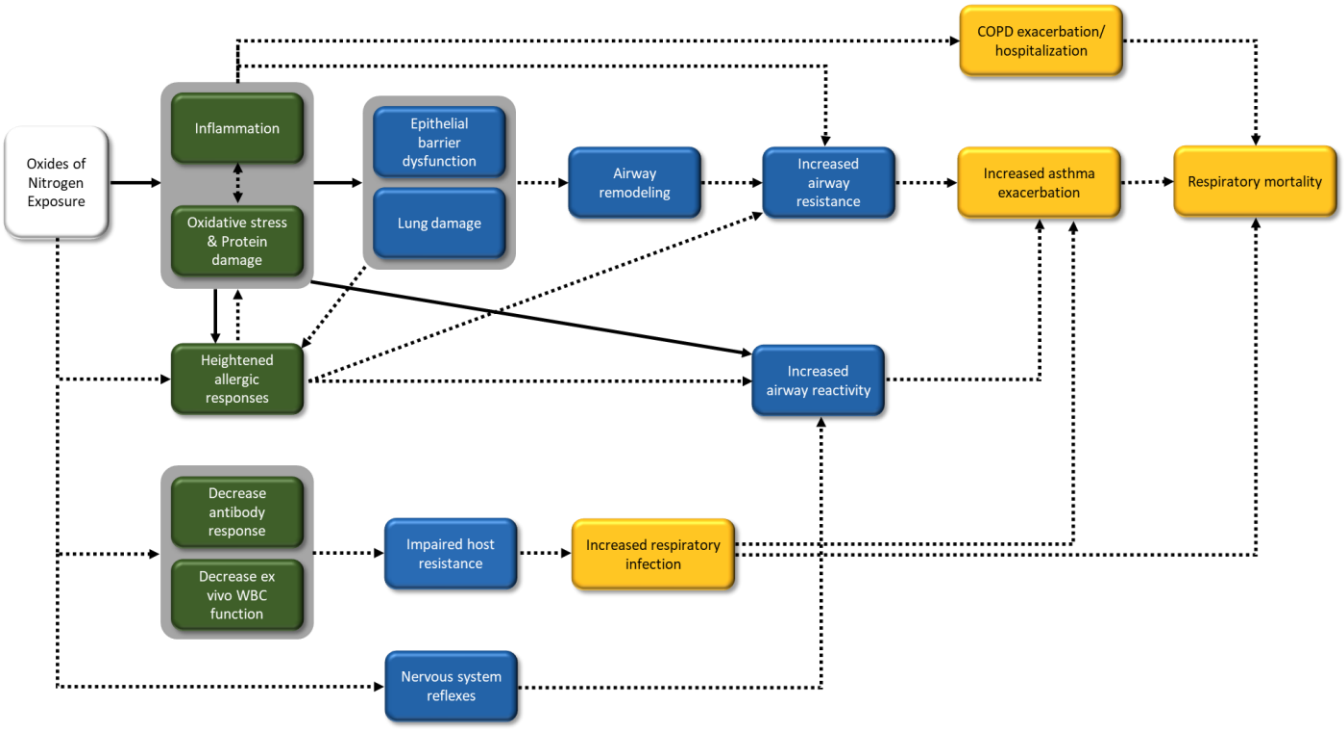
Increased reactivity of the airways to a constricting stimulus is a hallmark of asthma. Meta analyses of controlled human exposure studies of asthmatics at rest have reported an increase in the fraction of individuals that experience increased airway reactivity post-exposure to NO_2 (Brown, 2015; Goodman et al., 2009). Increased reactivity could underlie the exacerbation of asthma symptoms which, if severe enough, can lead to emergency department visits or hospitalization.

Another pathway for respiratory-related effects resulting from short-term NO_2 exposure relates to suppression of host defense mechanisms. Animal toxicological studies consistently show that NO_2 exposure suppresses ex vivo white blood cell function and reduces humoral antibody responses. While not consistent, there are at least some instances of reduced macrophage function demonstrated in controlled human exposure studies (Devlin et al., 1999; Frampton et al., 1989). This suppression of immune function with NO_2 exposure can thus plausibly result in more frequent or severe lung infections. This is of particular importance for those with underlying lung disease like asthma or COPD as exacerbations of symptoms are known to triggered by lung infection.In those with underlying disease or who are otherwise immunocompromised, infection can lead to the need for clinical care, hospitalization, or in severe cases mortality.

A final pathway that may potentially be relevant to respiratory effects involves NO_2-induced changes to nervous system reflexes. As discussed in the 2016 Oxides of Nitrogen ISA, some animal studies have reported increases in respiratory rate and decreased tidal volume that is consistent with activation of respiratory irritation responses (Murphy et al., 1964). However, human exposure studies

have not replicated the increased respiratory rates. In addition, human studies showed that atropine and β agonists did not impact NO_2-mediated increased airway resistance but the response was blocked by a histamine-suppressive agent suggesting that neuronal relfexes may not be relevant to airway resistance changes in humans (von Nieding and Wagner, 1979).

Taken together, the available evidence indicates multiple potentially intertwined pathways that could plausibly result in the respiratory health effects shown in epidemiologic studies to be associated with NO_2 exposures. The NO_2-induced pathways supported by the strongest evidence are those involving oxidative stress and inflammation and heightened allergic response.



Note: The boxes represent the effects for which there is experimental or epidemiologic evidence related to oxides of nitrogen exposure, and the arrows indicate a proposed relationship between those effects. Solid arrows denote evidence of essentiality as provided, for example, by an inhibitor of the pathway used in an experimental study involving oxides of nitrogen exposure. Dotted arrows denote a possible relationship between effects. Shading around multiple boxes is used to denote a grouping of these effects. Arrows may connect individual boxes, groupings of boxes, and individual boxes within groupings of boxes. Progression of effects is generally depicted from left to right and color coded (white, exposure; green, initial effect; blue, intermediate effect; orange, effect at the population level or a key clinical effect). Here, population-level effects generally reflect results of epidemiologic studies. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below. The structure of the biological plausibility sections and the role of biological plausibility in contributing to the weight-of-evidence analysis used in this ISA as discussed in Section 3.11.

Figure 3-20 Potential biological plausibility pathways for respiratory effects associated with short-term exposure to oxides of nitrogen.

3.10 Summary of Lifestages and Populations

Some populations and lifestages may be at greater risk of oxides of nitrogen-related health effects than the general population. Higher risks could be due to intrinsic,[7] extrinsic,[8] and/or exposure-related factors. In assessing the recent evidence, these factors are identified, evaluated, and characterized to inform conclusions on the populations and lifestages that may be at increased risk. The systematic approach used to evaluate factors that may increase the risk of a population or specific lifestage to an air pollutant-related health effect is described in more detail in the Preamble to the ISAs (U.S. EPA, 2015a) and Volume 2 of the Integrated Review Plan for the Primary National Ambient Air Quality Standards for Oxides of Nitrogen (U.S. EPA, 2024). The evaluation of factors that could place particular groups at increased risk focuses on health effect studies that conduct stratified analyses to compare populations or lifestages exposed to similar air pollutant concentrations within the same study design. For the evaluation of these studies, important considerations include whether the stratified analyses were planned a priori or were post hoc analyses, whether the study conducted multiple comparisons, and whether there were small sample sizes in individual strata. These study design issues can increase the probability of finding associations by chance or reduce the power to detect associations in subgroup analyses. Other epidemiologic studies that do not stratify results but instead examine a specific population or lifestage can provide supporting evidence for the pattern of associations observed in studies that formally examine effect measure modification. Additionally, experimental studies that examine health effects exclusively in human subjects or animal models with a particular factor, such as pre-existing disease, are also important lines of evidence to evaluate because they inform judgments of the coherence and biological plausibility of effects observed in epidemiologic studies, as well as the independent effect of NO_2 exposure.

The objective of this section is to integrate information from preceding sections and describe the basis for characterizing the evidence for particular factors to increase risk of NO_2-related respiratory effects as adequate, suggestive, inadequate, or no effect. The classification provides a consistent basis for evaluating the evidence that a population or lifestage may be at increased risk and for describing the overall confidence in the evidence. Consistent with the characterization of evidence for health effects in the general population, the greatest emphasis is placed on patterns in results across studies. These classifications are not direct assessments of causality but rather can inform consideration of whether a NAAQS provides an adequate margin of safety across groups in the population.[9] For NO_2, judgments

[7]Intrinsic factors such as genetics, lifestage, or disease status can contribute to larger or more serious responses to a particular pollutant exposure, and physiological differences between groups (e.g., differences in breathing patterns between children and adults) can contribute to higher internal pollutant doses in some populations.
[8]Extrinsic factors (e.g., nutritional status) and exposure-related factors (e.g., working outdoors, living near roadways or other pollution sources) can also contribute to increased risk, and many of the characteristics commonly used to classify populations potentially at increased risk (e.g., income, educational attainment) are surrogates for the combined impact of several intrinsic, extrinsic, and exposure-related factors.
[9]Although the at-risk classifications are not direct assessments of causality, evidence for at-risk groups or factors inform causality determinations in several ways. Consistent evidence that at least one population subgroup is at risk of an NO_2-related health effect provides strong support for causality determinations. Evidence for effect

about factors that may place some groups at increased risk emphasize evidence for asthma exacerbation, given the strong evidence for that outcome. The factors that are evaluated in this section include pre-existing diseases and conditions (see Section 3.10.1), sociodemographic factors (see Section 3.10.2), and diet (see Section 3.10.3). Table 3-1 at the end of this section summarizes the evidence for each factor to increase risk of NO_2-related health effects.

3.10.1 Pre-Existing Disease/Conditions

Individuals with pre-existing disease or medical conditions may be considered at greater risk for some air pollution-related health effects because they may be in a compromised biological state depending on the disease/condition and severity. The large proportions of the United States population affected by various chronic diseases or medical conditions underscore the importance of characterizing the potential risk of NO_2-related health effects for affected populations. The following sections integrate the evidence evaluating potential increased risk due to pre-existing asthma (see Section 3.10.1.1) and obesity (see Section 3.10.1.2).

3.10.1.1 Pre-existing Asthma

An estimated 8.0% of adults and 9.3% of children in the United States have asthma (Blackwell et al., 2014; Bloom et al., 2013), which is the leading chronic illness affecting children. The 2016 Oxides of Nitrogen ISA concluded that evidence was adequate to conclude that people with asthma are at increased risk for NO_2-related health effects. The strongest evidence supporting increased risk in people with asthma came from consistent cross-disciplinary evidence for independent effects of NO_2 on asthma exacerbation, along with controlled human exposure studies that provide compelling evidence that people with asthma are at greater risk for NO_2-related respiratory effects than people without asthma. Recent evidence supports these conclusions, further demonstrating increased risk of NO_2 effects in people with asthma.

As discussed in the 2016 Oxides of Nitrogen ISA, controlled human exposure studies demonstrate increased airway responsiveness and allergic inflammation in adults with asthma exposed to NO_2 (see Sections 3.3.3.1 and 3.3.3.3.1). Recent experimental animal studies further support NO_2-induced allergic inflammation (see Section 3.3.3.3.3), which may promote asthma exacerbation through increases in airway reactivity, reductions in lung function, and/or increased airway resistance. The experimental evidence is coherent with – and provides biological plausibility for the independent nature of – the NO_2-

modification can also explain heterogeneity in results across studies, which could reduce uncertainties regarding inconsistent evidence. Finally, at-risk factors such as genetics or pre-existing conditions can provide supporting information on mechanisms contributing to NO_2-related health effects. Notably, the lack of evidence for differential effects in subgroups where there is otherwise evidence of an NO_2-related health effect in the general population does not weaken the overall evidence supporting a causality determination.

related increases in asthma symptoms in children (see Section 3.3.2.1) and asthma hospital admissions and ED visits among subjects of all ages and children (see Section 3.3.1) observed across a large body of epidemiologic studies.

In addition to strong evidence for asthma exacerbation, the controlled human exposure studies discussed previously also demonstrate clear differences in responses to NO_2 exposure among participants with and without asthma. Whereas increased airway responsiveness has been induced in adults with asthma following NO_2 exposures at rest in the range of 100−300 ppb, results for healthy adults are inconsistent and increased airway responsiveness is only reported for NO_2 exposures >1,000 ppb. Epidemiologic evidence does not clearly show differences in NO_2-related respiratory effects between children with asthma and children in the general population, undifferentiated by asthma status.

Overall, recent evidence continues to support the evidence available in the 2016 Oxides of Nitrogen ISA and is adequate to conclude that individuals with pre-existing asthma are at greater risk of NO_2-related respiratory effects based on the substantial and consistent evidence across scientific disciplines.

3.10.1.2 Pre-existing Obesity

Obesity is a condition defined by abnormal or excessive body fat accumulation. Nearly 20% of children and over 40% of adults are estimated to be obese based on CDC growth charts (Stierman et al., 2021). Being obese or overweight could increase the risk of NO_2-related health effects through multiple mechanisms including persistent, low-grade inflammation. Poor diet and chronic diseases often occur with obesity and could be part of the pathway by which obesity increases the risk of NO_2-related health effects or could act in combination with obesity to increase risk. The 2016 Oxides of Nitrogen ISA did not consider any short-term exposure studies that evaluated obesity as a factor that could modify the risk of respiratory effects.

A recent epidemiologic study of Medicaid beneficiaries reported a larger NO_2-related increase in hospital admissions for asthma in neighborhoods with high average BMIs compared to neighborhoods with medium to low average BMIs (Wei et al., 2022). There is significant uncertainty in interpreting an area-level obesity variable, which does not inform associations among individuals with high BMIs. Particularly at an area-level, BMI is strongly correlated with several sociodemographic and behavioral factors, including diet, neighborhood resources, and neighborhood safety. Accordingly, the limited evidence is inadequate to determine whether individuals with obesity are at increased risk for NO_2-related respiratory effects.

3.10.2 Sociodemographic Factors

3.10.2.1 Lifestage

Lifestage refers to a distinguishable time frame in an individual's life characterized by unique and relatively stable behavioral and/or physiological characteristics that are associated with development and growth (U.S. EPA, 2014). Differential health effects of NO_2 across lifestages could be due to several factors. With regard to children, the human respiratory system is not fully developed until 18−20 years of age; therefore, it is biologically plausible for children to have increased intrinsic risk for respiratory effects if exposures are sufficient to contribute to potential perturbations in normal lung development. Older adults, typically considered those 65 years of age or greater, have weakened immune function, impaired healing, decrements in pulmonary and cardiovascular function, and greater prevalence of chronic disease (Blackwell et al., 2014), which may contribute to, or worsen, respiratory effects related to NO_2 exposure. The following sections describe the evidence for children (see Section 3.10.2.1.1) and older adults (see Section 3.10.2.1.2) being at increased risk of NO_2-related respiratory effects.

3.10.2.1.1 Children

Epidemiologic evidence from high-quality studies across diverse locations (i.e., United States, Canada, Europe, Asia, and Australia) consistently demonstrates that short-term increases in ambient NO_2 concentration are associated with larger increases in asthma-related hospital admissions, ED visits, or outpatient visits among children than adults [(Bi et al., 2023; Wei et al., 2022; Alhanti et al., 2016; Son et al., 2013; Sinclair et al., 2010; Ko et al., 2007; Hinwood et al., 2006; Peel et al., 2005; Atkinson et al., 1999; Anderson et al., 1998); see Figure 3-4]. Although not all studies documented an intention to compare lifestages a priori, each evaluated a large number of hospital admissions or ED visits and had ample statistical power to detect associations in stratified analyses. Most results are based on comparisons between children ages 0−14 years and people ages 15−64 years, and these show NO_2-associated increases in asthma hospital admissions that are 1.8 to 3.4-fold greater in children (Son et al., 2013; Ko et al., 2007; Atkinson et al., 1999; Anderson et al., 1998). Other studies examining hospital admissions or ED visits for asthma reported that a 25 ppb increase in 1-hour max NO_2 concentrations was associated a ~9 to 13% increase in risk for children ages 5–18 years and no change in risk for adults ages 35–64 years (Wei et al., 2022) or 65+ years (Bi et al., 2023; Alhanti et al., 2016). Overall, the consistent epidemiologic evidence for larger NO_2-related asthma exacerbation in children relative to adults is adequate to conclude that children are at increased risk for NO_2-related health effects. This is also supported by recent animal evidence reporting increased NO_2-induced allergic asthma phenotype in mice gestationally exposed to NO_2 when sensitized and challenged with OVA later in life (Yue et al., 2018; Yue et al., 2017).

3.10.2.1.2 Older Adults

The 2016 Oxides of Nitrogen ISA indicated that older adults are at increased risk for NO_2-related respiratory effects. A limited number of studies assessed NO_2-related risk of asthma exacerbation among older and younger adults and generally demonstrated larger associations (one to threefold) in adults ages 65 years or older than among individuals ages 15−64 years or 15−65 years (Ko et al., 2007; Villeneuve et al., 2007; Migliaretti et al., 2005; Anderson et al., 1998). However, results from studies in the 2016 Oxides of Nitrogen ISA were not entirely consistent, as some studies did not observe increased risk in older adults (Son et al., 2013; Hinwood et al., 2006). Recent epidemiologic studies provide additional conflicting evidence, including a study that reported no association between short-term NO_2 exposure and ED visits for asthma across adult age groups [18–49, 50–64, and 65+ years; (Bi et al., 2023)] and a study that reported associations between NO_2 exposure and risk of ED visits for asthma in younger adults (19–39 and 40–64 years) but not in adults 65 years and older (Alhanti et al., 2016). Controlled human exposure studies examining older adults were limited to healthy adults, and thus do not inform the evidence base for asthma exacerbation.

Epidemiologic studies of mortality from respiratory causes also examined effect modification by age. A pooled analysis of 16 European cohorts described in the 2016 Oxides of Nitrogen ISA reported that NO_2-respiratory mortality associations were stronger among older adults (≥60 years) than younger adults (<60 years) (Dimakopoulou et al., 2014). Recent studies that compared associations in older adults (65+ years) to all other ages (i.e., <64 years) reported that associations increased in magnitude with increasing age (Gariazzo et al., 2023; Li et al., 2021; Chen et al., 2018). Other studies compared age-stratified NO_2-related risk of respiratory mortality across age groups that only included older adults (e.g., 65–74, 75–84, and 85+ years) and did not observe evidence of effect modification across those groups (Huang et al., 2021; Perez et al., 2015).

Whereas evidence from the 2016 Oxides of Nitrogen ISA generally supported larger NO_2-related risk of hospital admissions and ED visits for asthma in older adults compared to younger adults, the recent evidence is less consistent. Across a limited number of studies that examined effect modification by age in respiratory mortality studies, associations were consistently larger in adults ages 65 or older than in younger adults. However, given high correlations with other traffic-related pollutants, it is uncertain whether NO_2 exposure has an independent effect on respiratory mortality. Together, given the inconsistency in the asthma exacerbation evidence in older adults and the uncertainty in the independent nature of the NO_2-respiratory mortality relationship, the evidence is suggestive that older adults are at increased risk for NO_2-related health effects.

3.10.2.2 Sex

A vast number of health conditions and diseases have been shown to differ by sex, with some indication that there may be sex-specific differences in the relationship between air pollution and health

effects. The 2010 United States census indicates an approximately equal distribution of males and females in the United States: 49.2% male and 50.8% female (Howden and Meyer, 2011). However, the distribution varies by age with a greater prevalence of females above 65 years of age compared to males. Similarly, there are complex sex-specific disparities in disease status that similarly vary by age. For example, asthma prevalence and severe exacerbations are more common in boys than girls throughout early childhood. However, those patterns are reversed in adolescence and into adulthood, with females becoming more likely to have asthma and experience related symptoms. Thus, the public health implications of potential sex-based differences in air pollution-related health effects may vary among age groups within the population.

A few studies that were evaluated in the 2016 Oxides of Nitrogen ISA examined sex differences in the associations between short-term NO_2 exposure and asthma exacerbation. Evidence equally pointed to increased risk of hospital admissions for asthma in female children (Lin et al., 2004), increased risk of wheeze in male children (Mann et al., 2010), and no difference in risk between sexes for airway inflammation, pulmonary function, or respiratory symptoms in children with asthma (Sarnat et al., 2012; Liu et al., 2009). Evidence was similarly inconsistent across several recent studies of hospital admissions or ED visits for asthma (see Section 3.3.1.1.5), including no difference in NO_2-related risk between sexes (Cisneros et al., 2021; Szyszkowicz et al., 2018) and increased risk for males (Wei et al., 2022; Alhanti et al., 2016). Collectively, the results are equivocal.

Analyses of short-term NO_2 exposure and respiratory mortality revealed more consistent sex-specific patterns (see Section 3.8.3). Several recent studies in Canada (Shin et al., 2022) and China (Huang et al., 2021; Li et al., 2021; Chen et al., 2018) examined effect modification by sex and reported generally consistent evidence of stronger NO_2-respiratory mortality associations among females. In contrast, a nationwide study in Italy reported comparable positive associations between short-term NO_2 exposure and respiratory mortality across sexes (Gariazzo et al., 2023).

Collectively, the evidence for sex-specific differences in NO_2-related risk of respiratory effects is inconsistent. Differences in risk between males and females are not consistently observed for associations of short-term NO_2 exposure with asthma exacerbation, the respiratory effect for which evidence most strongly indicates independent relationships with short-term NO_2 exposure (see Section 3.3.4). In contrast, studies of respiratory mortality related to short-term NO_2 exposure provide some limited but generally consistently evidence of increased risk among females. Because it is uncertain whether NO_2 exposure has an independent effect on respiratory mortality, the evidence is suggestive that females are at increased risk for respiratory effects of short-term NO_2 exposure.

3.10.2.3 Race/Ethnicity

Disparities in health outcomes across racial groups are likely to reflect race as a proxy measure for a complex set of factors that can include differences in nutrition, housing, income, health care, etc.

This section describes racial and ethnic disparities in NO_2-related respiratory effects, while some of the ensuing sections address other factors for which race may be a proxy (i.e., SES and diet).

The evidence on race and ethnicity-modified risk of NO_2-related respiratory effects is largely inconsistent. A single study evaluated in the 2016 Oxides of Nitrogen ISA reported that short-term NO_2 exposures were associated with a larger risk of ED visits for Black children compared to Hispanic children, but no difference was observed between Black children and white children (Grineski et al., 2010). Analyses from several recent studies variably indicated 1) no differences in risk of asthma exacerbation across racial/ethnic groups (Wei et al., 2022) (Sharma et al., 2023); 2) higher risk among white children compared to Black, Hispanic, or Asian children (Cisneros et al., 2021); and 3) higher risk among non-white children compared to white children (Alhanti et al., 2016). Given the inconsistent results across epidemiologic studies, the evidence is inadequate to determine whether certain racial or ethnic groups are at increased risk for NO_2-related respiratory effects.

3.10.2.4 Socioeconomic Status

SES is a composite measure that usually consists of economic status measured by income, social status measured by education, and work status measured by occupation. Given the variety of indicators that are used to assess SES, and the underlying characteristics of many lower SES communities (e.g., higher prevalence of psychosocial stressors (Diez Roux and Mair, 2010; O'Neill et al., 2003)), it can be difficult to synthesize results across studies. Of the limited number of respiratory effects studies in the 2016 oxide of nitrogen ISA that examined potential effect modification by SES or social stressors, each used different indicators of SES or social stress. Some studies indicated increased risk of NO_2-related asthma exacerbation and incidence in groups of children with high exposure to community violence (Clougherty et al., 2007) or a lack of health insurance (Grineski et al., 2010), but no difference in risk across census block income levels (Lin et al., 2004). A limited number of recent studies of asthma exacerbation similarly observed inconsistent results. One study observed stronger associations between NO_2 exposure and pediatric hospital admissions or ED visits for asthma in neighborhoods with indicators of higher SES (Sharma et al., 2023), while another recent study reported the opposite pattern (Wei et al., 2022). Notably, given the underlying population of Medicaid beneficiaries, the results from Wei et al. (2022) may not be directly comparable to Sharma et al. (2023). Given the inconsistent results across epidemiologic studies, the evidence is inadequate to determine whether low SES populations are at increased risk for NO_2-related respiratory effects.

3.10.3 Diet

Diet is an important influence on health and therefore could plausibly influence air pollutant-related health effects. While there are no recent studies that examined dietary modification of short-term

NO_2 exposure and respiratory effects, experimental studies evaluated in the 2016 Oxides of Nitrogen ISA provide evidence that dietary intake of vitamins modifies airway responsiveness, pulmonary inflammation, and oxidant balance following short-term exposure to NO_2. The strongest evidence for diet modifying NO_2-related health effects comes from a controlled human exposure study that demonstrated that healthy adults with diets supplemented with Vitamin C had smaller increases in airway responsiveness following NO_2 exposure compared to adults with a normal diet (Mohsenin, 1987b). This finding is supported by experimental evidence in humans and rodents that higher dietary Vitamin E and/or C reduces NO_2-induced pulmonary inflammation and modulates the oxidant/antioxidant balance (Mohsenin, 1991; Hatch et al., 1986; Elsayed and Mustafa, 1982; Sevanian et al., 1982b; Selgrade et al., 1981; Ayaz and Csallany, 1978). Airway responsiveness is a hallmark of asthma exacerbation, while pulmonary inflammation and formation of oxidation products are early events in the biologically plausible pathways by which short-term NO_2 exposure can result in asthma exacerbation (see Section 3.9). Despite the consistency and coherence of evidence, the number of studies is limited, particularly for changes that are indicative of health effects (i.e., airway responsiveness). Therefore, there is suggestive evidence that low dietary antioxidant intake increases the risk for NO_2-related health effects.

Table 3-1 Summary of evidence for increased risk of respiratory effects from short-term oxides of nitrogen exposure

	Key Evidence	Key References
Adequate evidence		
People with asthma	Consistent evidence for increased risk for NO_2-related asthma exacerbation.	Section 3.3
	Consistent evidence of NO_2-induced increases in airway responsiveness in adults with asthma.	Brown (2015) Goodman et al. (2009) Kjaergaard and Rasmussen (1996) Folinsbee (1992)
Children	Consistent epidemiologic evidence that associations between short-term NO_2 exposure and asthma exacerbation are stronger in children relative to adults	†Bi et al. (2023) †Wei et al. (2022) †Alhanti et al. (2016) Son et al. (2013) Sinclair et al. (2010) Ko et al. (2007) Hinwood et al. (2006) Peel et al. (2005) Atkinson et al. (1999) Anderson et al. (1998)

	Key Evidence	Key References
Suggestive evidence		
Older adults	Inconsistent epidemiologic evidence of age-specific differences in NO_2-related asthma exacerbations in adults, but consistently stronger NO_2-respiratory mortality associations observed among older adults compared to younger adults	†Gariazzo et al. (2023) †Li et al. (2021) †Chen et al. (2018) Dimakopoulou et al. (2014)
Females	Inconsistent epidemiologic evidence of sex-specific differences in NO_2-related asthma exacerbations, but consistently stronger NO_2-respiratory mortality associations observed among females	†Shin et al. (2022) †Chen et al. (2018) †Li et al. (2021) †Huang et al. (2021)
Diet	Limited evidence from experimental studies for Vitamin C modification of NO_2-related increases in airway responsiveness. Additional evidence that changes in NO_2-related oxidant balance may be modified by vitamin intake, but results are not necessarily indicative of health effects.	Mohsenin (1987b) Hatch et al. (1986) Mohsenin (1991) Elsayed and Mustafa (1982) Ayaz and Csallany (1978) Sevanian et al. (1982b) Selgrade et al. (1981)
Inadequate evidence		
Obesity	A recent study reported larger NO_2-related increase in hospital admissions for asthma in neighborhoods with high average BMIs compared to neighborhoods with medium to low average BMIs. Notable uncertainty in spatial scale of BMI variable.	†Wei et al. (2022)
Race/ethnicity	Evidence of effect modification of associations between short-term NO_2 exposure and asthma exacerbation was inconsistent across racial and ethnic groups.	Section 3.3.1.1.5
Socioeconomic status	Inconsistent results across epidemiologic studies of short-term NO_2 exposure indicate both smaller and larger risks of asthma exacerbation in low SES populations relative to high SES populations.	Section 3.3.1.1.5

List of abbreviations in alphabetical order: BMI = body mass index; NO_2 = nitrogen dioxide; SES = socioeconomic status.
†Studies published since the 2016 Oxides of Nitrogen ISA.

3.11 Summary and Causality Determinations

The 2016 Oxides of Nitrogen ISA concluded that there is a "causal relationship" between short-term exposure to NO_2 and respiratory effects. This conclusion was based primarily on coherence along

multiple lines of evidence and biological plausibility for effects on asthma exacerbation. Evidence from controlled human exposure studies for NO_2-induced increases in airway responsiveness in adults with asthma was sufficient to support the biological plausibility for the effects of NO_2 exposure on asthma exacerbation. Clinically relevant increases in airway responsiveness and doubling reduction in provocative dose following NO_2 exposures not much higher than peak ambient concentrations supported an effect on asthma exacerbation of ambient NO_2 exposures. Experimental studies in rodents and humans also demonstrated NO_2-induced allergic inflammation. The experimental evidence was coherent with epidemiologic evidence of associations between short-term NO_2 exposure, and a range of outcomes associated with asthma exacerbation in children, including increased asthma hospital admissions and ED visits, respiratory symptoms, and pulmonary inflammation. Uncertainty in the epidemiologic evidence as to whether NO_2 has an effect independent from other traffic-related pollutants was mitigated by the experimental evidence for an independent effect of NO_2 on asthma exacerbation. There was some support for NO_2-related exacerbation of COPD, respiratory infection, respiratory mortality, and respiratory effects in healthy populations. However, because of inconsistency within and across lines of evidence and consequent uncertainty about the independent effects of NO_2 exposure, evidence for other non-asthma respiratory effects did not strongly contribute to the determination of a causal relationship.

Consistent with the previous evidence, recent epidemiologic studies continue to support NO_2-related asthma exacerbation at the population level. Recent epidemiologic evidence includes more multicity and nationwide studies, including several in the United States and Canada with large numbers of cases, that observed NO_2-related increases in hospital admissions and ED visits for asthma, as well as smaller panel studies linking short-term NO_2 exposures to respiratory symptoms and medication use in children and adults with asthma. Particularly strong evidence comes from recent studies of asthma medication use that utilize digital tracking of inhaler use and GPS data to geolocate participants for exposure assignment (Su et al., 2024; Su et al., 2022), multicity and national studies of asthma ED visits in the United States and Canada (Bi et al., 2023; Krall et al., 2018; Alhanti et al., 2016; Weichenthal et al., 2016), and a nationwide study of asthma hospital admissions among United States Medicaid beneficiaries that implemented negative exposure controls and did not detect evidence or residual of unmeasured confounding (Wei et al., 2022). Across studies of hospital admissions and ED visits, there is consistent evidence demonstrating stronger associations among children compared to adults (see Section 3.3.1.1.5; Figure 3-4), and there continues to be evidence of effects of prolonged short-term exposures, with a few recent studies demonstrating associations with individual exposure lags of up to eight days (see Section 3.3.1.1.3). Recent studies also reported positive associations corresponding to previously unexamined exposure assignment techniques, including CMAQ modeling estimates and LUR models (see Section 3.3.1.1.2).

Controlled human exposure studies add further support for an independent effect of short-term NO_2 exposures on the physiological processes that underly asthma exacerbation. As in 2016, controlled human exposure studies of individuals with asthma showed increases in a marker of eosinophilic inflammation (ECP). Several meta-analyses of controlled human exposure studies evaluated in the 2016

Oxides of Nitrogen ISA that indicated increased airway responsiveness in adults with asthma exposed to NO_2 at rest. Exposures as low as 100 ppb resulted in approximately 70% of study participants experiencing increased airway responsiveness to nonspecific stimuli (Brown, 2015; Goodman et al., 2009; Kjaergaard and Rasmussen, 1996; Folinsbee, 1992). A clinically relevant doubling reduction in provocative dose in response to NO_2 was also reported, with evidence for the reproducibility of response in individuals (Brown, 2015). The evidence for clinically relevant increases in airway responsiveness in adults with asthma following relatively low NO_2 exposures provides key support that ambient concentrations of NO_2 can exacerbate asthma. These findings are coherent with epidemiologic studies reporting associations between short-term NO_2 exposures and endpoints related to asthma exacerbation. The lack of strong experimental evidence supporting direct NO_2-induced increases in asthma symptoms does not necessarily diminish this coherence given the ethical considerations that may preclude controlled human exposure studies from enrolling the participants at greatest risk from asthma exacerbations.

Findings of allergic mediators in controlled human exposure studies are coherent with recent toxicological studies that demonstrated NO_2-induced increases in markers of allergic inflammation in animal models (see Section 3.3.3.3.3), and epidemiologic studies that reported ambient or personal NO_2-related increases in eNO (see Section 3.3.3.3.3.1), most of which were evaluated in the 2016 Oxides of Nitrogen ISA. Further, the epidemiologic evidence provides support for NO_2-related decrements in lung function in children with asthma (see Section 3.3.3.2.1). The most consistent evidence for NO_2-related lung function decrements comes from well-designed, well-conducted studies that measured lung function under supervised conditions and assessed exposure in subjects' locations (Martins et al., 2012; Delfino et al., 2008; McCreanor et al., 2007). As discussed in Section 3.9, the collective findings support biologically plausible pathways by which NO_2-induced inflammation and heightened allergic response may promote asthma exacerbation through increases in airway reactivity, reductions in lung function, and/or increased airway resistance.

There continues to be uncertainty as to whether the observed epidemiologic associations between short-term NO_2 and asthma exacerbation represent an independent effect or serve as an indicator for effects of the traffic-related pollution mixture or components of that mixture. Of the limited number of studies that examined potential confounding by $PM_{2.5}$ or traffic-related pollutants (e.g., UFP, CO, or VOCs), associations between short-term NO_2 exposures and asthma hospital admissions and ED visits remained relatively unchanged in copollutant models (see Section 3.3.1.1.6). Notably, many studies do not mutually adjust for highly correlated pollutants because resulting multicollinearity may produce unstable effect estimates. Although copollutant models have well-recognized limitations for distinguishing independent pollutant associations, particularly for highly correlated pollutants, the evidence integrated from copollutant models and coherence with results from experimental studies support an effect of ambient NO_2 exposure on asthma exacerbation independent of other traffic-related pollutants.

Evidence for non-asthma respiratory effects continues to be inconsistent within and across disciplines. Recent epidemiologic studies support and extend previous findings of NO_2-associated increases in risk of COPD exacerbation, including evidence for increased ED visits and hospital admissions for COPD (see Section 3.4.1.1), increased respiratory symptoms and medication use among adults with COPD (see Section 3.4.2.1), and reduced lung function in adults with COPD (see Section 3.4.3.1.1). Although the epidemiologic evidence is generally consistent, a limited number of controlled human exposure studies do not clearly indicate respiratory symptoms, lung function changes, or pulmonary inflammation following NO_2 exposure in adults with COPD. Animal studies report increased inflammation following short term NO_2 exposures that could provide a plausible mechanism underlying COPD exacerbations however, uncertainty, including the lack of animal studies relating to other COPD-relevant endpoints and lack of coherence with controlled human exposure studies, remain. The lack of coherent evidence across disciplines, along with limited evaluation of potential copollutant confounding in the epidemiologic evidence base, contributes to overall uncertainty regarding the independent relationship between short-term NO_2 exposure and COPD exacerbation.

Experimental evidence in animals supports immunosuppression following short-term NO_2 exposure, but there is limited coherence from other lines of evidence. Although there are no recent PECOS-relevant studies, animal toxicological studies evaluated in the 2016 Oxides of Nitrogen ISA indicated that short-term exposures to NO_2 compromise host resistance to bacterial and viral infection (see Sections 3.5.3.2 and 3.5.3.3) in addition to inducing decreased antibody responses and impaired ex vivo WBC function (see Section 3.5.3.4). In contrast to animal models, controlled human exposure studies do not provide support for NO_2 effects on antibody response or susceptibility to bacterial or viral infection. Additionally, although there is some epidemiologic support for NO_2-related increases in ED visits for respiratory infections, there is limited evidence of consistent associations across specific infection types, and evidence is inconsistent for other outcomes, including hospital admissions and self-reported, laboratory-confirmed, or physician-diagnosed respiratory infection.

In epidemiologic studies in healthy populations, ambient NO_2 concentrations were consistently associated with increased respiratory symptoms and pulmonary inflammation in children without pre-existing respiratory disease. Epidemiologic evidence was inconsistent for healthy adults. There has been limited evaluation of potential copollutant confounding in the epidemiologic studies, raising some uncertainty about the independent effect of NO_2. Additionally, controlled human exposure and animal studies provide limited and inconsistent results. Overall, the lack of coherence between epidemiologic, controlled human exposure, and experimental findings raises uncertainty as to whether the observed epidemiologic associations are indicative of an independent effect of NO_2.

A large number of recent epidemiologic studies support and extend conclusions from the 2016 Oxides of Nitrogen ISA and provide consistent evidence of an association between short-term NO_2 exposure and respiratory mortality. Positive associations were observed across studies that used more spatially refined exposure assessment methods (see Section 3.8.4) and comprised greater geographic

diversity, including several multicity studies in North America and analyses of a large dataset collected from over 360 cities in 16 different countries across the world (Schwarz et al., 2024; Meng et al., 2021). The collective evidence supports a consistent pattern of stronger associations at earlier lags (e.g., zero and one days) with decreasing magnitudes of associations at later lags. The recent evidence base also includes expanded evaluation of the C-R relationship that suggests an approximately linear relationship between short-term NO_2 exposure and respiratory mortality across the concentration distribution. Although recent studies observed NO_2-related increases in respiratory mortality that were robust to adjustment for PM_{10}, $PM_{2.5}$, SO_2, or O_3, evaluation of confounding by traffic-related pollutants remains limited, and high correlations of NO_2 with other pollutants complicate the interpretation of the available copollutant models. There is also uncertainty in the spectrum of respiratory effects that could ultimately lead from exposure to mortality. The primary causes of non-malignant respiratory mortality are COPD and respiratory infection (e.g., influenza and pneumonia) (NHLBI, 2017), but these conditions are not clearly related to NO_2 exposure.

Overall, there is sufficient evidence to conclude that there is a *causal relationship* between short-term oxides of nitrogen exposure and respiratory effects. Coherence of findings integrated across controlled human exposure, epidemiologic, and animal toxicological studies supports a relationship between short-term NO_2 exposure and asthma exacerbation. NO_2-induced increases in allergic inflammation and airway responsiveness in controlled human exposure studies of adults with asthma comprise the key evidence that NO_2 exposure can independently exacerbate asthma and support the epidemiologic evidence for NO_2-related asthma hospital admissions and ED visits, as well as increased respiratory symptoms and pulmonary inflammation in populations with asthma. The evidence for the relationship between short-term exposure to NO_2 and respiratory effects is summarized in Table 3-2, using the framework for causality determinations described in Section 3.3.4 of Volume 2 of the Integrated Review Plan for the Integrated Science Assessment for Oxides of Nitrogen – Health Criteria (U.S. EPA, 2024).

Table 3-2 Summary of evidence indicating a causal relationship between short-term oxides of nitrogen exposure and respiratory effects

Rationale for Causality Determination[a]	Key Evidence[b]	Key References	Nitrogen Dioxide Concentrations Associated with Effects
Asthma Exacerbation			
Consistent evidence from multiple, high-quality controlled human exposure studies. Rules out chance, confounding, and other biases with reasonable confidence	NO_2 increases airway responsiveness in adults with asthma exposed at rest following nonspecific or allergen challenge in several individual studies and meta-analyses.	Brown (2015) Goodman et al. (2009) Kjaergaard and Rasmussen (1996) Folinsbee (1992) Section 3.3.3.1.1	100 ppb for 1 h 200−300 ppb for 30 min
	Clinical relevance supported by findings of a doubling reduction in provocative dose in response to NO_2.	Brown (2015)	100 ppb for 1 h, 140 ppb for 30 min
Generally consistent epidemiologic evidence from multiple, high-quality studies with relevant NO_2 concentrations	Generally consistent evidence for increases in hospital admissions and ED visits for asthma incidence associated with 1-hr max and 24-hr avg NO_2 concentrations, including multicity and national studies in the United States and Canada. Consistent evidence demonstrating stronger associations among children compared to adults.	†Bi et al. (2023) †Krall et al. (2018) †Alhanti et al. (2016) †Weichenthal et al. (2016) †Wei et al. (2022) Section 3.3.1.1	Range of means across studies: 1-hr max: 9.2–56.0 ppb 24-hr avg: 5.1–37.2 ppb
	In children with asthma, generally consistent evidence for NO_2 associations with:		
	• Increased respiratory symptoms	†Hao et al. (2022) Gent et al. (2009) von Klot et al. (2002) Section 3.3.2.1 Section 5.2.2.3 of U.S. EPA (2016)	Range of means across studies: 24-hr avg: 7.6–38.8 ppb 1-hr max: 7.2–79.5 ppb
	• Reduced lung function	Delfino et al. (2008) Martins et al. (2012) McCreanor et al. (2007) Section 3.3.3.2.1 Section 5.2.2.2 of U.S. EPA (2016)	Range of means across studies: *Personal Monitor* 24-hr avg: 6.3–28.6 ppb *Central Site Monitor* 24-hr avg: 11.2–37.6 ppb 1-hr max: 7.2–57.0 ppb

Rationale for Causality Determination[a]	Key Evidence[b]	Key References	Nitrogen Dioxide Concentrations Associated with Effects
	• Increased pulmonary inflammation	Delfino et al. (2006) Delfino et al. (2013) Martins et al. (2012) Section 3.3.3.3.1 Section 5.2.2.5 of U.S. EPA (2016)	Range of means across studies: 24-hr avg: 11.2–38.8 ppb
Consistent positive epidemiologic evidence for associations between NO_2 exposure and asthma exacerbation across exposure assessment methods	Asthma-related effects associated with NO_2 across a range of exposure method, including population-weighted and unweighted averages from multiple monitors, CMAQ models, and hybrid prediction models, as well as concentrations measured in subjects' locations (personal total and ambient, school outdoor) in panel studies.	Section 3.3.1.1.2 Section 3.3.2.1 Section 3.3.3.2.1 Section 3.3.3.3.1	
Uncertainty regarding potential copollutant confounding	Limited evidence that associations between short-term NO_2 exposure and asthma exacerbation were robust to adjustment for PM2.5 or traffic-related pollutants (e.g., UFP, CO, or VOCs). Moderate to high correlations in most studies limit confidence in copollutant model results. Uncertainties reduced by support for an independent effect in animal studies.	Section 3.3.1.1.6	
Evidence supporting biological plausibility	Evidence from experimental studies for NO_2-induced oxidative stress, inflammation, and allergic responses in humans and animals provides biologically plausibility for epidemiologic findings of asthma exacerbation	Section 3.9	
COPD Exacerbation			
Generally consistent epidemiologic evidence of severe COPD exacerbation from a limited number of high-quality multicity studies at relevant concentrations	Increases in hospital admissions and ED visits for COPD associated with increases in NO_2 concentrations measured at central site monitors	†Krall et al. (2018) †Szyszkowicz et al. (2018) †Weichenthal et al. (2016) Faustini et al. (2013) Section 3.4.1	Range of means across studies: 1-hr max: 45.9 ppb 24-hr avg: 11.9–35.1 ppb

Rationale for Causality Determination[a]	Key Evidence[b]	Key References	Nitrogen Dioxide Concentrations Associated with Effects
Supporting evidence from a limited number of epidemiologic studies of less severe exacerbation and reduced lung function in adults with COPD	Limited epidemiologic evidence for increased respiratory symptoms and medication uses among adults with COPD. A few studies that provide stronger inference indicate an association between short-term NO_2 exposure and reduced lung function in adults with COPD.	†Evangelopoulos et al. (2021) †Magzamen et al. (2018) †Sinharay et al. (2017) †Nurhussien et al. (2022) Section 3.4.2.1 Section 3.4.3.1	Range of means across studies: Personal monitors, 24-hr avg: 6.8–14.0 ppb
Uncertainty regarding potential copollutant confounding	Uncertainty in the independent nature of NO_2 associations in epidemiologic studies due to limited evaluation of potential copollutant confounding.	Section 3.4.1.1	
Uncertainty due to limited coherence with experimental evidence.	Controlled human exposure studies do not clearly indicate respiratory symptoms, lung function changes, or pulmonary inflammation following NO_2 exposure in adults with COPD.	Section 3.4.2.2 Section 3.4.3.1.2 Section 3.4.3.2.2	
Respiratory Infection			
Consistent animal toxicological evidence with relevant NO_2 exposures	Mortality from bacterial or viral infection in animals with NO_2 exposures of 1,500 ppb and higher; no mortality at lower concentrations.	Ehrlich et al. (1977) Ehrlich et al. (1979) Ehrlich (1980) Graham et al. (1987) Section 3.5.3.2 Section 3.5.3.3	1,500−5,000 ppb for 3 h 1,500 ppb with 4,500 ppb spike of 1−7 h
	Decreased resistance to bacterial and viral lung infection in NO_2-exposed animals.	Section 3.5.3.2 Section 3.5.3.3	≥5,000 ppb
Inconsistent epidemiologic evidence and uncertainty regarding NO_2 independent effects	Associations with ED visits for respiratory infections. Limited evidence across specific infection types. Inconsistent evidence for hospital admissions and parental reports or physician-confirmed infections. Limited evaluation of potential copollutant confounding.	†Krall et al. (2018) †Szyszkowicz et al. (2018) Section 3.5.1 Section 3.5.2	Range of means across studies: 1-hr max: 9.22–20.5 ppb 24-hr avg: 11.9 ppb
Respiratory Effects in Populations Without Pre-Existing Disease			

Rationale for Causality Determination[a]	Key Evidence[b]	Key References	Nitrogen Dioxide Concentrations Associated with Effects
Limited epidemiologic evidence and uncertainty regarding NO_2 independent effects	Consistent evidence for respiratory symptoms in children without pre-existing respiratory disease. No examination of potential confounding by traffic-related copollutants.	Section 3.6.1.1 Section 5.2.7.1 of U.S. EPA (2016)	Range of means across studies: 1-hr max: 18.0–41.7 ppb 24-hr avg: 7.0–18.0 ppb
	Lung function in children or adults without pre-existing respiratory disease not consistently associated with NO_2 measured at subjects' locations or at central site monitors.	Section 3.6.3.1	
Limited and inconsistent evidence from controlled human exposure studies	Increases in airway responsiveness found in healthy adults above 1,000 ppb NO_2, not lower concentrations.	Folinsbee (1992) Kjaergaard and Rasmussen (1996) Section 3.6.2.2	1,000−2,000 ppb for 3 h
	Limited evidence does not support respiratory symptoms or lung function in healthy adults exposed to NO_2.	Section 3.6.1.2 Section 3.6.3.2	200−4,000 ppb for 2 to 5 h
Respiratory Mortality			
Generally consistent epidemiologic evidence from multiple, high-quality studies with relevant NO_2 concentrations	Multicity studies in the United States, Canada, Europe, and Asia consistently observe associations of respiratory mortality with 24-hr avg NO_2 at lag 0−1 or 0–2 days. Results based on NO_2 concentrations measured at central-site monitors or estimated using chemical transport models or hybrid prediction models.	†Liu et al. (2022) †Meng et al. (2021) †Schwarz et al. (2024) Chiusolo et al. (2011) Bellini et al. (2007) Section 3.8	Range of means across studies: 1-hr max: 5.5 ppb 24-hr avg: 7.8–35.1 ppb
Uncertainty regarding potential copollutant confounding.	A small number of multicity studies in China and Italy reported positive associations that were relatively unchanged in models adjusting for PM_{10}, $PM_{2.5}$, SO_2, and O_3. Important traffic-related copollutants not considered (e.g., EC or UFPs). Moderate to high correlations with some copollutants limit confidence in copollutant model results.	Section 3.8.2 Figure 3-15	

Rationale for Causality Determination[a]	Key Evidence[b]	Key References	Nitrogen Dioxide Concentrations Associated with Effects
Uncertainty due to limited coherence with respiratory morbidity evidence	A lack of coherence between experimental and epidemiologic studies of short-term NO_2 exposure and respiratory and morbidity outcomes that support pathways for mortality, including COPD and respiratory infection.	Section 3.4 Section 3.5	

List of abbreviations in alphabetical order: avg = average; CMAQ = Community Multiscale Air Quality; CO = carbon monoxide; COPD = chronic obstructive pulmonary disease; EC = elemental carbon; ED = emergency department; h = hour; max = maximum; min = minutes; NO_2 = nitrogen dioxide; O_3 = ozone; $PM_{2.5}$ = particulate matter with a nominal mean aerodynamic diameter less than or equal to 2.5 μm; PM_{10} = particulate matter with a nominal mean aerodynamic diameter less than or equal to 10 μm; ppb = parts per billion; SO_2 = sulfur dioxide; UFP = ultrafine particles; VOC = volatile organic carbon.

[a]Based on application of the causality framework described in Section 3.3.4 of Volume 2 of the Integrated Review Plan for the Integrated Science Assessment for Oxides of Nitrogen – Health Criteria (U.S. EPA, 2024).

[b]Describes the key evidence and provides references contributing to the causality determination. References to earlier sections indicate where the full body of evidence is described.

†Studies published since the 2016 Oxides of Nitrogen ISA

3.12 References

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