EPA/600/R-25/232
July 2026
www.epa.gov/isa

Integrated Science Assessment for Oxides of Nitrogen – Health Criteria

Chapter 4: Respiratory Effects – Long-Term Exposure

External Review Draft

July 2026

U.S. Environmental Protection Agency

Disclaimer

This document is an external review draft for peer review purposes only. This information is distributed solely for the purpose of pre-dissemination peer review under applicable information quality guidelines. It has not been formally disseminated by the U.S. Environmental Protection Agency. It does not represent and should not be construed to represent any agency determination or policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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.

Front Matter

Executive Summary

Integrated Synthesis

Chapter 1. Atmospheric Chemistry and Ambient Concentrations

Chapter 2. Exposure and Dosimetry of Inhaled Oxides of Nitrogen

Chapter 3. Respiratory Effects – Short-Term Exposure

Chapter 4. Respiratory Effects – Long-Term Exposure

Chapter 5. Cardiovascular Effects – Short-Term Exposure

Chapter 6. Cardiovascular Effects – Long-Term Exposure

Chapter 7. Reproductive and Developmental Effects

Chapter 8. Metabolic Syndrome and Diabetes

Chapter 9. Nervous System Effects

Chapter 10. Total Mortality – Short-Term Exposure

Chapter 11. Total Mortality – Long-Term Exposure

Chapter 12. Cancer

Chapter 13. Health Effects with Limited Evidence

Chapter 14. Process for Developing the Integrated Science Assessment for Oxides of Nitrogen – Health Criteria

Appendix A. Appendix Tables

Table of Contents

Disclaimer ii
Document Guide iii
Table of Contents iv
List of Tables vi
List of Figures vii
Acronyms and Abbreviation viii
CHAPTER 4 Respiratory Effects – Long-Term Exposure 1
4.1 Introduction 1
4.1.1 Summary of Previous ISA 1
4.1.2 Chapter Organization and Conventions 2
4.2 Scope 4
4.3 Asthma Development and Severity 6
4.3.1 Development of Asthma 6
4.3.2 Asthma Severity 30
4.3.3 Synthesis Across Lines of Evidence 34
4.4 Chronic Obstructive Pulmonary Disease Development and Exacerbation 35
4.4.1 Epidemiologic Studies of Development of COPD 35
4.4.2 Animal Toxicological Studies of the Development of COPD 47
4.4.3 Synthesis Across Lines of Evidence 49
4.5 Respiratory Infection 50
4.5.1 Epidemiologic Studies of Respiratory Infection 50
4.5.2 Epidemiologic Studies of COVID-19 55
4.5.3 Experimental Support for Respiratory Infection 58
4.5.4 Synthesis Across Lines of Evidence 60
4.6 Lung Function and Development 60
4.6.1 Epidemiologic Studies of Lung Function and Development 60
4.6.2 Animal Toxicological Studies of Lung Function and Development 79
4.6.3 Synthesis Across Lines of Evidence 80
4.7 Interstitial Lung Disease 81
4.7.1 Epidemiologic Studies of Interstitial Lung Disease 81
4.7.2 Conclusions and Remaining Uncertainties 83
4.8 Respiratory Mortality 83

4.8.1 Concentration-Response 87
4.8.2 Lifestages and Populations 91
4.8.3 Copollutants 96
4.8.4 Additional Sources of Confounding 99
4.8.5 Exposure Assignment 101
4.8.6 Conclusions and Remaining Uncertainties 103
4.9 Biological Plausibility 104
4.10 Lifestages and Populations 107
4.10.1 Sociodemographic Factors 108
4.11 Summary and Causality Determination 113
4.12 References 123

List of Tables

Table 4-1 Summary of evidence for increased risk of respiratory effects from oxides of nitrogen exposure 112
Table 4-2 Summary of evidence indicating a causal relationship between long-term oxides of nitrogen exposure and respiratory effects 118
Table 4-3 Epidemiologic studies evaluating effect-measure modification of the relationship between long-term NO_2 exposure and childhood asthma incidence by sex 16
Table 4-4 Epidemiologic studies evaluating effect-measure modification of the relationship between long-term NO_2 exposure and COPD incidence by age 41
Table 4-5 Epidemiologic studies evaluating effect-measure modification of the relationship between long-term NO_2 exposure and COPD incidence by sex 43
Table 4-6 Epidemiologic studies evaluating effect-measure modification of the relationship between long-term NO_2 exposure and COPD incidence by pre-existing diseases 45
Table 4-7 Copollutant-adjusted associations between long-term exposure to nitrogen dioxide and chronic obstructive pulmonary disease or chronic bronchitis incidence 46
Table 4-8 Associations between long-term exposure to oxides of nitrogen and respiratory infection 52
Table 4-9 Association between long-term exposure to oxides of nitrogen and COVID-19 56
Table 4-10 Longitudinal and cross-sectional studies of long-term exposure to oxides of nitrogen and lung function in children and adults 73
Table 4-11 Associations between long-term exposure to oxides of nitrogen and interstitial lung disease incidence or severity 83
Table 4-12 Summary of epidemiologic studies evaluating potential non-linearities in the relationship between long-term NO_2 exposure and respiratory mortality 90
Table 4-13 Epidemiologic studies evaluating effect-measure modification of the relationship between long-term nitrogen dioxide exposure and respiratory mortality by sex 93
Table 4-14 Epidemiologic studies evaluating effect-measure modification of the relationship between long-term nitrogen dioxide exposure and respiratory mortality by age 95

List of Figures

Figure 4-1 Associations of long-term exposure to nitrogen dioxide with asthma incidence in cohort studies of children. 10
Figure 4-2 Concentration-response curve between annual average nitrogen dioxide concentrations from industrial sources and asthma incidence in children; estimated from a restricted cubic spline model. 11
Figure 4-3 Associations between long-term nitrogen dioxide exposure and asthma incidence in children in single pollutant models and models adjusted for copollutants. 13
Figure 4-4 Associations of long-term exposure to nitrogen dioxide with asthma incidence in cohort studies of adults. 21
Figure 4-5 Concentration-response curve between 3-year moving average nitrogen dioxide concentrations and adult-onset asthma in the Danish Nurse Cohort. 22
Figure 4-6 Concentration-response curve between nitrogen dioxide concentrations and adult-onset asthma in an analysis of pooled data from three European cohorts. 22
Figure 4-7 Associations between long-term nitrogen dioxide exposure and asthma incidence in adults in single pollutant models and models adjusted for copollutants. 24
Figure 4-8 Associations of long-term exposure to nitrogen dioxide with COPD incidence. 37
Figure 4-9 Concentration-response curve between 3-year moving average nitrogen dioxide concentrations and COPD incidence in the Danish Nurse Cohort. 38
Figure 4-10 Concentration-response curve between nitrogen dioxide concentrations and COPD incidence in an analysis of pooled data from three European cohorts. 39
Figure 4-11 Concentration-response curve between nitrogen dioxide concentrations and COPD incidence in an analysis of data from an administrative cohort in Ontario, Canada. 39
Figure 4-12 Associations of oxides of nitrogen with lung function from longitudinal and cross-sectional studies of children. 63
Figure 4-13 Associations of oxides of nitrogen with lung function from longitudinal and cross-sectional studies of adults. 66
Figure 4-14 Concentration-response relationship of annual average nitrogen dioxide concentration with the risk of incident idiopathic pulmonary fibrosis in the UK Biobank Study. 82
Figure 4-15 Associations of long-term exposure to nitrogen dioxide with respiratory mortality. 87
Figure 4-16 Concentration-response curve between annual average nitrogen dioxide concentrations in 2010 and respiratory mortality in a pooled analysis of data from eight European cohorts. 88
Figure 4-17 Concentration-response curve between 12-month moving average nitrogen dioxide concentration and respiratory mortality in an analysis of Medicare beneficiaries. 89
Figure 4-18 Concentration-response curve between five-year moving average nitrogen dioxide concentration and respiratory mortality in the Ontario Health Study. 90
Figure 4-19 Associations between long-term nitrogen dioxide exposure and respiratory mortality in single pollutant models and models adjusted for particulate matter. 98
Figure 4-20 Associations between long-term nitrogen dioxide exposure and respiratory mortality in single pollutant models and models adjusted for ozone, greenspace, or traffic noise. 99
Figure 4-21 Potential biological plausibility pathways for respiratory effects associated with long-term exposure to oxides of nitrogen. 106

Acronyms and Abbreviation

ALRI	acute lower respiratory infection
ARDS	acute respiratory distress syndrome
AQCD	Air Quality Criteria Document
AQS	air quality system
avg	average
BAMSE	Children, Allergy, Environment, Stockholm, Epidemiology
BC	black carbon
BMI	body mass index
C	Carbon
CHS	Children's Health Study
CI	confidence interval
CMAQ	community multiscale air quality
CO	carbon monoxide
COI	Childhood Opportunity Index
COPD	chronic obstructive pulmonary disease
COVID-19	Coronavirus Disease 2019
C-R	concentration-response
CREW	Children's Respiratory and Environmental Workgroup
d	day(s)
df	degrees of freedom
DNA	deoxyribonucleic acid
DNC	Danish Nurse Cohort
e.g.	exempli gratia (for example)
EC	elemental carbon
ED	emergency department
ELAPSE	Effects of Low-Level Air Pollution
ELF	epithelial lining fluid
eNO	exhaled nitric oxide
EPA	U.S. Environmental Protection Agency
ESCAPE	European Study of Cohorts for Air Pollution Effects
etc.	et cetera
F	female(s)
FEF	forced expiratory flow
FEV_1	forced expiratory volume in one second
FVC	forced vital capacity
g	gram(s)
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GPS	Global Positioning System
HAA	high attenuation area
HONO	nitrous acid
H_2SO_4	sulfuric acid
hr	hour(s)
HR	hazard ratio
i.e.	id est (that is)
ICU	intensive care unit
IDW	inverse distance weighted
IgE	immunoglobulin E
ILA	interstitial lung abnormalities
ILD	Interstitial lung disease
IPF	idiopathic pulmonary fibrosis
IQR	interquartile range
IRS	intensive respiratory support
ISA	Integrated Science Assessment
KPSC	Kaiser Permanente Southern California
LRTI	lower respiratory tract infection
LUR	land use regression
M	male
m	milligram
m	meter(s)
Max	maximum
MeBr	methyl bromide
MESA	Multi-Ethnic Study of Atherosclerosis
Min	minimum
min	minute(s)
mL	milliliter
mo	month(s)
n	sample size
N	population size
NA	not applicable
NO	nitric oxide
NO_2	nitrogen dioxide
NO_X	NO_2 + NO
NR	not reported
O_3	ozone
OC	Organic carbon
OP	Organophosphate
OR	odds ratio
OVA	ovalbumin
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
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
RHINE	Respiratory Health in Northern Europe
RR	relative risk or risk ratio
SCHIFs	shape-constrained health impact functions
SD	standard deviation
SE	standard error
SES	socioeconomic status
SLAMS	State and Local Air Monitoring Stations
SO_2	sulfur dioxide
U.K.	United Kingdom

U.S.	United States	β	beta
UFP	ultrafine particles	µg	microgram
URI	upper respiratory infection	µg/m^3	micrograms per cubic meter
vs.	versus	µm	micrometer
wk	week(s)	ρ	rho
yr	year(s)		

CHAPTER 4 Respiratory Effects – Long-Term Exposure

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

This chapter characterizes the scientific evidence that supports causality determinations for long-term oxides of nitrogen exposure 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 (see 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, 2015). 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.

4.1 Introduction

4.1.1 Summary of Previous ISA

The U.S. Environmental Protection Agency's (EPA) 2016 Oxides of Nitrogen ISA – Health Criteria (hereafter referred to as the 2016 Oxides of Nitrogen ISA) concluded that the available scientific evidence supported a "likely to be causal" relationship between long-term NO_2 exposure and respiratory effects (U.S. EPA, 2016). The evidence underpinning this causality determination is briefly summarized below.

The "likely to be causal relationship" with long-term exposure to nitrogen dioxide (NO_2) represented a change from the "suggestive but not sufficient to infer a causal relationship" determination

in the 2008 Oxides of Nitrogen ISA. The change to the causality determination was driven by stronger epidemiologic evidence demonstrating consistent associations between long-term ambient NO_2 concentrations and asthma development in children. The largest remaining uncertainty in the epidemiologic evidence was a limited of consideration of potential confounding by traffic-related copollutants. However, animal toxicological and controlled human exposure evidence for increased airway responsiveness and allergic inflammation, which are key events in the proposed mode of action for asthma development, was coherent with epidemiologic evidence for asthma incidence and provided some support for an independent effect of long-term exposure to NO_2 on the development of asthma. Because this experimental evidence was limited in quantity, there was some remaining uncertainty regarding an independent effect of long-term NO_2 exposure on asthma development. Epidemiologic studies also provided consistent evidence for NO_2-related lung function decrements and decreased lung development in children. Animal toxicology data on NO_2-induced changes in lung function were inconsistent, whereas evidence from controlled human exposure studies was predominantly null; thus, there was a lack of coherence among the multiple lines of evidence for effects on lung function. Studies examining other respiratory health effects were limited in number and largely inconsistent, with a lack of coherence between different lines of evidence. Together, consistent epidemiologic and experimental evidence for the development of asthma, with some remaining uncertainty regarding the independent effect of NO_2, the evidence was sufficient to conclude that there was "likely to be a causal relationship" between long-term NO_2 exposure and respiratory effects.

4.1.2 Chapter Organization and Conventions

This chapter evaluates recent evidence examining the relationship between long-term oxides of nitrogen exposure and respiratory effects. Its conclusions are drawn from the integration of the full body of evidence available in the 2016 Oxides of Nitrogen ISA and the evidence that has become available since the previous ISA. The following sections provide an overview of study inclusion criteria for this chapter (see Section 4.2), summaries of recent health effects evidence (see Sections 4.3 to 4.8), a discussion of biological plausibility (see Section 4.9), summary of lifestages and populations (see Section 4.10, Table 4-1), and a discussion of the causality determination for long-term oxides of nitrogen exposure and respiratory effects (see Section 4.11, Table 4-2). 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.

4.1.2.1 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 last 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" sections, 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, long-term exposure to oxides of nitrogen is assumed to be approximately 30 days or greater. 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 the comparability of results across epidemiologic studies, this ISA presents effect estimates for associations with health outcomes scaled to the same increment of oxide of nitrogen concentration.[1] For long-term exposure metrics, effect estimates are scaled to 10-parts per billion (ppb) increase in NO_2, a 15-ppb increase in NO, and a 20-ppb increase in NO_X. These increments were derived by calculating the United States (U.S.) nationwide percentile distributions for annual average concentrations and then calculating the approximate difference between the median (a typical pollution

[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 ppb.

year) and the 95th percentile (a more polluted year) concentrations among monitors in the State and Local Air Monitoring Stations (SLAMS) network. Long-term averages of ambient oxides of nitrogen concentrations are less variable across time, and do not differ widely among multi-month averages, annual averages, or multi-year averages. Thus, all long-term exposure metrics were scaled to the same increment.

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 U.S. or other countries. Therefore, the ISA presents effect estimates based on $\mu g/m^3$ of NO_X as they are reported in their respective studies.

4.1.2.2 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 last 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.

4.2 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 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

[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 recent 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).

Oxides of Nitrogen ISA inform the scope of this chapter. Studies that were included in the 2016 Oxides of 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, cohort, cross-sectional studies, and quasi-experimental, with appropriate timing of exposure for the health outcome of interest.

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]

[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 are considered for inclusion in the ISA if they meet additional study quality criteria described in Chapter 14.

[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 the 2015 Preamble to the ISAs (U.S. EPA, 2015) and in Volume 2 of the Integrated Review Plan for the Primary National Ambient Air Quality Standards for Oxides of Nitrogen (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.

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.

4.3 Asthma Development and Severity

Asthma is typified by reversible airway obstruction, airway hyperresponsiveness, and in some cases, airway remodeling. Epidemiologic studies examining associations between long-term oxides of nitrogen exposure and the development of asthma employ longitudinal study designs and primarily focus on children and asthma incidence. Some studies examine the severity of asthma as judged by symptom questionnaires, frequency of asthma medication use, and in the more severe cases, emergency department (ED) visits and hospital admissions for asthma-related causes. While animal studies are not able to directly measure disease onset or severity of disease, the presence of subclinical markers that underlie asthma can be measured in studies of longer-term exposures to NO_2. The increase in airway responsiveness in naive animals can promote progression to an asthma-like phenotype. This is frequently measured as increased airway responsiveness, the presence of Th-2 mediated inflammation, and evidence of airway remodeling and mucus secretion. Several animal models of allergen sensitivity have also been used to mimic stimulation of the IgE-mediated eosinophilic inflammation that is common in asthma. NO_2-induced worsening of these responses is used as a surrogate for worsening of disease severity.

4.3.1 Development of Asthma

As described above, the development of asthma can be measured through the increased incidence of asthma in longitudinal studies. While asthma can begin at any age, it often starts during childhood. The reliability of asthma diagnosis improves in children ages five years and older. This section evaluates evidence of NO_2 associations with asthma onset in children (see Section 4.3.1.1) and adults (see Section 4.3.1.2), as well as NO_2-induced changes in animal models examining airway responsiveness, allergic responses, and pulmonary inflammation that, over time, could underlie the development of asthma (see Section 4.3.1.3).

4.3.1.1 Epidemiologic Studies of Asthma Incidence in Children

The 2016 Oxides of Nitrogen ISA evaluated several cohort studies that comprised a strong evidence base demonstrating generally positive associations between long-term NO_2 exposure and asthma incidence in children. The strongest evidence was provided by cohort studies in North America and Europe that followed participants from birth and reported positive associations between physician-

diagnosed asthma and long-term average NO_2 concentrations assigned to participants via land use regression (LUR) models, dispersion models, or inverse distance weighting (IDW) of central site monitors (Ranzi et al., 2014; Gruzieva et al., 2013; Carlsten et al., 2011a; Clark et al., 2010; Gehring et al., 2010). Associations were observed across various periods of exposure, including the first year of life, the year prior to asthma diagnosis, and cumulative lifetime exposure. Although associations were positive in most cohort studies, a study in Oslo, Norway did not observe associations of long-term average NO_2 concentrations with asthma diagnosed at any age examined (Oftedal et al., 2009). Oftedal et al. (2009) reported comparable mean annual average NO_2 concentrations to the studies that reported a positive association between long-term NO_2 exposure and asthma incidence. The general consistency across the studies evaluated in the 2016 Oxides of Nitrogen ISA was supported by a pooled analysis of six birth cohort studies (n = 5,115 children), which reported that annual average NO_2 exposure assigned to each child's birth address by LUR was associated with higher asthma incidence up to ages seven to eight years [odds ratio (OR): 1.48 (95% CI: 1.06, 2.06); (MacIntyre et al., 2014a)]. Across studies, LUR models were observed to have good accuracy with respect to predicting the spatial pattern of ambient NO_2 concentrations in the study area (r^2 = 0.66 to 0.76), and follow-up participation ranged from 63 to 82%, with no indication of selective drop-out due to exposure or asthma status. A key remaining uncertainty was confounding by particulate matter with a nominal mean aerodynamic diameter less than or equal to 2.5 micrograms ($PM_{2.5}$) and traffic-related pollutants such as carbon monoxide (CO), elemental carbon (EC), and ultrafine particles (UFP). No studies of NO_2 and asthma development examined copollutant models with traffic-related copollutants. Evidence from recent studies is summarized below.

A large number of recent studies expand the existing evidence base and continue to support a positive association between long-term NO_2 exposure and asthma development in children (see Figure 4-1). Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 4-1. Recent evidence comes from a mix of traditional recruitment-based and administrative cohort studies using a range of exposure assessment methods, including LUR, dispersion models, and fixed-site monitors. Administrative cohort studies provide large population-based samples that are less susceptible to selection bias and attrition, but typically have fewer individual-level covariates than traditional cohort studies. Whereas traditional cohorts typically assess asthma incidence via a series of questionnaires on diagnosis history, administrative cohorts utilize medical records to identify physician claims and hospitalization. An analysis comparing methods for ascertaining asthma status in epidemiologic studies indicates good agreement between administrative health data and self-report (Rousseau et al., 2023). Notably, the use of medical records to ascertain asthma incidence does not necessarily distinguish exacerbation of mild/undiagnosed asthma from asthma onset. If the case definition does not capture a portion of asthma cases, observed effects could be partially reflective of an acute outcome (i.e., exacerbation of mild or undiagnosed asthma). In this case, observed effects could potentially be confounded by short-term increases in NO_2 or other air pollutants. Administrative studies that include physician claims and outpatient records in addition to hospitalization and ED records are more likely to capture a larger portion of incident asthma cases.

Recent evidence also includes more thorough evaluation of potential copollutant confounding and considers several other policy-relevant topics, including the shape of the concentration-response (C-R) relationship and comparisons of various exposure windows. A short summary of the recent evidence is provided below, followed by additional discussion of the shapes of concentration-response (C-R) relationships in available studies (see Section 4.3.1.1.1), potential confounding by copollutants (see Section 4.3.1.1.2) and other sources (see Section 4.3.1.1.3), the lifestages and populations evaluated (see Section 4.3.1.1.4), exposure assignment approaches (see Section 4.3.1.1.5), and comparisons of various exposure windows (see Section 4.3.1.1.6).

Several recent administrative birth cohort studies provide consistent evidence of an association between long-term NO_2 exposure and incident asthma among children followed from birth up to 19 years of age.

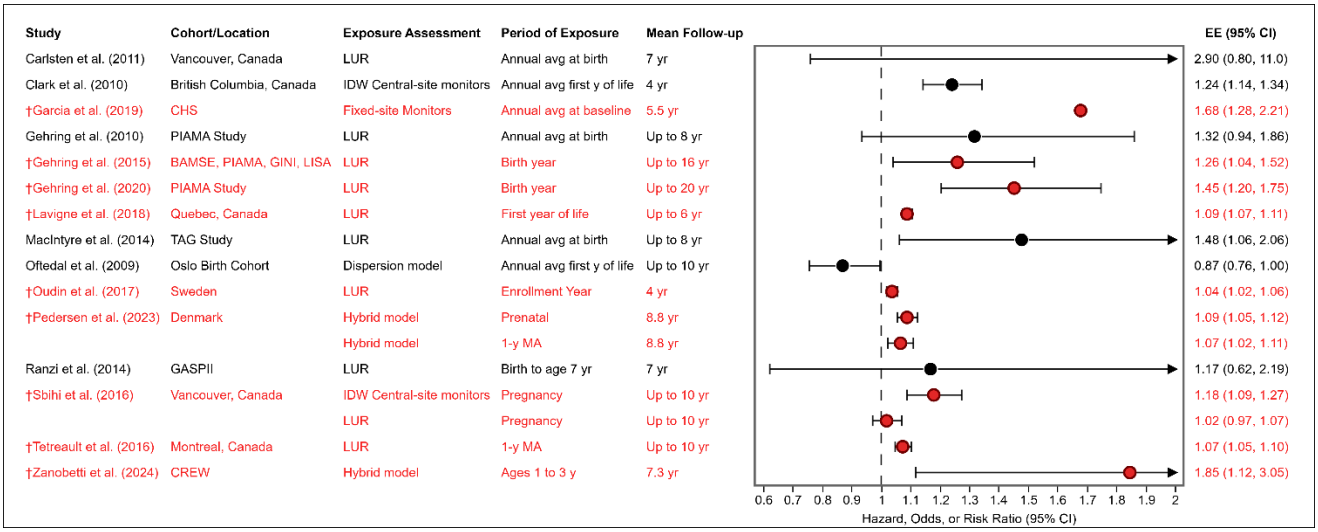
- This evidence includes a nationwide study in Denmark of over 1.1 million children and 65,000 asthma cases (Pedersen et al., 2023), as well as a number of studies in Ontario (Lavigne et al., 2018), Quebec (Buteau et al., 2020; Tétreault et al., 2016a), and Vancouver, Canada (Sbihi et al., 2017; Sbihi et al., 2016), and a multicity study in Sweden (Oudin et al., 2017). Most studies had at least eight years of follow-up. Associations with asthma onset were observed with both prenatal and postnatal NO_2 exposure.
- In a large administrative cohort study in Vancouver, Canada, Sbihi et al. (2017) used group-based trajectory modeling to identify four asthma trajectories based on patterns of healthcare use for asthma among children followed from birth up to 10 years of age. The empirically identified trajectories included non-asthma, early-onset chronic asthma (i.e., peak asthma prevalence by four years of age), late-onset chronic asthma (i.e., peak asthma prevalence by six years of age), and transient asthma (i.e., asthma diagnosis by one year of age followed by no asthma activity after age six). The authors reported that relative to the non-asthma trajectory, an interquartile range (IQR) increase in NO_2 concentrations averaged across pregnancy was associated with increases in the risk of each asthma trajectory. The strongest association was observed for early-onset chronic asthma (relative risk (RR): 1.51 [95% CI: 1.21, 1.88]).
- In most of the recent administrative cohort studies, asthma incidence was ascertained by identifying the first use of medical services (e.g., hospital admissions, ED visits, outpatient visits). In contrast, one recent cohort study in Sweden used data on dispensed medications for asthma to ascertain incidence, which may capture subjects with less severe or better-controlled asthma than hospital records (Oudin et al., 2017). The authors reported an increase in odds of asthma incidence associated with a 5.32 ppb increase in annual average NO_2 concentrations at baseline (OR: 1.02 [95% CI: 1.01, 1.03]).

Recent recruitment-based cohort studies also reported generally consistent evidence of a positive association between long-term NO_2 exposure and asthma incidence in children.

- Consistent with results from previous analyses of the southern California Children's Health Study (CHS) (McConnell et al., 2010; Jerrett et al., 2008) and the Dutch Prevention and Incidence of Asthma and Mite Allergy (PIAMA) cohort (Gehring et al., 2010), recent studies of the same cohorts reported positive associations between long-term NO_2 exposure and asthma incidence in children. Gehring et al. (2020) extended analyses of the PIAMA cohort to include children up to 20 years old and reported that annual average NO_2 concentrations at

birth address or in the year before each questionnaire were associated with increased odds of asthma incidence (OR: 1.20 per 4.9 ppb increase in NO_2 [95% CI: 1.10, 1.32] and 1.15 [95% CI: 1.04, 1.27], respectively). Similarly, annual average NO_2 concentrations at baseline were positively associated with asthma incidence in a recent extension of the CHS (Garcia et al., 2019). This study, which measured annual average NO_2 concentrations at baseline and followed schoolchildren for eight years from 4th to 12th grade, included three cohorts of the CHS rather than the single cohort analyzed in previous iterations.

- Pooled analyses of eight U.S. birth cohorts (Zanobetti et al., 2024) and four European birth cohorts (Gehring et al., 2015) provide further strong evidence of a positive association between long-term NO_2 exposure and asthma incidence. Notably, Gehring et al. (2015) used allergen-specific immunoglobulin E (IgE) tests in a subset of participants to distinguish between non-allergic and allergic asthma cases. The authors only observed increased NO_2-related risk of non-allergic asthma. This finding is consistent with an analysis of the Children, Allergy, Environment, Stockholm, Epidemiology (BAMSE) birth cohort that distinguished between asthma subtypes and reported stronger NO_2 associations with incident non-allergic asthma (Gruzieva et al., 2013).
- In addition to cohort studies that compared spatial variation in long-term average oxides of nitrogen concentrations and asthma incidence, Yu et al. (2024) leveraged decreasing air pollution trends in Stockholm, Sweden to evaluate how reductions in air pollution relate to the risks of developing asthma. The authors analyzed participants of the BAMSE birth cohort who did not have doctor-diagnosed asthma at eight years of age, and observed a reduction in asthma incidence by 24 years of age associated with a reduction in annual average NO_X concentrations from the first year of life to the 8-year follow-up visit (OR: 0.94 [95% CI: 0.83, 1.05] per 6.58 ppb reduction in NO_X). Notably, because baseline concentrations may be associated with pollutant reductions and asthma incidence, the authors controlled for baseline concentrations in the regression models. Additionally, consistent with analyses by Gehring et al. (2015) and Gruzieva et al. (2013), in models stratified by asthma type, Yu et al. (2024) only observed NO_X-associated reductions in asthma risk among participants with non-allergic asthma (OR: 0.86 [95% CI: 0.75, 1.00] per 6.58 ppb reduction in NO_X).
- Although most recent recruitment-based cohort studies noted positive associations between long-term NO_2 exposure and asthma onset in children, a small, pooled cohort of children from five U.S. cities reported an inverse association between two-year average NO_2 concentrations and asthma prevalence at age nine (Dearborn et al., 2023). This cohort, which included only 294 participants, focused on children with a history of bronchiolitis in infancy. The inverse association may be explained by a strong negative correlation between NO_2 and ozone (O_3) (r = -0.65), given that O_3 was positively associated with asthma incidence.
- Because transient wheezing is common in infants and often resolves as the child ages (Martinez et al., 1995), asthma diagnoses in infants and young children may have lower reliability. As a child progresses in age, the reliability of diagnosis of asthma would be expected to improve, as would the strength of inference regarding associations with NO_2 exposure. Some recent recruitment-based and administrative cohort studies have conducted sensitivity analyses restricting to asthma diagnoses after age four or five. In analyses restricted to these older groups, associations were comparable to, or slightly larger than, associations based on the full study populations [i.e., children of all ages; (Pedersen et al., 2023; Gehring et al., 2020; Tétreault et al., 2016a)].
- A few recent cross-sectional studies in Europe (Fuertes et al., 2020) and Australia (Knibbs et al., 2018) provide supporting evidence of an association between long-term NO_2 exposure and asthma prevalence in children.



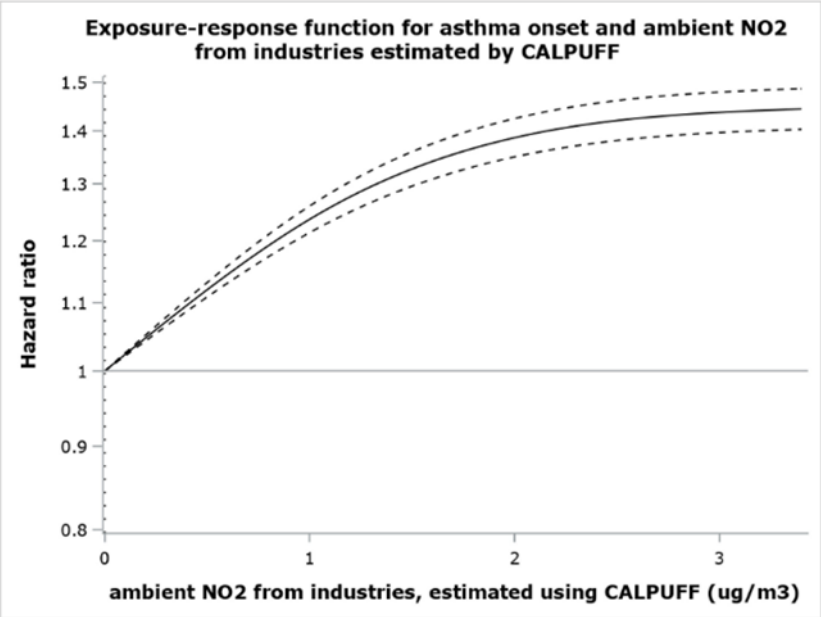
List of abbreviations in alphabetical order: Avg = average; BAMSE = Children, Allergy, Environment, Stockholm, Epidemiology (in Swedish); CHS = Children's Health Study, Southern California; CI = confidence interval; CREW = Environmental Influences on Child Health Outcomes (ECHO) Children's Respiratory and Environmental Workgroup consortium, U.S. birth cohorts; EE = effect estimate; GASPII = The Gene and Environment Prospective Study in Italy; GINI = German Infant Nutritional Intervention; IDW = inverse distance weighting; LISA = Influence of life-style factors on the development of the immune system and allergies in East and West Germany; LUR = land use regression; MA = moving average; PIAMA = The Prevention and Incidence of Asthma and Mite Allergy, Netherlands; TAG = Traffic, Asthma, and Genetics, international birth cohort; 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. Results are standardized to a 10-ppb increase in NO_2.
†Studies published since the 2016 Oxides of Nitrogen ISA.

Figure 4-1 Associations of long-term exposure to nitrogen dioxide with asthma incidence in cohort studies of children.

4.3.1.1.1 Concentration-Response

To date, there has been limited evaluation of the shape of the C-R relationship between long-term NO_2 exposure and asthma incidence in children. A few recent studies used cubic spline models to account for potential non-linear effects of long-term NO_2 exposures on asthma-onset (Pedersen et al., 2023; Buteau et al., 2020). In a nationwide administrative birth cohort study in Denmark, Pedersen et al. (2023) used a natural cubic spline model with four knots to assess the relationship of prenatal and post-natal annual average NO_2 concentrations and asthma incidence in children up to 19 years old. The C-R curves corresponding to prenatal exposure were linear across the entire concentration distribution. For postnatal exposure, represented as the time-weighted annual average NO_2 concentrations corresponding to all residential addresses during pre- and post-natal life, the C-R curve was linear through approximately 15 $\mu g/m^3$ (~8 ppb), at which point the relationship plateaued. (Buteau et al., 2020) utilized a dispersion model to estimate NO_2 concentrations from industrial sources in Quebec, Canada. In a restricted cubic spline model with three knots, the shape of the relationship between industrial-related NO_2 concentrations and asthma incidence was approximately linear at low concentrations and plateaued at higher concentrations (see Figure 4-2). Comparison of Akaike information criterion (AIC) values for the linear

and non-linear models supported a non-linear relationship, which is consistent with visual inspection of the spline plot. While neither study that examined spline models provides evidence of a threshold at lower concentrations, a recent cohort study in Sweden analyzed models that were stratified by NO_2 concentration above and below 15 µg/m^3 (~8 ppb), and only observed a positive association between NO_2 and asthma incidence in the higher exposure group (Oudin et al., 2017). The mean concentration of NO_2 in this study was 10.2 µg/m^3 (5.4 ppb) but the authors did not report the number of participants in each of the stratified exposure groups.



List of abbreviations in alphabetical order: NO_2 = nitrogen dioxide; µg/m^3 = microgram per cubic meter.
Source: Adapted from Buteau et al. (2020). Copyright permission pending.

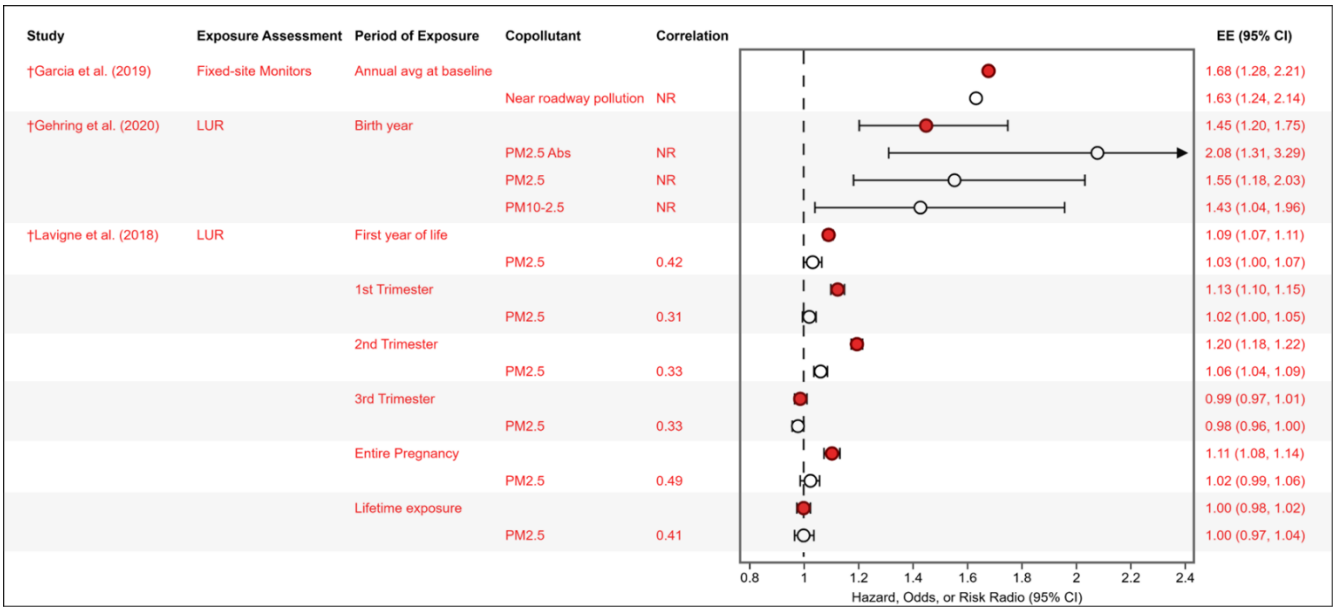
Figure 4-2 Concentration-response curve between annual average nitrogen dioxide concentrations from industrial sources and asthma incidence in children; estimated from a restricted cubic spline model.

4.3.1.1.2 Copollutants

NO_2 in ambient air is often highly correlated with other traffic-related copollutants; therefore, it can be difficult to distinguish an independent association between long-term NO_2 exposures and respiratory disease. In the 2016 Oxides of Nitrogen ISA, no studies of NO_2 and asthma development examined copollutant models with traffic-related copollutants. A few recent studies provide initial evidence that associations between long-term NO_2 exposure and asthma incidence in children persist in models adjusting for PM and near-road pollution (see Figure 4-3). A large administrative birth cohort in Ontario, Canada that included over 100,000 incident asthma cases in children ages zero to six years reported positive association between asthma development and NO_2 concentrations during certain developmental windows (see Section 4.3.1.1.6; (Lavigne et al., 2018)). For most exposure windows in

which NO_2 concentrations were associated with asthma onset, the observed associations were attenuated, but still positive after adjustment for $PM_{2.5}$ in a copollutant model. For example, second trimester NO_2 concentrations were associated with a 20% increase in asthma incidence (95% CI: 18, 22) in a single pollutant model, but only a 6% increase (95% CI: 4, 9) in a model that controlled for $PM_{2.5}$ concentrations.

Smaller, traditional longitudinal cohort studies observed positive associations that were relatively unchanged in copollutant models adjusting for PM or near-road pollution. In a recent analysis of the CHS cohort described in Section 4.3.1.1, Garcia et al. (2019) observed that decreases in asthma incidence associated with lower annual average monitored NO_2 concentrations at baseline (incidence rate ratio (IRR): 0.60 [95% CI: 0.45, 0.78] per 10 ppb decrement) were comparable after adjustment for near-road pollution estimated using a line source dispersion model including variable such as distance to roadways, vehicle counts, and vehicle oxides of nitrogen emissions (IRR: 0.61 [95% CI: 0.47, 0.80]). The authors did not report correlations between NO_2 concentrations and modeled near-road pollution, but they would be expected to be high considering vehicle oxides of nitrogen emissions rates were a model input. Similarly, the association between annual average NO_2 concentrations at birth address and asthma incidence observed in an extended analyses of the PIAMA cohort were unchanged in models adjusted for $PM_{2.5}$ or $PM_{10–2.5}$, and larger in magnitude following adjustment for $PM_{2.5}$ absorbance – a surrogate for traffic-related particle pollution (Gehring et al., 2020).



List of abbreviations in alphabetical order: Abs = absorbance; Avg = average; CI = confidence interval; EE = effect estimate; LUR = land use regression; NO2 = nitrogen dioxide; NR = not reported; $PM_{2.5}$ = particulate matter with a nominal mean aerodynamic diameter less than or equal to 2.5 µm.
Notes: Red = recent studies and those not included in 2016 Oxides of Nitrogen ISA. Filled circles = results from single pollutant models; unfilled circles = results from copollutant models. Results are standardized to a 10-ppb increase in NO_2.
†Studies published since the 2016 Oxides of Nitrogen ISA.

Figure 4-3 Associations between long-term nitrogen dioxide exposure and asthma incidence in children in single pollutant models and models adjusted for copollutants.

4.3.1.1.3 Additional Sources of Confounding

In addition to the potential copollutant confounding, other factors, including risk factors for chronic respiratory conditions, can also confound the relationship between long-term NO_2 exposure and asthma incidence in children. Confounders considered by recent studies included child and parental demographics and socioeconomic status (SES) factors, parental health-behaviors, familial health history, housing characteristics, and residential area characteristics. Recent studies accounted for confounding in a similar manner: typically controlling for age by using age as a timescale in Cox proportional hazards models, stratifying models by sex, matching cases and controls by age and sex, and adjusting multivariate models using individual-level and area-level covariates.

At the individual-level, studies controlled for factors including child attributes (e.g., sex, birth year, race, and ethnicity, atopy at 12 months, child respiratory illness before age 2, participation in sports, day-care attendance), birth attributes (e.g., parity, preterm birth, delivery mode, breast-feeding, birth weight, gestational length), maternal attributes (e.g., smoking during pregnancy, maternal education, maternal age at delivery, body mass index (BMI) during early pregnancy), paternal attributes (e.g., smoking habits, paternal education), familial health history (e.g., history of asthma and/or hay fever in child's mother, father, or sibling), SES factors (e.g., household income, medical insurance coverage), and

housing characteristics (e.g., presence of mold and dampness, furry pets, use of gas for cooking, unvented gas water heater, year that residence was built, secondhand smoke exposure). Area-level factors that were adjusted for included environmental characteristics (e.g., season, location, residential greenness, temperature, relative humidity), approximations for socioeconomic status (e.g., household income, education, occupation, neighborhood deprivation, racial composition), and an approximation for secondhand smoke exposure (e.g., smoking rates). In some studies (i.e., administrative cohorts), individual-level data are more limited and area-level covariates were often used as surrogates for individual-level information. The area-level variables included in recent studies were considered at the neighborhood level (e.g., population size less than 10,000) (Dearborn et al., 2023; Buteau et al., 2020; Sbihi et al., 2017; Sbihi et al., 2016; Clark et al., 2010) and at the postal code level (Lavigne et al., 2018).

Following varying levels of adjustment for the confounders noted above, recent studies have consistently observed positive associations between NO_2 exposure and asthma risk (Pedersen et al., 2023; Buteau et al., 2020; Gehring et al., 2020; Garcia et al., 2019; Knibbs et al., 2018; Oudin et al., 2017; Sbihi et al., 2017; Tétreault et al., 2016a; Gehring et al., 2015; McConnell et al., 2010). In some cases, authors reported similar magnitudes of association in minimally- and fully-adjusted models. For example, in analyses of the CHS cohort, Garcia et al. (2019) and Gehring et al. (2020) reported comparable associations in minimally- and full-adjusted models. This indicates that there was limited confounding by the additional covariates that were included in the fully adjusted models. In contrast, a few other studies that compared models with different levels of adjustment noted that associations remained positive, but the magnitude of effect estimates decreased with the inclusion of more variables. Thus, although the attenuation of effect estimates suggests the presence of confounding by the additional variables included in these models, the positive association in the fully-adjusted model indicates that the relationship between long-term NO_2 exposure and asthma incidence in children is independent of those factors. For example, Oudin et al. (2017) reported an odds ratio of 1.10 ([95% CI: 1.08, 1.12]) per 10 ppb increase in NO_2 in the minimally adjusted model and 1.04 ([95% CI: 1.02, 1.06]) in the model further adjusted for parental and environmental factors. Similarly, Tétreault et al. (2016a) observed a positive association between a 10 ppb increase in NO_2 and an increased hazard ratio in the basic model (hazard ratio [HR]: 1.13 [95% CI: 1.11, 1.15]) that was reduced in magnitude, but remained positive, after additional adjustment for child's sex and socioeconomic variables (HR: 1.07 [95% CI: 1.05, 1.10]).

4.3.1.1.4 Lifestages and Population Differences

Sex

A limited number of studies that were evaluated in the 2016 Oxides of Nitrogen ISA examined sex differences in the association between long-term NO_2 exposure and asthma incidence in children. Evidence was inconsistent, with one study each indicating increased risk for females (Clark et al., 2010), or increased risk for males [(Carlsten et al., 2011b); quantitative data not provided]. While neither of the studies indicated that comparisons were planned a priori, the proportion of sexes was approximately

equal, providing fairly large numbers of males and females to compare. A limited number of recent studies examined the potential effect modification of long-term NO_2 exposure and asthma incidence by sex. In a pooled analysis of eight longitudinal birth cohorts participating in the Children's Respiratory and Environmental Workgroup (CREW), Zanobetti et al. (2024) reported stronger associations between average NO_2 concentrations during the first three years of life and asthma incidence by age 11 in males relative to females. There was no sex-specific difference in the magnitude of associations with asthma incidence in the first four years of life. Lavigne et al. (2018) did not observe any sex-specific differences in the strength of the association between annual average NO_2 concentrations and asthma incidence in a large administrative birth cohort in Ontario, Canada. As a whole, evidence continues to be inconsistent for sex-related differences in the risk of asthma incidence associated with long-term exposure to NO_2 (see Table 4-3).

Table 4-3 Epidemiologic studies evaluating effect-measure modification of the relationship between long-term NO_2 exposure and childhood asthma incidence by sex

Study	Location/Cohort (Years)	EMM Reference Factor	EMM Comparison Factor	Direction of EMM[a]	Hazard Ratio (95% CI)	Spatial Scale of Effect Modifier
Carlsten et al. (2011b)	Vancouver, Canada (1995–2002)	Male	Female	↓	EMM Reference Factor: NR EMM Comparison Factor: NR	Individual level
Clark et al. (2010)	British Columbia, Canada (1999–2003)	Male	Female	↑	EMM Reference Factor: 1.18 (1.05, 1.32) EMM Comparison Factor: 1.34 (1.17, 1.54)	Individual level
†Lavigne et al. (2018)	Quebec, Canada (2006–2013)	Male	Female	–	EMM Reference Factor: 1.06 (1.04, 1.09) EMM Comparison Factor: 1.06 (1.04, 1.09)	Individual level
†Zanobetti et al. (2024)	ECHO CREW (1987–2018)	Male (4 yrs)	Female (4 yrs)	–	EMM Reference Factor: 2.09 (1.06, 4.11) EMM Comparison Factor: 1.94 (1.01, 3.74)	Individual level
		Male (11 yr)	Female (11 yrs)	↓	EMM Reference Factor: 2.25 (1.27, 3.96) EMM Comparison Factor: 1.58 (0.92, 2.72)	

List of abbreviations in alphabetical order: CREW = Children's Respiratory and Environmental Workgroup consortium, U.S. birth cohorts; CI = confidence interval; ECHO = Environmental Influences on Child Health Outcomes; EMM = effect measure modification; NO_2 = nitrogen dioxide; NR = not reported; ppb = parts per billon; yrs = years.
Results are standardized to a 10-ppb increase in 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.

†Studies published since the 2016 Oxides of Nitrogen ISA.

Sociodemographic Factors

In addition to sex, the recent CREW analysis also evaluated EMM by several other sociodemographic factors, including education, race/ethnicity, and neighborhood characteristics (Zanobetti et al., 2024). In models stratified by race/ethnicity, non-white and Hispanic participants had a higher NO_2-related risk of asthma development in the first 11 years of life relative to non-Hispanic white participants. The authors also observed a stronger NO_2-asthma association among participants born to mothers who did not finish high school compared to those whose mothers had higher educational attainment (i.e., high school or college/graduate school). EMM by these individual-level characteristics were consistent with results from models stratified by neighborhood-level variables, including the percentage of the Black population and the educational domain of the Childhood Opportunity Index (COI). The magnitudes of associations were larger among participants living in neighborhoods in the 90th percentile of the percentage Black population (relative to the 10th percentile) and in the 10th percentile of educational opportunity and resources (relative to the 90th percentile).

Genetics

There is a well-recognized heritable component to asthma – children who have at least one parent with asthma are significantly more likely to develop asthma than children born to asthma-free parents. A recent administrative birth cohort study in Ontario, Canada analyzed the modifying effect of maternal asthma or atopy history on the association between prenatal NO_2 exposure and asthma incidence in children by 6 years of age (Lavigne et al., 2018). Lavigne et al. (2018) observed that children born to mothers with a history of asthma were at slightly higher risk of developing asthma per 10 ppb increase in NO_2 concentrations during the second trimester (HR: 1.09 [95% CI: 1.03, 1.16]) than children born to mothers without asthma (HR: 1.06 [95% CI: 1.04, 1.09]). Similar differences were reported for maternal atopy (HR: 1.12 [95% CI: 1.05, 1.20] vs. HR: 1.06 [95% CI: 1.04, 1.09]).

4.3.1.1.5 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. Thus, a key issue in evaluating the strength of inference about NO_2-related asthma development from epidemiologic studies is the extent to which the NO_2 exposure assessment method used in a study captured the variability in exposure among study subjects. Chapter 2 provides a thorough discussion of the bias resulting from particular exposure assessment methods common to the epidemiologic studies of long-term exposure and its impact on the inferences that can be drawn about health effects. In the 2016 Oxides of Nitrogen ISA, the set of studies examining asthma among children used a variety of exposure assessment techniques including LUR models, monitors outside each subject's home, fixed-site community monitors, monitoring site measurements combined by IDW, and dispersion models. These studies of asthma incidence demonstrate various tradeoffs in an effort to design exposure assessments that might characterize exposure for

individual subjects. However, the robustness of the evidence across exposure assessment methods provides a strong basis for drawing inferences about the relationship between long-term NO_2 exposure and asthma development in children.

Recent studies similarly employ a variety of exposure assessment methods, including the methods used in studies examined in the 2016 Oxides of Nitrogen ISA, as well as a novel hybrid prediction model that combines data from LUR models, dispersion models, and monitoring data to estimate spatially and temporally resolved exposure data across the entirety of Denmark (Pedersen et al., 2023). Consistent with findings from the 2016 Oxides of Nitrogen ISA, recent studies have reported that long-term NO_2 concentrations estimated from each of the exposure assessment methods were consistently associated with increased asthma incidence in children. A recent administrative birth cohort study in Vancouver, Canada compared results corresponding to NO_2 concentrations estimated from LUR models or IDW averages of three monitors (Sbihi et al., 2016). In children up to 6 years old, the authors observed that asthma incidence was positively associated with IDW NO_2 estimates, but not LUR estimates. While LUR estimates provided better spatial resolution than IDW estimates, the LUR model only performed moderately well in cross-validation ($R^2 = 0.56$).

4.3.1.1.6 Exposure Windows

Epidemiologic studies to date provide evidence that long-term exposure to NO_2 during various lifestages or time periods throughout childhood is consistently associated with asthma incidence. Cohort studies of asthma incidence in children often consider outcomes in relation to annual average NO_2 exposures at birth, cumulative lifetime exposures, or time-varying exposures in the year(s) prior to the outcome. Cumulatively, recent studies have reported that asthma development is positively associated with NO_2 concentrations measured or modeled during each of the aforementioned time periods. Often, NO_2 concentrations during the various exposure periods evaluated are highly correlated with one another, making it difficult to identify a single exposure window of concern. Nonetheless, a few recent studies have compared results from different exposure windows in the same study. Most notably, Lavigne et al. (2018) used LUR models to estimate NO_2 concentrations across a range of potentially sensitive exposure windows, including each trimester of pregnancy, the entire prenatal period, the first year of life, and cumulative lifetime. The authors reported moderate to strong correlations between each of the exposure periods ($r = 0.48$ to 0.78) but still observed varying strengths of associations across the exposure metrics. The strongest associations were reported for average NO_2 concentrations during the second trimester (HR: 1.20 [95% CI: 1.18, 1.22] per 10 ppb), which corresponds to several important stages of lung and airway development in utero (Schittny, 2017). Asthma incidence was also associated with NO_2 concentrations during the first trimester (HR: 1.13 [95% CI: 1.10, 1.15]), the entire pregnancy period (HR: 1.11 [95% CI: 1.08, 1.14]), and the first year of life (HR: 1.09 [95% CI: 1.07, 1.11]), but not during the third trimester (HR: 0.99 [95% CI: 0.97, 1.01]) or as measured cumulatively over a child's lifetime (HR: 1.00 [95% CI: 0.98, 1.02]). In most other recent studies that compared prenatal or at-birth exposures

to time-varying exposures, authors reported positive associations of comparable magnitude for each exposure period (Pedersen et al., 2023; Gehring et al., 2020; Knibbs et al., 2018; Tétreault et al., 2016a; Gehring et al., 2015). In contrast, in a recent analysis of the CREW study, Zanobetti et al. (2024) observed that asthma incidence was only associated with NO_2 concentrations estimated after birth (i.e., in the first years of life). The authors reported null associations with prenatal exposures (quantitative results not provided).

4.3.1.1.7 Conclusions and Remaining Uncertainties

In summary, a large body of epidemiologic evidence supports an association between long-term NO_2 exposure and asthma incidence in children. Evidence is generally consistent across a mix of traditional recruitment-based and administrative cohort studies using a range of exposure assessment methods, including LUR, dispersion models, hybrid prediction models, and fixed-site monitors. Recent studies adjusted for a wide range of potential confounders using individual-level and area-level covariates (see Section 4.3.1.1.3) and reported consistent positive associations across varying levels of adjustment. Evidence suggests that long-term exposure to NO_2 during various lifestages or time periods throughout childhood, including prenatal, at-birth, or time-varying exposures, is consistently associated with asthma incidence. Although it can be challenging to disentangle the effects of NO_2 exposure across exposure windows due to highly correlated exposures, a recent study examining exposure across trimesters of pregnancy reported that increased asthma incidence in childhood was most strongly associated with average NO_2 concentrations during the second trimester, which corresponds to several important stages of lung and airway development in utero (Schittny, 2017). A few recent studies observed associations between NO_2 exposure and asthma incidence that were robust to adjustment for modeled near-road pollution or traffic-related particle pollution. In each case, the authors did not report correlations between NO_2 and the corresponding copollutant. Given the likelihood that the copollutants are highly correlated with NO_2 concentrations, there is uncertainty concerning the applicability and interpretation of those copollutant models due to potential issues with multicollinearity.

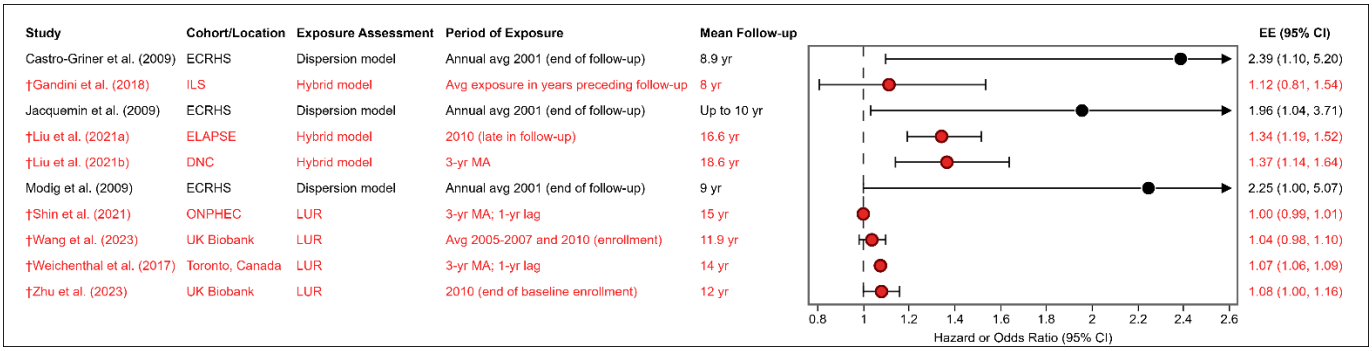
4.3.1.2 Epidemiologic Studies of Asthma Incidence in Adults

The 2016 Oxides of Nitrogen ISA evaluated a limited number of studies of adult-onset asthma. The available evidence consisted of analyses of the European Community Respiratory Health Survey (ECRHS) cohort. Several studies reported positive associations between NO_2 concentrations estimated from dispersion models and asthma incidence in various subsets of adults in the ECRHS cohort (Castro-Giner et al., 2009; Jacquemin et al., 2009; Modig et al., 2009). The strength of inference from these studies is limited because findings are based on one cohort, and none of the studies considered confounding by $PM_{2.5}$ or traffic-related pollutants.

Several recent studies expand the existing evidence base and provide additional support for a positive association between long-term NO_2 exposure and adult-onset asthma (see Figure 4-4). Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 4-2. Recent evidence includes a greater diversity of cohorts and geographic locations, and larger sample sizes, which in turn produce more precise effect estimates. Positive associations were observed in relation to NO_2 exposures estimated at residences with LUR or hybrid prediction models. Recent evidence also includes evaluation of potential copollutant confounding and consideration of the shape of the C-R relationship. A short summary of the recent evidence is provided below, followed by an additional discussion of the concentration-response relationships in available studies (see Section 4.3.1.2.1), analyses of copollutants (see Section 4.3.1.2.2) and other potential confounders (see Section 4.3.1.2.3), and the lifestages and populations evaluated (see Section 4.3.1.2.4). Section 4.3.1.2.5 discusses conclusions from these studies and summarizes remaining uncertainties.

- Recent studies provide consistent evidence of a positive association between long-term NO_2 exposure and adult-onset asthma. Evidence of positive associations comes from cohort studies conducted in Canada (Weichenthal et al., 2017a), Italy (Gandini et al., 2018b), the United Kingdom (U.K.) (Wang et al., 2023b; Zhu et al., 2023), and Denmark (Liu et al., 2021d), as well as a pooled analysis of multiple cohorts from Sweden and Denmark (Brunekreef et al., 2021; Liu et al., 2021a).
- In each recent study, adult-onset asthma was ascertained through record linkages to health administrative databases. Asthma incidence was defined as the first use of medical services (e.g., hospital admissions, ED visits, outpatient visits) for asthma in participants without a history of asthma at baseline. Associations were observed with long-term average NO_2 concentrations estimated using LUR or hybrid prediction models.
- One recent study utilized data from the UK Biobank study to examine the progression of chronic lung disease in adults without chronic lung disease at baseline. Wang et al. (2023b) reported a small increase in asthma incidence associated with a 2.7 ppb increase in 4-year average NO_2 concentrations prior to follow-up (4% increase [95% CI: -2%, 10%]). The authors noted a much larger increase in NO_2-related risk of chronic lung multimorbidity in participants who developed asthma during the course of follow-up. Specifically, in participants with incident asthma, long-term NO_2 exposure was associated with a 53% increase (95% CI: 25, 87) in the risk of developing a second chronic lung disease, either chronic obstructive pulmonary disease (COPD) or lung cancer.
- Although most recent cohort studies noted positive associations between long-term NO_2 exposure and adult-onset asthma, a null association was observed in a recent population-based administrative cohort of 5.1 million adults in Ontario, Canada (Shin et al., 2021). Strengths of this study included a long follow-up time (15 years), a large number of incident asthma cases (n = 218,005), and minimal risk of selection bias due to the inclusion of the majority of the population captured under a universal healthcare system. In contrast, a smaller administrative cohort study of 1.1 million adults in Toronto, Ontario reported a 7% increase (95% CI: 6, 9) in asthma incidence per 10-ppb increase in 3-year moving average NO_2 concentrations (Weichenthal et al., 2017a).
- Recent studies used various averaging times and exposure time periods to assess the relationship between long-term NO_2 exposure and adult-onset asthma. Common approaches

included 3-year moving average concentrations in time-varying regression models (Liu et al., 2021d; Shin et al., 2021; Weichenthal et al., 2017a) and annual or multi-year average concentrations assigned at baseline (Wang et al., 2023b; Zhu et al., 2023; Brunekreef et al., 2021; Liu et al., 2021a; Gandini et al., 2018b). Positive associations were observed in relation to both approaches, and studies that conducted sensitivity analysis reported that results from time-varying models were robust to alternative averaging times (Liu et al., 2021d; Weichenthal et al., 2017a) and results corresponding to exposures assigned at baseline were comparable to results using time-varying exposure (Liu et al., 2021a).

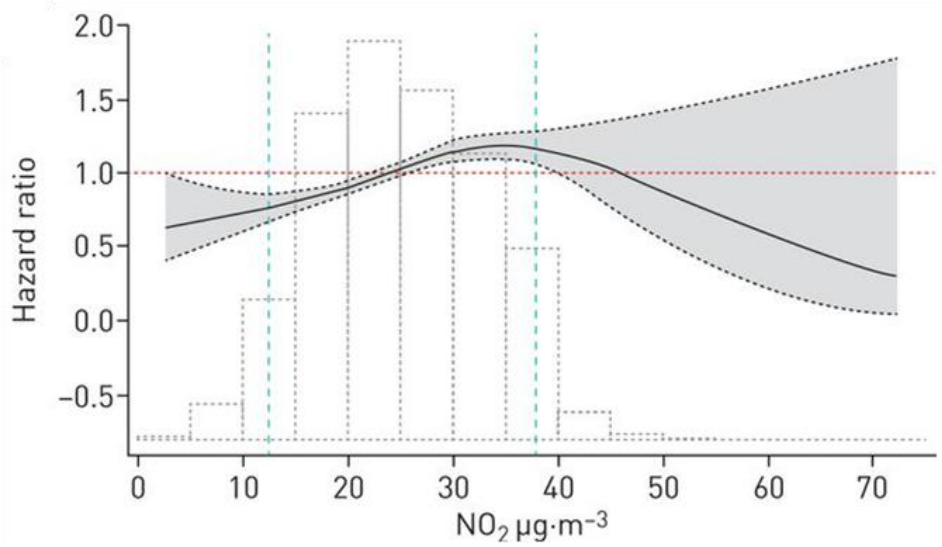


List of abbreviations in alphabetical order: Avg = average; CI = confidence interval; DNC = Danish Nurses Cohort; ECRHS = European Community Respiratory Health Survey; ELAPSE = Effects of Low-level Air Pollution: a Study in Europe; ILS = Italian Longitudinal Study; LUR = land use regression; MA = moving average; ONPHEC = The Ontario Population Health and Environment Cohort; 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. Results are standardized to a 10-ppb increase in NO_2.
†Studies published since the 2016 Oxides of Nitrogen ISA.

Figure 4-4 Associations of long-term exposure to nitrogen dioxide with asthma incidence in cohort studies of adults.

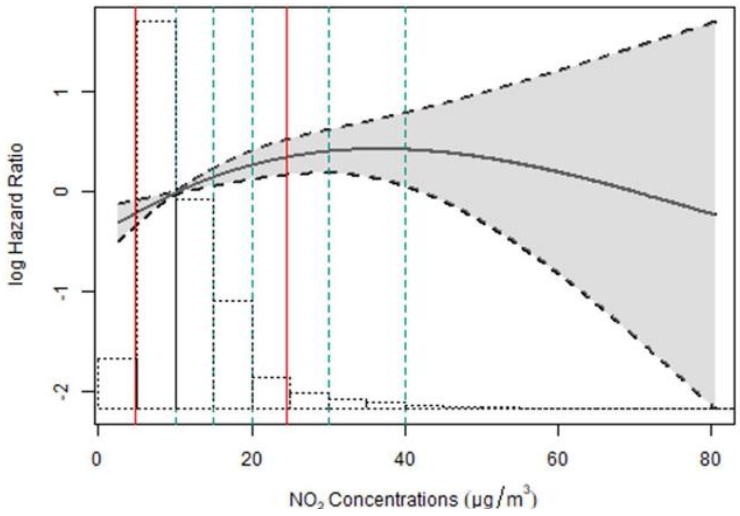
4.3.1.2.1 Concentration-Response and Effects at Low Concentrations

No studies in the 2016 Oxides of Nitrogen ISA evaluated the shape of the C-R relationship between long-term NO_2 exposure and adult-onset asthma. A few recent studies examined potential non-linearities in the C-R relationship through natural cubic spline models with either three (Liu et al., 2021d) or four (Liu et al., 2021a) knots. The shape of the C-R relationship between the 5th and 95th percentile of NO_2 concentrations was approximately linear in both studies (see Figure 4-5 and Figure 4-6). Both curves indicate a plateau or inverse association at concentrations above the 95th percentile of NO_2 concentrations, but there is less confidence in the shape of the C-R relationships at these higher concentrations due to fewer observations, as indicated by wider 95% CIs. Each study also analyzed potential thresholds using AIC values to compare the fit of regression models with and without thresholds. Consistent with the visual inspection of the C-R curves, the AIC values indicated that the no-threshold model was a better fit than each of the threshold models. The results from these studies are not entirely independent, given that they examined overlapping study populations – the Danish Nurse Cohort (DNC) (Liu et al., 2021d) and pooled data from three European cohorts, including the DNC (Liu et al., 2021a).



List of abbreviations in alphabetical order: NO_2 = nitrogen dioxide; µg/m^3 = micrograms per cubic meter.
Notes: green dashed lines indicate the 5th and 95th percentiles of NO_2 concentrations; red dashed line indicates the null value.
Source: Adapted from Liu et al. (2021a). Copyright permission pending.

Figure 4-5 Concentration-response curve between 3-year moving average nitrogen dioxide concentrations and adult-onset asthma in the Danish Nurse Cohort.



List of abbreviations in alphabetical order: NO_2 = nitrogen dioxide; µg/m^3 = micrograms per cubic meter.
NOTE: Red solid lines indicate the 5th and 95th percentiles of NO_2 concentrations. Green dashed lines indicate cutoff values for analyses not reflected in this figure.
Source: Adapted from Liu et al. (2021d). Copyright permission pending.

Figure 4-6 Concentration-response curve between nitrogen dioxide concentrations and adult-onset asthma in an analysis of pooled data from three European cohorts.

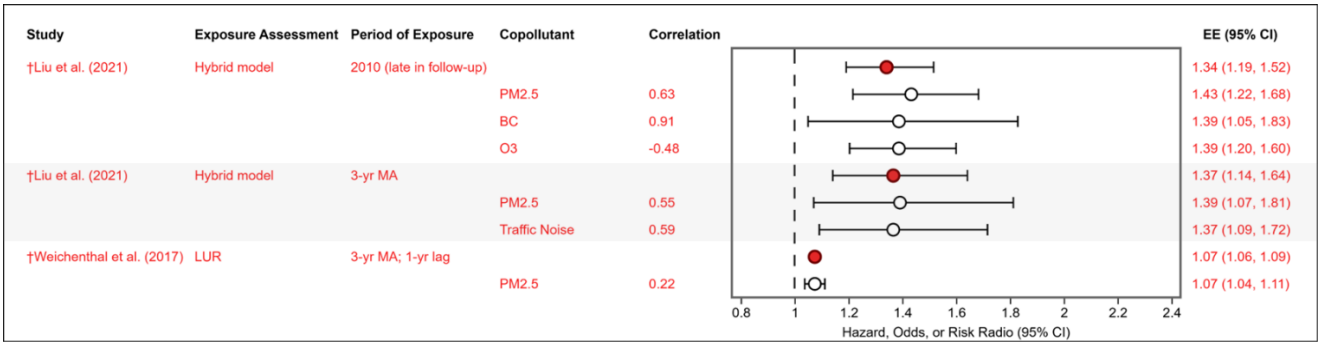
Liu et al. (2021d) and Liu et al. (2021a) also employed subset analyses to examine the strength of the relationship between long-term NO_2 exposure and asthma incidence in adults below increasingly

stringent concentration cutoffs. Consistent with the findings from the spline and threshold models, the magnitude of the observed associations in the full population was comparable to the associations below each of the cutoffs of 40 µg/m^3 (21.3 ppb) and 20 µg/m^3 (10.6 ppb). Liu et al. (2021d) further analyzed lower cutoffs and noted a positive association that was stronger but less precise than the unrestricted analysis in participants exposed to concentrations less than 15 µg/m^3 (8 ppb; HR: 2.13 [95% CI: 1.18, 3.85]) and an association that was null and imprecise among participants exposed to concentrations less than 10 µg/m^3 (5.3 ppb; HR: 0.80 [95% CI: 0.20, 3.27]).

As a whole, the evidence suggests an approximately linear relationship between long-term NO_2 exposure and adult-onset asthma from the 5th to 95th percentile of NO_2 concentrations (as low as 5 µg/m^3 [2.66 ppb]). Evidence examining a potential threshold is inconsistent. While the spline plots and statistical analyses of model fit do not support the presence of a threshold, a subset analysis indicated a null association at concentrations below 10 µg/m^3 (5.3 ppb), albeit with reduced precision.

4.3.1.2.2 Copollutants

As discussed in Section 4.4.1.3, exposure to other air pollutants may confound the relationship between long-term NO_2 exposure and respiratory disease. Among the limited number of studies in the 2016 Oxides of Nitrogen ISA that examined adult-onset asthma, none evaluated potential copollutant confounding. A few recent studies of adult-onset asthma have analyzed copollutant models adjusted for traffic-related pollutants, as well as other air pollutants or environmental stressors (Liu et al., 2021d; Liu et al., 2021a; Weichenthal et al., 2017a). Across studies, observed associations between long-term NO_2 exposure and asthma incidence in adults are relatively unchanged in copollutant models, including models adjusted for $PM_{2.5}$ (Liu et al., 2021d; Liu et al., 2021a; Weichenthal et al., 2017a), black carbon (BC) (Liu et al., 2021a), O_3 (Liu et al., 2021a), distance from roadway (Weichenthal et al., 2017a), and traffic noise (Liu et al., 2021d) (see Figure 4-7). Liu et al. (2021a) reported a strong correlation between long-term NO_2 and BC concentrations ($\rho = 0.91$), complicating the interpretation of those results. Collinearity resulting from high correlations between NO_2 concentrations and the potential confounder can result in highly unstable effect estimates for NO_2 or the covariate (Dominici et al., 2010).



List of abbreviations in alphabetical order: CI = confidence interval; LUR = land use regression; MA = moving average; $PM_{2.5}$ = particulate matter with a nominal mean aerodynamic diameter less than or equal to 2.5 µm; yr = year.
Notes: Red = recent studies and those not included in 2016 Oxides of Nitrogen ISA. Filled circles = results from single pollutant models; unfilled circles = results from copollutant models. Results are standardized to a 10-ppb increase in NO_2.
†Studies published since the 2016 Oxides of Nitrogen ISA.

Figure 4-7 Associations between long-term nitrogen dioxide exposure and asthma incidence in adults in single pollutant models and models adjusted for copollutants.

4.3.1.2.3 Additional Sources of Confounding

Confounders considered by recent studies included personal demographics, health factors, behavioral factors, and socioeconomic variables. Recent studies have addressed these potential confounders using age as a timescale in Cox proportional hazards models and adjusted models using individual-level and area-level factors. Factors controlled for at the individual-level included personal demographics (e.g., age, sex), health factors (e.g., BMI, comorbidities), behavioral characteristics (e.g., smoking status and duration, physical activity, diet, alcohol consumption), and socioeconomic variables (e.g., marital status, occupational status, education level, household income). Adjustment for area-level factors included socioeconomic factors (e.g., household income, unemployment, education, recent immigrant population), potential access to healthcare (e.g., density of primary care physicians), and indirect measures of health factors (e.g., smoking and obesity). Area-level variables included in recent studies were considered at the neighborhood level (e.g., population size less than 10,000) (Shin et al., 2021; Weichenthal et al., 2017a), municipality level (Liu et al., 2021a), and at the regional level (Zhu et al., 2023).

After adjusting for the aforementioned potential confounders, recent studies have consistently observed positive associations between NO_2 exposure and asthma risk (Zhu et al., 2023; Liu et al., 2021d; Liu et al., 2021a; Weichenthal et al., 2017a). Liu et al. (2021d) observed that the magnitude of the association between a 10-ppb increase in NO_2 and asthma incidence was similar between the minimally adjusted model (HR: 1.46 [95% CI: 1.20, 1.78]) and the model adjusted for behavioral characteristics, health factors, and socioeconomic variables (HR: 1.41 [95% CI: 1.16, 1.71]). Liu et al. (2021a) similarly observed comparable magnitudes of associations between long-term NO_2 concentrations and asthma incidence in a minimally adjusted model (HR: 1.49 [95% CI: 1.29, 1.73]), a model adjusted for behavioral

characteristics and socioeconomic status (HR: 1.46 [95% CI: 1.26, 1.69]), and a model further adjusted for area-level income (HR: 1.43 [95% CI: 1.24, 1.66]). Compared to a minimally adjusted model (HR: 1.20 [95% CI: 1.15, 1.25]), Weichenthal et al. (2017a) also observed minimal attenuation of HRs in a model adjusting for neighborhood-level socioeconomic status (HR: 1.15 [95% CI: 1.12, 1.17]), and a model further adjusting for individual comorbidities (HR: 1.15 [95% CI: 1.07, 1.22]). Zhu et al. (2023) reported smaller but comparable HRs and 95% CIs in an unadjusted model (HR: 1.08 [95% CI: 1.01, 1.14]) and a model adjusting for socioeconomic factors, behavioral characteristics, and health factors (HR: 1.08 [95% CI: 1.00, 1.16]). Null observations between NO_2 exposure and asthma risk were also observed (Wang et al., 2023b; Shin et al., 2021).

Similar to NO_2, recent studies have consistently observed positive associations between NO_X exposure and asthma risk in both parsimonious and fully-adjusted models (Zhu et al., 2023; Liu et al., 2021d). Parsimonious models include crude models with no adjustments and models adjusted for age and study year. Fully adjusted models further controlled for individual (BMI, occupation), lifestyle (physical activity, diet), and other exposures (smoking).

4.3.1.2.4 Lifestages and Populations

Sex

Few studies have examined potential sex-specific differences in the association between long-term NO_2 exposure and adult-onset asthma. A limited number of recent studies provide contrasting results. In a nationwide, cohort study of 35+ year-old adults in Italy, Gandini et al. (2018b) reported a positive but imprecise association between NO_2 concentrations and asthma incidence in males (HR: 1.26 [95% CI: 0.73, 2.16] per 10 ppb), but not in females (HR: 1.00 [95% CI: 0.67, 1.49]). In contrast, Weichenthal et al. (2017a) reported a 7% increase in the incidence of adult-onset asthma in both males (95% CI: 4, 11) and females (95% CI: 3, 13) associated with a 10 ppb increase in 3-year average NO_2 concentrations in a large administrative cohort study in Toronto, Canada.

Lifestage

No studies in the 2016 Oxides of Nitrogen ISA examined the potential effect modification of long-term NO_2 exposure and adult-onset asthma by age. As discussed previously, Weichenthal et al. (2017a) analyzed asthma incidence in adults in a large cohort study in Toronto, Canada. The authors examined regression models stratified by age (i.e., 30–59, 60–74, and 75+ years) to assess the potential modification of NO_2-related risk of asthma incidence across age groups. The authors reported comparable hazard ratios for 30–59-year-olds (1.10 [95% CI: 1.05, 1.15]) and 60–74-year-olds (1.07 [95% CI: 1.03, 1.13]), and observed a null association for 75+ years olds (1.00 [95% CI: 0.92, 1.09]). Notably, there were many fewer cases in the oldest age group (n = 6,453) compared to the middle (n = 21,754) and youngest groups (n = 58,934).

Genetics

One recent study utilized data on risk alleles from the UK Biobank Study to calculate polygenic risk scores that categorized participants as being at "low" or "high" genetic risk for asthma (Zhu et al., 2023). In a sample of nearly 300,000 adults who were asthma-free at enrollment, Zhu et al. (2023) observed a stronger association between annual average NO_2 concentrations at baseline and asthma incidence in participants with low genetic risk (HR: 1.14 [95% CI: 1.02, 1.27] per 10 ppb) compared to participants with high genetic risk (HR: 1.04 [95% CI: 0.94, 1.14]). One possible explanation for this finding is that people with a high genetic risk of asthma may be less susceptible to, or less influenced by, environmental exposures. Alternatively, there is a possibility of selection bias if the identification of adults without asthma with high genetic risk led to a sample of participants who were more resistant to specific factors leading to asthma-onset.

Behavioral/Lifestyle Factors

In the UK Biobank study discussed above, Zhu et al. (2023) also examined effect modification by lifestyle factors. Participants were assigned a set of binary lifestyle scores corresponding to their level of physical activity, smoking status, body mass index, and diet. Participants with at least two "healthy" lifestyle factors were classified as having a "healthy lifestyle" and everyone else was classified as having an "unfavorable lifestyle." In stratified models, NO_2 concentrations were associated with asthma incidence in the unfavorable lifestyle group (HR: 1.10 [95% CI: 1.02, 1.18]), but not in the healthy lifestyle group (HR: 0.98 [95% CI: 0.85, 1.14]).

Socioeconomic Status

No studies in the 2016 Oxides of Nitrogen ISA examined the potential effect modification of long-term NO_2 exposure and adult-onset asthma by SES factors. In a recent study, Liu et al. (2021a) analyzed the effect of employment status and education level on the relationship between long-term NO_2 exposure and adult-onset asthma using a large, pooled dataset from three European cohorts in Denmark and Sweden. The authors reported stronger associations between NO_2 exposure and asthma incidence among unemployed participants or participants with lower educational attainment than employed participants or participants with higher educational attainment, respectively (quantitative results not provided).

4.3.1.2.5 Conclusions and Remaining Uncertainties

Several recent epidemiologic studies support and extend the limited evidence evaluated in the 2016 Oxides of Nitrogen ISA, providing generally consistent evidence of positive associations between long-term NO_2 exposure and asthma onset in adults. Relative to evidence evaluated in the 2016 Oxides of Nitrogen ISA, recent studies include larger sample sizes and greater geographic diversity. Consistent with the epidemiologic evidence for asthma incidence in children, studies of asthma incidence in adults adjusted for a wide range of potential confounders using individual-level and area-level covariates (see

Section 4.3.1.1.3) and reported consistent positive associations across varying levels of adjustment. Positive associations were also observed across various averaging times and exposure time periods, including multi-year averages assigned at baseline and moving averages in time-varying regression models. The magnitudes of the observed associations were similar across studies that compared different exposure averaging times and time periods. A limited number of studies have examined the shape of the C-R relationship across the exposure distribution, and each noted an approximately linear relationship between NO_2 exposure and asthma incidence.

A number of uncertainties remain in the evidence base related to asthma onset in adults. Most available studies are administrative cohorts that use health records to ascertain asthma incidence. The first-ever hospitalization or ED visit for asthma is likely to underestimate the total number of new-onset asthma cases, as only a fraction of cases require medical intervention (Andersen et al., 2012). Additionally, to the extent that ED visits and hospital admissions represent, to some degree, acute exacerbations of asthma rather than asthma onset, short-term exposure to NO_2 or other air pollutants could potentially confound the relationship between long-term exposures and asthma onset in these studies. Finally, although a limited number of studies examining potential copollutant confounding reported that the observed associations between long-term NO_2 exposure and asthma incidence in adults were generally robust to adjustment for $PM_{2.5}$, BC, O_3, distance from roadway, or traffic noise, many of these copollutants or exposure surrogates were highly correlated with long-term NO_2 concentrations, complicating the interpretation of those results.

4.3.1.3 Animal Toxicological Studies of Asthma Development

Common mammalian animal models do not develop chronic lung conditions like asthma and thus, may be limited in their ability to fully recapitulate disease development. Despite this, animal toxicological studies can measure markers of a pro-asthmatic phenotype including changes in airway extracellular matrix deposition or other markers of airway remodeling, abnormal or aberrant mucus production, infiltration of inflammatory cells, and production of pro-inflammatory signals. Changes in these markers in animal models following prolonged periods of NO_2 exposure indicate that activation of similar biological pathways in humans, over time, could promote the NO_2-dependent development of asthma. The animal studies presented in the 2016 Oxides of Nitrogen ISA showed that prolonged exposure to NO_2 resulted in changes consistent with asthma development and were similar to those from rodent studies of shorter NO_2 exposure duration (see Chapter 3, Section 3.3.3.3). Evidence from recent studies is summarized below, followed by additional discussion of dose-response relationships in these studies (see Section 4.3.1.3.1). Section 4.3.1.3.2 discusses conclusions from these studies and summarizes remaining uncertainties. Study specific details, exposure conditions, and endpoints examined are highlighted in the text below and described in more detail in Appendix A – Appendix Table 4-3.

- Increased airway reactivity was induced in guinea pigs with exposure to 2,000–4,000 ppb NO_2 for six weeks, but not at 60 or 500 ppb (Kobayashi and Miura, 1995).

- Increased airway reactivity was induced with lower NO_2 exposure concentrations (1,000 ppb–2,000 ppb) with longer exposure duration (12 weeks) (Kobayashi and Miura, 1995).
- Exposure of a variety of animal species (dogs, rabbits, guinea pigs, rats, hamsters, and mice) to 1,000 or 5,000 ppb NO_2 for up to 18 months resulted in comparable pulmonary histologic areas of enlarged airspaces, edema, and mild to moderately thickened septa with chronic inflammatory cells as control animals exposed to air for the same duration (Wagner et al., 1965).
- (Furiosi et al., 1973) found evidence of bronchial epithelial hypertrophy in the respiratory bronchioles and a cuboidal phenotype in the squamous proximal bronchiolar epithelium of monkeys following 14 months of exposure to 2 ppm NO_2. Less obvious lesions were seen in the lungs of rats exposed in a similar manner.
- Rodents exposed to long-term NO_2 (1–27 months) in the range of 340–5,000 ppb showed increased markers of inflammation and lung damage including epithelial hyperplasia and increased extracellular matrix (Hayashi et al., 1987; Kubota et al., 1987; Chang et al., 1986; Crapo et al., 1984; Yamamoto and Takahashi, 1984; Gregory et al., 1983; Sherwin and Richters, 1982), although histological effects were not seen in a study that exposed rats to 9 weeks of 500 ppb NO_2 with two, 1-hour peaks of 1,500 ppb NO_2 (Mercer et al., 1995). Another study that exposed rats to 40, 400, and 4,000 ppb NO_2 for 27 months found that lung histologic changes were not seen in the 40 ppb NO_2 but were present in the other groups as early as 18 months into exposure (Kubota et al., 1987).
- Exposure of rats to 1,000 ppb or 5,000 ppb NO_2 for 1.7 to 15 weeks found increased markers of pulmonary injury and signs of increased oxidant defense at 1.7 and 2.7 weeks of exposure but generally resolved after longer exposure periods (6–15 weeks) (Gregory et al., 1983).
- Wistar rats exposed to 40–4,000 ppb NO_2 for 9–27 months showed decreased antioxidant enzyme activity (Glutathione peroxidase, aryl S-transferase, aralkyl S-transferase, and epoxy S-transferase) and increased markers of lipid peroxidation (Sagai et al., 1984). In general, effects were detectable after nine months of exposure to 4,000 ppb NO_2 but were present with exposure to either 400 ppb or 4,000 ppb NO_2 with longer exposure durations (18 months).

Several recent animal studies examined baseline airway reactivity in non-sensitized mice.

- Lu et al. (2023b) exposed 7-week-old mice to four hours/day of 5 ppm NO_2 for 30 days and found that the mice had statistically significant increases in resistance on inhalation or exhalation and a non-significant decrease in dynamic compliance. Increased markers of asthma-relevant airway remodeling in the absence of allergen sensitization were reported including airway thickening, goblet cell proliferation, and mucus production. These changes corresponded to increased ovalbumin (OVA) specific IgE, lung tissue IL-4/IFNg ratios, and increased IL-6 and TNFa tissue staining. The levels of total IgE and measures of oxidative stress were largely unchanged with NO_2 exposure in unsensitized mice.
- Studies of gestational exposure showed that exposure of pregnant mice to 2.5 ppm NO_2 throughout gestation did not affect the baseline airway responsiveness in the offspring mice (Yue et al., 2018; Yue et al., 2017).
- In addition, one recent study looked at airway morphology and mucus-related gene expression in the following 27 days of exposure to approximately 1 ppm or 2.6 ppm NO_2. Exposures resulted in increased histologic markers of inflammation including airway

epithelial damage, infiltration of inflammatory cells and visible mucus secretion (Han et al., 2017). This corresponded to increased gene expression of MUC5AC, IL-1β, and ICAM.

4.3.1.3.1 Dose Response

Impacts of long-term NO_2 exposure on airway responsiveness in non-sensitized animals generally appear to show a dose-response relationship, with impacts seen most consistently at high exposure concentrations (2,000–5,000 ppb) and seen at lower concentrations with longer exposures. Kobayashi and Miura (1995) found that guinea pigs exposed to 60–4,000 ppb NO_2 for six or 12 weeks of NO_2 had both a dose-dependent and time-dependent increase in airway reactivity. After six weeks of NO_2 exposure, airway reactivity was significantly increased in the group exposed to 2,000 or 4,000 ppb NO_2. When the exposure period was increased to 12 weeks, animals exposed to 1,000 ppb had statistically significant increased airway reactivity, but this was not seen with exposure to 500 or 60 ppb NO_2. Similar results were seen in studies of histological lung epithelial damage. A study by Kubota et al. (1987) found histologic signs of inflammation in the lungs of rats exposed to 4,000 ppb NO_2 starting at 18 months. Rats exposed to 400 ppb NO_2 showed noticeable histologic signs of lung inflammation beginning at 27 months of exposure, but these effects were not seen with 40 ppb NO_2 exposure for the same duration. Another rat study showed that exposure to 500 ppb NO_2 for seven months resulted in swelling of cilia in the terminal bronchioles and resulted in the decrease or loss of cilia at higher exposure concentrations (1,000 or 4,000 ppb), suggestive of a dose-dependent worsening of epithelial damage (Yamamoto and Takahashi, 1984). In exposure of rats to NO_2 for up to 27 months, increased oxidative stress and lipid peroxidation was seen in the lungs of animals exposed to 4,000 ppb NO_2 as early as nine months into exposure whereas significant increases in oxidative stress markers were not seen in the 400 ppb group until 18 months (Sagai et al., 1984). In the same study, exposure to 40 ppb NO_2 did not show any changes in oxidative stress or lipid peroxidation at any time point investigated up to 27 months. A recent study of NO_2 exposure in rats showed that 28 days of exposure to approximately 1,000 ppb or 2,600 ppb NO_2 showed increased markers of pulmonary inflammation and mucus production (histologic changes and inflammatory gene expression) that were worsened in the 2,600 ppb exposure group compared to the 1,000 ppb group (Han et al., 2017). Together, there is evidence of worsening asthma-related endpoints with increased exposure concentration and exposure duration. Effects were seen following exposure concentrations as low as 400 ppb with exposure durations of 18 or 27 months and following exposures of 0.5 ppm for seven months.

4.3.1.3.2 Conclusions and Remaining Uncertainties

Together, animal toxicological studies generally indicate an increase in asthma-related markers including airway reactivity, inflammation, lung damage, and airway histologic changes in response to prolonged NO_2 exposure in adult animals. Exposure to NO_2 during gestation did not impact baseline

airway reactivity in the absence of allergen sensitization in the single study that investigated gestational exposures. The existing information often comes from air control and NO_2 exposure arms that are built-in controls for studies testing the impact on airway reactivity in sensitized mice. Studies have shown impacts of NO_2 exposure on airway reactivity and underlying histologic changes consistent with an asthma-like phenotype. Species differences in models of airway reactivity should be considered. Guinea pig models show similar physiology and airway smooth muscle control as humans and may be better for modeling human airway reactivity than mice or rats (Canning, 2003).

4.3.2 Asthma Severity

Epidemiologic studies from the 2016 Oxides of Nitrogen ISA provided support for a relationship between long-term exposures to NO_2 and respiratory symptoms, especially in children. The strongest evidence came from prospective studies that consistently found associations between long-term average NO_2 exposures and asthma severity scores, asthma symptoms, medication use, and asthma-related hospitalizations. Similarly, animal models of respiratory allergen sensitization consistently showed increased responses in animals following prolonged exposure to NO_2. Some uncertainty remained regarding the potential for copollutant confounding in epidemiologic studies, as well as the role of short-term NO_2 exposure effects when analyzing longer exposure durations.

4.3.2.1 Epidemiologic Studies of Asthma Severity

Longitudinal epidemiologic studies in the 2016 Oxides of Nitrogen ISA reported consistent associations between long-term ambient NO_2 exposure metrics and an array of respiratory symptoms in school-age children. As relating specifically to populations with asthma, the evidence base was limited. A prospective CHS study examined prolonged bronchitic symptoms in children with asthma and reported stronger associations with NO_2 variation within communities than for NO_2 variation between communities (McConnell et al., 2003). In copollutant models, the effects of annual variation in NO_2 were only modestly attenuated after adjustment for either $PM_{2.5}$ or a traffic-related copollutant such as EC or organic carbon (OC). This finding was supported by European cohort studies that observed increases in asthma symptoms and wheeze associated with higher annual average NO_2 or NO_X concentrations at birth (Gruzieva et al., 2013; Gehring et al., 2010), and a longitudinal study conducted in Connecticut and Western Massachusetts, in which residential indoor NO_2 was associated with asthma symptoms and medication use in children with asthma (Belanger et al., 2013).

Evidence for adults was similarly limited. In the ECRHS cohort, (Jacquemin et al., 2009) reported that associations between NO_2 concentrations estimated from dispersion models and all examined asthma symptoms were positive. An analysis of the Danish Diet, Cancer, and Health cohort reported that long-term NO_2 exposure was associated with a greater risk for re-admission in people with a previous asthma

hospital admission (Andersen et al., 2012). Andersen et al. (2012) similarly used a dispersion model to assign exposures at participants' residences.

A few recent studies have examined respiratory symptoms and ED visits or hospital admissions for asthma in children and/or adults previously diagnosed with asthma. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 4-4. Recent studies ascertained respiratory symptoms from validated questionnaires, and information on ED visits and hospital admissions for asthma were obtained either from linkages to administrative health databases or via self-report. In general, recent evidence provides some additional support for previously limited findings that long-term NO_2 exposure is associated with measures of asthma severity. A key limitation of the evidence is that increased symptoms, ED visits, and hospital admissions may represent acute exacerbations of asthma. The studies included in this section do not adjust for short-term exposure to NO_2 or other air pollutants, which could potentially confound the relationship between long-term exposures and measures of asthma severity. A summary of the recent evidence is provided below.

- In a large administrative birth cohort of children with asthma in Quebec, Canada, long-term NO_2 exposure was associated with aggregated hospital admissions and ED visits for asthma (Tétreault et al., 2016b). An increase in the risk of exacerbation was observed when time-dependent NO_2 concentrations were used to estimate exposure (HR: 1.201 [95% CI: 1.123, 1.285]), but not when NO_2 exposure was assigned at birth residences (HR: 1.002 [95% CI: 0.992, 1.012]). This may indicate that asthma exacerbation is related to prolonged exposure to NO_2, rather than prenatal exposure. Alternatively, time-varying annual average NO_2 exposures may be more strongly correlated with short-term exposures, and the observed association could reflect acute exacerbations to some degree.
- A recent cohort study reported positive associations between annual average NO_2 concentrations and respiratory symptoms in children with asthma over a 12-month follow-up period (Berhane et al., 2016). Similar to findings from a previous analysis of a single CHS cohort (McConnell et al., 2003), an extended analysis of 4,602 children from three CHS cohorts reported NO_2-related increases in bronchitic symptoms among children with asthma (Berhane et al., 2016). Berhane et al. (2016) observed greater increases in NO_2-related bronchitic symptoms in females compared to males at age 10, but no sex differences at age 15.
- A recent cross-sectional study of self-reported ED visits for asthma in a nationwide U.S. National Health and Nutrition Examination Survey (NHANES) analysis of children and adults (Mendy et al., 2019) provides supporting evidence of an association between long-term NO_2 exposure and asthma severity.
- In addition to the Mendy et al. (2019) NHANES analysis that included both children and adults, other recent studies examined long-term NO_2 exposure and asthma severity in adults (Keirsbulck et al., 2023) or respiratory symptoms in adults with asthma (Garcia et al., 2024). Keirsbulck et al. (2023) analyzed data from a 5-year population-based cohort study of adults in France and reported a 13% increase (95% CI: 11, 15) in asthma symptom scores associated with a 10 ppb increase in annual average NO_2 concentrations at baseline. The authors examined effect modification by sex and reported comparable increases in NO_2-related risk in males and females (quantitative results not provided). In an extended follow-up of the CHS

cohorts, Garcia et al. (2024) reported an association between self-reported bronchitic symptoms in adults (mean age = 32.0 years) and NO_2 concentrations averaged from birth year until 17 years (OR: 1.60 [95% CI: 1.13, 2.28] per 10 ppb increase in NO_2). Associations were stronger among adults who were diagnosed with asthma as children (OR: 2.07 [95% CI: 1.33, 3.20]) compared to those who were not (OR: 1.49 [95% CI: 1.05, 2.11]).

- Across studies of asthma severity, associations were observed with long-term exposures estimated using a variety of exposure assessment approaches, including LUR models (Keirsbulck et al., 2023; Tétreault et al., 2016b), outdoor monitors (Berhane et al., 2016), and the CMAQ Downscaler Model (Mendy et al., 2019).

4.3.2.2 Animal Toxicological Studies of Asthma Severity

As noted in Section 4.3.1.3, rodent models do not develop asthma but are used to replicate phenotypes of asthma including increased airway reactivity, histologic changes consistent with airway obstruction and mucus production, and subclinical markers of inflammation, oxidative stress, and IgE production. Previously reviewed animal toxicological studies of prolonged NO_2 exposure concurrent with or subsequently followed by allergen sensitization consistently showed more severe biomarkers of asthma severity including increased airway responsiveness, heightened inflammation and oxidative stress, and increased induction of IgE-mediated immune responses (U.S. EPA, 2016). Several recent studies support the previous findings of heightened responses to allergen sensitization with NO_2 exposure. Evidence from recent studies is summarized below, followed by additional discussion of dose-response relationships in these studies (see Section 4.3.2.2.1). Section 4.3.2.2.2 discusses conclusions from available studies and summarizes remaining uncertainties. Study specific details, exposure conditions, and endpoints examined are highlighted in the text below and in Appendix A – Appendix Table 4-5.

- OVA sensitization resulted in increased qualitative and semi-quantitative measures of histologic inflammation and mucus production in the lungs that worsened in animals subjected to prolonged exposure (four to six hours per day for 30 to 42 days) to NO_2 (2.6 to 5 ppm) and which corresponded to increases in inflammatory cell influx (Lu et al., 2024; Lu et al., 2023b; Lu et al., 2023a; Han et al., 2017). These analyses often reached statistical significance compared to air-exposed, OVA-sensitized animals. In contrast, one study showed that neutrophil percentage in lavage fluid was unchanged after four weeks of NO_2 exposure (six hours per day) at 2 ppm (de Broucker et al., 2015).
- Activation of cellular inflammatory signaling pathways, including the transcription factors ERK, Lck, and NF-kB, was seen in rats exposed to five hours per day of 2.6 ppm NO_2 for 42 days in a model of allergen sensitization (Han et al., 2017). Similarly, increases in lung inflammatory mediators such as IL-1β (Lu et al., 2024; Han et al., 2017), IL-6 (Lu et al., 2023b; Lu et al., 2023a; Han et al., 2017), 8-isoprostane (de Broucker et al., 2015), IL-17 (Lu et al., 2024), and TNF-α (Lu et al., 2024; Lu et al., 2023b; Lu et al., 2023a) were observed after long-term (four to six hours per day for 30 to 42 days) NO_2 exposure (2 to 5 ppm). The increased inflammatory signaling was greater in sensitized animals exposed to long-term NO_2 compared to air-exposed sensitized mice, often achieving statistical significance.
- Mucus production and histological signs of airway remodeling (i.e., peri-bronchial collagen deposition) were also greater in OVA-sensitized animals exposed to 2.6 to 5 ppm NO_2 for

four to six hours per day for 30 to 42 days compared to air-exposed sensitized animals (Lu et al., 2023b; Lu et al., 2023a; Han et al., 2017).

- Airway responsiveness following OVA challenge generally increased in sensitized mice following 5 ppm NO_2 exposures for 30 to 38 days compared to controls which corresponded to increases in OVA-specific IgE (Lu et al., 2024; Lu et al., 2023b; Lu et al., 2023a).
- OVA sensitization leads to a shift to increased Th2 responses (IL-4 production) and decreased Th1 responses (IFN-g). This shift is consistently exacerbated with NO_2 exposure (2.6 to 5 ppm for 30 to 42 days) with studies showing either statistically significant or nonsignificant trends (Lu et al., 2024; Lu et al., 2023b; Lu et al., 2023a; Han et al., 2017).
- Gestational exposure (approximately 21 days) of mice to 2.5 ppm NO_2 increased airway reactivity, Th2 inflammation, and antibody responses to OVA in the offspring (Yue et al., 2018; Yue et al., 2017).

4.3.2.2.1 Dose Response

Animal toxicological studies reviewed here do not directly investigate the concentration- or time-relationship between the NO_2 exposure and the effects on markers of asthma development or severity. Animal toxicological studies generally use a high exposure concentration of NO_2 (2.5 to 5 ppm) for varying lengths of time (roughly one to two months) but consistently see worsened asthma-like phenotypes.

4.3.2.2.2 Conclusions and Remaining Uncertainties

Recent studies reinforce conclusions from the 2016 Oxides of Nitrogen ISA that NO_2 exposure generally worsens asthma-like responses that are seen when animals are sensitized to a foreign antigen. This includes worsened airway reactivity, increased cellular infiltration, and promotion of Th2 inflammatory signaling. The recent evidence also supports existing evidence that suggests that gestational exposure to NO_2 can lead to persistent effects in offspring mice that are subsequently sensitized to an allergen. This evidence together supports both the increases in development of asthma as well as the severity of asthma symptoms that are seen in epidemiology studies.

Some uncertainties remain for the development of asthma-like phenotypes in animal models. These include limitations that are inherent to animal models, including physiological and biological differences between rodents and humans. Because of this, the extrapolation of animal exposure concentrations to those that would be expected to cause similar effects in humans is uncertain. The majority of studies expose animals to NO_2 either prior to or concurrent with allergen sensitization allowing for the effects of NO_2 to impact sensitization as well as the allergen challenge. There is a lack of information on the impacts of NO_2 exposures that occur predominantly after sensitization, which might better replicate the response of those with existing asthma. Recent information provides insight into the

role of gestational exposure to NO_2 and health effects of the offspring, but this information is still limited in quantity and could be further strengthened with more supporting studies.

4.3.3 Synthesis Across Lines of Evidence

Recent evidence integrated across health outcomes and scientific disciplines further supports conclusions from the 2016 Oxides of Nitrogen ISA indicating a relationship between long-term NO_2 exposure and asthma development. Several recent epidemiologic cohort studies provide additional, generally consistent evidence of a positive association between long-term NO_2 exposure and asthma incidence in children across a mix of traditional recruitment-based and administrative cohort studies. Positive associations were observed in studies that utilized a range of exposure assessment methods, including LUR, dispersion models, hybrid prediction models, and fixed-site monitors, and adjusted for a wide range of potential confounders. The available epidemiologic evidence suggests that asthma incidence is associated with long-term exposure to NO_2 during various lifestages or time periods throughout childhood, including prenatal, at-birth, or time-varying exposures. For adults, the epidemiologic evidence is more limited, but generally consistent positive associations are also observed between long-term NO_2 exposure and asthma onset. The collective epidemiologic evidence also provides some support for an association between long-term NO_2 exposure and measures of asthma severity in children and adults. A key limitation of the evidence for asthma severity is the lack of adjustment for short-term exposure to NO_2 or other air pollutants given that increased symptoms, ED visits, and hospital admissions may represent acute exacerbations of asthma.

Although animal models do not develop asthma, experimental animal studies can measure markers of a pro-asthmatic phenotype that support the epidemiologic results and provide biological plausibility for NO_2-induced asthma development in humans. In the pathophysiology of asthma, recurrent pulmonary inflammation, allergic sensitization, and subsequent development of airway hyperresponsiveness play important roles (see Section 4.9). Together, animal studies of allergen sensitization generally indicate an increase in asthma-related markers, including airway reactivity, inflammation, lung damage, and airway histologic changes in response to prolonged NO_2 exposure. Recent experimental animal studies in particular support and extend the previously limited evidence that repeat exposure to NO_2 alone can increase airway responsiveness, inflammation, and oxidative stress in a variety of non-primate animal species. Impacts of long-term NO_2 exposure on airway responsiveness in non-sensitized animals generally appears to show a dose response. Additionally, controlled human exposure studies of repeated short-term exposures support for NO_2-induced development of inflammatory and allergic responses in healthy adults (see Chapter 3, Section 3.6.4.2). Similar increases in inflammation along with markers of lung damage and airway remodeling are seen in unsensitized animal models of repeat NO_2 exposures. A few recent studies also demonstrated that gestational exposure to NO_2 resulted in detrimental impacts on offspring response to allergen sensitization later in life, which is

coherent with epidemiologic studies that demonstrate associations between prenatal NO_2 exposure and asthma incidence in children.

A key remaining uncertainty in the epidemiologic evidence is potential copollutant confounding, particularly by traffic-related pollutants. Although a few recent studies observed associations between NO_2 exposure and asthma incidence that were robust to adjustment for modeled near-road pollution or traffic-related particle pollution, the copollutant models may be susceptible to multicollinearity due to the high correlations typically seen between long-term NO_2 concentrations and the examined copollutants. However, together with the expanded support and biological plausibility from the experimental animal studies, the collective evidence indicates an effect of ambient NO_2 exposure on asthma development independent of other traffic-related pollutants.

4.4 Chronic Obstructive Pulmonary Disease Development and Exacerbation

Chronic obstructive pulmonary disease (COPD) is a lung disease characterized by persistent respiratory symptoms and airflow limitation due to destruction of alveolar tissue and airway remodeling. Reduced airflow is associated with decreased lung function, and clinical symptoms demonstrating exacerbation of COPD include cough, sputum production, and shortness of breath. Severe exacerbation can lead to ED visits or hospital admissions.

4.4.1 Epidemiologic Studies of Development of COPD

Few studies in the 2016 Oxides of Nitrogen ISA examined COPD development in relation to long-term exposure to NO_2. In an analysis of the Danish Diet, Cancer, and Health Cohort, Andersen et al. (2011) reported that COPD incidence was associated with 35-year mean concentrations of NO_2 estimated by a validated LUR model for residential locations (HR: 1.28 [95% CI: 1.07, 1.54] per 10-ppb increase). In contrast, the association of 5-year NO_2 concentrations with incident COPD hospital admissions was null in a much larger study in Vancouver, Canada (Gan et al., 2013).

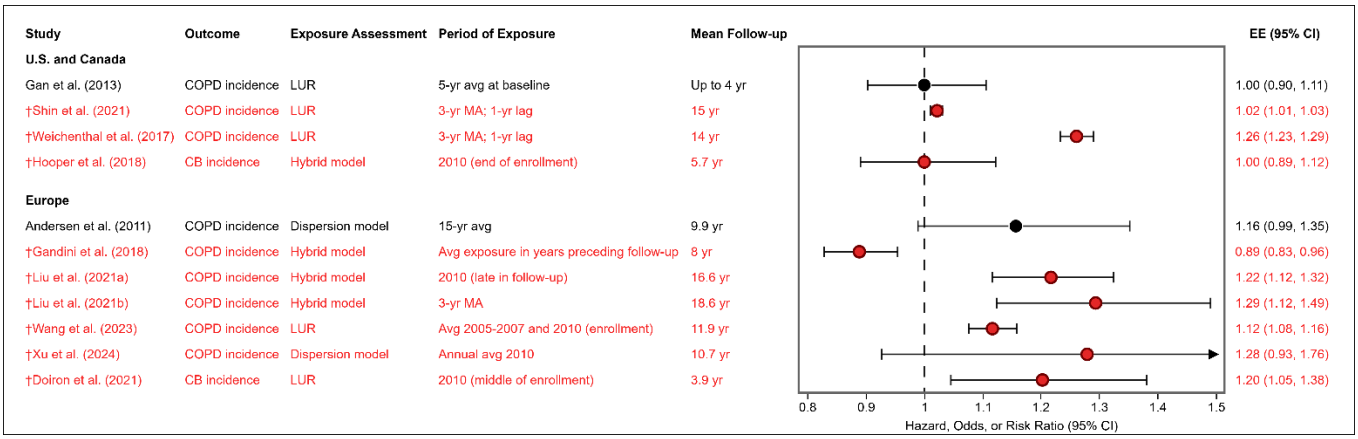
Several recent studies expand the existing evidence base and provide generally consistent evidence of an association between long-term NO_2 exposure and COPD incidence in adults (see Figure 4-8). Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 4-6. The majority of recent studies include longitudinal cohorts that track COPD incidence through repeated questionnaires or administrative cohorts that ascertain COPD incidence through outpatient care and hospital admissions databases. As described previously (see Section 4.4.1), the former study type typically includes more information on individual-level covariates, whereas the latter includes larger sample sizes and less threat of selection bias. In addition to cohort studies, a few recent cross-sectional studies evaluated prevalent

COPD cases as determined by lung function measurements that correspond to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria. Across studies, NO_2 exposure was most often assigned using LUR models or hybrid LUR models utilizing satellite data and kriging. Most studies reported good model performance, with cross-validation R^2 values ranging from 0.59 to 0.85. A short summary of the recent evidence is provided below, followed by additional discussion of concentration-response relationships in available studies (see Section 4.4.1.1), the lifestages and populations evaluated (see Section 4.4.1.2), and copollutant impacts (see Section 4.4.1.3). Section 4.4.1.4 discusses conclusions from available studies and summarizes remaining uncertainties.

- Recent studies provide generally consistent evidence of a positive association between long-term NO_2 exposure and COPD or chronic bronchitis incidence. Evidence of positive associations comes from cohort studies conducted in Canada (Shin et al., 2021; Weichenthal et al., 2017a), the United Kingdom (Wang et al., 2023b), Netherlands (Doiron et al., 2021), and Denmark (Liu et al., 2021c), as well as pooled analyses of multiple cohorts from Sweden and Denmark (Brunekreef et al., 2021; Liu et al., 2021b) and northern Europe Xu et al. (2024).
- Although most recent cohort studies reported positive associations between long-term NO_2 exposure and COPD incidence, null associations were observed in an Italian cohort study utilizing data from a population-based national health survey (Gandini et al., 2018b) and an analysis of the U.S.-based Sister Study, which recruited the sisters of women with breast cancer (Hooper et al., 2018). The lack of an association does not appear to be explained by the all-female study population examined by (Hooper et al., 2018), as other studies that evaluated models stratified by sex consistently reported positive associations among females (Shin et al., 2021; Weichenthal et al., 2017a).
- An analysis using pooled data from three European cohorts (Brunekreef et al., 2021; Liu et al., 2021b) and an administrative cohort comprising 5.1 million adults in Ontario, Canada (Shin et al., 2021) provide particularly strong inference. Shin et al. (2021) conducted an administrative cohort study of over 5.1 million adults and ~340,000 COPD cases in Ontario, Canada using a LUR regression model with high spatial resolution to assign time-varying exposure at each participant's residence. Although Liu et al. (2021b) evaluated a much smaller study population, the authors were able to control for a larger number of individual-level covariates than Shin et al. (2021) and applied a hybrid LUR model incorporating satellite and CTM data. Additionally, both studies examined a number of policy-relevant topics, including evaluation of C-R relationships, populations potentially at increased risk, and copollutant confounding (see Sections 4.4.1.1 through 4.4.1.3).
- A recent study utilized data from the UK Biobank study to examine the progression of chronic lung disease in adults without chronic lung disease at baseline. (Wang et al., 2023b) reported an increase in COPD incidence associated with a 10 ppb increase in 4-year average NO_2 concentrations prior to follow-up (12% increase [95% CI: 8%, 16%]). The authors also examined NO_2-related risk of chronic lung multimorbidity in participants who developed COPD during the course of follow-up. Whereas long-term NO_2 exposure was associated with an increase in the risk of participants with incident asthma developing a second chronic lung disease, either COPD or lung cancer, NO_2 exposure was not associated with the risk of chronic lung multimorbidity in participants with COPD.
- A few recent cross-sectional analyses in the United States (Hooper et al., 2018), United Kingdom (Doiron et al., 2019), and Canada (Bourbeau et al., 2022) provide supporting

evidence, demonstrating consistent associations between long-term NO_2 exposure and COPD or chronic bronchitis prevalence. A strength of some of these studies is the use of lung function measures to ascertain COPD case status according to definitions from the Global Lung Function Initiative (e.g., FEV_1/FVC ratio <0.7) (Bourbeau et al., 2022; Doiron et al., 2019). Spirometry is recognized as the gold standard for diagnosing COPD and could reduce outcome misclassification from other methods such as questionnaires and hospital records. However, inference is not as strong for cross-sectional studies of COPD prevalence, which cannot establish temporality between exposure and disease onset.

- One other recent cross-sectional study examined lung function and computerized tomography (CT) lung measurements in 296 adults with COPD who resided in areas near cement plants in South Korea (Kwon et al., 2020). The authors did not observe strong evidence of an association between long-term NO_2 exposure and lung function but did report imprecise increases in emphysema index (β: 1.53 [95% CI: -1.60, 4.67]) and mean wall area percent (1.26% [95% CI: -1.47, 4.00]) associated with 3.0 ppb higher annual average NO_2 concentrations. Both measures are structural features of COPD and can indicate disease severity.



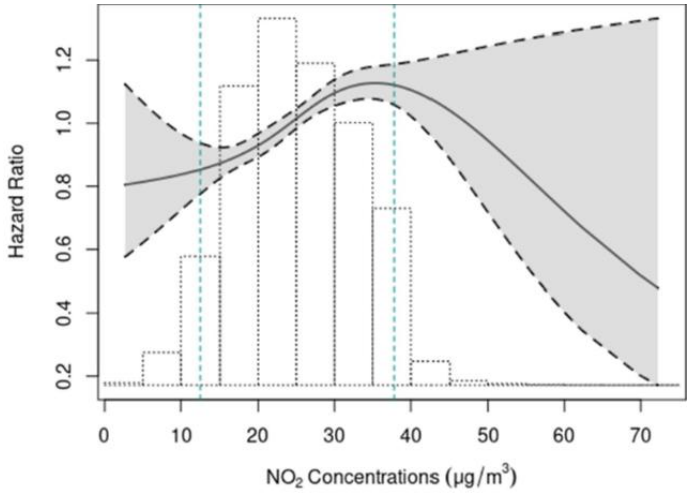
List of abbreviations in alphabetical order: Avg = average; CB = chronic bronchitis; CI = confidence interval; COPD = chronic obstructive pulmonary disease; IDW = inverse distance weighting; LUR = land use regression; MA = moving average; NO_2 = nitrogen dioxide; yr = year.
Notes: Black = studies from the 2016 Oxides of Nitrogen ISA; Red = recent studies and those not included in 2016 Oxides of Nitrogen ISA. Results are standardized to a 10-ppb increase in NO_2.
†Studies published since the 2016 Oxides of Nitrogen ISA.

Figure 4-8 Associations of long-term exposure to nitrogen dioxide with COPD incidence.

4.4.1.1 Concentration-Response

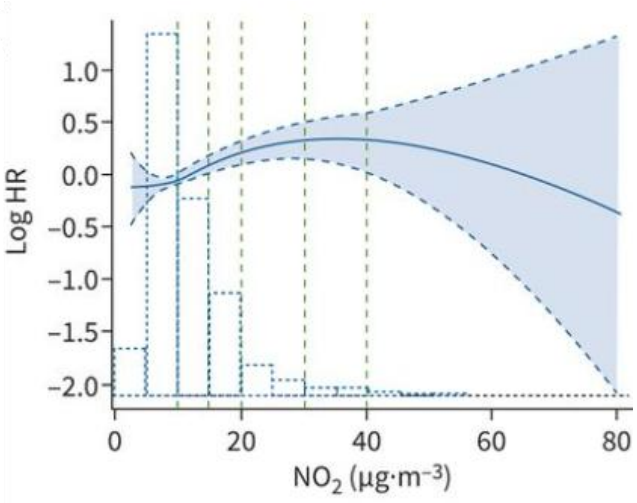
The 2016 Oxides of Nitrogen ISA did not evaluate studies that examined potential non-linearities or thresholds in the association between long-term NO_2 exposure and COPD incidence. A few recent studies have employed spline models, shape-constrained health impact functions (SCHIFs), and subset analyses to further explore the nature of the relationship between NO_2 exposure and COPD incidence across the NO_2 concentration distribution. Liu et al. (2021b) and Liu et al. (2021c) examined non-linearities in the C-R relationship using natural cubic spline models with three or four knots, respectively. Visual inspection of the spline plots from each study indicates an approximately linear C-R relationship

between the 5th and 95th percentile of NO_2 concentrations (see Figure 4-9 and Figure 4-10). Both curves indicate a plateau or inverse association at concentrations above the 95th percentile of NO_2 concentrations, but there is less confidence in the shape of the C-R relationships at these higher concentrations due to fewer observations, as indicated by wider 95% CIs. The results from these studies are not entirely independent given that they examined overlapping study populations – the DNC (Liu et al., 2021b) and pooled data from three European cohorts, including the DNC (Liu et al., 2021c). Whereas previous studies utilized spline models, Shin et al. (2021) employed a SCHIF model to assess potential non-linearities in the association between long-term NO_2 exposure and COPD incidence. The SCHIF model applies non-linear transformations of exposure based on a sigmoidal function that allows for a variety of monotonically non-decreasing shapes (Nasari et al., 2016). In contrast to results from Liu et al. (2021b) and Liu et al. (2021c), the SCHIF C-R curve presented in Shin et al. (2021) suggests a threshold at approximately 30 ppb, above which the C-R relationship appears to be approximately linear (see Figure 4-11).



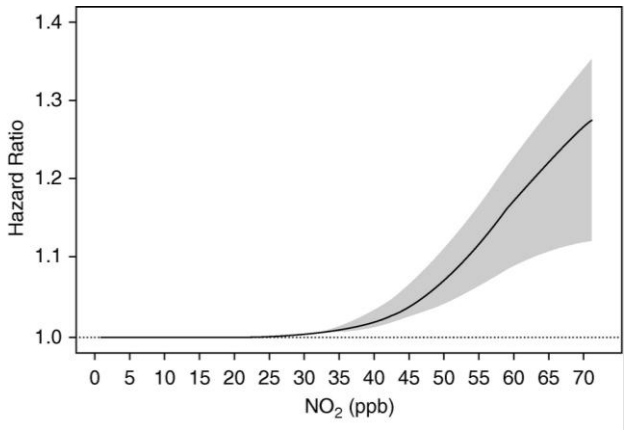
List of abbreviations in alphabetical order: CI = confidence interval; NO_2 = nitrogen dioxide; $\mu g/m^3$ = micrograms per cubic meter.
Note: Green dashed lines indicate the 5th and 95th percentiles of NO_2 concentrations; black dashed lines indicate the 95% CI.
Source: Adapted from Liu et al. (2021b). Copyright permission pending.

Figure 4-9 Concentration-response curve between 3-year moving average nitrogen dioxide concentrations and COPD incidence in the Danish Nurse Cohort.



List of abbreviations in alphabetical order: CI = confidence interval; NO_2 = nitrogen dioxide; $\mu g/m^3$ = micrograms per cubic meter.
Note: Blue dotted lines indicate the 95% CI.
Source: Adapted from Liu et al. (2021c). Copyright permission pending.

Figure 4-10 Concentration-response curve between nitrogen dioxide concentrations and COPD incidence in an analysis of pooled data from three European cohorts.



List of abbreviations in alphabetical order: NO_2 = nitrogen dioxide; ppb = parts per billion.
Source: Adapted from Shin et al. (2021). Copyright permission pending.

Figure 4-11 Concentration-response curve between nitrogen dioxide concentrations and COPD incidence in an analysis of data from an administrative cohort in Ontario, Canada.

Liu et al. (2021b) and Liu et al. (2021a) also employed subset analyses to examine associations between COPD incidence and long-term average NO_2 concentrations below increasingly stringent concentration cutoffs. Consistent with the findings from the spline models from the same studies, the magnitude of the observed associations in the full population was comparable to the associations in each of the analyses with concentration cutoffs from 40 $\mu g/m^3$ (21.28 ppb) down to 20 $\mu g/m^3$ (10.64 ppb). Liu

et al. (2021b) further analyzed thresholds down to 10 µg/m^3 (5.32 ppb) and noted a slightly attenuated and much less precise association among participants exposed to concentrations less than 10 µg/m^3 (HR: 1.20 [95% CI: 0.42, 3.42]). The limited number of observations in the more restricted datasets limits the statistical power to detect an association at lower concentrations.

4.4.1.2 Lifestages and Population

There was limited evaluation of populations potentially at increased risk of NO_2-related COPD incidence in studies included in the 2016 Oxides of Nitrogen ISA. In one study, Andersen et al. (2011) examined potential effect modification by pre-existing conditions and behavioral factors in an analysis of COPD incidence among participants in the Danish Diet, Cancer, and Health Cohort. The positive association observed between long-term NO_2 exposure and COPD incidence was stronger in people with diabetes or asthma compared to participants without these diseases, but no difference in association was observed with smoking. A few recent studies have further explored factors that may increase risk, including lifestage, sex, and pre-existing conditions.

4.4.1.2.1 Lifestage

Recent evidence examining EMM by age is inconsistent (see Table 4-4). Shin et al. (2021) and Weichenthal et al. (2017a) analyzed overlapping data from administrative cohorts in Ontario, Canada. One cohort included 5.1 million adults across all of Ontario between 2001 and 2015 (Shin et al., 2021), whereas the other included data on 1.1 million residents of Toronto, Ontario from 1996 to 2012 (Weichenthal et al., 2017a). Both studies reported decreasing trends in the strength of association between long-term NO_2 exposure and COPD incidence with increasing age. For example, Weichenthal et al. (2017a) observed the strongest association among adults less than 60 years old (HR: 1.38 per 10-ppb increase [95% CI: 1.29, 1.47]), a slightly weaker association among adults between 60 and 74 years old (HR: 1.21 [95% CI: 1.08, 1.35]), and a further attenuated association among adults 75 years (HR: 1.05 [95% CI: 0.98, 1.013]). In contrast, Xu et al. (2024) evaluated EMM of the association between long-term NO_2 exposure and incidence of self-reported chronic bronchitis or COPD in a small prospective cohort study of 5,355 adult participants of the Respiratory Health in Northern Europe (RHINE) study and observed a positive association only among older adults (50+ years; IRR: 1.76 [95% CI: 1.05, 2.94] per 10 ppb increase in NO_2 concentrations). Additionally, comparable associations were observed in younger (<65 years) and older (65+ years) adults in a cross-sectional analysis of COPD prevalence in the UK Biobank Study (Doiron et al., 2019).

Table 4-4 Epidemiologic studies evaluating effect-measure modification of the relationship between long-term NO_2 exposure and COPD incidence by age

Study	Cohort (Years)	EMM Reference Factor	EMM Comparison Factor	Direction of EMM[a]	Hazard Ratio (95% CI)	Spatial Scale of Effect Modifier
Age						
†Shin et al. (2021)	Ontario, Canada administrative cohort (2001–2015)	35–44 yr	45–54 yr	↓	EMM Reference Factor: 1.327 (1.29, 1.366) EMM Comparison Factor: 1.177 (1.152, 1.202)	Individual level
			55–64 yr	↓	1.074 (1.054, 1.094)	
			65–74 yr	↓	0.993 (0.975, 1.01)	
			75–85 yr	↓	0.945 (0.925, 0.965)	
†Weichenthal et al. (2017a)	Toronto, Canada administrative cohort (1996–2012)	<60 yr	60–74 yr	↓	EMM Reference Factor: 1.38 (1.29, 1.47) EMM Comparison Factor: 1.21 (1.08, 1.35)	Individual level
			≥75 yr	↓	1.05 (0.98, 1.13)	
†Doiron et al. (2019)	UK Biobank (2006–2010)	<65 yr	≥65 yr	–	EMM Reference Factor: 1.22 (1.18, 1.26)[b] EMM Comparison Factor: 1.26 (1.17, 1.36)[b]	Individual level
†Xu et al. (2024)	RHINE (2010–2023)	<50 yr	≥50 yr	↑	EMM Reference Factor: No association[c] EMM Comparison Factor: 1.76 (1.05, 2.94)[d]	Individual level

List of abbreviations in alphabetical order: CI = confidence interval; COPD = chronic obstructive pulmonary disease; EMM = effect measure modification; IRR = incidence rate ratio; NO_2 = nitrogen dioxide; RHINE = The Respiratory Health in Northern Europe study; ppb = parts per billion; yr = year.
Results are standardized to a 10-ppb increase in 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]Odds Ratio (COPD Prevalence).

[c]Quantitative results presented in figure only; IRR near 1.0.

[d]IRR.

†Studies published since the 2016 Oxides of Nitrogen ISA.

4.4.1.2.2 Sex

Evaluations of effect modification by sex were equivocal (see Table 4-5). The Shin et al. (2021) population-based analysis including all of Ontario demonstrated larger increases in NO_2-associated COPD incidence in males (4.0% per 10 ppb increase in 3-year moving avg NO_2 [95% CI: 2.7, 5.3]) relative to females (1.6% [95% CI: 0.3, 3.0]). In contrast, no sex-specific differences were observed in an analysis focused only on Toronto residents (Weichenthal et al., 2017a), or in the RHINE prospective cohort study in northern Europe [(Xu et al., 2024); quantitative results not provided]. Doiron et al. (2019) reported slightly larger COPD prevalence ORs associated with long-term NO_2 exposure among females.

Table 4-5 Epidemiologic studies evaluating effect-measure modification of the relationship between long-term NO_2 exposure and COPD incidence by sex

Study	Cohort (Years)	EMM Reference Factor	EMM Comparison Factor	Direction of EMM[a]	Hazard Ratio (95% CI)	Spatial Scale of Effect Modifier
†Shin et al. (2021)	Ontario, Canada administrative cohort (2001–2015)	Male	Female	↓	EMM Reference Factor: 1.040 (1.027, 1.053) EMM Comparison Factor: 1.016 (1.003, 1.030)	Individual level
†Weichenthal et al. (2017a)	Toronto, Canada administrative cohort (1996–2012)	Male	Female	–	EMM Reference Factor: 1.26 (1.17, 1.36) EMM Comparison Factor: 1.26 (1.14, 1.39)	Individual level
†Doiron et al. (2019)	UK Biobank (2006–2010)	Male	Female	↑	EMM Reference Factor: 1.20 (1.14, 1.26)[b] EMM Comparison Factor: 1.26 (1.20, 1.32)[b]	Individual level

List of abbreviations in alphabetical order: CI = confidence interval; COPD = chronic obstructive pulmonary disease; EMM = effect measure modification; NO_2 = nitrogen dioxide; RHINE = The Respiratory Health in Northern Europe study; ppb = parts per billion; yr = year.
Results are standardized to a 10-ppb increase 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]Odds Ratio (COPD Prevalence).

†Studies published since the 2016 Oxides of Nitrogen ISA.

4.4.1.2.3 Pre-existing Conditions

Whereas evidence from an analysis of the Danish Diet, Cancer, and Health Cohort that was evaluated in the 2016 Oxides of Nitrogen ISA demonstrated an increased risk of NO_2-related COPD incidence in people with pre-existing asthma or diabetes (Andersen et al., 2011), recent evidence is not consistent with those findings (see Table 4-6). Shin et al. (2021) reported a stronger association between NO_2 exposure and COPD incidence in participants without diabetes compared to those with diabetes. Similarly, Doiron et al. (2019) observed higher odds of COPD prevalence associated with long-term NO_2 exposure in UK Biobank participants without asthma relative to those with asthma. Notably, Shin et al. (2021) also evaluated effect modification by hypertensive status and observed a positive association between long-term NO_2 exposure and COPD incidence in participants without hypertension (HR: 1.115 per 10 ppb increase in 3-year moving avg NO_2 [95% CI: 1.101, 1.128]) but not in those with hypertension (HR: 1.001 [95% CI: 0.986, 1.015]).

Table 4-6 Epidemiologic studies evaluating effect-measure modification of the relationship between long-term NO_2 exposure and COPD incidence by pre-existing diseases

Study	Cohort (Years)	EMM Reference Factor	EMM Comparison Factor	Direction of EMM[a]	Hazard Ratio (95% CI)	Spatial Scale of Effect Modifier
Asthma						
Andersen et al. (2011)	Danish Diet, Cancer, and Health cohort (1993–2006)	No asthma	Asthma	↑	EMM Reference Factor: 1.17 (0.94, 1.45) EMM Comparison Factor: 1.76 (1.09, 2.83)	Individual level
†Doiron et al. (2019)	UK Biobank (2006–2010)	No asthma	Asthma	↓	EMM Reference Factor: 1.28 (1.23, 1.33)[b] EMM Comparison Factor: 1.12 (1.04, 1.20)[b]	Individual level
Diabetes						
Andersen et al. (2011)	Danish Diet, Cancer, and Health cohort (1993–2006)	No diabetes	Diabetes	↑	EMM Reference Factor: 1.21 (0.99, 1.47) EMM Comparison Factor: 2.28 (1.28, 4.07)	Individual level
†Shin et al. (2021)	Ontario, Canada administrative cohort (2001–2015)	No diabetes	Diabetes	↓	EMM Reference Factor: 1.066 (1.056, 1.077) EMM Comparison Factor: 1.037 (1.013, 1.062)	Individual level
Hypertension						
†Shin et al. (2021)	Ontario, Canada administrative cohort (2001–2015)	No hypertension	Hypertension	↓	EMM Reference Factor: 1.115 (1.101, 1.128) EMM Comparison Factor: 1.001 (0.986, 1.015)	Individual level

List of abbreviations in alphabetical order: CI = confidence interval; COPD = chronic obstructive pulmonary disease; EMM = effect measure modification; NO_2 = nitrogen dioxide; RHINE = The Respiratory Health in Northern Europe study; ppb = parts per billion; yr = year.
Note: Results are standardized to a 10-ppb increase in 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.
[b]Odds Ratio (COPD Prevalence).
†Studies published since the 2016 Oxides of Nitrogen ISA.

4.4.1.3 Copollutants

Long-term exposure to air pollutants that are correlated with NO_2 has the potential to confound associations between long-term NO_2 exposure and respiratory disease. The potential for copollutant confounding is especially high in relation to other traffic-related pollutants, which share a common source with NO_2 and have been linked to similar health effects. None of the studies of COPD development in the 2016 Oxides of Nitrogen ISA examined potential copollutant confounding. A few recent epidemiologic studies of long-term NO_2 exposure and COPD incidence have evaluated copollutant models. Across studies, positive associations reported between long-term NO_2 exposure and COPD incidence were robust to adjustment for long-term $PM_{2.5}$ exposure [(Liu et al., 2021c; Liu et al., 2021d; Weichenthal et al., 2017a); Table 4-7]. Liu et al. (2021d) also noted a slightly attenuated but still positive association in a copollutant model adjusted for BC. Strong correlations between long-term NO_2 and BC concentrations (r = 0.93) complicate the interpretation of this result due to collinearity.

In a DNC study, (Liu et al., 2021c) considered traffic noise as a potential confounder given its correlation to both NO_2 exposure and increased stress levels. The authors observed a positive association (HR: 1.29 per 10 ppb increase in 3-year moving average NO_2 [95% CI: 1.12, 1.49]) that was slightly attenuated, but still positive after adjustment for modeled residential traffic noise (HR: 1.18 [95% CI: 0.98, 1.42]). Notably, traffic noise may serve as a proxy measure of other traffic-related pollutants.

Table 4-7 Copollutant-adjusted associations between long-term exposure to nitrogen dioxide and chronic obstructive pulmonary disease or chronic bronchitis incidence

Study and Cohort	Outcome	Correlations (*r*)	Effect Estimate (95% CI)
†Weichenthal et al. (2017a) Toronto, Canada administrative cohort	COPD Incidence	$PM_{2.5}$: 0.22	HR: 1.26 (1.23, 1.29) +$PM_{2.5}$: 1.32 (1.22, 1.42)
†Liu et al. (2021d) DNC, DDCH, and CEANS	COPD Incidence	$PM_{2.5}$: 0.55 Traffic Noise: 0.59	HR: 1.29 (1.12, 1.49) +$PM_{2.5}$: 1.32 (1.09, 1.60) +Traffic Noise: 1.18 (0.98, 1.42)
†Liu et al. (2021a) DNC	COPD Incidence	$PM_{2.5}$: 0.63 BC: 0.91 O_3: -0.48	HR: 1.22 (1.12, 1.32) +$PM_{2.5}$: 1.24 (1.12, 1.37) +BC: 1.12 (0.94, 1.33) +O_3: 1.32 (1.20, 1.46)
†Doiron et al. (2021) Dutch Lifelines Cohort	Chronic Bronchitis Incidence	$PM_{2.5}$: 0.57	OR: 1.20 (1.05, 1.38) +$PM_{2.5}$: 1.26 (1.06, 1.50)

List of abbreviations in alphabetical order: BC = black carbon; CEANS = Cardiovascular Effects of Air Pollution and Noise in Stockholm; CI = confidence interval; COPD = chronic obstructive pulmonary disease; DDCH = Danish Diet, Cancer, and Health cohort study; DNC = Danish Nurse Cohort; NO_2 = nitrogen dioxide; O_3 = ozone; OR = odds ratio; $PM_{2.5}$ = particulate matter with a nominal mean aerodynamic diameter less than or equal to 2.5 μm; ppb = parts per billion.
Note: Results are standardized to a 10-ppb increase in NO_2.
†Studies published since the 2016 Oxides of Nitrogen ISA.

4.4.1.4 Conclusions and Remaining Uncertainties

In contrast to a limited and inconsistent evidence base from 2016 Oxides of Nitrogen ISA, several recent epidemiologic studies provide generally consistent evidence between long-term NO_2 exposure and COPD incidence. Positive associations were observed across various study designs, including traditional and administrative cohorts, and in relation to exposures assigned using LUR models, hybrid exposure models, and dispersion models. Although positive associations were reported in some North American studies, more consistent results were observed in European cohort studies. Most of the exposure models used demonstrated good model performance (i.e., R^2 values ranging from 0.59 to 0.85), though high cross-validation R^2 values do not necessarily reduce bias in health effect estimates (see Chapter 2 on Exposure and Dosimetry). The shape of the C-R relationship was inconsistent across a few studies that used flexible modeling terms to investigate potential non-linearities. While approximately linear relationships were observed in a pair of administrative cohort studies with partially overlapping study populations, a C-R plot from another administrative cohort study suggests a threshold at approximately 30 $\mu g/m^3$ (~16 ppb), above which the C-R relationship appears to be approximately linear. A few recent studies also evaluated potential effect modification by age, sex, or pre-existing disease, and reported mostly equivocal results.

A key remaining uncertainty is the independent nature of the observed epidemiologic associations. Although several recent studies reported that associations between long-term NO_2 exposure and COPD incidence were robust to adjustment for long-term exposure to $PM_{2.5}$ and were attenuated but remained positive in models adjusted for BC or traffic noise, high correlations between NO_2 and the examined copollutants add uncertainty to the observed results.

4.4.2 Animal Toxicological Studies of the Development of COPD

COPD shares similar subclinical features as asthma, including inflammation, oxidative stress, and airway remodeling. Since rodents do not develop COPD, animal models look to recapitulate the excess inflammation, airway remodeling, lung function decrement, and airspace enlargement that typify COPD. As discussed in Section 4.3.1.3, the existing evidence reviewed in previous ISAs provides support for increased lung inflammation and oxidative stress in response to long-term NO_2 exposure. The 2016 Oxides of Nitrogen ISA also evaluated several studies that showed airspace enlargement following prolonged NO_2 exposures. Increases in alveolar mean linear intercept (MLI), a measure of alveolar enlargement (Lafuma et al., 1987; Sherwin and Richters, 1982), histologic enlargement in airspaces (Blair

et al., 1969), and evidence of inflammatory septal wall thickening (Hayashi et al., 1987) were seen in rodent studies of long-term NO_2 exposure. These results are not seen in all animal studies, as decreased MLI (Gregory et al., 1983) and lack of effect on septal thickness (Mercer et al., 1995) have been reported. In general, airspace enlargement occurs with exposure to high NO_2 concentrations (>5 ppm).

Several recent studies are discussed in Section 4.3.1.3 that show increased inflammation and oxidative stress in rodent models of long-term NO_2 exposure (Lu et al., 2024; Lu et al., 2023b; Lu et al., 2023a; Han et al., 2017). No recent studies have looked at airspace enlargement or alveolar destruction in response to NO_2 exposure that would be consistent with the development of emphysema. Interestingly, one recent study did find decreased MLI (increased alveolarization) in the lungs of newborn rats after 7-day exposure to 20 ppm NO, although this was not seen at lower concentrations (5 ppm) or at 14 days post exposure (Duong-Quy et al., 2014). Exposure to 5.8 ppm HONO (nitrous acid) for six weeks caused increased MLI in the lungs of rats that corresponded to increased markers of inflammation (Ohyama et al., 2018). In another study by the same group, Ohyama et al. (2020) showed tissue architecture changes in the lungs of male guinea pigs exposed to HONO for four weeks with statistically significant increases in MLI seen with exposure to 1.7 ppm HONO. The recent evidence supports NO_2-induced subclinical events, including inflammation and mucus production, which could contribute to the development or worsening of COPD and existing evidence from animal models suggests that these correspond with emphysema-like airspace enlargement changes that could precipitate the development of emphysema. Evidence of effects related to COPD following exposure to other oxides of nitrogen are limited in number but with some coherent findings in studies of HONO exposure. Study specific details, exposure conditions, and endpoints examined are described in more detail in Appendix A – Appendix Table 4-7.

4.4.2.1.1 Conclusions and Remaining Uncertainties

The animal toxicological evidence related to COPD development is limited. There is consistent evidence that long-term NO_2 exposures can lead to pulmonary inflammation, which is a common hallmark of COPD. The evidence for emphysema-like changes is more limited with some, but not all, studies showing airspace enlargement following long-term exposure to NO_2, however few studies have evaluated measurements of alveolar airspace enlargement. There is also evidence that exposure to other oxides of nitrogen such as NO or HONO at relatively high concentrations for long periods of time can similarly lead to airspace enlargement. While animal models are considered reasonable models for emphysema-like phenotypes it should be noted that these models lack the chronic and progressive nature of COPD and animal models are also subject to inherent species differences that could potentially result in differences from human responses.

4.4.3 Synthesis Across Lines of Evidence

The 2016 Oxides of Nitrogen ISA included a limited number of epidemiologic studies that provided inconsistent evidence of an association between long-term exposure to NO_2 and the development of COPD. Experimental animal studies evaluated in previous ISAs provide consistent evidence for increased lung inflammation and oxidative stress in response to long-term NO_2 exposure. Additionally, there were a small number of studies in rodents that demonstrated alveolar airspace enlargement following long-term exposures to high NO_2 concentrations (i.e., >5 ppm), though the results were inconsistent.

Several recent epidemiologic studies expand the existing evidence base and generally support positive associations between long-term NO_2 exposure and COPD incidence. Positive associations were observed across various study designs, including recruitment-based and administrative cohorts, and in relation to exposures assigned using LUR models, hybrid exposure models, and dispersion models. The consistency of results across varying study designs and exposure assignment methods reduces the likelihood that the findings are due to chance or bias related to exposure measurement error. Additionally, studies have controlled for potential confounding across a range of individual and area-level covariates (see Appendix A – Appendix Table 4-6). Although several recent studies reported positive associations between long-term NO_2 exposure and COPD incidence that persisted in models adjusted for long-term exposure to $PM_{2.5}$, BC, or traffic noise, high correlations between NO_2 and the examined copollutants add uncertainty to the observed results. Additionally, potential confounding by other traffic-related copollutants was not thoroughly evaluated.

Recent experimental evidence in animals is limited but mostly consistent with previous findings. Although animal models do not develop COPD, the NO_2-induced increases in lung inflammation and damage, and airspace enlargement in adult animals, may underlie the development and progression of chronic lung disease and provide biological plausibility for NO_2-induced development of COPD (see Section 4.9). Increased lung inflammation and airspace enlargement have similarly been reported following HONO exposure in animal models. Notably, however, the high concentrations necessary to induce alveolar airspace enlargement in the available experimental animal studies may limit the relevance to human responses at relevant NO_2 concentrations. Thus, given some of the limitations in comparing the animal toxicological and epidemiologic evidence, a key remaining uncertainty is the independent nature of the observed epidemiologic associations.

4.5 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.

4.5.1 Epidemiologic Studies of Respiratory Infection

The 2016 Oxides of Nitrogen ISA evaluated a limited number of epidemiologic studies examining associations between long-term NO_2 exposures and the development of respiratory infection in children and adults. Results from a limited number of studies examining aggregated lower respiratory tract infections (LRTI) (i.e., bronchitis, bronchiolitis, pneumonia, influenza, and unspecified LRTI) in infants were equivocal (Aguilera et al., 2013; Sunyer et al., 2004). Focusing on specific respiratory infections in children, MacIntyre et al. (2014b) reported that LUR modeled NO_2 concentrations at birth were associated with pneumonia and otitis media incidence but not croup incidence among young children in a meta-analysis of ten European birth cohorts. The meta-analytic association observed for pneumonia and otitis media was attenuated and imprecise (i.e., wide 95% CIs) after adjusting for $PM_{2.5}$ or $PM_{2.5}$ absorbance. In a population-based case-control study of adults aged 65 years or older in Ontario, Canada, annual average NO_2 exposures estimated by three methods – central site monitors, LUR models, and IDW – were all associated with pneumonia hospital admissions (Neupane et al., 2010). There was no adjustment for short-term exposure effects, and it is unclear what the relative impacts on prevalent respiratory infections are for short-term versus long-term exposure.

Several recent cohort studies expand the existing evidence base for respiratory infection outcomes including pneumonia, aggregated LRTIs, acute respiratory distress syndrome (ARDS), and pulmonary tuberculosis (see Table 4-8). Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 4-8. A limited number of studies in children provide some evidence of associations between long-term exposure to oxides of nitrogen and pneumonia and aggregated LRTI. In recent studies that examined adult populations, there is some evidence of associations with pneumonia incidence, as well as individual studies that indicate associations between long-term NO_2 exposure and pulmonary tuberculosis, aggregated LRTI, aggregated RTI, and self-reported respiratory infection. A short, bulleted summary of the recent evidence is provided below, followed by an additional discussion of the lifestages and populations evaluated (see Section 4.5.1.1). Section 4.5.1.2 discusses conclusions from available studies and summarizes remaining uncertainties. Studies examining the associations between long-term NO_2 exposure and COVID-19-related health outcomes are discussed in Section 4.5.2.

Children

- A recent analysis of the Kaiser Air Pollution and Pediatric Asthma Study birth cohort adds to the limited evidence of an association between long-term exposure and pneumonia in children (Kennedy et al., 2018). Kennedy et al. (2018) observed an association between annual average log-transformed mobile-source NO_X concentrations at-birth estimated using the RLINE dispersion model and incident clinical diagnosis for pneumonia in the first two years of life (HR: 1.06 per 2.7-fold increase [95% CI: 0.97, 1.20]). The authors reported stronger and more precise associations for bronchiolitis (HR: 1.19 [95% CI: 1.12, 1.27]) and otitis media (HR: 1.17 [95% CI: 1.11, 1.22]).
- A cohort study from Norway examined the association between LUR-estimated long-term NO_2 concentrations and LRTI in infants in an analysis of a subgroup of the Norwegian Mother and Child Cohort Study (Madsen et al., 2017). The authors reported an imprecise positive association between long-term NO_2 exposure and LRTI in infants six to 18 months and a null association in infants zero to six months (see Table 4-8). Sensitivity analyses indicated that the association observed in the six to 18-month group was driven by associations in smaller cities, with null associations observed in large urban areas.

Adults

- Consistent with findings from Neupane et al. (2010), a limited number of recent cohort studies provide some additional evidence that long-term NO_2 exposure is positively associated with pneumonia incidence in adults (Wang et al., 2023a; Danesh Yazdi et al., 2021), although results were not always consistent across the concentration distribution (Danesh Yazdi et al., 2021). A large nationwide Medicare cohort study of over 60 million adults aged 65 years and older examined pneumonia hospital admissions in relation to annual average NO_2 concentrations estimated at the centroids of 1 x 1 km grid cells using a hybrid ensemble exposure prediction model (Danesh Yazdi et al., 2021). The authors used inverse probability weights (IPW) to control for potential confounding by accounting for the conditional probability that an individual was exposed to different NO_2 concentrations based on a given set of covariates. Danesh Yazdi et al. (2021) reported an inverse association between long-term NO_2 exposure and pneumonia hospital admissions in the full population that was positive after restricting the analysis to observations at lower concentrations (<20 ppb). The authors reported that pneumonia hospital admissions were positively associated with annual average ozone concentrations, which were inversely correlated with NO_2 concentrations (ρ = -0.186). However, this weak correlation is unlikely to explain the inverse association between NO_2 exposure and pneumonia hospital admissions. The discrepancy between the full population and restricted analysis could be due to a number of other factors, including differential exposure measurement error or unmeasured confounding.
- A recent analysis of data from the UK Biobank Study examined incident hospital admissions for pneumonia among nearly 450,000 adults (40 to 69 yrs) who did not have pneumonia within one year prior to enrollment (Wang et al., 2023a). The authors reported positive associations between long-term exposure to both NO_2 (HR: 1.22 per 10 ppb increase [95% CI: 1.17, 1.26]) and NO_X (HR: 1.06 per 16.52 $\mu g/m^3$ [95% CI: 1.04, 1.07]) concentrations estimated at baseline and pneumonia incidence. Notably, associations were considerably stronger in time-varying models with annual average concentrations (HR: 1.39 [95% CI: 1.36, 1.42] and HR: 1.18 [95% CI: 1.17, 1.20], respectively). Similar to the results reported by Danesh Yazdi et al. (2021), Wang et al. (2023a) observed a stronger positive association at annual average concentrations below 20 ppb. Unlike Danesh Yazdi et al. (2021), the association remained positive at higher concentrations.

- In a nested case-control study of patients with tuberculosis from the Kaiser Permanente of Northern California administrative cohort, Smith et al. (2016) similarly reported a positive association between long-term exposure to NO_2 and pulmonary tuberculosis (TB).
- Several other recent studies in adults examined less specific respiratory infection outcomes (Kaspersen et al., 2024; Zhang et al., 2024; Kirwa et al., 2021; Gandini et al., 2018b). Large nationwide, population-based administrative cohort studies in Denmark (Kaspersen et al., 2024) and Italy (Gandini et al., 2018b) reported positive associations between long-term exposure to NO_2 and incident RTI and LRTI, respectively. In a nested case-control analysis including over 180,000 incident cases of respiratory tract infections, three-month average NO_2 concentrations were associated with a large increase in RTI incidence (HR: 2.20 [95% CI: 2.11, 2.30] per 10 ppb increase in NO_2) (Kaspersen et al., 2024). Gandini et al. (2018b) observed a smaller, 10% increase in incident hospital admissions LRTI (95% CI: 0, 20) associated with a 10 ppb increase in CTM-estimated long-term NO_2 concentrations. In a smaller cohort study of female nurses enrolled in the Danish Nurse Cohort, Zhang et al. (2024) also reported a positive association between annual average NO_2 concentrations and incident LRTI (HR: 1.45 [95% CI: 1.27, 1.2465] per 10 ppb increase in NO_2).
- Another recent cohort study assessed self-reported RTI incidence through periodic questionnaires. Kirwa et al. (2021) assessed self-reported respiratory infection in the two weeks preceding each of five study evaluations over a 12-year study period as part of the Multiethnic Study of Atherosclerosis and Air Pollution (MESA Air). The authors reported that an IQR increase (increment NR) in annual average NO_2 or NO_X concentrations was associated with an imprecise increase in the prevalence of self-reported respiratory infection, including cold, influenza, febrile illness, sinus infection, bronchitis or pneumonia (7% [95% CI: -6, 22] and 4% [95% CI: -5, 14], respectively).

Table 4-8 Associations between long-term exposure to oxides of nitrogen and respiratory infection

Study and Cohort/Location	Exposure Assessment	Outcome	Effect Estimate (95% CI)
Children			
Aguilera et al. (2013) Spanish birth cohort (2003–2008)	LUR (NO_2)	Incident LRTI: Doctor diagnosed	RR: 1.10 (0.97, 1.24)
†Kennedy et al. (2018) Kaiser Air Pollution and Pediatric Asthma Study (2000–2010)	Dispersion model (NO_2)	Incident pneumonia, bronchiolitis, and otitis media: Doctor diagnosed	HRs Pneumonia: 1.06 (0.97, 1.20)[a] Bronchiolitis: 1.19 (1.12, 1.27)[a] Otitis media: 1.17 (1.11, 1.22)[a]
MacIntyre et al. (2014b) 10 ESCAPE Cohorts	LUR (NO_2)	Incident pneumonia, otitis media, and croup: Physician diagnosed	ORs Pneumonia: 1.64 (1.04, 2.58) Otitis media: 1.18 (1.04, 1.33) Croup: 0.93 (0.7, 1.23)
†Madsen et al. (2017)	LUR (NO_2)	Incident LRTI: Maternal reports	RRs 0–6 months: 0.98 (0.72, 1.34)

Study and Cohort/Location	Exposure Assessment	Outcome	Effect Estimate (95% CI)
Norwegian Mother and Child Cohort Study (1999–2008)			6–18 months: 1.10 (0.90, 1.34)
Sunyer et al. (2004) AMICS (1995–1997)	Indoor NO_2 via passive sampler	Incident LRTI: Maternal reports	<5 µg/m^3 (<2.66 ppb): Reference 5–10 µg/m^3 (2.7–5.3 ppb): 0.92 (0.67, 1.26) 10–30 µg/m^3 (5.3–16.0 ppb): 0.99 (0.73, 1.36) >30 µg/m^3 (>16.0 ppb): 0.69 (0.5, 0.95)
Adults – Pneumonia			
Neupane et al. (2010) Hamilton, Canada (2003–2005)	IDW of central-site monitors (NO_2)	Radiographic-confirmed pneumonia	OR: 1.93 (1.00, 3.73)
†Wang et al. (2023a) UK Biobank (2006–2019)	LUR (NO_2 and NO_X)	Incident Pneumonia: First hospital admission	HRs NO_2: 1.22 (1.17, 1.26) NO_X: 1.06 (1.04, 1.07)[b]
†Danesh Yazdi et al. (2021) U.S. Medicare (2000–2016)	Hybrid model (NO_2)	Pneumonia hospital admissions	Risk Difference: -0.0134 (-0.0158, -0.011)
Adults – Other			
†Gandini et al. (2018b) Italian Longitudinal Study (2001–2008)	Chemical transport model (NO_2)	Incident LRTI: First hospital admission	HR: 1.10 (1.00, 1.20)
†Kaspersen et al. (2024) Danish national administrative cohort (2004–2016)	Dispersion model (NO_2 and NO_X)	Incident RTI: First-time hospital contact (inpatient, outpatient, emergency)	ORs NO_2: 1.57 (1.52, 1.62) NO_X: 1.16 (1.15, 1.18)[c]
†Kirwa et al. (2021) MESA (2000–2012)	Spatiotemporal model (NO_2 and NO_X)	Self-reported RTI	RRs NO_2: 1.04 (0.95, 1.14)[d] NO_X: 1.07 (0.94, 1.22)[d]
†Smith et al. (2016) Kaiser Permanente of Northern California cohort (1996–2010)	Central site monitors (closest monitor; NO_2)	Incident Pulmonary TB: Clinical diagnosis and prescription for anti-TB medication; or positive TB culture	ORs Q1: Reference Q2: 1.17 (0.93, 1.46) Q3: 1.17 (0.93, 1.48) Q4: 1.27 (1.01, 1.61) Q5: 1.42 (1.1, 1.84)
†Zhang et al. (2024) Danish Nurses Cohort (1993–2018)	Hybrid model	Incident LRTI: First-time hospital contact (inpatient, outpatient, emergency)	HR: 1.45 (1.27, 1.65)

List of abbreviations in alphabetical order: AMICS = Asthma Multicentre Infant Cohort Study (Europe); CI = confidence interval; ESCAPE = The European Study of Cohorts for Air Pollution Effects; HR = hazard ratio; IDW = inverse distance weighting; MESA = Multi-Ethnic Study of Atherosclerosis; LRTI = lower respiratory tract infection; LUR = land use regression; NO_2 = nitrogen dioxide; NO_X = NO + NO_2; OR = odds ratio; ppb = parts per billion; RR = relative risk; RTI = respiratory tract infection; TB = tuberculosis; Q# = #th quartile; ; U.S. = United States; $\mu g/m^3$ = micrograms per cubic meter.
Note: Results are standardized to a 10-ppb increase in NO_2.
[a]Not standardized. Effect estimates per 2.7-fold increase in NO_2.
[b]Not standardized. Effect estimates per 16.52 $\mu g/m^3$ increase in NO_X.
[c]Not standardized. Effect estimates per 10 $\mu g/m^3$ increase in NO_X.
[d]Not standardized. Effect estimates per IQR increase (NR).
†Studies published since the 2016 Oxides of Nitrogen ISA.

4.5.1.1 Lifestages and Populations

There was little evaluation of populations potentially at increased risk of NO_2-related respiratory infection incidence within studies included in the 2016 Oxides of Nitrogen ISA. Evidence remains limited, but a few studies reported stratified results for lifestage, smoking status, and sex for several outcomes.

In a nested case-control study of patients with tuberculosis from the Kaiser Permanente of Northern California administrative cohort, Smith et al. (2016) performed subgroup analyses by stratification. Positive associations were observed when comparing the highest quintile of NO_2 exposure (>17.7 ppb) to the lowest quintile (<9.8 ppb) for both smokers and never smokers; however, the magnitude of the association was stronger in smokers (OR: 1.81 [95% CI: 1.13, 2.91]) when compared to never smokers (OR: 1.29 [95% CI: 0.97, 1.71]). This is in contrast to the findings of Wang et al. (2023a), who reported larger positive associations between both long-term NO_2 and NO_X exposure and pneumonia incidence in smokers when compared to never smokers in a large administrative cohort study in patients enrolled in the UK Biobank.

A few studies performed additional stratifications by sex and lifestage, but results were also inconsistent. Effect measure modification analyses performed in a large administrative cohort study of Medicare recipients found largely small negative associations between long-term NO_2 exposure and pneumonia hospitalization admissions (Danesh Yazdi et al., 2021). Associations remained negative for most subsets when stratified by race, Medicaid-eligibility, and age category; more detailed information on this study can be found in Section 4.5.1. In contrast, Wang et al. (2023a) reported positive associations for all subgroup analyses. A stronger association was observed in participants 65 years and older (HR: 1.27 per 10 ppb increase [95% CI: 1.21, 1.34]) compared to participants under 65 years (HR: 1.14 [95% CI: 1.09, 1.20]). There were no differences in the strength of associations in sex-specific analyses. Associations were also comparable in strength for participants with BMI <30 when compared to participants with BMI >30. Notably, the authors reported a stronger association in low-income participants, defined as an annual income less than 31,000 pounds sterling (GBP) (HR: 1.27 [95% CI: 1.22, 1.33]), when compared to participants with higher incomes (i.e., ≥31,000 GBP; HR: 1.11 per 5.79 ppb increase [95% CI: 1.04, 1.18]).

4.5.1.2 Conclusions and Remaining Uncertainties

In the small body of epidemiologic studies evaluated in the 2016 Oxides of Nitrogen ISA, there was some evidence that LUR-estimated long-term NO_2 exposure was associated with respiratory infections in school children and pneumonia hospital admissions in adults, ages 65 years or older. For studies of hospital admissions, it is not clear whether the association observed for long-term NO_2 exposure is independent of an association with short-term exposure. Recent studies of respiratory infections are similarly limited in number and provide some additional support for an association, particularly relating to pneumonia, bronchiolitis, and otitis media in children. The recent evidence for adults is not entirely consistent. In a study providing strong inference due to its large sample size and alternative methods for confounder control, long-term NO_2 concentrations were not associated with pneumonia incidence across the entire concentration distribution, though associations were observed at low concentrations (i.e., <20 ppb). The limited but consistent evidence of positive associations of NO_2 with LRTI provides some additional support for the inconsistent associations reported for pneumonia. However, the entirety of the recent evidence for pneumonia and LRTI incidence in adults comes from studies examining hospital admissions and does not address previous uncertainties regarding the role of short-term NO_2 exposures. Another key uncertainty is the limited evaluation of potential copollutant confounding.

4.5.2 Epidemiologic Studies of COVID-19

Coronavirus Disease 2019 (COVID-19), caused by the SARS-CoV-2 virus, emerged in late 2019 in Wuhan, China, and escalated to a global pandemic in March 2020. A growing number of studies have emerged examining the relationship between air pollution, including NO_2 exposure, and COVID-19-related outcomes. This section presents an evaluation of studies that examined the relationship between long-term NO_2 exposure and COVID-19-related outcomes, including SARS-CoV-2 infection, COVID-19 mortality, and hospital admissions. Additionally, a single study assessed development of long COVID-19, a chronic condition that can occur after SARS-CoV-2 infection (Yu et al., 2023a).

Several recent studies have evaluated long-term oxides of nitrogen exposure and COVID-19-related outcomes in adults. Studies are reported some evidence of positive associations (see Table 4-9), but there are methodological limitations in the available studies that warrant caution in the interpretation of the evidence. Additionally, a small number of ecological studies, all of which were at neighborhood or census tract scales, also reported positive associations between long-term exposure to NO_2 and COVID-19 mortality (Garcia et al., 2022; Marian et al., 2022; Konstantinoudis et al., 2021; Lipsitt et al., 2021) but are not the focus of this section due to study design limitations. A more detailed summary of the evidence is provided below, followed by conclusions and remaining uncertainties in Section 4.5.2.1.

Several large cohort studies analyzed inpatient claims data and electronic health records from the Kaiser Permanente Southern California (KPSC) patient cohort to assess associations between long-term exposure to oxides of nitrogen and COVID-19 morbidity and mortality (Jerrett et al., 2023; Chen et al., 2022b; Chen et al., 2021).

- Chen et al. (2021) and Chen et al. (2022b) analyzed data from the initial wave of the COVID-19 pandemic in the U.S., including hospital admissions and mortality among KPSC members who tested positive for COVID-19 between March 1 and August 31, 2020. Chen et al. (2022b) reported positive associations between long-term exposure to NO_2 and COVID-19-related hospital admissions, intensive respiratory support (IRS), and intensive care unit (ICU) admissions (see Table 4-9). Associations between long-term NO_2 exposure and mortality due to COVID-19 were weaker in magnitude and confidence intervals included the null (HR: 1.11 [95% CI: 0.88, 1.41] per 10 ppb increase in NO_2). In contrast, Chen et al. (2021) found that annual average near-road NO_X concentrations were not associated with the same outcomes. However, when decomposing near roadway NO_X concentrations exposure into freeway and non-freeway exposure, associations were observed with non-freeway NO_X. Non-freeway near-road NO_X concentrations may be an indicator for traffic-related pollution with a different composition than freeway sources.
- Consistent with Chen et al. (2022b), Jerrett et al. (2023) analyzed the same population across an extended follow-up period (June 2020 through January 2022) and reported an association between long-term NO_2 exposure and mortality due to COVID-19 that was comparable in magnitude but more precise (HR: 1.08 [95% CI: 1.03, 1.13] per 10 ppb increase in annual average NO_2 concentrations).

A large nationwide study in Denmark provided additional support for an association between long-term NO2 exposure and COVID-19 incidence and mortality.

- A recent large nationwide Danish study (n = 3,721,810) reported a positive association between 1-year average NO_2 concentrations and COVID-19 incidence, hospitalization, and mortality (Zhang et al., 2023). Results were consistent in sensitivity analyses utilizing different exposure windows (i.e., 3-year and 10-year averages). A major strength of this study was the minimal risk of selection bias due to the inclusion of a majority of the population captured under a universal healthcare system, which was further linked to an individual-level nationwide COVID-19 surveillance system.

In contrast, a study in New York City, N.Y., observed a null association between long-term NO_2 exposures and COVID-19 mortality and ICU admissions in the early stages of the pandemic [i.e., March through August 2020; (Bozack et al., 2022)].

Table 4-9 Association between long-term exposure to oxides of nitrogen and COVID-19

Study	Location/Cohort	Exposure Assessment	Effect Estimate (95% CI)[a]
†Zhang et al. (2023)	AIRCODEN Denmark	Dispersion model	**HR** COVID-19 Incidence: 2.08 (1.77, 2.44)

Study	Location/Cohort	Exposure Assessment	Effect Estimate (95% CI)[a]
			COVID-19 Hospitalization: 2.08 (1.51, 2.86) COVID-19 Mortality: 1.81 (0.90, 3.65)
†Jerrett et al. (2023)	Kaiser Permanente Southern California	Chemical Transport Model	COVID-19 Mortality: NO_2 HR: 1.08 (1.03, 1.13)
†Chen et al. (2021)	Kaiser Permanente Southern California	Dispersion Model	COVID-19 Hospitalization (OR): Total NO_X (1.5 ppb): 0.99 (0.96, 1.03) Freeway NO_X (1.3 ppb): 0.99 (0.96, 1.02) Non-Freeway NO_X (0.5 ppb): 1.01 (0.97, 1.05) COVID-19 Intensive Respiratory Support (OR): Total NO_X (1.5 ppb): 1.00 (0.95, 1.05) Freeway NO_X (1.3 ppb): 0.98 (0.93, 1.03) Non-Freeway NO_X (0.5 ppb): 1.07 (1.01, 1.13) COVID-19 ICU Admission (OR): Total NO_X (1.5 ppb): 0.99 (0.93, 1.06) Freeway NO_X (1.3 ppb): 0.96 (0.90, 1.02) Non-Freeway NO_X (0.5 ppb): 1.11 (1.03, 1.18) COVID-19 Mortality (HR): Total NO_X (1.5 ppb): 0.97 (0.91, 1.05) Freeway NO_X (1.3 ppb): 0.94 (0.88, 1.01) Non-Freeway NO_X (0.5 ppb): 1.10 (1.03, 1.18)
†Chen et al. (2022b)	Kaiser Permanente Southern California	Central Site Monitors	COVID-19 Hospitalization (OR): NO_2: 1.39 (1.22, 1.59) COVID-19 Intensive Respiratory Support (OR): NO_2: 1.64 (1.34, 2.01) COVID-19 ICU Admission (OR): NO_2: 1.64 (1.26, 2.12) COVID-19 Mortality (HR): NO_2: 1.11 (0.88, 1.41)
†Bozack et al. (2022)	NYC; Administrative Cohort	Hybrid Model	**RR (per 1 ppb NO_2)** Mortality: 1.00 (0.98, 1.03) ICU Admission: 1.01 (0.98, 1.05)

List of abbreviations in alphabetical order: AIRCODEN = AIR Pollution and COVID-19 Mortality and Morbidity in Denmark; CI = confidence interval; COVID-19 = Coronavirus disease 2019; HR = hazard ratio; ICU = intensive care unit; KPSC = Kaiser Permanente Southern California; NO_2 = nitrogen dioxide; NO_X = nitrogen oxides; NYC = New York City; OR = odds ratio; ppb = parts per billion; RR = relative risk; UK = United Kingdom; $\mu g/m^3$ = micrograms per cubic meter.
[a]Effect estimates are standardized to a 10 ppb increase in NO_2 or a 20 ppb increase in NO_X, unless otherwise noted.
†Studies published since the 2016 Oxides of Nitrogen ISA.

4.5.2.1 Conclusions and Remaining Uncertainties

An emerging body of evidence has examined associations between long-term oxides of nitrogen exposure and COVID-19 outcomes. While these studies provide some evidence of a positive association between long-term average NO_2 concentrations and SARS-CoV-2 infections and COVID-19 deaths reported positive associations, a number of methodological issues could influence results. While the included studies account for population density, they mostly do not consider other crucial factors that may strongly influence the spread of COVID-19. In particular, behavioral factors such as social distancing; vaccination status; implementation of, and adherence to, stay-at-home orders; and the use of N95 masks, as well as other preventive measures, are important for slowing the spread of SARS-CoV-2 and may be directly or indirectly related to exposure. There was no adjustment for these behavioral factors in the available evidence base. Two critiques of the air pollution exposure and COVID-19 literature conducted by Bourdrel et al. (2021) and Villeneuve and Goldberg (2020) additionally highlight the importance of control for race/ethnicity and other sociodemographic factors, which may strongly influence exposure or susceptibility to SARS-CoV-2. Additionally, these reviews indicate the potential for exposure misclassification and the likelihood of underreporting of cases and deaths, particularly in the early stages of the pandemic. Although most studies adjusted for race/ethnicity, the majority of studies that adjusted for sociodemographic factors used contextual variables, which may be subject to residual confounding. Taken together, there notable uncertainty regarding the influence of long-term oxides of nitrogen exposure on the spread, susceptibility, or severity of SARS-CoV-2.

4.5.3 Experimental Support for Respiratory Infection

Available animal toxicological evidence evaluated in the 2008 and 2016 Oxides of Nitrogen ISAs provide evidence that host resistance to bacterial and viral infection is compromised following long-term exposures to PECOS-relevant NO_2 concentrations in mice (U.S. EPA, 2016, 2008). There are no recent animal toxicological studies investigating the effects of long-term NO_2 exposure on susceptibility to bacterial or viral infection. Study specific details, exposure conditions, and endpoints examined in the older studies are highlighted in the text below and described in more detail in Appendix A – Appendix Table 4-9. Section 4.5.3.1 discusses the relevance of the animal models used for humans, and Section 4.5.3.2 discusses conclusions from these studies and summarizes remaining uncertainties.

Long-term NO_2 exposure has been shown to increase the susceptibility of experimental animals to bacterial infection. In (Henry et al., 1970), squirrel monkeys exposed to 5 ppm NO_2 for two months and then exposed to *Klebsiella pneumoniae* or influenza virus had reduced clearance of bacteria from the lungs, three days post-infection. Furthermore, two of the seven monkeys exposed to NO_2 died at 3- and 10-days post-infection while none of the air-exposed, infected controls succumbed to the infection. When influenza virus was given 24 hours prior to NO_2 exposure and after NO_2 exposure, one of the three exposed monkeys died 5-days post-infection. Ehrlich and Henry (1968) and Ehrlich (1980) also studied

the effects of NO_2 on *K. pneumoniae* infection in mice. Exposures were either continuous or intermittent (6, 18, or 24 hours/days) at a concentration of 0.5 ppm NO_2, and bacterial challenge was administered at 1, 3, 6, 9, and 12 months. Continuous exposure to 0.5 ppm NO_2 for three months or longer increased mortality rates after infection, whereas intermittent exposures increased mortality at 6, 9, and 12 months. Likewise, Miller et al. (1987) showed increased mortality in mice exposed to a base of 0.2 ppm NO_2 with two daily 1-hour peaks of 0.78 ppm and subsequent challenge with *Streptococcus zooepidemicus* at 16, 32, and 52 weeks.

Overall, animal toxicological evidence of NO_2-mediated exacerbation of respiratory infection suggests that host resistance to bacterial and viral infection is compromised following long-term exposures to PECOS-relevant NO_2 concentrations in monkeys and mice. While the number of studies is limited, results across studies, and across species, are consistent.

4.5.3.1 Relevance of Animal Responses to Human Responses

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 can aid in determining immunotoxicity in humans (IPCS, 2012). However, other factors (e.g., strengths and limitations of individual studies, exposure, breadth of evidence/endpoints, consistency) must be considered when determining immunotoxicity.

4.5.3.2 Conclusions and Remaining Uncertainties

Animal toxicological studies provide consistent evidence of effects related to exacerbation of respiratory infection, suggesting that host resistance to bacterial and viral infection may be compromised following long-term exposures to ambient-relevant NO_2 concentrations. The evidence is consistent across toxicological studies conducted in rodents and primates. Due to the limited number of studies available, important uncertainties and data gaps remain. For example, there was only one study investigating the potential effects of NO_2 on susceptibility to viral infections available for this review. Consequently, additional studies investigating susceptibility to bacterial and viral susceptibility along with many other related functional endpoints (e.g., antibody responses, delayed type hypersensitivity, ex vivo white blood cell function) are needed to fully understand the effects of long-term NO_2 exposures on the immune system.

4.5.4 Synthesis Across Lines of Evidence

Animal toxicological evidence suggests that host resistance to bacterial and viral infection is compromised following long-term exposures to PECOS-relevant NO_2 concentrations in monkeys and mice. While the number of studies is limited, results across studies and across species, are consistent. Host resistance assays used in the available literature are considered indicators of immunotoxicity in humans (IPCS, 2012). Epidemiologic evidence provides limited coherence. While a small number of studies reported evidence of an association between long-term NO_2 exposure and respiratory infections in infants or young children (e.g., pneumonia, otitis media, or bronchiolitis), results were mostly inconsistent. There were similarly inconsistent results for long-term NO_2 exposure and pneumonia incidence in adults within and across a limited number of studies. Across epidemiologic studies of respiratory infection there has been limited evaluation of potential copollutant confounding. Additionally, the entirety of the recent evidence for pneumonia and LRTI incidence in adults comes from studies examining hospital admissions, which raises uncertainty regarding potential confounding due to short-term NO_2 exposures given that hospital admissions could result from acute exacerbations of prevalent respiratory infections.

4.6 Lung Function and Development

The 2016 Oxides of Nitrogen ISA characterized evidence for the relationship between long-term exposures to NO_2 and lung function and development. Similar to the results for short-term impacts of NO_2 (see Chapter 3, Section 3.6.3), studies that inform long-term effects on lung function predominantly use spirometry to assess the ability of the lung to hold and move air, with measurements including FEV_1, FVC, and MMEF, but evaluate longer exposure durations. Lung function increases from birth to early adulthood and then declines with aging (Stanojevic et al., 2008; Zeman and Bennett, 2006; Thurlbeck, 1982). Thus, multiple spirometry measurements over time can inform lung development trajectory in younger populations or the rate of lung function decline in older populations.

4.6.1 Epidemiologic Studies of Lung Function and Development

In the following subsections, epidemiologic studies of lung development and lung function are evaluated separately. Studies of lung development and age-related decline include cohort studies with repeated measurements of lung function over time that characterize pulmonary growth in children or pulmonary decline in adults associated with long-term NO_2 exposure. Epidemiologic studies of lung function evaluate the effects of long-term NO_2 exposure on attained pulmonary function at a singular point in time. Section 4.6.1.1 discusses evidence for associations between long-term oxides of nitrogen exposure and lung function development and decline, and Section 4.6.1.2 discusses evidence for associations with lung function at a single point in time. A more detailed discussion of copollutant models

is presented in Section 4.6.1.3, and Section 4.6.1.4 discusses conclusions from available studies and summarizes remaining uncertainties.

4.6.1.1 Lung Development and Age-Related Decline

Children

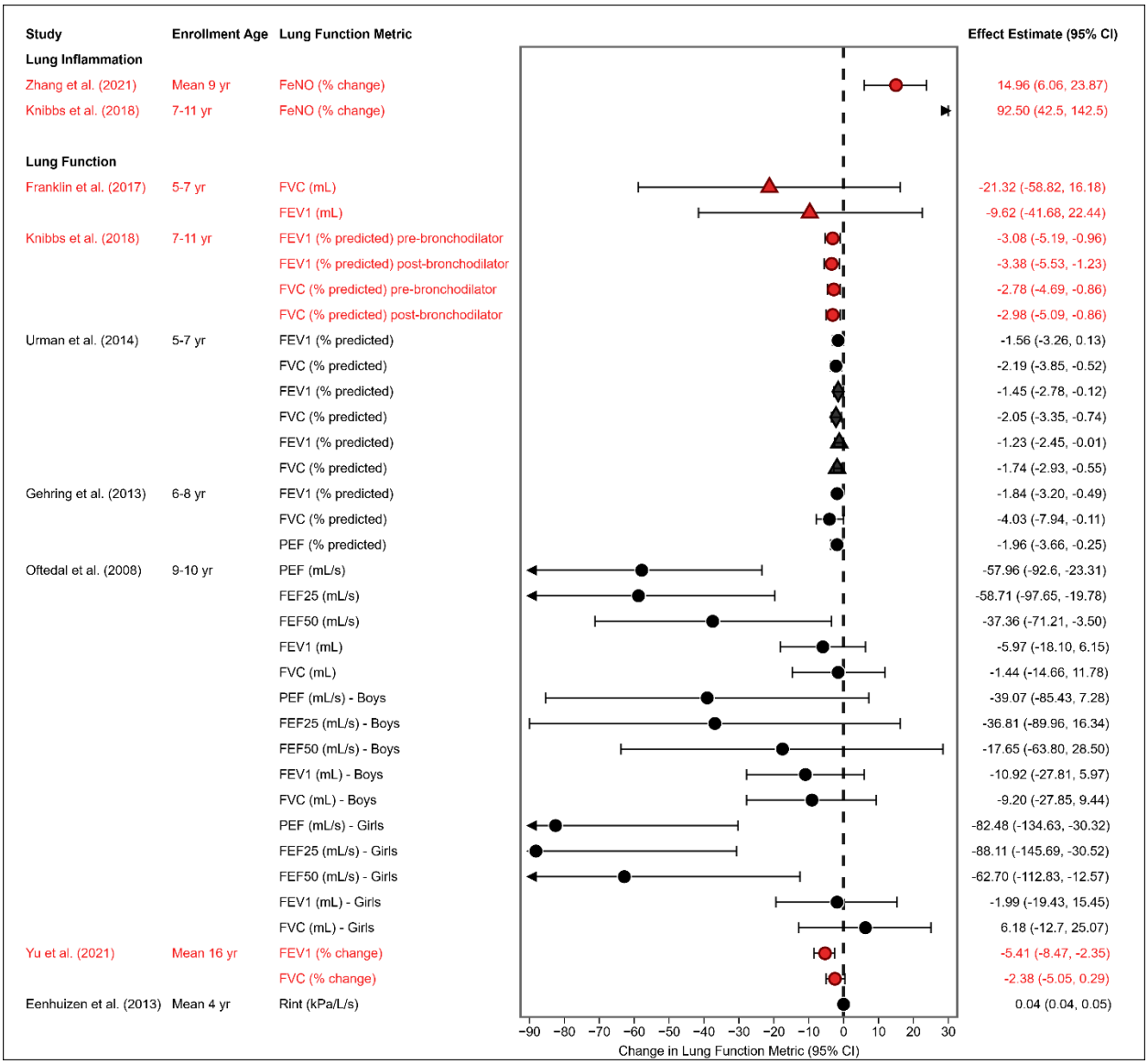
Longitudinal epidemiologic studies evaluated in the 2016 Oxides of Nitrogen ISA provided consistent evidence of associations between long-term NO_2 exposure and decrements in lung development as assessed by supervised spirometry. Strong evidence for NO_2-related decrements in lung development came from the examination of two cohorts of the Children's Health Study in southern California [i.e., the 1993 cohort in Gauderman et al. (2004) and combined analyses of the 1993 and 1996 cohorts in Gauderman et al. (2007) and Breton et al. (2011)]. In each analysis, children with higher long-term NO_2 exposures had deficits in lung development over the 8-year follow-up. Gauderman et al. (2007) observed that associations with NO_2 exposure assessed from central site monitors persisted with adjustment for distance to freeway, a proxy for near-roadway NO_X. However, inference from these copollutant model results is limited because of the potential for differential exposure measurement error. Another longitudinal cohort study in Mexico City, Mexico (Rojas-Martinez et al., 2007a, b) provided supporting evidence for an association between long-term NO_2 exposure and decreased lung function growth in children. Rojas-Martinez et al. (2007b) and Rojas-Martinez et al. (2007a) evaluated lung development in a prospective cohort of children aged 8 years at baseline with follow-up over three years. A 10 ppb increase in NO_2 was associated with an annual deficit in FEV_1 of 27 mL (95% CI: 22, 33) in girls and 22 mL (95% CI: 16, 28) in boys. The negative association for NO_2 persisted in models that accounted more directly for other traffic-related pollutants, including copollutants models adjusted with O_3 or PM_{10} and a model adjusting for short-term NO_2 (previous-day concentrations).

Recent studies contribute to the existing evidence base and provide additional support for a positive association between long-term NO_2 exposure and decrements in lung development in children (see Figure 4-12). Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 4-10. Recent evidence comes from traditional prospective cohort studies using exposure assessment methods that included dispersion models, central site monitors, and fixed-site monitors. Prospective cohort studies follow participants over time which allows for the establishment of temporality, where exposure precedes the development of the outcome. Evidence from the recent studies comes from clinically measured spirometry and biomarkers. Recent evidence also includes studies adjusting for potential confounding by short-term increases in NO_2 or other air pollutants. A short summary of the recent evidence is provided below.

- Several recent longitudinal studies provide generally consistent evidence of an association between long-term NO_X and NO_2 exposure and reductions in lung function development in

school age children and young adults. Studies in the United States (Zhang et al., 2021a; Ierodiakonou et al., 2015), Canada (Ierodiakonou et al., 2015), and Sweden (Yu et al., 2023b; Schultz et al., 2015) provide this evidence in cohorts followed over a 4- to 24-year timeframe. Associations with reductions in lung function development were observed through decreased FEV_1 and FVC, and through increases in fractional exhaled nitric oxide (FeNO). The reported associations were consistent across studies using different exposure assessment metrics.

- Studies providing the strongest inference on developmental impacts include a few studies that examined lung development over a 16- and 24-year period in a Swedish birth cohort (BAMSE) followed through adolescence and young adulthood (Yu et al., 2023b; Schultz et al., 2015). Among 1,931 adolescents (16 years of age) with spirometry measured at eight years and 16 years, Schultz et al. (2015) reported a decrease in FEV_1 associated with a 10 $\mu g/m^3$ increase in NO_X exposure in the first year of life (-12.5 mL [95%CI: -26.4, 1.4]), where the confidence intervals include the null. In stratified analyses, sex was reported to be an important effect modifier, with a stronger association in males, where a 10 $\mu g/m^3$ increase in NO_X was associated with a decrease in FEV_1 (-23.3 mL [95% CI: -44.8, -1.7]). In females, no association was reported between NO_X and FEV_1 (-0.3 mL [95% CI: -17.4, 16.8]). Yu et al. (2023b) examined the association in the BAMSE cohort at 24 years of age. Findings between age eight and 24 years were consistent with observations made at the earlier timepoint (16 years). In 1,104 young adults, a 6.17 $\mu g/m^3$ increase in NO_X was associated with decreased FVC (-3.29 mL/yr [95% CI: -6.23, -0.35]) and FEV_1 decline (-1.70 mL/yr [95% CI: -3.57, 0.16]).
- A multi-city study in North America also examined lung development in a cohort of children with asthma (Ierodiakonou et al., 2015). In 1,003 children with asthma (5 to 12 years old) from seven U.S. states and Canada, a 13-ppb increase in 4-month NO_2 was associated with decreased post bronchodilator FVC (-0.2% predicted [95% CI: -0.04, 0.01]) and FEV_1 (-0.2% predicted [95% CI: -0.4, -0.1]).
- One study in Southern California in the CHS cohort examined a marker of airway inflammation, FeNO, in a cohort of 3,607 school aged children (6 to 17 years of age) followed over seven years (Zhang et al., 2021a). Zhang et al. (2021a) used central site monitors to estimate annual average NO_2 exposure for participants who were assessed up to six times annually. An increase of annual average NO_2 by 10 ppb was associated with an increase in FeNO (14.96% [95%CI: 6.06, 23.87]). This study adjusted for potential confounding by short-term NO_2 exposure.



List of Abbreviations: CI = confidence interval; FeNO = fractional exhaled nitric oxide; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; PEF = peak expiratory flow; FEF_{25} = forced expiratory flow at 25% of forced vital capacity; FEF_{50} = forced expiratory flow at 50% of forced vital capacity; R_{int} = interrupter resistance; % change = percent change; % predicted = percent predicted (sex, age, height); mL = milliliters; mL/s = milliliters per second; kPa/L/s = kilopascal per liter per second; yr = year(s).
Note: Black = study from the 2016 Oxides of Nitrogen ISA; red = recent studies and those not included in 2016 Oxides of Nitrogen ISA. Circles = nitrogen dioxide (NO_2); Diamonds = nitric oxide (NO); Triangles = sum of NO_2 and nitric oxide (NO_X). Effect estimates are represented as a percent increase.

Figure 4-12 Associations of oxides of nitrogen with lung function from longitudinal and cross-sectional studies of children.

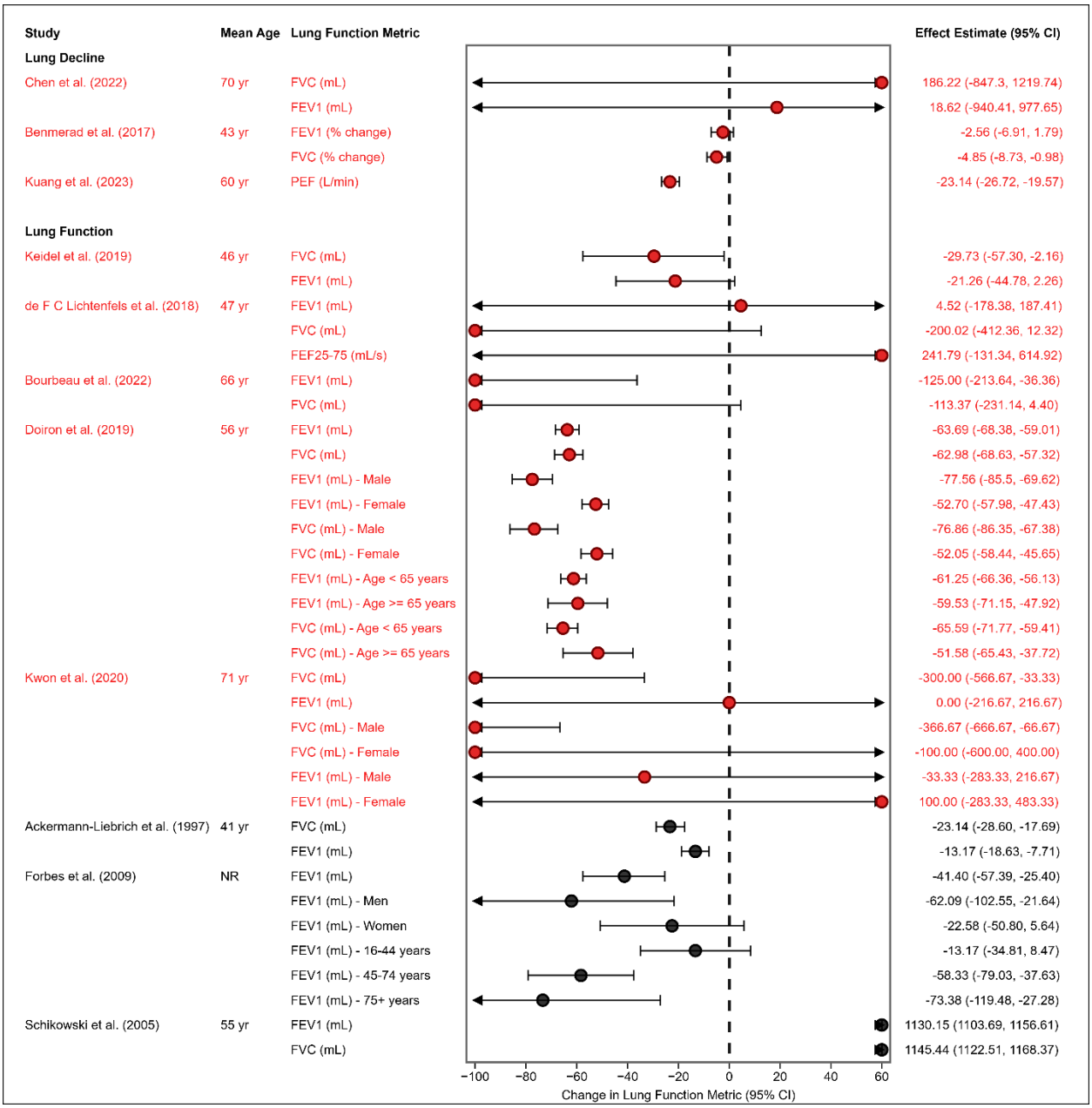
Adults

Evidence presented in the 2016 Oxides of Nitrogen ISA for lung function decline in adults was more limited and inconsistent, compared with studies in children. Götschi et al. (2008) assessed FEV_1 and FVC at baseline and after nine years of follow-up in 5,610 participants from 21 European centers in the European Community Respiratory Health Survey. Long-term NO_2 concentrations were not associated with a change in lung function level over the follow-up period. Similarly, long-term NO_2 exposure was not associated with a decline in FEV_1 over a 9-year follow-up in a Nottingham, U.K., cohort of 1,329 adults aged 18−70 years (Pujades-Rodriguez et al., 2009). Insufficient contrast in NO_2 exposure (IQR: 18.1 to 19.1 ppb) likely reduced the statistical power to detect an association for NO_2 in this study population. In contrast, a longitudinal cohort study of 5,682 females in Tokyo, Japan reported a trend of larger decrements in lung function over an 8-year follow-up in wards with higher long-term average NO_2 concentrations (Sekine et al., 2004). However, the grouping of study areas by NO_2 concentrations also factored in PM_{10} concentrations, precluding any conclusions on the independent effects of either pollutant.

Recent longitudinal studies continue to be limited and report inconsistent evidence of an association between long-term NO_2 exposure and changes in lung function in adults (see Figure 4-13). Recent evidence comes from traditional prospective cohort studies using exposure assessment methods that included a chemical transport model, spatiotemporal model, LUR, and a hybrid model using kriging and LUR. Similar to the studies in children, the evidence in adults includes clinically measured spirometry and biomarkers. In the recent studies, participants mean ages ranged from 43 to 70 years and follow-up periods lasted 2 to 28 years. Recent evidence also includes a more thorough evaluation of potential copollutant confounding. 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 4-10.

- Recent studies in older adults in China (Kuang et al., 2023), a cohort of older adults in Taiwan (Chen et al., 2022a), older women in Germany (Hüls et al., 2019), and in lung transplant recipients in France (Benmerad et al., 2017) provide inconsistent evidence of an association between long-term NO_2 exposure and changes in lung function over time. Differences in observations could be related to sensitivities by population and confounding by traffic-related copollutants.
- One study in the Ruhr region of Germany followed participants over a period of 28 years and reported an association between long-term NO_X and NO_2 exposure and reduced pulmonary function in older women (Hüls et al., 2019). In 601 women (mean age at baseline 55 years) followed over a period of 28 years, a 5.32 ppb increase in 2-year average NO_2 exposure was associated with decreased FEV_1 (-0.14 standard deviation [95% CI: -0.01, -0.26]) and FEV_1/FVC (-0.2 standard deviation [95% CI: -0.06, -0.33]). A 20 $\mu g/m^3$ increase in NO_X exposure was also associated with decreased FEV_1 (-0.08 standard deviation [95% CI: 0.00, -0.16]) and FEV_1/FVC (-0.09 standard deviation [95% CI: 0.00, -0.17]). Effect estimates were robust to adjustment with $PM_{2.5}$ but were attenuated with adjustment for PM_{10}. No association between long-term NO_X and NO_2 exposure and FVC was reported.

- Similar findings were reported in another study of older adults in China followed over a 4-year period. Kuang et al. (2023) reported long-term exposure to NO_2 to be associated with reduced lung function. In 13,614 individuals (mean age 60 years), a 10 ppb increase in annual average NO_2 exposure was associated with decreased PEF (-41.96 L/min [95% CI: -45.63, -38.29]). The reported association was robust to further model adjustments including behavioral and health factors (-40.64 L/min [95% CI: -44.31, -36.97]) and environmental factors (-40.83 L/min [95% CI: -44.78, -36.88]).
- A negative association with lung capacity outcomes was also reported in participants in Taiwan followed over two years. In this cohort of 543 older adults (mean age 70 years), lung function changes were reported in some measures, but not others. Chen et al. (2022a) observed associations between a 10 ppb increase in LUR-estimated annual average NO_2 concentrations and decreases in total lung capacity (TLC) (-3.39 L [95% CI: -5.15, -1.63]), residual volume (RV) (-6.22 L [95% CI: -9.39, -3.04]), the ratio of residual volume to total lung capacity (RV/TLC) (-2.94 [95% CI: -4.89, -1.00]), and diffusion capacity (-2.10 mL/min/mmHg [95% CI: -3.77, -0.44]). These results were robust to adjustment for $PM_{2.5}$. No association was observed between long-term exposure to NO_2 and changes in FEV_1 and FVC in single pollutant models or in models additionally adjusted for $PM_{2.5}$.
- A study in France reported different observations in copollutants models. In a cohort of 520 lung transplant recipients (mean age 43 years) followed over a 4-year period, Benmerad et al. (2017) assessed the association between long-term exposure to NO_2 and changes in lung function following lung transplantation. A 10 ppb increase in annual average NO_2 was associated with a 4.85% decrease in predicted FVC (95% CI: -8.73, -0.98) and a 2.56% decline in predicted FEV_1 (95% CI: -6.91, 1.79). In copollutant models, the observed association between long-term NO_2 and reduced FEV_1 was greater in magnitude, but less precise after adjustment for $PM_{2.5}$ (-7.17% predicted [95% CI: -12.36, -1.98]) or PM_{10} (-5.10% predicted [95% CI: -10.41, 0.21]). The reported associations between long-term exposure to NO_2 and FVC were attenuated to null after adjustment for $PM_{2.5}$ or PM_{10}. Similarly, long-term exposure to NO_2 was not associated with either FEV_1 or FVC in copollutant models with O_3.



List of Abbreviations: CI = confidence interval; FeNO = fractional exhaled nitric oxide; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; PEF = peak expiratory flow; FEF_{25} = forced expiratory flow at 25% of forced vital capacity; FEF_{50} = forced expiratory flow at 50% of forced vital capacity; R_{int} = interrupter resistance; % change = percent change; % predicted = percent predicted (sex, age, height); mL = milliliters; mL/s = milliliters per second; kPa/L/s = kilopascal per liter per second; yr = year(s).
Note: Black = study from the 2016 Oxides of Nitrogen ISA; red = recent studies and those not included in 2016 Oxides of Nitrogen ISA. Circles = nitrogen dioxide (NO_2). Effect estimates are represented as a percent increase.

Figure 4-13 Associations of oxides of nitrogen with lung function from longitudinal and cross-sectional studies of adults.

4.6.1.2 Lung Function

Children

Several epidemiologic studies evaluated in the 2016 Oxides of Nitrogen ISA provided generally consistent evidence of associations between long-term NO_2 exposure and lower lung function measures as assessed by supervised spirometry. While providing evidence of consistency, these cross-sectional studies are limited in addressing temporality as these studies examine the association at a singular timepoint, not allowing for the establishment of exposure coming before observed health outcomes. In an analysis of the 2002 cohort of the CHS, Urman et al. (2014) observed inverse associations between annual average NO_2 concentrations estimated from LUR and FEV_1 or FVC levels that persisted with adjustment for distance to freeway or near-roadway NO_X, and associations with near-roadway NO_X that were robust to adjustment for $PM_{2.5}$. Several European birth cohort studies also reported associations between higher long-term NO_2 or NO_X exposure and lower lung functions measures in children ages 4-8 years (Eenhuizen et al., 2013; Gehring et al., 2013; Schultz et al., 2012). In these studies, short-term exposure may confound the association between long-term exposure and lung function, as short-term exposure can be both correlated with long-term exposure and associated with changes in lung function. Although most studies did not adjust for short-term exposures, a cohort study in Oslo, Norway, examined associations of short- and long-term NO_2 exposures with PEF, $FEF_{25\%}$, and $FEF_{50\%}$ of forced vital capacity in 2,307 children ages nine to 10 years (Oftedal et al., 2008). In models that included both short- and long-term NO_2 concentrations estimated from dispersion models, only the association with long-term NO_2 persisted.

Recent studies contribute to the existing evidence base and provide further support for a positive association between long-term NO_2 exposure and decrements in lung function in children (see Figure 4-12). Evidence from recent cross-sectional studies used exposure assessment methods including central site monitors, dispersion models, LUR models, and a hybrid model that used monitoring station data, LUR, and a dispersion model. The evidence from recent studies comes from clinically measured spirometry and biomarkers. Recent evidence also includes studies adjusting for potential confounding by other traffic-related copollutants and short-term increases in other air pollutants and weather. 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 4-10.

- Recent studies providing evidence of an association between long-term NO_2 and NO_X exposure and decreases in lung function in school age children include an Australian multi-city study and several studies in California (Benka-Coker et al., 2020; Knibbs et al., 2018; Franklin and Fruin, 2017). Studies in Europe reported heterogenous results, noting NO_2 associated declines in some lung function measures, but not others (Yu et al., 2021; Schultz et al., 2015). A single Swiss study in infants provided some evidence of an association with a marker of airway inflammation (Decrue et al., 2022). Several studies reported the potential for modification by BMI (Zhao et al., 2021; LISS, 2020) and family socioeconomic status (Cakmak et al., 2016), though the reported modification by BMI was inconsistent.

- A large multi-city study in Australian children (Australian Child Health and Air Pollution Study) reported an association between long-term NO_2 and decreased lung function and a marker of airway inflammatory response (Knibbs et al., 2018). In 2,630 school aged children (mean age 10 years), a 10 ppb increase in annual average NO_2, estimated from nearest central monitor in the year prior, was associated with decreased FEV_1 (-3.38% predicted [95% CI: -5.53, -1.23]), decreased FVC (-2.98% predicted [95% CI: -5.09, -0.86]), and increased FeNO (92.50% [95% CI: 45.50, 142.50]). Similar observations were reported for estimated lifetime NO_2 exposure and other considerations of prior exposure. Annual NO_2 exposure was also evaluated using monthly estimates in the year prior using LUR. Only a significant increase in FeNO was reported when using exposure estimates from the LUR model.
- Consistent with results from a prior study in the United States, a few recent studies reported an inverse association with lung function. In a study in Fresno, California of 153 school age children (mean age = 9 years), a 3.3-ppb increase in a 3-month NO_2 exposure was associated with decreased FEV_1 (-0.31 L [95% CI: -0.55, -0.07]) and FVC (-0.35 L [95% CI: -0.59, -0.11]) (Benka-Coker et al., 2020). The 3-month exposure was reported as the average of daily maximum 1-hour NO_2 concentrations from the nearest monitoring station. The reported associations were stronger in magnitude in multipollutant models that simultaneously adjusted for O_3, $PM_{2.5}$, PM_{10}, carbamates, methyl bromide, and organophosphates. Similar results were also reported in a recent study of children in the southern California CHS. Among 1,397 children (14-17 years of age), annual average NO_X exposure estimated using a dispersion model was reported to be associated with decreased FVC and FEV_1 (Franklin and Fruin, 2017). A 20 ppb increase in annual NO_X was associated with a trend toward decreased FVC (-21.32 mL [95% CI: -58.82, 16.18]) and FEV_1 (-9.62 mL [95% CI: -41.68, 22.44]) in a single pollutant models, and a decrease in FVC (-50.88 mL [95% CI: -97.59, -4.18]) and trend of a decrease in FEV_1 (-31.03 mL [95% CI: -70.08, 8.02]) in a models adjusted for noise with a spline.
- Recent studies in Europe also reported negative associations with lung function measures in adolescents. In a Swedish birth cohort study (BAMSE; Children, Allergy, Environment, Stockholm, Epidemiology) of 2,278 adolescents (16 years of age), Schultz et al. (2015) reported an association between a 10 $\mu g/m^3$ increase in NO_X exposure in the first year and decreased FEV_1 (-15.80 mL [95% CI: -33.60, 2.00]), though the confidence interval overlaps the null. In an analysis stratified by sex, there was a stronger association in males, where a 10 $\mu g/m^3$ increase in NO_X concentrations was associated with decreased FEV_1 (-30.40 mL [95% CI: -59.10, -1.70]). In females, the association was null (0.90 mL [95% CI: -20.30, 22.10]). A study of 706 adolescents (mean age 16 years) in the PIAMA birth cohort in the Netherlands, also reported NO_2 exposure to be associated with decreased FEV_1 and FVC (Yu et al., 2021). A 10 ppb increase in NO_2 was associated with a 5.41% decrease in FEV_1 (95% CI: -8.47, -2.35). This association was attenuated slightly after adjustment for UFP (-4.19% difference [95% CI: -8.33, -0.05]). A decrease in FVC was also observed in the single pollutant model; however, this observation was not robust to adjustment with UFP. Additionally, a study of 241 school age children (mean age = 6 years) in the Basel-Bern Infant Lung Development in Switzerland, also reported long-term NO_2 exposure to be associated with decreased FEV_1 (Usemann et al., 2019). A 10 ppb increase in NO_2 exposure between birth and six years of age was associated with decreased FEV_1 (-37.70 mL [95% CI: -72.30, -3.10]).

The influence of modifiers, including weight grouping and socioeconomic status, on the association between long-term NO_2 exposure and lung function was also examined in several studies.

- In a recent study in Germany in 2,224 adolescents (mean age 15 years), a 10 ppb increase in annual average NO_2 was associated with decreased FEV_1 in the underweight (-11.02% [95%

CI: -21.29, -0.76]) and overweight/obese (-15.21% [95% CI: -24.90, -5.51]) groups (Zhao et al., 2021). The same change in NO_2 was also associated with decreased FVC in the underweight (-11.02% [95% CI: -20.15, -1.90]) and overweight/obese (-13.30% [95% CI: -22.43, -4.18]) groups. Additionally, PEF was reported to be decreased in the underweight group (-19.77% change [95% CI: -31.17, -8.36]). Weight categories were created from BMI percentiles, where underweight included individuals with a BMI <10th percentile, normal weight included individuals with a BMI between the 10th and 90th percentile, and the overweight or obese group included individuals with a BMI >90th percentile.

- A multi-city study in China reported a different association with weight groups based on BMI (LISS, 2020). In 6,740 school age children (seven to 14 years of age), a 7.3 ppb increase in 4-year average NO_2 concentrations was associated with reduced PEF (-65.56 mL/s [95% CI: -114.80, -16.32]) and MMEF (-40.22 mL/s [95% CI: -77.27, -3.18]) in the normal weight group. In the overweight and obese groups, no associations between long-term NO_2 and FVC, FEV_1, PEF, or MMEF were reported.
- A single study in the Children's Health Study cohort examined the modifying effect of SES (household income) on the association between NO_2 and lung function (Cakmak et al., 2016). In 3,591 children nine to 11 years old in Windsor, Canada, in the lowest income group (< $35,000 CAD), a 10 ppb increase in annual average NO_2 was associated with a decline in FEV_1 (-8.55% predicted [95% CI: -15.75, -1.34]) and a decline in FVC (-4.98% predicted [95% CI: -10.07, 0.11]). Associations were positive for the middle-income group ($35,000 to $80,000 CAD, 4.14% predicted [95% CI: -1.48, 9.76]) and null for the highest income group (>$80,000 CAD, -0.84% predicted [95% CI: -3.39, 1.72]).
- A single study in infants reported NO_2-associated changes in a marker of airway inflammatory response that was modified by birth status (Decrue et al., 2022). In this Swiss study of 771 infants, a 5.32 ppb increase in NO_2 in the second trimester was associated with increased FeNO in preterm infants (1.5 ppb [95% CI: -0.2, 3.1], <32 weeks).

Adults

A limited number of studies evaluated in the 2016 Oxides of Nitrogen ISA provided generally consistent evidence of associations between long-term NO_2 exposure and lung function in adults. Annual average NO_2 concentrations were inversely associated with FEV_1 and FVC levels in a cross-sectional analysis of healthy never-smokers in Switzerland (Ackermann-Liebrich et al., 1997); FEV_1 levels in a population-based cross-sectional survey of adults in England (Forbes et al., 2009); and FEV_1, FVC, and FEV_1/FVC a cross-sectional analysis of women living in the Rhine-Ruhr Basin of Germany (Schikowski et al., 2005). Conversely, long-term NO_2 exposure was not associated with FEV_1 levels in a cross-sectional analysis of 2,599 adults aged 18 to 70 years in Nottingham, U.K. (Pujades-Rodriguez et al., 2009).

Recent studies add to the existing evidence base and provide additional support for a negative association between long-term NO_2 exposure and lung function in adults (see Figure 4-13). Recent evidence comes from cross-sectional studies that use LUR models to characterize exposure and clinically measured spirometry and biomarkers. 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 4-10.

- Recent multi-city and national studies in Europe and Canada provide evidence of an association between long-term exposure to NO_2 and declines in multiple spirometry measures (Bourbeau et al., 2022; Doiron et al., 2019; Keidel et al., 2019). Another study in Germany provided evidence of an association with declines in lung function at an early time-point when NO_X and NO_2 exposures were higher (Hüls et al., 2019). Results from studies in the Netherlands and South Korea reported more heterogeneous results, noting NO_2-associated declines in one lung function measure but not others (Kwon et al., 2020; de F C Lichtenfels et al., 2018).
- Cross-sectional studies continue to report associations between long-term NO_2 exposure and reduced lung function. In a large study in the U.K. of 303,887 adults (mean age = 56 years), a 10 ppb increase in annual average NO_2 concentrations was associated with decreased FEV_1 (-63.69 mL [95% CI: (-68.36, -59.01]), FVC (-62.98 mL [95% CI: -68.63, -57.32]), and FEV_1/FVC (-4.27 [95% CI: -4.85, -3.70]) (Doiron et al., 2019). In subgroup analyses that reported on modification by suggested vulnerability factors (i.e. sex, age, BMI, and income), greater declines in lung function were observed in males, some age groups, obese individuals, and individuals with lower income. Individuals with an income less than GBP 31,000 had a larger decrease in all three lung function variables (FEV_1, FVC, FEV_1/FVC), males were reported to have larger decreases in FEV_1 and FVC, individuals <65 years of age had a larger decrease in FEV_1, individuals 65 years of age and older has a larger decrease in FEV_1/FVC, and obese individuals had a larger decrease in FEV_1.
- A multi-country study in Europe also reported long-term NO_2 exposure to be associated with reduced lung function (Keidel et al., 2019). In 6,502 adults (mean age 46 years), a 10 ppb increase in annual average NO_2 concentrations was associated with decreased FVC (-29.73 mL [95% CI: -57.30, -2.16]) and reduced FEV_1 (-21.26 mL [95% CI: -44.78, 2.26]). In models additionally adjusted for education and occupation, associations with long-term NO_2 exposure were slightly larger, indicating that socioeconomic factors may confound the association between long-term NO_2 and lung function.
- A Canadian study in 1,452 adults (mean age = 66 yrs) reported lower FEV_1, FVC, FEV_1/FVC associated with long-term NO_2 exposure (Bourbeau et al., 2022). A 10 ppb increase in annual average NO_2 was associated with decreased FEV_1 (-125.00 mL [95% CI: -213.64, -36.36]). Decreases, where the confidence interval overlapped the null, in FVC (-113.37 mL [95% CI: -231.14, 4.40]) and FEV_1/FVC (-1.20 [95% CI: -2.72, 0.33]) were also reported.
- A study in Germany examined associations cross-sectionally at two time points, at baseline and 25 to 28 years later when concentrations of NO_X and NO_2 in ambient air were lower, and reported associations that varied between time periods (Hüls et al., 2019). In 601 women (mean age at baseline 55 years), a negative association was observed with lung function measures at baseline, where a 10 $\mu g/m^3$ increase in 2-year average NO_2 concentrations was reported to be associated with decreased FEV_1 (-0.16 standard deviation [95% CI: -0.24, -0.09]), FVC (-0.11 standard deviation [95% CI: -0.17, -0.04]), and FEV_1/FVC (-0.09 standard deviation [95% CI: -0.16, -0.03]). A 20 $\mu g/m^3$ increase in 2-year average NO_X was reported to be associated with decreased FEV_1 (-0.09 standard deviation [95% CI: -0.15, -0.04]), FVC (-0.06 standard deviation [95% CI: -0.11, -0.02]), and FEV_1/FVC (-0.05 standard deviation [95% CI: -0.09, 0.00]). No associations were reported in analyses conducted 25 to 28 years later when NO_X and NO_2 concentrations in ambient air were lower.
- Inconsistent evidence between lung function measures was also observed. In a study in the Netherlands of 1,017 adults (mean age = 47 years), a 10-ppb increase in annual NO_2 was associated with a decline in FVC (-200.02 mL [95% CI: -412.35, 12.32]). No association was reported with FEV_1 (4.52 mL [95% CI: -178.38, 187.41]). A South Korean study of 457

adults (mean age = 71 years) also reported inconsistent associations between long-term NO_2 exposure and some lung function measures. A 10 ppb increase in annual NO_2 was associated with a decrease in FVC (-0.30 L [95% CI: -0.57, -0.03]), but no association was observed with FEV_1 (0.00 L [95% CI: -0.22, 0.22]) and no differences were observed in models stratified by sex (Kwon et al., 2020).

4.6.1.3 Copollutants

Determining the independent association between long-term NO_2 exposures and lung function can be challenging due to the high correlation between NO_2 and other copollutants. Other factors that may confound the association include short-term exposure to copollutants and changes in weather, particularly temperature and relative humidity. In the 2016 Oxides of Nitrogen ISA, a few studies of NO_2 and lung development and lung function in children examined copollutant models. No prior studies in adults examined this potential source of confounding. Other potential confounders to the relationship between long-term NO_2 exposure and lung function include short-term exposure to traffic pollutants, oxides of nitrogen, and weather conditions as both impact lung function and may influence long-term NO_2 exposure. In the 2016 Oxides of Nitrogen ISA, no studies examined models adjusted for short-term exposures.

Several recent studies examined potential confounding by copollutants and provide initial evidence that the associations between long-term NO_2 exposure and lung function in children persist in models adjusting for PM or noise. The evidence for confounding in adults was inconsistent (see Table 4-10).

- A cross-sectional study of 1,397 children in California reported negative associations between NO_X and FEV_1 and FVC that were robust to copollutant adjustments in multipollutant models (Benka-Coker et al., 2020). In single pollutant models, a 3.3-ppb increase in a 3-month average NO_2 was associated with decreased FEV_1 (-0.31 L [95% CI: -0.55, -0.07]) and FVC (-0.35 L [95% CI: -0.59, -0.11]). There were similar observations in multipollutant models, where greater declines in FEV_1 and FVC were reported. In models adjusted for multipollutant exposures including, $PM_{2.5}$, PM_{10}, O_3, and multiple pesticides, the negative associations with FEV_1 (-0.74 L [95% CI: -1.24, -0.24]) and FVC (-0.81 L [95% CI: -1.30, -0.32]) were reported to be strengthened.
- Another cross-sectional study of 706 adolescents in the Netherlands also reported negative associations between NO_2 and FEV_1 and FVC (Yu et al., 2021). The observed association between 10 ppb increase in annual average NO_2 and FEV_1 was slightly attenuated, from a -5.41% difference (95% CI: -8.47, -2.35) to a -4.19% difference (95% CI: -8.33, -0.05), but remained negative after UFP adjustment in a copollutant model. The negative association with FVC was not robust to adjustment with UFP.
- In a single study, noise was also reported to be an important confounder when examining the association between long-term NO_X exposure and lung function (Franklin and Fruin, 2017). In a cross-sectional study of 1,397 children in Southern California, a 20 ppb increase in annual average NO_X was associated with a trend toward decreased FVC (-21.32 mL [95% CI:

-58.82, 16.18]) in single pollutant models. In a copollutant model adjusted for noise with a spline, the reported association was strengthened (-50.88 mL [95% CI: -97.59, -4.18]). Greater NO_X associated declines in FVC were reported with other considerations of noise (i.e. linear, cubic, and categorical); however, these estimates had confidence intervals that contained the null. Similarly, a trend toward a negative association with FEV_1 was reported in a single pollutant model (-9.62 mL [95% CI: -41.68, 22.44]) and strengthened in the model adjusted for noise with a spline (-31.03 mL [95% CI: -70.08, 8.02]).

Additionally, recent studies report on associations between long-term NO_2 exposures and lung function while controlling for short-term confounders. The choice and number of confounders have not been uniform across studies, making comparisons difficult. The evidence for short-term confounding in adults was limited and inconsistent, while the recent evidence suggests that the association between long-term NO_2 exposure and reduced lung function in children is robust after adjusting for these variables (see Table 4-10).

- In a longitudinal study of 3,607 school-aged children (mean age nine years at baseline) followed over seven years in Southern California, an increase of annual average NO_2 concentrations by 10 ppb was associated with a 14.96% increase in FeNO (95% CI: 6.06, 23.87) in the minimally adjusted model (Zhang et al., 2021a). This association was strengthened in the model that adjusted for smoking, 7-day average temperature, 7-day average NO_2 (23.93% [95% CI: 15.00, 32.85]).
- Negative associations between long-term NO_2 exposure and reduced lung function were also reported in the models adjusted for potential confounders in cross-sectional studies. In studies of 706 to 2,224 adolescents, negative associations with FEV_1, FVC, and PEF were reported in models adjusted for short-term air pollution (Yu et al., 2021; Zhao et al., 2021). In studies of 153 to 6,740 school age children, negative associations with FEV_1, FVC, and PEF were reported in models adjusted for combinations of temperature and precipitation (Benka-Coker et al., 2020; LISS, 2020; Knibbs et al., 2018).

Table 4-10 Longitudinal and cross-sectional studies of long-term exposure to oxides of nitrogen and lung function in children and adults

Study Reference	Cohort	Copollutant	Correlation value	Modeling Method	Endpoint	Effect Estimate (95% CI)[a] and Direction of Association[b]	
Children in the General Population - Lung Development							
NO_2							
Rojas-Martinez et al. (2007a) Enrollment: 1996 Follow-up: 1996–1999		-	NR	Mutually adjusted two or three pollutant models	FVC – Girls[c]		Main: -48.00 (-55.00, -41.00)
		O_3				≈	Copollutant: -45.00 (-53.00, -37.00)
		PM_{10}				↓	Copollutant: -41.00 (-49.00, -33.00)
		O_3, PM_{10}				↓	Copollutant: -40.00 (-48.00, -32.00)
		-			FVC – Boys[c]		Main: -45.00 (-53.00, -37.00)
		O_3				≈	Copollutant: -44.00 (-53.00, -36.00)
		PM_{10}				≈	Copollutant: -41.00 (-49.00, -32.00)
		O_3, PM_{10}				≈	Copollutant: -41.00 (-50.00, -32.00)
		-			FEV_1 – Girls[c]		Main: -32.00 (-39.00, -26.00)
		O_3				≈	Copollutant: -33.00 (-40.00, -26.00)
		PM_{10}				↓	Copollutant: -27.00 (-34.00, -20.00)
		O_3, PM_{10}				≈	Copollutant: -30.00 (-37.00, -22.00)
		-			FEV_1 – Boys[c]		Main: -26.00 (-33.00, -19.00)
		O_3				≈	Copollutant: -30.00 (-37.00, -23.00)

Study Reference	Cohort	Copollutant	Correlation value	Modeling Method	Endpoint	Effect Estimate (95% CI)[a] and Direction of Association[b]	
		PM_{10}				≈	Copollutant: -21.00 (-28.00, -13.00)
		O_3, PM_{10}				≈	Copollutant: -25.00 (-33.00, -18.00)
Children in the General Population - Lung Function							
NO_2							
Gehring et al. (2013)	Manchester Asthma and Allergy Study (MAAS), Children, Allergy, Milieu, Stockholm, Epidemiological Survey (BAMSE), European Study of Cohorts for Air Pollution Effects (ESCAPE), German Infant Study on the influence of Nutrition Intervention plus environmental and genetic influences on allergy (GINIplus), Lifestyle-related factors on the Immune System and the development of Allergies in childhood (LISAplus) study 2000–2007	-		Mutually adjusted two pollutant models	FEV_1		Main: -1.84 (-3.20, -0.49)
		$PM_{2.5}$	0.71			↓	Copollutant: -0.49 (-2.35, 1.37)
		-			FVC		Main: -4.03 (-7.94, -0.11)
		$PM_{2.5}$	0.71			≈	Copollutant: -2.69 (-5.2, -0.18)
		-			PEF		Main: -1.96 (-3.66, -0.25)
		$PM_{2.5}$	0.71			≈	Copollutant: -1.07 (-3.75, 1.61)
Urman et al. (2014)	Children's Health Study (CHS) 2007–2008	-	NR	Mutually adjusted two pollutant models	FEV_1		Main: -1.56 (-3.26, 0.13)
		NO_x				≈	Copollutant: -1.67 (-3.37, 0.02)
		-			FVC		Main: -2.19 (-3.85, -0.52)
		NO_x				≈	Copollutant: -2.28 (-3.95, -0.62)

Study Reference	Cohort	Copollutant	Correlation value	Modeling Method	Endpoint	Effect Estimate (95% CI)[a] and Direction of Association[b]	
†Benka-Coker et al. (2020)	Fresno Asthmatic Children's Environment Study (FACES) 2000–2008	-	-	Mutually adjusted multipollutant models	FEV_1[c]		Main: -0.31 (-0.55, -0.07)
		O_3, $PM_{2.5}$, PM_{10}, C, MeBr, OP	-0.63, 0.88, 0.72, 0.08, 0.07, -0.53			↑	Copollutant: -0.74 (-1.24, -0.24)
		-	-		FVC[c]		Main: -0.35 (-0.59, -0.11)
		O_3, $PM_{2.5}$, PM_{10}, C, MeBr, OP	-0.63, 0.88, 0.72, 0.08, 0.07, -0.53			↑	Copollutant: -0.81 (-1.30, -0.32)
		-	-		FEV_{25-75}[c]		Main: -0.02 (-0.28, 0.24)
		O_3, $PM_{2.5}$, PM_{10}, C, MeBr, OP	-0.63, 0.88, 0.72, 0.08, 0.07, -0.53			≈	Copollutant: -0.10 (-0.64, 0.45)
†Yu et al. (2021)	Prevention and Incidence of Asthma and Mite Allergy (PIAMA) Birth Cohort Enrollment: 1996–1997 Follow-up: At 16 yrs of age	-	NR	Mutually adjusted two pollutant models	FEV_1		Main: -5.41 (-8.47, -2.35)
		UFP				≈	Copollutant: -4.19 (-8.33, -0.05)
		-			FVC		Main: -2.38 (-5.05, 0.29)
		UFP				↓	Copollutant: -0.23 (-3.86, 3.39)
NO_x							
†Franklin and Fruin (2017)	Southern California Children's Health Study Enrollment: 2002–2003 Follow-up: 2011–2012	-		Mutually adjusted two pollutant models	FVC		Main: -21.32 (-58.82, 16.18)
		Noise, linear	0.53			↑	Copollutant: -40.74 (-83.53, 2.06)
		Noise, cubic	0.53			↑	Copollutant: -42.94 (-88.11, 2.23)
		Noise, categories	0.53			↑	Copollutant: -43.68 (-89.40, 2.05)
		Noise, splines	0.53			↑	Copollutant: -50.88 (-97.59, -4.18)
		-			FEV_1		Main: -9.62 (-41.68, 22.44)

Study Reference	Cohort	Copollutant	Correlation value	Modeling Method	Endpoint	Effect Estimate (95% CI)[a] and Direction of Association[b]	
		Noise, linear	0.53			↑	Copollutant: -25.15 (-61.33, 11.04)
		Noise, cubic	0.53			↑	Copollutant: -26.18 (-64.10, 11.75)
		Noise, categories	0.53			↑	Copollutant: -29.85 (-68.16, 8.46)
		Noise, splines	0.53			↑	Copollutant: -31.03 (-70.08, 8.02)
Adults in the General Population - Lung Decline							
NO_2							
†Benmerad et al. (2017)	Cohort in Lung Transplantation (COLT), Systems prediction of Chronic Lung Allograft Dysfunction (SysCLAD) Enrollment: 2009–2013 Follow-up: 6 months or more after lung transplant	-	NR	Mutually adjusted two pollutant models	FEV_1		Main: -2.56 (-6.91, 1.79)
		$PM_{2.5}$				↑	Copollutant: -7.17 (-12.36, -1.98)
		PM_{10}				≈	Copollutant: -5.10 (-10.41, 0.21)
		O_3				↓	Copollutant: 3.78 (-1.83, 9.39)
		-			FVC		Main: -4.85 (-8.73, -0.98)
		$PM_{2.5}$				≈	Copollutant: -3.33 (-7.94, 1.28)
		PM_{10}				↓	Copollutant: -0.83 (-5.51, 3.86)
		O_3				≈	Copollutant: -3.69 (-8.56, 1.19)
†Hüls et al. (2019)	Study on the influence of Air pollution on Lung function, Inflammation and Ageing (SALIA) Enrollment: 1985–1994 Follow-up: 2007–2010 and 2012–2013	-	NR	Mutually adjusted two pollutant models	FEV_1 [c]		Main: 0.14 (0.01, 0.26)
		$PM_{2.5}$				≈	Copollutant: 0.19 (0.05, 0.33)
		PM_{10}				≈	Copollutant: 0.12 (-0.02, 0.26)
		-			FVC [c]		Main: 0.00 (-0.12, 0.12)
		$PM_{2.5}$				≈	Copollutant: 0.02 (-0.11, 0.15)

Study Reference	Cohort	Copollutant	Correlation value	Modeling Method	Endpoint	Effect Estimate (95% CI)[a] and Direction of Association[b]	
		PM_{10}				≈	Copollutant: 0.03 (-0.11, 0.16)
†Chen et al. (2022a)	Enrollment: 2016–2018 Follow-up: 2016–2018	-		Mutually adjusted two pollutant models	FVC		Main: 0.19 (-0.85, 1.22)
		$PM_{2.5}$	0.45			≈	Copollutant: -0.17 (-1.34, 1.01)
		-			FEV_1		Main: 0.02 (-0.94, 0.98)
		$PM_{2.5}$	0.45			≈	Copollutant: 0.02 (-1.08, 1.12)
		-			TLC		Main: -3.39 (-5.15, -1.63)
		$PM_{2.5}$	0.45			≈	Copollutant: -3.15 (-5.48, -0.81)
		-			RV		Main: -6.22 (-9.39, -3.04)
		$PM_{2.5}$	0.45			≈	Copollutant: -6.18 (-10.35, -2.01)
		-			DIco		Main: -2.10 (-3.77, -0.44)
		$PM_{2.5}$	0.45			≈	Copollutant: -2.38 (-4.30, -0.47)
NO_x							
†Hüls et al. (2019)	Study on the influence of Air pollution on Lung function, Inflammation and Ageing (SALIA) Enrollment: 1985–1994 Follow-up: 2007–2010 and 2012–2013	-	NR	Mutually adjusted two pollutant models	FEV_1 [c]		Main: 0.14 (0.01, 0.26)
		$PM_{2.5}$				≈	Copollutant: 0.19 (0.05, 0.33)
		PM_{10}				≈	Copollutant: 0.12 (-0.02, 0.26)
		-			FVC [c]		Main: 0.00 (-0.12, 0.12)
		$PM_{2.5}$				≈	Copollutant: 0.02 (-0.11, 0.15)
		PM_{10}				≈	Copollutant: 0.03 (-0.11, 0.16)
Adults in the General Population - Lung Function							

Study Reference	Cohort	Copollutant	Correlation value	Modeling Method	Endpoint	Effect Estimate (95% CI)[a] and Direction of Association[b]	
NO_2							
†Chen et al. (2022a)	Enrollment: 2016–2018 Follow-up: 2016–2018	-		Mutually adjusted two pollutant models	FVC		Main: 6.95 (2.46, 11.43)
		$PM_{2.5}$	0.45			↓	Copollutant: 1.27 (-5.97, 8.50)
		-			FEV_1		Main: 4.66 (0.47, 8.84)
		$PM_{2.5}$	0.45			↓	Copollutant: -1.27 (-7.91, 5.38)
		-			TLC		Main: -0.17 (-7.34, 7.00)
		$PM_{2.5}$	0.45			≈	Copollutant: 3.58 (-7.95, 15.10)
		-			RV		Main: -4.04 (-16.13, 8.04)
		$PM_{2.5}$	0.45			≈	Copollutant: 8.06 (-12.50, 28.63)
		-			DIco		Main: 6.29 (-0.68, 13.27)
		$PM_{2.5}$	0.45			≈	Copollutant: 2.94 (-8.47, 14.36)

List of abbreviations in alphabetical order: BAMSE = Children, Allergy, Milieu, Stockholm, Epidemiological Survey; C = carbamates; CI = confidence interval; CHS = Children's Health Study; DIco = diffusing capacity of the lung for carbon monoxide; ESCAPE = European Study of Cohorts for Air Pollution Effects; FACES = Fresno Asthmatic Children's Environment Study; FEV1 = forced expiratory volume in 1 second; FEV25-75 = forced expiratory flow at 25-75% of pulmonary volume; FVC = forced vital capacity; GINIplus = German Infant Study on the influence of Nutrition Intervention plus environmental and genetic influences on allergy; LISAplus = Lifestyle-related factors on the Immune System and the development of Allergies in childhood study; MeBr = methyl bromide; NO_2 = nitrogen dioxide; NO_X = nitrogen oxides; NO = nitric oxide; NR = not reported; O_3 = ozone; OP = organophosphate; PIAMA = Prevention and Incidence of Asthma and Mite Allergy Birth Cohort; $PM_{2.5}$ = particulate matter with a diameter less than 2.5 micrometers; PM_{10} = particulate matter with a diameter less than 10 micrometers; RV = residual volume; SALIA = Study on the influence of Air pollution on Lung function, Inflammation and Ageing; TLC = total lung capacity; UFP = ultrafine particles.

[a]Effect estimates are standardized and are presented as a 10 ppb change in NO_2 for annual average averaging time; 15 ppb change in NO for annual average averaging time; and 20 ppb change in NO_X for annual average averaging time.

[b]Up facing arrow (and yellow shading) indicate that the effect of NO_2 is greater (e.g., larger risk) with the copollutant evaluated than in the single pollutant model. Down facing arrow (and blue shading) indicate that the effect of NO_2 is smaller with the copollutant evaluated than in the single pollutant model. An almost equal sign (and grey shading) indicates that the effect of NO_2 is comparable between the single pollutant analysis and copollutant analysis.

[c]Estimates are not standardized.

†Studies published since the 2016 Oxides of Nitrogen ISA.

4.6.1.4 Conclusions and Remaining Uncertainties

Recent longitudinal studies continue to provide consistent evidence of an association between long-term NO_2 exposure and decrements in lung development in school-age children. Inverse associations between long-term NO_2 exposure and spirometry measures and a marker of airway inflammation were also reported in studies accounting for potential confounding by short-term exposure. Examination of the potential for confounding by other copollutants and trends in weather was limited and these remain areas of uncertainty. In adult populations, recent studies continue to report inconsistent evidence of an association between long-term NO_2 exposure and changes in lung function. Studies in adults that examined the potential for confounding by copollutants and short-term NO_2 exposure continued to report an inconsistent relationship. The potential for confounding of the independent effect of NO_2 by weather trends remains.

Recent cross-sectional studies also reported consistent evidence of decreased lung function associated with long-term NO_2 in school-age children. Evidence for this relationship was observed in studies adjusting for potential confounding by traffic-related pollutants, noise, pesticides, and weather. A single study also provided evidence for a potential increase in an airway inflammatory marker in infants. A few studies explored potential modification of the association by BMI, but the results were inconsistent. In adult populations, recent studies provide evidence of an association between long-term NO_2 and a decline in lung function. The number of studies examining potential confounding by copollutants, weather trends, and short-term exposures was reported in a limited number of studies, and these remain areas of uncertainty.

4.6.2 Animal Toxicological Studies of Lung Function and Development

No recent animal studies have examined repeated lung function measurements after long-term exposure to NO_2, thus the evidence evaluated in this ISA remains the same as that evaluated in the 2008 and 2016 Oxides of Nitrogen ISAs. In general, the evidence base is limited in number and the results are inconsistent. Rats exposed to 2,900 ppb NO_2 continuously for five days a week for nine months showed decreased lung compliance and a reduced lung volume compared to air exposed rats (Arner and Rhoades, 1973). Exposure of rats to a daily paradigm of 500 ppb NO_2 for 16 hours, with intermittent 6-hour peaks of 1,500 ppb NO_2 and two hours without NO_2 exposure, showed decreased frequency of breathing after 78 weeks which was paralleled by nonsignificant increases in tidal volume, expiratory resistance, inspiratory time, and expiratory time, although none of these reached statistical significance (Tepper et al., 1993). Other studies in mice and rats did not find altered lung function or lung volumes with NO_2 exposures up to 52 weeks (Mercer et al., 1995; Miller et al., 1987). Stevens et al. (1988) exposed 1-day and 7-week-old rats to 500, 1,000, and 2,000 ppb NO_2 continuously with two daily peaks at three times

the baseline concentration (1,500, 3,000, and 6,000 ppb) for one to seven weeks and observed different results among age groups. Rats exposed from one day of age had increased lung compliance after three weeks of exposure that returned to control levels by six weeks (1,000 ppb with 3,000 ppb peaks). In rats exposed from seven weeks of age, compliance was decreased after six weeks of exposure at 1,000 and 2,000 ppb NO_2. In an 8-week study, Lafuma et al. (1987) reported increased lung volumes in animals exposed to 2,000 ppb (8 hours/day, 5 days/week), but vital capacity and compliance were unaffected. Study specific details, exposure conditions, and endpoints examined are described in more detail in Appendix A – Appendix Table 4-11.

4.6.2.1 Conclusions and Remaining Uncertainties

The evidence base for long-term NO_2 exposure and impacts on lung function remains limited as far as the number of studies available, but the existing animal toxicological studies do not suggest robust impacts on lung function from long-term NO_2 exposures. The majority of the data come from studies that use a baseline exposure to a lower NO_2 concentration with intermittent peaks of higher NO_2 exposures.

4.6.3 Synthesis Across Lines of Evidence

Recent longitudinal studies continue to indicate an association between long-term NO_2 exposure and reductions in lung function and lung development in school-aged children. Similar associations were also observed in recent cross-sectional studies (Decrue et al., 2022; Yu et al., 2021; Benka-Coker et al., 2020; Knibbs et al., 2018; Franklin and Fruin, 2017; Schultz et al., 2015). Strong evidence that is consistent with the existing evidence base comes from longitudinal studies with extensive follow-up periods (i.e., up to 24 years) and a multi-city study in North America. The evidence base from epidemiologic studies in adults remains limited and inconsistent. In children, much of the evidence is for associations between long-term NO_2 exposure and FEV_1, which reflects the mechanical properties of the airways and airway obstruction, and for FVC, which represents lung volume. NO_2-associated declines in lung function were observed in populations in multiple geographic locations, studies using different exposure characterization methods, and studies adjusting for short-term NO_2 exposures.

Uncertainty remains about whether long-term NO_2 exposure has an independent effect on lung function or lung development. In children, a limited number of studies assess health endpoints at multiple timepoints to report on the longitudinal impacts on lung development. Some cross-sectional studies support associations with reductions in lung function that were robust to copollutant adjustments. In copollutant models with UFP, NO_2 remained associated with declines in FEV_1. In a copollutant model with noise, NO_2 remained associated with declines in FEV_1 and FVC. In a multi-pollutant model adjusting for O_3, $PM_{2.5}$, PM_{10}, C, MeBr, and OP, NO_2 remained associated with declines in FEV_1 and FVC.

The animal toxicological evidence for long-term NO_2 exposure and impacts on lung function remains limited in the number of studies available and does not suggest robust impacts on lung function. Therefore, the available animal toxicological studies do not help to address uncertainty regarding the independent nature of the associations observed in epidemiologic studies.

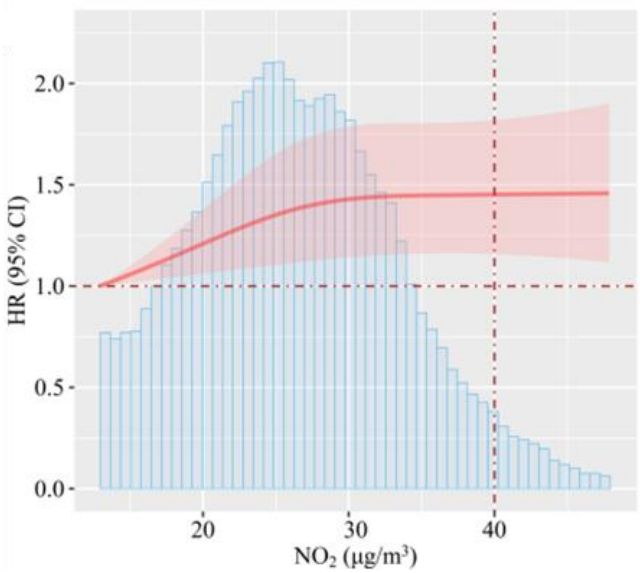
4.7 Interstitial Lung Disease

Interstitial lung disease (ILD) is a group of chronic lung diseases that cause inflammation and scarring (i.e., fibrosis) in the interstitium – the tissue and space between the alveoli. The stiffness caused by inflammation and scarring in the lungs can eventually impair a person's ability to breathe and adequately transfer oxygen to the bloodstream. ILDs often result in irreversible and progressive lung damage.

4.7.1 Epidemiologic Studies of Interstitial Lung Disease

There were no epidemiologic studies examining long-term exposure to NO_2 and ILD in the 2016 Oxides of Nitrogen ISA. A few recent studies provide initial evidence of a positive association between long-term NO_2 exposure and ILDs (see Table 4-11), including idiopathic pulmonary fibrosis (IPF) and interstitial lung abnormalities (ILAs). Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 4-12.

- Notable evidence comes from an analysis of data from the UK Biobank Study, a large prospective cohort study with data linkages to medical records in the UK (Cui et al., 2023). The authors defined incident IPF as the first record of hospital admission for ILD during a 10-year follow-up in a sample of over 400,000 participants without IPF at baseline. Cui et al. (2023) reported that a 10-ppb increment in annual average NO_2 concentration at baseline was associated with a 22% increase in incident hospital admissions for IPF (95% CI: 6, 40). In an analysis examining the interaction of pollutant exposure and genetic risk of IPF, the authors observed a synergistic effect on the additive scale, suggesting that the effects of NO_2 were stronger in participants at high-risk of IPF. The authors also used a restricted cubic spline model to assess potential non-linearities in the relationship between long-term NO_2 concentrations and the incidence of ILF. Visual inspection of the C-R curve revealed an approximately linear relationship up to 28 $\mu g/m^3$ (14.9 ppb), at which point the relationship plateaus (see Figure 4-14).



List of abbreviations in alphabetical order: CI = confidence interval; HR = hazard ratio; NO_2 = nitrogen dioxide; $\mu g/m^3$ = micrograms per cubic meter.
Note: Horizontal red dashed line indicates the null value.
Source: Adapted from Cui et al. (2023). Copyright permission pending.

Figure 4-14 Concentration-response relationship of annual average nitrogen dioxide concentration with the risk of incident idiopathic pulmonary fibrosis in the UK Biobank Study.

- A much smaller study of 118 IPF patients in Athens, Greece examined acute exacerbations of IPF in relation to 10-year average NO_2 concentrations at baseline (Tomos et al., 2021). Tomos et al. (2021) observed higher odds of presenting with at least one IPF exacerbation over the course of a 6-year follow-up associated with higher baseline NO_2 concentrations. Given the small sample size, the reported odds ratio was notably imprecise (2.20 [95% CI: 1.31, 3.70] per 10 ppb). Additionally, while IPF exacerbations may be indicative of greater disease severity, acute exacerbations could also be related to short-term exposures. Therefore, to the extent that spatially resolved long-term NO_2 exposure estimates are correlated with short-term exposure, the lack of adjustment for short-term exposure to NO_2 or other air pollutants may confound the relationship between long-term exposures and IPF exacerbation.
- In addition to studies examining IPF, one recent analysis of the MESA Air study utilized cardiac CT scans, which image approximately 70% of total lung volume, to assess ILAs and high attenuation areas (HAAs) in 5,495 adult participants from six study centers across the U.S. (Sack et al., 2017). ILAs and HAAs represent qualitative and quantitative measures of subclinical ILD, respectively. The study had board-certified radiologists read CT scans, but inter-rater reliability scores indicated moderate agreement (kappa = 0.47). Similar to evidence for IPF, Sack et al. (2017) reported an imprecise increase in the odds of ILAs (OR: 1.23 per 10 ppb increase [95% CI: 0.87, 1.76]) and a small increase in HAAs with confidence intervals inclusive of the null (0.17% per 10-ppb increase [95% CI: -0.16 to 0.51]) in relation to 10-year average NO_2 concentrations. ILAs (OR: 1.62 per 40-ppb increase [95% CI: 0.97, 2.71]) and HAAs (0.45% per 40-ppb increase [95% CI: -0.02, 0.92]) were more strongly associated with long-term NO_X exposures.

Table 4-11 Associations between long-term exposure to oxides of nitrogen and interstitial lung disease incidence or severity

Study and Cohort/Location	Exposure Assessment	Outcome	Effect Estimate (95% CI)
†Cui et al. (2023) UK Biobank (2006–2020)	LUR	Incident IPF: Hospital admissions for IPF	HR 1.22 (1.06, 1.40)
†Tomos et al. (2021) Attikon University Hospital, Greece (2013–2019)	Dispersion model	IPF acute exacerbation: Worsening of dyspnea within the last 30 days, exclusion of identifiable causes, confirmed by chest CT	OR 2.20 (1.31, 3.70)
†Sack et al. (2017) MESA (2000–2012)	Spatiotemporal model	ILAs: full lung CT scan HAAs: cardiac CT scan	OR ILA (NO_2): 1.23 (0.87, 1.76) ILA (NO_X): 1.62 (0.97, 2.71)[a] Percent change HAA (NO_2): 0.17% (-0.16, 0.51) HAA (NO_X): 0.55% (0.08, 1.00)[a]

List of abbreviations in alphabetical order: CI = confidence interval; CT = computed tomography; HAA = high attenuation area; HR = hazard ratio; ILA = interstitial lung abnormality; IPF = idiopathic pulmonary fibrosis; LUR = land use regression; MESA = Multi-Ethnic Study of Atherosclerosis; NO_2 = nitrogen dioxide; OR = odds ratio.
Note: Results are standardized to a 10-ppb increase in NO_2.
[a]Not standardized. Effect estimates per 40-ppb increase in NO_X.
†Studies published since the 2016 Oxides of Nitrogen ISA.

4.7.2 Conclusions and Remaining Uncertainties

Recent epidemiologic studies provide emerging evidence of an association between long-term NO_2 exposure and ILD. There are a number of uncertainties in the evidence base, including a limited number of studies – several of which have small sample sizes and imprecise effect estimates – and a lack of studies examining potential copollutant confounding.

4.8 Respiratory Mortality

As in short-term exposure studies, the relationship between long-term NO_2 exposure and respiratory mortality provides additional evidence for NO_2-related respiratory effects, specifically as it relates to a continuum of effects from subclinical effects underlying the development and exacerbation of disease to clinical indicators of disease and disease severity. In the 2016 Oxides of Nitrogen ISA, the evidence for long-term exposure and respiratory mortality was inconsistent. A limited number of cohort studies conducted in the United States, including AHSMOG (Abbey et al., 1999), ACS CPS-II (Jerrett et al., 2013), and the California Teachers Study (Lipsett et al., 2011), all reported null associations. Additionally, a large analysis of pooled data from 16 cohorts in the multicenter European Study of

Cohorts for Air Pollution Effects (ESCAPE) also observed a null association between long-term NO_2 exposure and respiratory disease mortality (Dimakopoulou et al., 2014). In contrast, a larger body of cohort studies conducted in Canada, Europe, and Asia observed generally positive associations with mortality due to respiratory disease, though many of these estimates were imprecise (i.e., wide 95% confidence intervals). Although not entirely consistent, a few studies that examined long-term NO_2 exposure in relation to COPD mortality provided some evidence of a positive association. Studies evaluating the associations between long-term exposure to NO_2 and respiratory mortality employed various exposure assessment methods such as central site monitors, LUR, or dispersion modeling. There was no distinguishable pattern of results that could be attributed to the use of different exposure assessment methods. The respiratory mortality studies reviewed in the 2016 Oxides of Nitrogen ISA did not provide thorough analyses of potential confounding by traffic-related copollutants.

A large number of recent cohort studies expand the existing evidence base and provide more consistent evidence of an association between long-term NO_2 exposure and mortality due to respiratory disease (see Figure 4-15). Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 4-13. Although the recent evidence is not entirely consistent, a number of recent nationwide administrative cohort studies and a pooled analysis of multiple European cohorts with individual-level data provide strong evidence supporting a positive relationship. Recent studies most frequently assign exposures using LUR models, in some cases using additional predictor variables such as satellite data, though some studies include central site monitors or hybrid prediction models that combine data from various exposure assessment methods. A bulleted summary of the recent evidence is provided below, followed by an additional discussion of concentration-response relationships in available studies (see Section 4.8.1), the lifestages and populations evaluated (see Section 4.8.2), copollutant analyses (see Section 4.8.3) and additional sources of confounding (see Section 4.8.4), and approaches to exposure assignment (see Section 4.8.5). Section 4.8.6 discusses conclusions from available studies and summarizes remaining uncertainties.

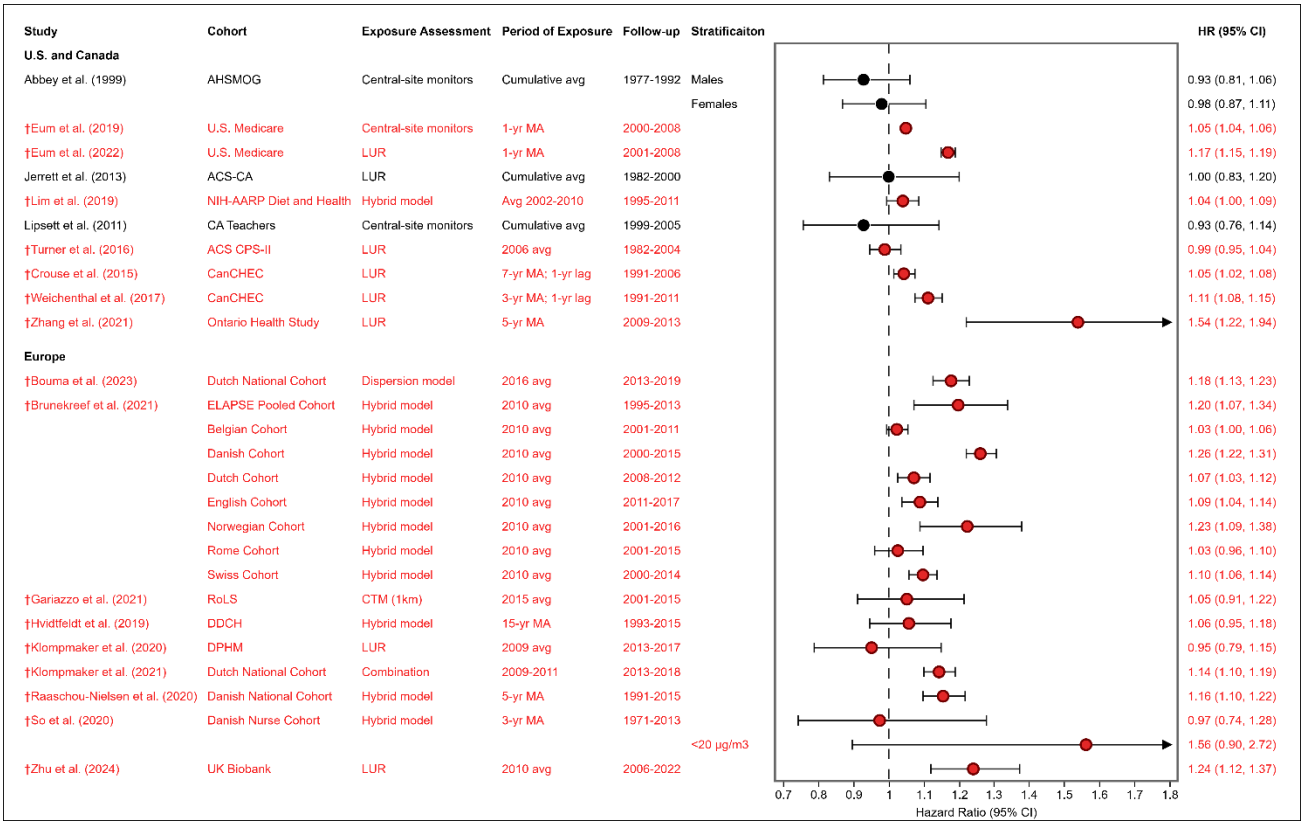
- To date, the most extensive evaluation of long-term NO_2 exposure and respiratory mortality is the recent multicenter Effects of Low-Level Air Pollution: A Study in Europe (ELAPSE) project (Brunekreef et al., 2021; Strak et al., 2021). This two-pronged study includes analyses of two sets of cohorts: a pooled analysis of data from eight well-established ESCAPE cohorts and analysis of seven large administrative cohorts in Europe. This study provides strength of inference from a large, pooled dataset of over 325,000 participants with individual-level data and analyses of nationally representative administrative data on over 28 million adults. The authors reported positive associations between long-term NO_2 concentrations and risk of respiratory mortality in the pooled analysis as well as in each of the administrative cohorts. Brunekreef et al. (2021) also evaluated a number of other policy-relevant topics, including thresholds, C-R relationships, and copollutant confounding (see Sections 4.8.1 through 4.8.5).
- Recent large nationwide studies in the United States (Eum et al., 2022; Eum et al., 2019) and Canada (Weichenthal et al., 2017b; Crouse et al., 2015) also reported generally consistent positive associations between long-term NO_2 exposure and respiratory disease mortality. A Medicare study of over 50 million beneficiaries (65+ years old) provides strong inference due

to its large number of person-years and respiratory mortality cases, as well as a high resolution nationwide LUR model with high validity (spatial $R^2 = 0.81$; temporal $R^2 = 0.73$) and low error (mean error = 2.4 ppb) (Eum et al., 2022). Eum et al. (2022) reported a large increase in respiratory disease mortality associated with a 10 pbb increase in 12-month moving average NO_2 concentrations (HR: 1.17 [95% CI: 1.15, 1.19]). An earlier iteration of this study analyzed a subset of Medicare patients living near central-site monitors (~14 million) and observed a smaller positive association (HR: 1.050 [95% CI: 1.044, 1.056]) with NO_2 concentrations measured at the nearest monitor (Eum et al., 2019). Crouse et al. (2015) and Weichenthal et al. (2017b) reported positive associations of similar magnitude in analyses of respiratory mortality among adults captured in separate waves of the Canadian Census Health and Environment Cohort (CanCHEC; 1991 to 2006 and 2001 to 2011, respectively).

- In contrast with other nationwide studies in the U.S. and Canada, a recent analysis of data from the American Cancer Society (ACS) Cancer Prevention Study II (CPS-II) demonstrated a null association between long-term NO_2 exposure and respiratory mortality among an analytic cohort of over 600,000 adults (30+ years old) (Turner et al., 2016). A strength of this study was its larger number of individual-level covariates compared to the administrative cohort studies, including data on education, BMI, and smoking status. One notable limitation was the temporal misalignment between the exposure and outcome (see Section 4.8.5). Turner et al. (2016) assigned exposure as the annual average of LUR-estimated NO_2 concentrations in 2006, two years after the end of follow-up (1982 to 2004).
- Recent regional studies in the United States and Canada provide additional support for a positive association between long-term NO_2 exposure and mortality. Lim et al. (2019) conducted a large analysis of over 500,000 participants in the NIH-AARP Diet and Health study, which included demographic and lifestyle data from 50- to 71-year-old voluntary respondents to a mailed questionnaire. After linking the participants to death registries, the authors reported a 4% increase in respiratory mortality (95% CI: -1, 8) associated with a 10 ppb increase in average NO_2 concentrations from 2002 to 2008. Similar to Turner et al. (2016), this study had temporally misaligned exposure data given that follow up began in 1995. In a smaller regional study in Ontario, Canada with only 550 recorded deaths due to respiratory diseases, Zhang et al. (2021b) observed an increase in respiratory mortality associated with an increase in 5-yr moving average NO_2 concentrations estimated using a high-resolution hybrid prediction model.
- A series of studies leveraging data from the UK Biobank reported consistent positive associations between respiratory mortality and long-term NO_2 concentrations estimated using ESCAPE LUR models (Li et al., 2024; Tao et al., 2024; Zhu et al., 2024). These studies, which evaluated largely overlapping samples of participants from the UK Biobank, each assessed specific topics related to the association between long-term NO_2 exposure and respiratory mortality, including specific causes of respiratory mortality and potential EMM relating to integrated social and ecological neighborhood environment. Results from those specific analyses are discussed in Section 4.8.2. Notably, each of these studies included follow-up through 2022, but did not consider significant changes in behavioral, air pollution, or mortality trends that resulted from the COVID-19 pandemic, which may have biased the study results.
- Several recent European studies examined individual cohorts contained within the aforementioned ELAPSE project, including analyses of the DNC (So et al., 2020); the Danish, Diet, Cancer, and Health cohort (Hvidtfeldt et al., 2019); nationwide administrative cohorts in the Netherlands (Bouma et al., 2023; Klompmaker et al., 2021a; Klompmaker et al., 2021b) and Denmark (So et al., 2022; Raaschou-Nielsen et al., 2020), and a city-wide

administrative cohort in Rome (Gariazzo et al., 2021). Consistent with ELAPSE, studies of the individual cohorts indicated positive associations of varying magnitude between long-term NO_2 exposure and respiratory disease mortality. Whereas Brunekreef et al. (2021) assigned exposure using an LUR model fit across the entirety of Western Europe, other studies of individual cohorts generally used more localized exposure models. Further discussion of exposure models used for the studies detailed in this section is provided in Section 4.8.5.

- Although the majority of recent European cohort studies reported increases in respiratory mortality associated with long-term NO_2 exposure, a Dutch cohort study utilizing data from a population-based national health survey reported a null association (Klompmaker et al., 2020). Notably, the authors assigned exposures using LUR models. Klompmaker et al. (2021a) analyzed data from a larger nationwide administrative Dutch cohort that was included in the ELAPSE project to compare three different exposure assessment methods– a LUR model, a dispersion model, and a hybrid LUR model supplemented with CTM and satellite data– and reported positive associations corresponding to exposures estimated from the dispersion model and the hybrid LUR model, but not the traditional LUR model (see Section 4.8.5).
- In addition to examining mortality from all respiratory disease, several of the previously discussed studies also examined mortality due to specific respiratory disease, including COPD, asthma, and acute lower respiratory infection (ALRI; e.g., pneumonia and influenza). The most consistent evidence was observed for long-term NO_2 exposure and ALRI. Each study that examined ALRI or its components reported positive associations with NO_2 concentrations, including studies in the United States, Canada, and Europe (Eum et al., 2022; So et al., 2022; Eum et al., 2019; Lim et al., 2019; Turner et al., 2016). Evidence for COPD mortality was also generally consistent, though some studies reported null associations. Positive NO_2-COPD mortality associations were observed in both sets of ELAPSE cohorts (Brunekreef et al., 2021), Medicare and AARP studies in the United States (Eum et al., 2022; Lim et al., 2019), the CanCHEC study (Crouse et al., 2015), the UK Biobank study (Li et al., 2024), and in a nationwide administrative cohort in Denmark (So et al., 2022). In contrast, null associations were reported in the ACS CPS-II study (Turner et al., 2016) and the Medicare study restricted to beneficiaries living near monitors (Eum et al., 2019). Finally, a large nationwide analysis of administrative data in Denmark reported an increase in mortality due to asthma associated with increases in long-term exposure to NO_2 (So et al., 2022).



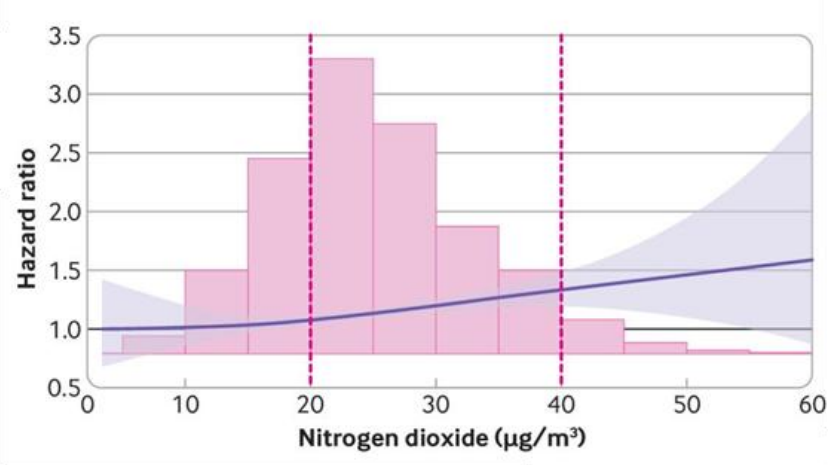
List of abbreviations in alphabetical order: ACS = American Cancer Society; AHSMOG = Adventist Health and Smog Study; Avg = average; CA = California; CanCHEC = The Canadian Census Health and Environment Cohorts; CI = confidence interval; CPS = Cancer Prevention Study; CTM = chemical transport model; ELAPSE = Effects of Low-Level Air Pollution: A Study in Europe; LUR = land use regression; MA = moving average; 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. Results are standardized to a 10 ppb increase in NO_2.
†Studies published since the 2016 Oxides of Nitrogen ISA.

Figure 4-15 Associations of long-term exposure to nitrogen dioxide with respiratory mortality.

4.8.1 Concentration-Response

There was limited evaluation of the shape of the C-R relationship between long-term NO_2 exposure and respiratory mortality in studies examined in the 2016 Oxides of Nitrogen ISA. Several recent studies have used cubic spline models, SCHIF models, and subset analyses to account for potential non-linear effects of long-term NO_2 exposure. As part of the ELAPSE project, Brunekreef et al. (2021) and Strak et al. (2021) examined non-linearities in a pooled analysis of data from ESCAPE cohorts and an analysis of large administrative cohorts in Europe. Using data from each of seven administrative cohorts, Brunekreef et al. (2021) modeled C-R relationships using natural cubic splines with 4 knots. The C-R curves differed for each cohort, but in most cohorts the relationship was approximately linear at lower NO_2 concentrations with some evidence of a plateau or inverse relationship at higher concentrations. Brunekreef et al. (2021) also analyzed potential thresholds in each administrative cohort using AIC values

to compare the fit of regression models with and without thresholds. The no-threshold model was the best fit in five of the seven cohorts, whereas models with thresholds at 10 µg/m^3 (5.32 ppb) and 20 µg/m^3 (10.64 ppb) were identified as the best fit for the Danish and Swiss cohorts, respectively. The general linear no-threshold trend was supported by a meta-analysis of all seven cohorts that included a subset analysis to examine associations below increasingly stringent concentration cutoffs. The meta-analytic effect estimates for subsets of the cohorts exposed to annual average NO_2 concentrations <30 µg/m^3 (<15.96 ppb; HR: 1.201 per 10 ppb increase in NO_2 [95% CI: 1.109, 1.300]) and <20 µg/m^3 (<10.64 ppb; 1.178 [95% CI: 0.910, 1.526]) were larger than estimates from the full cohorts (HR: 1.112 [95% CI: 1.046, 1.182]), which reflects the plateau observed at higher concentrations in many of the spline models. Results were comparable in the pooled analysis of data from eight ESCAPE cohorts from six European countries. Strak et al. (2021) observed a linear relationship between long-term NO_2 exposure and respiratory mortality in a natural cubic spline model with four knots (see Figure 4-16). In an analysis of the same data, Brunekreef et al. (2021) reported that a no-threshold model was a better fit than models with various thresholds.

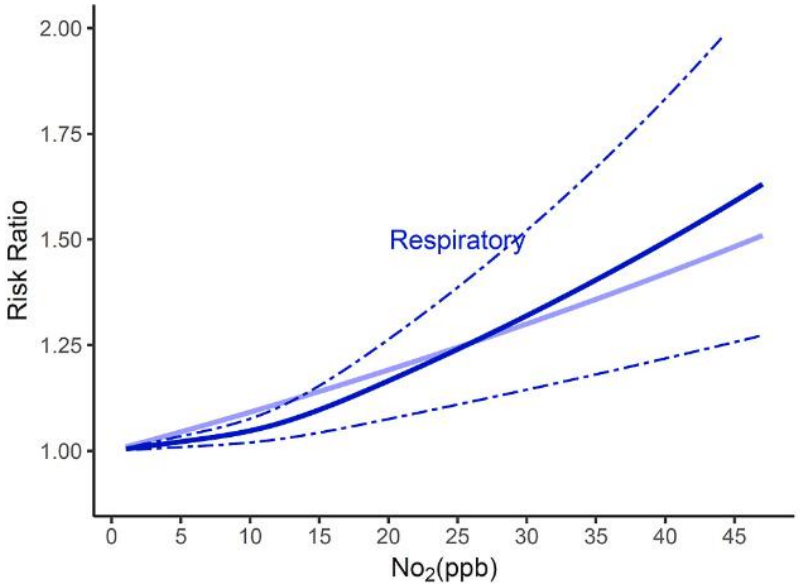


List of abbreviations in alphabetical order: CI = confidence interval; NO_2 = nitrogen dioxide; µg/m^3 = micrograms per cubic meter.
Note: Shaded blue area represents 95% CIs; histogram represents the distribution of exposure concentrations
Source: Adapted from Strak et al. (2021). Copyright permission pending.

Figure 4-16 Concentration-response curve between annual average nitrogen dioxide concentrations in 2010 and respiratory mortality in a pooled analysis of data from eight European cohorts.

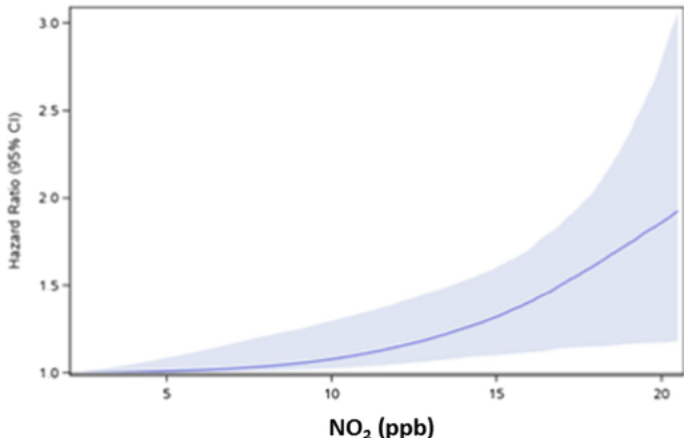
Examination of C-R relationships in other recent studies provided evidence of various non-linear relationships (see Table 4-12). In an analysis of over 50 million Medicare beneficiaries, Eum et al. (2022) observed a slightly sub-linear relationship between 12-month moving average NO_2 concentrations and respiratory mortality in a restricted cubic spline model with three knots (see Figure 4-17). A similar sub-linear relationship was observed in a SCHIF model applied to data from the Ontario Health Study (Zhang et al., 2021b) (see Figure 4-18). Visual inspection of the spline plots suggests limited evidence of an association corresponding to NO_2 concentrations below 10 to 15 µg/m^3 (5.32 to 7.98 ppb), above which the C-R relationship appears to be approximately linear. A contrasting relationship was identified in an

analysis of the Danish Nurses Cohort (So et al., 2020), one of the cohorts included in the Strak et al. (2021) pooled analysis. Visual inspection of a restricted cubic spline model with four knots suggests a positive relationship at lower concentrations and an inverse relationship at higher concentrations. The observed shape is consistent with the overall findings from the study, which reported a null association between NO_2 concentrations and respiratory mortality in the full cohort (HR: 0.97 per 10-ppb increase in 3-yr moving avg NO_2 concentrations [95% CI: 0.74, 1.28]), but noted increasingly stronger but less precise associations below decreasing concentration cutoffs (<30 µg/m^3 [<15.96 ppb]: 1.28 [95% CI: 0.86, 1.89]; <20 µg/m^3 [<10.64 ppb]: 1.56 [95% CI: 0.90, 2.72] ; <15 µg/m^3 [<7.98 ppb]: 2.41 [95% CI: 1.09, 5.34]).



List of abbreviations in alphabetical order: CI = confidence interval; NO_2 = nitrogen dioxide; ppb = parts per billion.
Note: The darker curved solid line represents the non-linear relationship estimated using restricted cubic spline (3 knots), the dotted lines represent the 95% CIs for the non-linear association, and the muted solid line represents the linear association.
Source: Adapted from Eum et al. (2022). Copyright permission pending.

Figure 4-17 Concentration-response curve between 12-month moving average nitrogen dioxide concentration and respiratory mortality in an analysis of Medicare beneficiaries.



List of abbreviations in alphabetical order: NO_2 = nitrogen dioxide; ppb = parts per billion.
Source: Adapted from Zhang et al. (2021b). Copyright permission pending.

Figure 4-18 Concentration-response curve between five-year moving average nitrogen dioxide concentration and respiratory mortality in the Ontario Health Study.

Table 4-12 Summary of epidemiologic studies evaluating potential non-linearities in the relationship between long-term NO_2 exposure and respiratory mortality

Study	Cohort (Follow-Up)	NO_2 Exposure: 5^{th} to 95^{th} Percentile	Modeling Method	Shape of C-R Relationship
Medicare Cohorts				
†Eum et al. (2022)	U.S. Medicare (2001–2008)	1.03–47.04 ppb[a]	Natural cubic spline with 3 knots	Sublinear: Flatter slope <15 ppb
Canadian Cohorts				
†Zhang et al. (2021b)	Ontario Health Study (2009–2017)	5.25–27.10 ppb[b]	Shape Constrained Health Impact Function (SCHIF)	Sublinear: Null <5 ppb; Flatter slope <10 ppb
ESCAPE Cohorts				
†Brunekreef et al. (2021); †Strak et al. (2021)	Eight Pooled ESCAPE Cohorts (~1995–2013)	8.6–19.4 µg/m^3 (4.58–10.32 ppb)	Natural cubic spline with 4 knots	Approximately Linear
†So et al. (2020)	Danish Nurses Cohort (1993–2013)	3.7–57.0 µg/m^{3a} (1.97–30.32 ppb)	Natural cubic spline with 4 knots	Parabolic
ELAPSE/European Administrative Cohorts				

Study	Cohort (Follow-Up)	NO_2 Exposure: 5th to 95th Percentile	Modeling Method	Shape of C-R Relationship
†Brunekreef et al. (2021)	Belgian National Administrative Cohort (2001–2011)	20.3–44.6 μg/m^3 (10.80–23.73 ppb)	Natural cubic spline with 4 knots	Approximately Linear; Plateau > ~8 ppb
	Danish National Administrative Cohort (2000–2015)	9.4–35.7 μg/m^3 (5.00–18.99 ppb)	Natural cubic spline with 4 knots	Parabolic
	Dutch National Administrative Cohort (2008–2012)	19.4–42.9 μg/m^3 (10.32–22.82 ppb)	Natural cubic spline with 4 knots	Parabolic
	English National Administrative Cohort (2011–2017)	16.4–39.0 μg/m^3 (4.58–10.32 ppb)	Natural cubic spline with 4 knots	Approximately linear
	Norwegian National Administrative Cohort (2001–2016)	4.1–30.0 μg/m^3 (8.72–15.96 ppb)	Natural cubic spline with 4 knots	Parabolic
	Rome Administrative Cohort (2001–2015)	21.4–42.5 μg/m^3 (11.38–22.61 ppb)	Natural cubic spline with 4 knots	U-shaped
	Swiss National Administrative Cohort (2000–2014)	12.5–42.5 μg/m^3 (6.65–22.61 ppb)	Natural cubic spline with 4 knots	U-shaped

List of abbreviations in alphabetical order: C-R = concentration-response; ELAPSE = Effects of Low-Level Air Pollution: A Study in Europe; ESCAPE = European Study of Cohorts for Air Pollution Effects; MeBr = methyl bromide; NO_2 = nitrogen dioxide; ppb = parts per billion; SCHIF = Shape Constrained Health Impact Function; U.K. = United Kingdom; U.S. = United States; μg/m^3 = micrograms per cubic meter.
[a]Range.
[b]25th to 75th percentile.
Results are standardized to a 10-ppb increase in NO_2.
†Studies published since the 2016 Oxides of Nitrogen ISA.

4.8.2 Lifestages and Populations

There was limited evaluation of populations potentially at increased risk of NO_2-related mortality in studies included in the 2016 Oxides of Nitrogen ISA. Respiratory disease mortality rates increase with increasing age, and therefore the association between long-term NO_2 exposure and respiratory mortality disproportionately affects older adults. However, no studies that observed positive associations between NO_2 exposure and respiratory mortality examined potential effect modification by age. Effect modification by sex was considered in a multi-region study in Japan that followed over 60,000

participants for 10 years (Katanoda et al., 2011). Katanoda et al. (2011) reported stronger associations in females (HR: 1.25 per 10 ppb increase in 10-yr avg NO_2 [95% CI: 1.18, 1.33]) relative to males (HR: 1.11 [95% CI: 1.05, 1.18]). A few recent studies have considered a range of at-risk factors, including lifestage, sex, race/ethnicity, and behavioral factors.

Sex

Consistent with findings from Katanoda et al. (2011), a few recent studies have also observed stronger associations between long-term NO_2 exposure and respiratory mortality in females (see Table 4-13). In the large Medicare analysis of over 50 million adults, (Eum et al., 2022) reported that increased risk of NO_2-related mortality was larger among females (HR: 1.11 per 10 ppb [95% CI: 1.10, 1.13]) relative to males (HR: 1.07 [95% CI: 1.05,1.09]). An analysis of the Ontario Health Study noted much larger but less precise sex-specific differences in NO_2-related mortality risk in exploratory stratified analyses (Zhang et al., 2021b). Raaschou-Nielsen et al. (2020) observed a similar trend in a national administrative cohort in Denmark, though the difference between females (MRR: 1.22 per 5.32 ppb [95% CI: 1.14, 1.30]) and males (MRR: 1.10 [95% CI: 1.04, 1.16]) was not as substantial.

Table 4-13 Epidemiologic studies evaluating effect-measure modification of the relationship between long-term nitrogen dioxide exposure and respiratory mortality by sex

Study	Cohort (Years)	EMM Reference Factor	EMM Comparison Factor	Direction of EMM[a]	Hazard Ratio (95% CI)	Spatial Scale of Effect Modifier
†Eum et al. (2022)	U.S. Medicare (2001–2008)	Male	Female	↑	EMM Reference Factor: 1.07 (1.05, 1.09) EMM Comparison Factor: 1.11 (1.10, 1.13)	Individual level
Katanoda et al. (2011)	Three-prefecture Cohort Study (1983–1995)	Male	Female	↑	EMM Reference Factor: 1.11 (1.05, 1.18) EMM Comparison Factor: 1.25 (1.18, 1.33)	Individual level
†Raaschou-Nielsen et al. (2020)	Danish National Administrative Cohort (1991–2015)	Male	Female	↑	EMM Reference Factor: 1.10 (1.04, 1.16) EMM Comparison Factor: 1.22 (1.14, 1.30)	Individual level
†Zhang et al. (2021b)	Ontario Health Study (2009–2013)	Male	Female	↑	EMM Reference Factor: 1.21 (0.90, 1.61) EMM Comparison Factor: 2.18 (1.49, 3.18)	Individual level

List of abbreviations in alphabetical order: CI = confidence interval; EMM = effect measure modification; NO_2 = nitrogen dioxide; ppb = parts per billion; U.S. = United States.
Results are standardized to a 10 ppb increase in 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.
†Studies published since the 2016 Oxides of Nitrogen ISA.

Lifestage

The same studies also evaluated differences in NO_2-related respiratory mortality risk across age groups. Results were not as consistent as for sex but suggest that long-term NO_2 exposure is more strongly associated with respiratory mortality among older adults (see Table 4-14). Evidence of effect modification was observed in the aforementioned Medicare analysis (Eum et al., 2022) and Danish administrative cohort (Raaschou-Nielsen et al., 2020), with the strongest associations observed in the oldest age groups examined (i.e., 80 to 84 years (Raaschou-Nielsen et al., 2020) and 75+ years (Eum et al., 2022). In contrast, Zhang et al. (2021b) reported a larger increase in NO_2-associated respiratory mortality among adults younger than 65 years compared to those 65 years and older. However, there were only 158 respiratory mortality deaths in the younger age group, which reduced the precision of the HR.

Table 4-14 Epidemiologic studies evaluating effect-measure modification of the relationship between long-term nitrogen dioxide exposure and respiratory mortality by age

Study	Cohort (Years)	EMM Reference Factor	EMM Comparison Factor	Direction of EMM[a]	Hazard Ratio (95% CI)	Spatial Scale of Effect Modifier
†Eum et al. (2022)	U.S. Medicare (2001–2008)	<75 yrs	≥75 yr	↑	EMM Reference Factor: 1.03 (1.01, 1.05) EMM Comparison Factor: 1.10 (1.09, 1.12)	Individual level
†Raaschou-Nielsen et al. (2020)	Danish National Administrative Cohort (1991–2015)	30-49 yr	50–59 yr	↑	EMM Reference Factor: 0.98 (0.59, 1.63) EMM Comparison Factor: 1.06 (0.95, 1.18)	Individual level
			60–69 yr	↑	1.16 (1.09, 1.23)	
			70–79 yr	↑	1.14 (1.08, 1.20)	
			80–85 yr	↑	1.30 (1.20, 1.41)	
†Zhang et al. (2021b)	Ontario Health Study (2009–2013)	<65 yrs	≥65 yr	↓	EMM Reference Factor: 2.35 (1.54, 3.58) EMM Comparison Factor: 1.37 (1.05, 1.80)	Individual level

List of abbreviations in alphabetical order: CI = confidence interval; EMM = effect measure modification; NO_2 = nitrogen dioxide; ppb = parts per billion; U.S. = United States; yr = years.
Results are standardized to a 10-ppb increase in avg 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.

†Studies published since the 2016 Oxides of Nitrogen ISA.

Behavioral Factors

Zhang et al. (2021b) also considered the impact of behavioral factors, such as diet and smoking status. Associations between NO_2 exposure and respiratory mortality were stronger among participants who reported eating fewer than five servings of fruits and vegetables daily. The authors also observed an increasing trend in NO_2-related respiratory mortality risk corresponding to smoking status. HRs were greater in magnitude among current smokers (1.17 per 1 ppb increase in NO_2 [95% CI: 1.10, 1.24]) and former smokers (1.03 [95% CI: 1.00, 1.07]) compared to never smokers (1.01 [95% CI: 0.96, 1.07]).

Socioeconomic Status

A few recent studies evaluated effect modification by income. Zhang et al. (2021b) analyzed regression models stratified by five levels of household income and did not identify clear patterns of heterogeneity in the associations of NO_2 exposure and respiratory mortality across income groups. Raaschou-Nielsen et al. (2020) similarly examined models stratified by quintiles of income but used neighborhood-level income data rather than individual-level data. The authors observed an increasing strength of associations between NO_2 exposure and respiratory mortality among participants living in areas with higher neighborhood-level income.

In a recent UK Biobank analysis, Tao et al. (2024) used principal component analysis to integrate variables across four dimensions (i.e., SES, ecological environment, housing, and crime) to designate U.K. neighborhoods as disadvantaged, intermediate, and advantaged. In stratified analyses incorporating neighborhood designations, the observed association between a one standard deviation increase in long-term NO_2 concentrations (increment NR) and respiratory mortality was strongest in the disadvantaged neighborhoods (HR: 1.124 [95% CI: 1.045, 1.210]) relative to the intermediate (HR: 1.027 [95% CI: 0.944, 1.119]) and advantaged neighborhoods (HR: 1.074 [95% CI: 0.904, 1.275]).

Race/Ethnicity

Eum et al. (2022) was the only recent study to examine effect modification by race/ethnicity. The authors reported stronger associations between long-term NO_2 exposure and respiratory mortality among Black (HR: 1.33 per 10-ppb NO_2 [95% CI: 1.30, 1.35]), Asian (HR: 1.25 [95% CI: 1.20, 1.29]), and Hispanic (HR: 1.17 [95% CI: 1.14, 1.21]) participants compared to white participants (HR: 1.09 [95% CI: 1.07, 1.10]).

4.8.3 Copollutants

As discussed previously, NO_2 is often highly correlated with traffic-related pollutants, which may consequently confound the relationship between long-term NO_2 exposure and respiratory disease. The studies that reported positive associations between long-term NO_2 exposure and respiratory mortality in

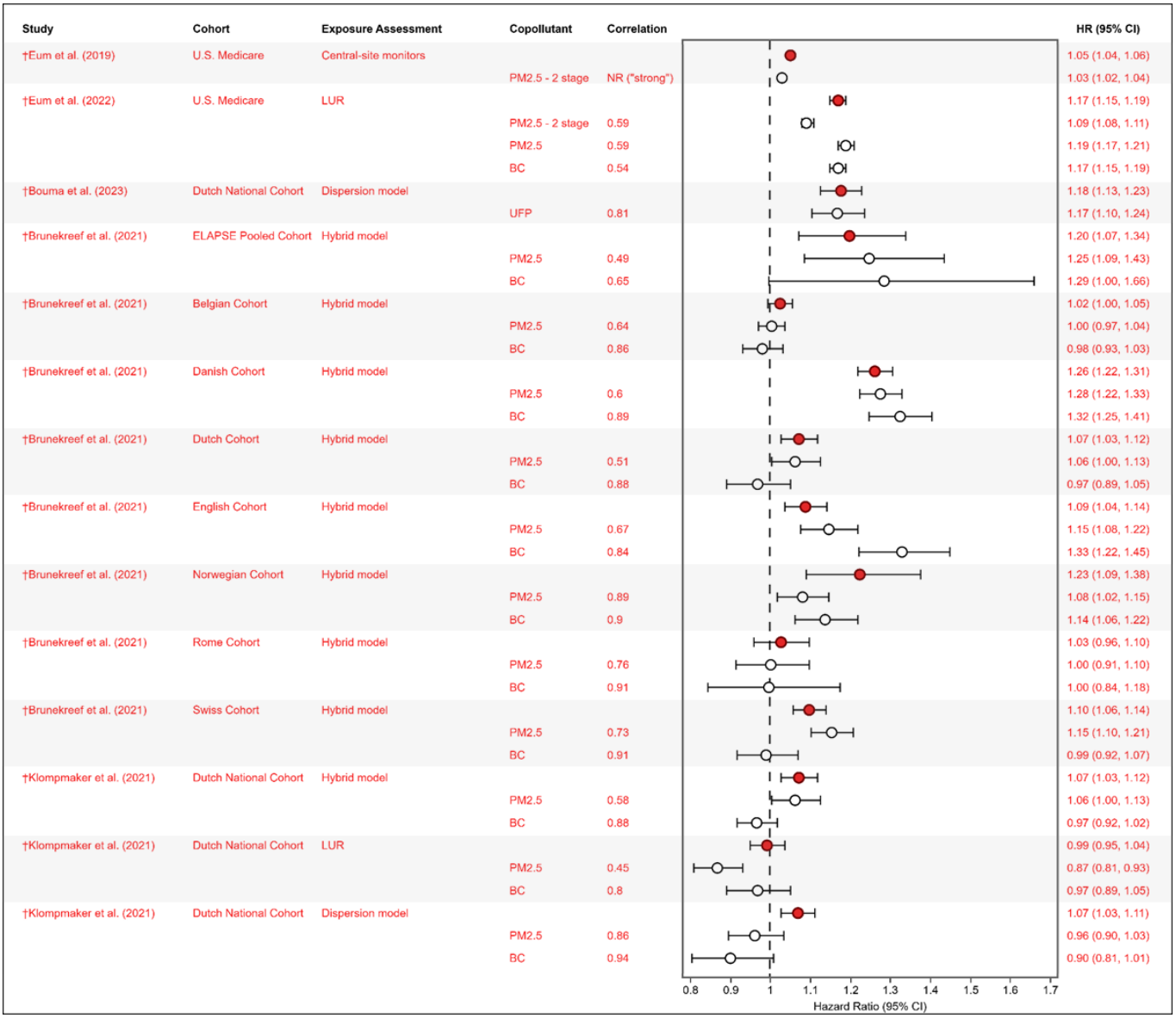
the 2016 Oxides of Nitrogen ISA did not evaluate potential copollutant confounding. Several recent studies have examined copollutant models and provide generally consistent evidence that the NO_2-respiratory mortality relationship remains positive after adjustment for $PM_{2.5}$ or O_3 in copollutant models (see Figure 4-19 and Figure 4-20). Evidence for potential confounding by BC is less consistent, though it is also complicated by particularly high correlations with NO_2 that complicate the interpretation of two-pollutant models.

High correlations between NO_2 concentrations and air pollutants can misleadingly decrease or increase the magnitude or precision of the effect estimate for NO_2 or the copollutant in two pollutant models. In order to address this concern, Eum et al. (2022) used a two-stage model to adjust for potential confounding by $PM_{2.5}$ in a large nationwide Medicare study. The authors first regressed 12-month moving average NO_2 concentrations on 12-month moving average $PM_{2.5}$ concentrations and then used the residuals as the exposure measure in the respiratory mortality regression model. Compared to the result from the single pollutant model (HR: 1.17 per 10 ppb [95% CI: 1.15, 1.19]), the strength of the association between long-term NO_2 exposure and respiratory mortality was attenuated but still positive in the two-stage model (1.09 [95% CI: 1.08, 1.11]). In a sensitivity analysis, the observed association in a traditional copollutant model adjusted for $PM_{2.5}$ was comparable to the single pollutant model (1.19 [95% CI: 1.17, 1.21]).

Other recent studies used traditional regression models to adjust for potential copollutant confounders. In nationwide cohort studies conducted in the United States (Eum et al., 2022; Eum et al., 2019) and Denmark (So et al., 2022; Raaschou-Nielsen et al., 2020), observed associations between long-term NO_2 exposure and respiratory mortality remained positive after adjustment for $PM_{2.5}$, BC, or O_3. Examination of the same copollutants in the ELAPSE project analysis of seven administrative cohorts demonstrated less consistent results (Brunekreef et al., 2021). Positive associations persisted in five of the seven cohorts after adjustment for $PM_{2.5}$ or O_3, and the associations were attenuated and null after adjustment for BC in four of the seven cohorts, including the Danish cohort (Klompmaker et al., 2021a). Notably the correlations between NO_2 and BC ranged from 0.84 to 0.91 across cohorts (NO_2 to $PM_{2.5}$: 0.51 to 0.89; NO_2 to O_3: -0.65 to -0.80). In the only recent study to evaluate potential copollutant confounding by UFPs, Bouma et al. (2023) reported a robust association between NO_2 and respiratory mortality in a national administrative cohort study in the Netherlands.

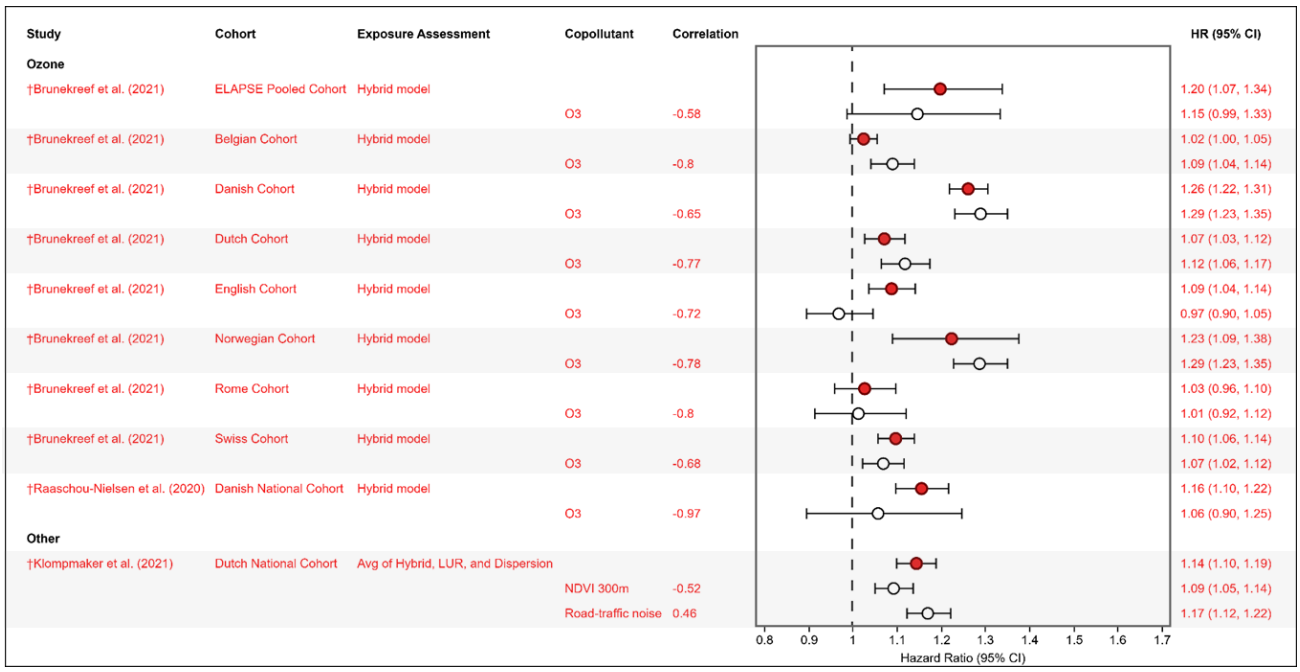
One additional study examined potential confounding by non-pollutant environmental stressors. In an analysis of a national administrative cohort in the Netherlands, Klompmaker et al. (2021b) evaluated copollutant models that adjusted for the modeled average density of green space surrounding each participant's residence. Greenness is often correlated with air pollutants and can exhibit direct and indirect effects on mortality (Brochu et al., 2022). Klompmaker et al. (2021b) reported that the positive association between long-term NO_2 concentrations and respiratory mortality (HR: 1.144 per 10 ppb increment [95% CI: 1.101, 1.189]) was attenuated but still positive after adjustment for greenness (HR: 1.093 [95% CI: 1.050, 1.138]). The authors also considered traffic noise as a potential confounder given

its correlation to both NO_2 exposure and increased stress levels. Traffic noise may also serve as a proxy measure of other traffic-related pollutants. The observed association was slightly increased after adjustment for modeled residential traffic noise (HR: 1.171 [95% CI: 1.122, 1.221]).



List of abbreviations in alphabetical order: BC = black carbon; CI=confidence interval; HR = hazard ratio; LUR = land use regression; NR = not reported; $PM_{2.5}$ = particulate matter with a nominal mean aerodynamic diameter less than or equal to 2.5 µm; U.S. = United States.
Notes: Red = recent studies and those not included in 2016 Oxides of Nitrogen ISA. Filled circles = results from single pollutant models; unfilled circles = results from copollutant models. Results are standardized to a 10 ppb increase in NO_2.
†Studies published since the 2016 Oxides of Nitrogen ISA.

Figure 4-19 Associations between long-term nitrogen dioxide exposure and respiratory mortality in single pollutant models and models adjusted for particulate matter.



List of abbreviations in alphabetical order: CI = confidence interval; LUR = land use regression; NDVI = Normalized Difference Vegetation Index; m = meter; O_3 = ozone.
Notes: Red = recent studies and those not included in 2016 Oxides of Nitrogen ISA. Filled circles = results from single pollutant models; unfilled circles = results from copollutant models. Results are standardized to a 10 ppb increase in NO_2.
†Studies published since the 2016 Oxides of Nitrogen ISA.

Figure 4-20 Associations between long-term nitrogen dioxide exposure and respiratory mortality in single pollutant models and models adjusted for ozone, greenspace, or traffic noise.

4.8.4 Additional Sources of Confounding

In addition to copollutant confounding, other factors have the potential to confound the relationship between long-term NO_2 exposure and respiratory mortality. Potential confounders considered by recent studies include individual attributes, health behaviors, residential area characteristics, and temporal trends of NO_2 and respiratory disease. Many recent studies accounted for confounding by using age as a timescale in Cox proportional hazards models, stratifying models by sex, and adjusting models for individual-level and area-level covariates. Covariates that were frequently controlled for at the individual-level included demographics (e.g., race, region of origin, marital status), indicators of socioeconomic status (e.g., household income, employment, education), health status (e.g., BMI, waist circumference), and health behaviors (e.g., diet, alcohol consumption, smoking, physical activity, environmental tobacco smoke). In studies that included individual-level factors (e.g., prospective cohort studies), area-level factors were often used to control for environmental factors thought to structurally influence exposure and health outcomes through historical policies. Studies with administrative cohorts also used area-level covariates as surrogates for individual-level covariates, as certain individual-level data were not available in administrative datasets. Area-level factors also included demographics (e.g.,

racial population percentages, immigrant population percentage), socioeconomic factors (e.g., unemployment rate, poverty, rented dwellings, one-parent households, criminal records, income, education, occupation), and health environment behaviors from surveys like the Behavioral Risk Factor Surveillance System survey. Area-level factors were typically considered at a census-tract or neighborhood level (Bouma et al., 2023; So et al., 2022; Brunekreef et al., 2021; Gariazzo et al., 2021; Klompmaker et al., 2021a; Klompmaker et al., 2021b; Strak et al., 2021; Zhang et al., 2021b; Klompmaker et al., 2020; Raaschou-Nielsen et al., 2020; Hvidtfeldt et al., 2019). Larger geographic regions including postal code (e.g., ZIP code) and region within a country were also common (Bouma et al., 2023; Eum et al., 2022; So et al., 2022; Klompmaker et al., 2021a; Klompmaker et al., 2021b; Klompmaker et al., 2020; Eum et al., 2019; Turner et al., 2016).

Epidemiologic studies often report results from multiple regression models that include increasingly stringent control for potential confounders. With adjustment for the confounders noted above, the observations in recent studies have been largely consistent in observing positive associations between long-term NO_2 exposure and mortality. Most studies observed robust associations in fully adjusted models (Bouma et al., 2023; Eum et al., 2022; So et al., 2022; Brunekreef et al., 2021; Klompmaker et al., 2021a; Klompmaker et al., 2021b; Strak et al., 2021; Zhang et al., 2021b; Klompmaker et al., 2020; Raaschou-Nielsen et al., 2020; Eum et al., 2019; Crouse et al., 2015).

In recent studies that reported results from minimally adjusted models, the most common adjustments were for age, sex, and calendar year. Studies that adjusted for these confounders observed a positive association between a 10 ppb increase in NO_2 concentrations and an increased hazard ratio or rate ratio (HR range: 1.24 to 1.45 for respiratory disease mortality (Brunekreef et al., 2021; Raaschou-Nielsen et al., 2020; Hvidtfeldt et al., 2019). After further adjustment for additional individual-level confounders, including socioeconomic, health status, and health behaviors, the observation of a positive association between long-term NO_2 exposure and respiratory disease mortality remained. Brunekreef et al. (2021) and Hvidtfeldt et al. (2019) observed that the addition of these individual-level confounders slightly decreased the magnitude of the positive association, while Raaschou-Nielsen et al. (2020) observed that the addition of these variables slightly increased the magnitude of the positive association. These studies reported similar levels of precision between NO_2 estimates in the base model and in models adjusted for individual-level confounders, as noted through similar confidence interval widths. Other studies with individual-level covariates also observed a positive association in fully-adjusted models (So et al., 2022; Zhang et al., 2021b).

Recent studies also observed consistent evidence of a positive association between long-term NO_2 exposure and respiratory disease mortality following further adjustment for neighborhood-level confounders. In an analysis of the U.S. Medicare population, Eum et al. (2022) reported that the association between long-term NO_2 exposure and respiratory disease mortality (HR: 1.24 [95% CI: 1.23, 1.26] per 10 ppb increase in NO_2) was attenuated but still positive after adjustment for neighborhood-level and state-level SES (HR: 1.09 [95% CI: 1.08, 1.11]). Thus, although this provides evidence of

confounding by SES factors in this population, a positive association was observed independent of the area-level covariates included in the fully-adjusted model. In a related study of Medicare beneficiaries, Eum et al. (2019) observed that the addition of county-level behavioral risk patterns increased the magnitude of the positive association between a 10 ppb increase in NO_2 and respiratory disease mortality from 1.030 (95% CI: 1.023, 1.038) to 1.069 (95% CI: 1.056, 1.082).

Studies conducted in Canada and Europe also observed a positive association between long-term NO_2 exposure and respiratory disease mortality following further adjustment for neighborhood-level socioeconomic status and urbanicity variables. Zhang et al. (2021b) conducted a study in Canada and observed that the adjustments for neighborhood-level SES and urbanicity did not alter the magnitude of the positive association between a 1 ppb increase in NO_2 and an increased hazard ratio for respiratory disease mortality (HR: 1.044). A study by Brunekreef et al. (2021) observed that the magnitude of the positive association between NO_2 and respiratory disease mortality increased with the neighborhood-level adjustment compared to the individual-level model that did not adjust for individual-level income. However, in line with other studies, Brunekreef et al. (2021) observed that the magnitude of the positive association in the fully adjusted model was decreased compared to the base model. Other studies also observed a decrease in the magnitude of the positive association between NO_2 and respiratory disease mortality. These decreases were in comparison to base and individual-level adjusted models (So et al., 2022; Raaschou-Nielsen et al., 2020; Hvidtfeldt et al., 2019). So et al. (2022) observed the magnitude of the positive association between a 10 ppb increase in NO_2 and an increased hazard ratio decrease from 1.22 (95% CI: 1.17, 1.27) in the base model to 1.16 (95% CI: 1.13, 1.19) in the fully adjusted model. Raaschou-Nielsen et al. (2020) also observed the magnitude of the positive association between a 10-ppb increase in NO_2 and respiratory mortality decrease from 1.24 (95% CI: 1.21, 1.27) in the basic model to 1.16 (95% CI: 1.10, 1.22) in the fully adjusted model. With the further adjustment for neighborhood-level, Hvidtfeldt et al. (2019) also observed a decrease in the magnitude of the positive association between a 10 ppb increase in NO_2 and an increased hazard ratio from 1.45 (95% CI: 1.30, 1.62) in the basic model to 1.06 (95% CI: 0.95, 1.18) in the fully adjusted model. Further, fully adjusted models were observed to have increased precision of the estimated associations, based on the decreased size of the confidence interval compared to the basic models (So et al., 2022; Raaschou-Nielsen et al., 2020; Hvidtfeldt et al., 2019).

4.8.5 Exposure Assignment

Exposure Assessment

Studies of long-term NO_2 exposure and respiratory mortality that were evaluated in the 2016 Oxides of Nitrogen ISA employed a variety of exposure assessment methods such as central site monitors, LUR, or dispersion modeling. While there was some inconsistency in results across studies, there were no strong patterns observed across exposure assessment methods. Recent studies similarly employ a variety of exposure assessment methods. In addition to utilizing the same methods used in

studies evaluated in the 2016 Oxides of Nitrogen ISA, some recent studies also assign exposures estimated from hybrid LUR models that incorporate satellite and/or CTM data and hybrid prediction models that combine data from multiple sources, including satellite, monitoring, and dispersion modeling data. Similar to LUR and dispersion models, these exposure assessment methods are used to create spatially refined exposure estimates that are assigned at the residential level. Positive associations between long-term NO_2 exposure and respiratory mortality were observed in studies using each of the aforementioned exposure assessment techniques. A few recent studies compare exposure assessment methods by assigning exposures using multiple methods to evaluate how the monitored or modeled exposure estimates influence results.

In the ELAPSE study, Brunekreef et al. (2021) assigned exposures using estimates from a Europewide hybrid LUR model (ELAPSE LUR model). Because the Europewide model may be limited in its ability to capture more localized small-scale spatial variation (Klompmaker et al., 2021a), Brunekreef et al. (2021) conducted a sensitivity analysis comparing results from the main exposure model to results estimated from localized ESCAPE LUR models for each site in the pooled cohort. Respiratory mortality was positively associated with long-term NO_2 concentrations estimated from the ELAPSE LUR model (HR: 1.144 per 10-ppb increase [95% CI: 0.958, 1.366]) and the cohort-specific ESCAPE LUR models (HR: 1.104 [95% CI: 0.958, 1.272]). Results were variable across the administrative cohorts. In most cases associations remained positive.

A recent study analyzing data from a national Dutch administrative cohort similarly compared associations between respiratory mortality and long-term NO_2 exposures estimated from ESCAPE and ELAPSE LUR models as well as from a national dispersion model (Klompmaker et al., 2021a). The authors observed comparable increases in respiratory mortality associated with a 10-ppb increase in NO_2 concentrations modeled using the ESCAPE LUR model (HR: 1.073 [95% CI: 1.028, 1.119]) and the dispersion model (HR: 1.069 [95% CI: 1.028, 1.111]). In contrast, Klompmaker et al. (2021a) reported that respiratory mortality was not associated with NO_2 concentrations estimated using the ESCAPE LUR model (HR: 0.992 [95% CI: 0.949, 1.038]). While the differences in the observed associations could be explained by model-specific differences in exposure measurement error, it is difficult to directly compare model performance because the LUR models use different validation methods.

Exposure Windows

Many recent studies use time-varying exposure estimates to ensure that assigned NO_2 concentrations correspond to the time-period in which participants are at risk of the given health outcome, in this case respiratory mortality. Recent studies have assigned time-varying NO_2 concentrations across a range of averaging times, including 1-year moving averages (Eum et al., 2022; Eum et al., 2019), 3-year moving averages (So et al., 2020; Weichenthal et al., 2017b), 5-year moving averages (Zhang et al., 2021b; Raaschou-Nielsen et al., 2020), 7-year moving averages (Crouse et al., 2015), and 15-year moving averages (Hvidtfeldt et al., 2019). Other studies utilize estimates from exposure models that do not span the entirety of a cohort's follow-up period and therefore assign fixed single- or multi-year average

concentrations from before (Klompmaker et al., 2021b; Klompmaker et al., 2020), during (Bouma et al., 2023; So et al., 2022; Brunekreef et al., 2021; Klompmaker et al., 2021a; Lim et al., 2019), or even after follow-up (Gariazzo et al., 2021; Turner et al., 2016). Temporally misaligned exposure data could result in exposure measurement error since exposures that are assigned after (or well before) an outcome occurs may not be representative of exposures earlier (or much later) in the study period. Studies that utilize temporally misaligned exposure data rely on the assumption that spatial contrasts of NO_2 concentrations remain relatively constant over time (Brunekreef et al., 2021), which would preserve the relationship between NO_2 exposure and mortality despite using misaligned data. de Hoogh et al. (2018) evaluated the spatiotemporal stability of the ELAPSE LUR model used in a few recent studies (So et al., 2022; Brunekreef et al., 2021; Klompmaker et al., 2021a; Klompmaker et al., 2021b) and observed strong correlations between the 2010 model and modeled estimates for 2005 ($R^2 = 0.92$) and 2000 ($R^2 = 0.91$). As a sensitivity analysis, So et al. (2022) assigned baseline and time-varying exposures by back- and forward-extrapolating the ELAPSE LUR model estimates using a low resolution (26 km x 26 km) exposure model that provides monthly average concentrations dating back to 1990. The authors analyzed Danish national administrative cohort data from 2000 until 2017 and reported comparable positive associations between respiratory mortality and NO_2 concentrations assigned using the primary exposure model (i.e., 2010 average), back-extrapolated baseline exposure (i.e., 2000 average), and back- and forward-extrapolated time-varying exposure estimates that covered the entire study period.

4.8.6 Conclusions and Remaining Uncertainties

A large number of recent epidemiologic studies provide generally consistent evidence of a positive association between long-term NO_2 exposure and respiratory mortality. Whereas evidence presented in the 2016 Oxides of Nitrogen ISA was somewhat inconsistent, and null associations were observed in several U.S. cohorts, recent studies reported NO_2-related increases in respiratory mortality across a range of geographic locations, including the United States, Canada, and Europe. Considering the null associations observed in U.S. studies evaluated in the 2016 Oxides of Nitrogen ISA, the overall evidence is more consistent across European cohort studies compared to U.S. studies. Across all geographic locations, associations were observed in both traditional and administrative cohort studies with varying levels of control for a range of potential individual- and area-level confounders, including demographics, socioeconomic status, health status, and health behaviors. Additionally, studies used a variety of exposure assessment methods, including central site monitors, LUR, dispersion models, and hybrid exposure models, and reported comparable magnitudes of associations. In a subset of studies that examined mortality due to specific respiratory diseases, increases long-term NO_2 exposure were consistently associated with increases in ALRI and COPD mortality.

The recent evidence base also includes expanded evaluation of the C-R relationship between long-term NO_2 exposure and respiratory mortality. The shape of the C-R relationship was inconsistent across several studies that used flexible modeling terms to investigate potential non-linearities. C-R

curves from analyses across several cohorts contrastingly indicated approximately linear, supralinear, sub-linear, or linear-threshold relationships. It's unclear from the available data whether these differences were the result of differences in the underlying populations, methodology, exposure distributions, or other factors.

A number of recent studies evaluated potential effect modification by several factors, including sex, age, behavioral factors, SES, and race/ethnicity. Consistent with limited evidence from the 2016 Oxides of Nitrogen ISA, several recent studies provide further evidence that NO_2 associations with respiratory mortality are larger in magnitude among females compared to males. Although the evidence is not as consistent as for modification by sex, studies that stratified by age generally reported stronger associations in older adults (e.g., 75+ years) compared to younger adults. Results for other factors were either inconsistent or limited to single studies. Further evaluation of at-risk factors in the context of all respiratory effects is discussed in Section 4.10.

A notable remaining uncertainty in the epidemiologic evidence base is the potential for copollutant confounding. Several studies reported that associations between long-term NO_2 exposure and respiratory mortality remained relatively unchanged in copollutant models adjusting for $PM_{2.5}$ or O_3 (i.e., similar in magnitude or attenuated slightly, but still positive). In contrast, results for BC were much less consistent, as the NO_2-respiratory mortality associations observed in several cohorts were attenuated and no longer positive in copollutant models. Evaluation of potential copollutant confounding by other traffic-related pollutants remains limited, and high correlations between NO_2 and the examined copollutants complicates the interpretation of the available results.

4.9 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 long-term NO exposure on the respiratory 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 may induce inflammation. Several plausible pathways exist by which long-term exposure to NO_2 can result in the respiratory effects seen in epidemiology studies (see Figure 4-21). The structure of the biological plausibility figures is described in IS.4.1.1.

The first pathway by which long-term NO_2 exposure can result in respiratory effects is through the induction of inflammation and oxidative stress. Studies reviewed in the 2008 and 2016 Oxides of Nitrogen ISAs and previous criteria documents, as well as more recent studies, show increased histologic markers of inflammation and oxidative stress in animals following long-term exposure to NO_2 (see

Section 4.3.1.3). Chronic or repetitive inflammation can lead to lung damage and subsequent remodeling that can contribute to lung disease onset and progression. For example, damage to the alveolar epithelium can lead to loss of alveolar septa and increased alveolar airspace typical of emphysema, a common feature of COPD. In addition, tissue damage and subsequent repair in the airways can lead to extracellular matrix remodeling around the airway, which can lead to airway narrowing and correlates with asthma severity. Indeed, studies have shown that histologic markers of inflammation that result from long-term exposure to NO_2 are accompanied by increases in signs of tissue damage, increased airspace enlargement, histologic signs of airway remodeling (Lu et al., 2023b; Lu et al., 2023a; Yue et al., 2018; Han et al., 2017; Yue et al., 2017; Blair et al., 1969). Pulmonary inflammation can also result in excess mucus production which can impact those with airway obstructive disease like COPD or asthma. Evidence from animal toxicological studies of long-term NO_2 exposure supports the exposure-driven enhancement of mucus production both with and without allergen sensitization although the effects appear more robust in models of allergen sensitization (Lu et al., 2023b; Lu et al., 2023a; Yue et al., 2018; Han et al., 2017).

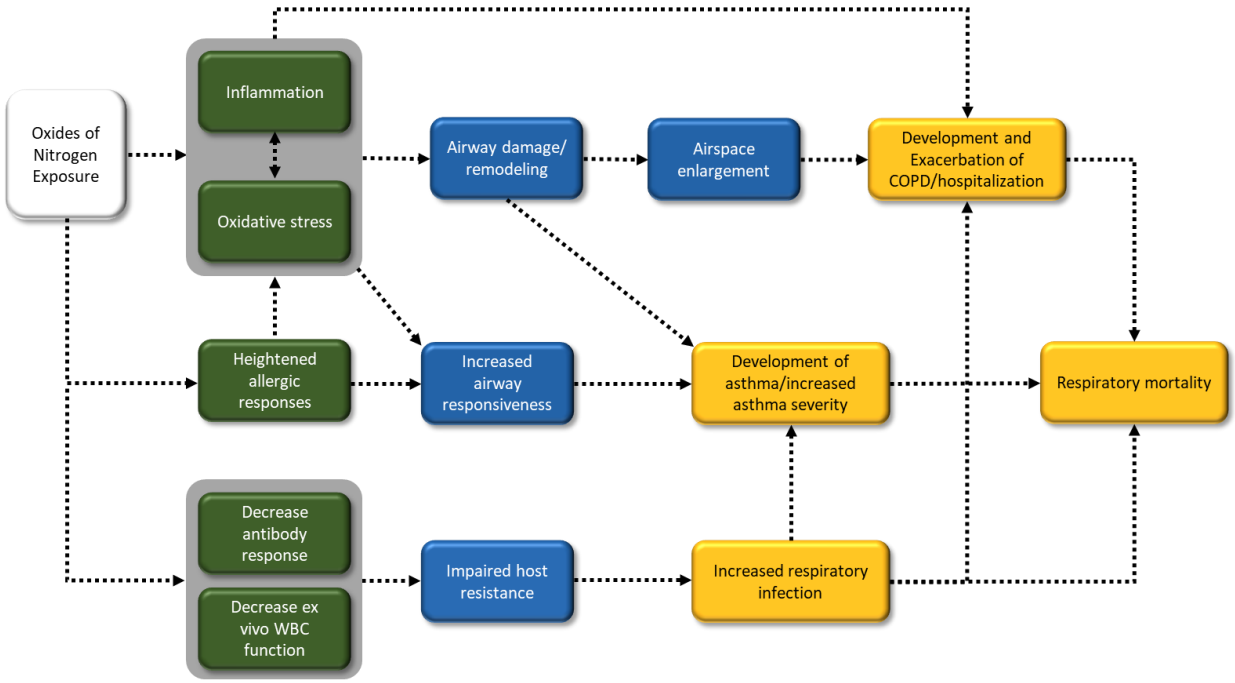
Chronic airway inflammation can cause irritation of the airways and structural changes in airway walls that enhance the contractile response of the smooth muscle to bronchoconstrictive stimuli, resulting in increased airway resistance (Cockcroft and Davis, 2006). Increased reactivity of airways, leading to increased airway resistance, is an underlying feature of asthma and is partly responsible for asthma-related respiratory symptoms and, when not properly controlled, asthma-related mortality. Experimental studies of long-term NO_2 exposure have shown increased airway resistance concurrent with increased markers of inflammation and inflammatory signaling in the lung (Lu et al., 2024; Lu et al., 2023b; Lu et al., 2023a; Yue et al., 2018).

The second pathway that can potentially explain respiratory effects in response to long-term NO_2 exposure is the augmentation of allergic responses. Animal models use sensitization and subsequent challenge with a foreign antigen, like OVA, to induce an asthma-like phenotype including production of allergen-specific antibodies, increased inflammation, and a shift towards production of pro – allergic (Th-2) signals. Increases in these endpoints can explain the increases in airway reactivity that are seen in animal models. Indeed, studies of long-term NO_2 exposure have shown an increase in inflammatory and allergic processes including increases in pro Th-2 inflammatory mediators in conjunction with suppression of Th-1 inflammatory markers (Lu et al., 2024; Lu et al., 2023b; Lu et al., 2023a; Yue et al., 2018; Han et al., 2017; Yue et al., 2017). These changes also correspond to increases in measures of airway reactivity to a bronchoconstricting agent (Lu et al., 2024; Lu et al., 2023b; Lu et al., 2023a; Yue et al., 2018; Yue et al., 2017). Heightened allergic responses and subsequent airway reactivity changes can plausibly contribute to the NO_2-associated increases in the development of asthma and respiratory mortality that have been reported in epidemiology studies.

The third pathway from long-term NO_2 exposure to respiratory effects is mediated through changes in host defense. As discussed in Section 4.5.3, previously reviewed studies have shown that long-term NO_2 exposure resulted in reduced bacterial clearance (Henry et al., 1970; Ehrlich and Henry, 1968),

suggesting that NO_2 exposure over time impairs the ability of the host to deal with infection. In addition, animals exposed to long-term NO_2 showed increased mortality in response to bacterial infection (Miller et al., 1987; Ehrlich, 1980; Henry et al., 1970; Ehrlich and Henry, 1968). A similar finding of increased mortality after long-term NO_2 exposure was seen in response to viral infection (Henry et al., 1970). Respiratory infection can cause exacerbation of underlying lung conditions; thus, it is also plausible that the immunosuppressive effects of NO_2 in response to pathogens could act indirectly to lead to worsening of COPD or asthma in affected individuals, potentially resulting in serious illness or death.

Together, there is evidence of several pathways that could result in the respiratory morbidity and mortality seen in epidemiology studies. This includes through the induction of inflammation and oxidative stress, heightening of allergic responses, and suppression of host resistance to pathogen infection.



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 Oxides of Nitrogen ISA are discussed in Section 4.11.

Figure 4-21 Potential biological plausibility pathways for respiratory effects associated with long-term exposure to oxides of nitrogen.

4.10 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,[5] extrinsic,[6] 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, 2015) 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 exposure to oxides of nitrogen.

The objective of this section is to integrate information in preceding sections and describe the basis for characterizing the evidence for particular factors to increase risk of oxides of nitrogen-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.[7] Consistent with the characterization of evidence for

[5]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.
[6]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., race, SES, educational attainment) are surrogates for the combined impact of several intrinsic, extrinsic, and exposure-related factors.
[7]Although the at-risk classifications are not direct assessments of causality, evidence for at-risk groups or factors also inform causality determinations in several ways. Consistent evidence that at least one population subgroup is at risk of an oxides of nitrogen-related health effect provides strong support for causality determinations. Evidence for effect 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 oxides of nitrogen-related health effects. Notably, the lack of

health effects in the general population, the greatest emphasis is placed on patterns in results across studies. For oxides of nitrogen, judgments about factors that may place some groups at increased risk emphasize evidence for asthma development, given the strong evidence for that outcome. The factors evaluated are discussed in the ensuing Section. Table 4-1 at the end of this Section summarizes the evidence for each factor to increase risk of oxides of nitrogen-related health effects.

4.10.1 Sociodemographic Factors

4.10.1.1 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 oxides of nitrogen across lifestages could be due to several factors. With regard to children, the human respiratory system is not fully developed until 18 to 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 4.10.1.1.1) and older adults (see Section 4.10.1.1.2) being at increased risk of NO_2-related respiratory effects.

4.10.1.1.1 Children

A large body of epidemiologic evidence– including recent studies, as well as studies evaluated in the 2016 Oxides of Nitrogen ISA– supports an association between long-term NO_2 exposure and asthma incidence in children (see Section 4.3.1; Figure 4-1). Consistent evidence is provided by traditional recruitment-based and administrative cohort studies using a range of exposure assessment methods. Recent studies adjusted for a wide range of potential confounders using individual-level and area-level covariates (see Section 4.3.1.1.3) and reported consistent positive associations across varying levels of adjustment. Experimental studies in animals generally indicate an increase in asthma-related markers, including airway reactivity, inflammation, lung damage, and airway histologic changes in response to allergen challenge following prolonged NO_2 exposure. These changes are seen in models of gestational exposure to NO_2 where the offspring animals are not exposed after birth. Specifically, these studies support the biological plausibility of associations between long-term NO_2 exposure and asthma

evidence for differential effects in subgroups where there is otherwise evidence of an oxides of nitrogen-related health effect in the general population does not weaken the overall evidence supporting a causality determination.

development and indicate that early-life NO_2 exposure can cause structural and functional changes that could potentially contribute to airway obstruction and increased airway responsiveness. Therefore, the evidence is adequate to conclude that children are at increased risk for respiratory effects of long-term NO_2 exposure.

4.10.1.1.2 Older Adults

To date, there are a small number of studies that have examined effect modification by older age groups in studies of long-term NO_2 exposure and respiratory effects. A few recent, large administrative cohort studies in the United States (Eum et al., 2022) and Denmark (Raaschou-Nielsen et al., 2020) observed stronger associations between long-term NO_2 exposure and respiratory mortality in older age groups (see Table 4-14). In contrast, a smaller prospective cohort study in Ontario reported a larger increase in NO_2-associated respiratory mortality among adults younger than 65 years compared to those 65 years and older (Zhang et al., 2021b). However, the Ontario study was likely underpowered to estimate differences in age-stratified associations given the small number of deaths observed in the younger age group. For other health outcomes, results were either inconsistent or too limited to draw conclusions. A few studies of long-term NO_2 exposure and COPD incidence provided inconsistent evidence of effect modification by age (Shin et al., 2021; Doiron et al., 2019; Weichenthal et al., 2017a), and a UK Biobank study observed a stronger association between long-term NO_2 exposure and pneumonia incidence in participants 65 years and older compared to participants under 65 years (Wang et al., 2023a).

In summary, there is some evidence that older adults may be at increased risk of NO_2-related respiratory mortality. However, there are a limited number of studies and uncertainty as to whether NO_2 exposure has an independent effect on respiratory mortality. Therefore, the evidence is suggestive that older adults are at increased risk for respiratory effects of long-term NO_2 exposure.

4.10.1.2 Sex

A vast number of health conditions and diseases have been shown to differ by sex, with some indication that there may be differences by sex in the relationship between air pollution and health effects. The 2010 U.S. census indicates an approximately equal distribution of males and females in the U.S.: 49.2% male and 50.8% female (Howden and Meyer, 2011). However, the distribution varies by age with a greater prevalence of females above 65 years of age compared to males. 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 limited number of studies that were evaluated in the 2016 Oxides of Nitrogen ISA examined differences in the association between long-term NO_2 exposure and asthma incidence in children based on sex. Evidence was inconsistent, with one study each indicating increased risk for females (Clark et al., 2010), or increased risk for males (Carlsten et al., 2011b). While neither of the studies indicated that comparisons were planned a priori, the proportion of sexes was approximately equal, providing fairly large numbers of males and females to compare. Recent studies were similarly inconsistent. While a pooled analysis of longitudinal birth cohorts in the United States reported stronger associations between long-term average NO_2 concentrations and asthma incidence by age 11 in males relative to females, no sex-specific difference in the magnitude of associations was observed with asthma incidence in the first four years of life (Zanobetti et al., 2024). Notably, asthma diagnosis can be unreliable in the first four years of life, which may have contributed to differential results. Similarly, a large administrative birth cohort in Ontario, Canada did not observe any sex-specific differences in the strength of the association between annual average NO_2 concentrations and asthma incidence by age six (Lavigne et al., 2018).

A limited number of recent studies of asthma onset in adults similarly provide inconsistent evidence of effect modification by sex. A nationwide, population-based administrative cohort study of 35+ year-old adults in Italy demonstrated a positive but imprecise association between long-term NO_2 exposure and asthma incidence in males, but not females (Gandini et al., 2018a). In contrast, Weichenthal et al. (2017a) reported identical magnitudes of increased risk of adult-onset asthma in both males and females associated with long-term average NO_2 concentrations in a large administrative cohort study in Toronto, Canada.

Evidence of effect modification by sex was also equivocal for associations between long-term NO_2 exposure and COPD incidence (see Section 4.4.1.2.2) or respiratory infection (see Section 4.5.1). The most consistent evidence for any respiratory outcome comes from a few studies, including large, national administrative cohorts, that observed stronger associations between long-term NO_2 exposure and respiratory mortality in females relative to males (Eum et al., 2022; Zhang et al., 2021b; Raaschou-Nielsen et al., 2020; Katanoda et al., 2011).

Collectively, the evidence for sex-specific differences in NO_2-related risk of respiratory effects is inconsistent. Differences between males and females are not consistently observed for associations of long-term NO_2 exposure with asthma incidence, the respiratory effect for which evidence most strongly indicates independent relationships with long-term NO_2 exposure (see Section 4.3.3). In contrast, the risk of respiratory mortality related to long-term NO_2 exposure is consistently increased 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 long-term NO_2 exposure.

4.10.1.3 Race/Ethnicity

There has been limited evaluation of effect modification by race/ethnicity in studies of long-term NO_2 exposure and respiratory effects. A limited number of recent studies reported increased risk of NO_2-related asthma incidence (Zanobetti et al., 2024) and respiratory mortality (Eum et al., 2022) among non-white and Hispanic participants relative to white participants. Given the limited evidence and different outcomes examined, the evidence is inadequate to determine whether certain racial or ethnic groups are at increased risk for respiratory effects of long-term NO_2 exposure.

4.10.1.4 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. Persons with lower SES generally have been found to have a higher prevalence of pre-existing diseases, less access to resources such as healthcare, and possibly increased nutritional deficiencies. Neighborhoods comprising low SES populations are characterized as having fewer resources and more psychosocial stressors and air pollution sources (Diez Roux and Mair, 2010; O'Neill et al., 2003). Thus, community-level SES may influence health. Given the variety of indicators that are used to assess SES, it can be difficult to synthesize results across studies.

Of the limited number of respiratory effects studies that evaluated potential effect modification by SES or social stressors in the 2016 Oxides of Nitrogen ISA, none examined long-term exposure to NO_2. A few recent studies provide generally consistent evidence for increased risk of long-term NO_2 exposure on respiratory effects in low SES populations relative to higher SES populations. The strongest evidence for SES-modified associations between long-term NO_2 exposure and respiratory effects comes from a few studies of asthma incidence in children and adults. A recent analysis of pooled U.S. cohort data indicated that long-term NO_2 exposure was associated with a greater risk of asthma incidence for children whose mothers did not finish high school compared to those whose mothers had higher educational attainment (Zanobetti et al., 2024). The authors similarly observed a greater risk of asthma incidence among children living in areas that scored lower in the educational domain of the Childhood Opportunity Index (COI). Consistent with limited evidence for children, a recent pooled European cohort study reported that the risk of asthma onset in adults was modified by educational attainment, with a greater risk observed in adults with lower educational attainment (Liu et al., 2021a).

A few recent studies of respiratory mortality provided inconsistent evidence of SES-related modification of associations with long-term NO_2 exposure. Across cohort studies in Denmark (Raaschou-Nielsen et al., 2020), Ontario (Zhang et al., 2021b), and the United Kingdom (Tao et al., 2024), there was evidence of higher NO_2-related mortality risk among participants living in areas with high neighborhood-level income (Raaschou-Nielsen et al., 2020), no difference in risk across individual-level household income quintiles (Zhang et al., 2021b), and increased risk in disadvantaged neighborhoods relative to

advantaged neighborhoods (Tao et al., 2024). Together, the limited number of studies, diversity of individual- and community-level SES indicators examined across studies, array of countries where studies were conducted, and uncertainty in the independent effect of NO_2 on respiratory mortality limit the strength of the conclusions that can be drawn. Given the limited but consistent evidence for SES-modified associations with asthma development, the evidence is suggestive that low SES populations are at increased risk for respiratory effects of long-term NO_2 exposure.

Table 4-1 Summary of evidence for increased risk of respiratory effects from oxides of nitrogen exposure

	Key Evidence	Key References
Adequate evidence		
Children	A large body of epidemiologic evidence supports an association between long-term NO_2 exposure and asthma incidence in children. Experimental studies in animals indicate that gestational NO_2 exposure can cause changes in markers of a pro-asthmatic phenotype that could promote the development of asthma in humans.	Section 4.3.1
Suggestive evidence		
Older adults	Limited but generally consistent evidence that older adults may be at increased risk of NO_2-related respiratory mortality. Uncertainty as to whether long-term NO_2 exposure has an independent effect on respiratory mortality	†Eum et al. (2022) †Raaschou-Nielsen et al. (2020)
Females	Inconsistent epidemiologic evidence of sex-specific differences in NO_2-related asthma incidence, but consistently stronger NO_2-respiratory mortality associations observed among females	Katanoda et al. (2011) †Eum et al. (2022) †Zhang et al. (2021b) †Raaschou-Nielsen et al. (2020)
Low socioeconomic status	Limited but consistent evidence of a stronger association between long-term NO_2 exposure and asthma incidence among individuals with lower educational attainment	†Zanobetti et al. (2024) †Liu et al. (2021a)
Inadequate evidence		
Race/Ethnicity	Limited evidence across outcomes	†Zanobetti et al. (2024) †Eum et al. (2022)

List of abbreviations in alphabetical order: NO_2 = nitrogen dioxide.
†Studies published since the 2016 Oxides of Nitrogen ISA.

4.11 Summary and Causality Determination

The 2016 Oxides of Nitrogen ISA concluded that there is "likely to be a causal relationship" between long-term exposure to NO_2 and respiratory effects. This conclusion was based primarily on evidence for asthma development. Specifically, epidemiologic studies consistently observed increases in asthma incidence in children associated with NO_2 exposures estimated at or near children's homes or schools. Potential confounding by copollutants of greatest concern– $PM_{2.5}$ and traffic-related copollutants– was largely unexamined, contributing uncertainty regarding the independent nature of the observed associations. However, experimental evidence indicating AHR induced by long-term NO_2 exposure and development of an allergic phenotype with continuous or repeated NO_2 exposures over weeks to months provided coherence with the epidemiologic evidence of associations between long-term NO_2 exposure and increased incidence in asthma in children and supported biological plausibility for independent effects of long-term NO_2 exposure on asthma development. Due to the limited number of experimental studies, however, some uncertainty regarding the independent effect of NO_2 and the strength of the biological plausibility remained, which limited the strength of the causality determination. Other lines of evidence were less robust. Longitudinal epidemiologic studies reported associations between long-term NO_2 exposure and decrements in lung function and lung development in children, but the findings were not clearly supported by animal toxicological studies.

Epidemiologic studies published since the 2016 Oxides of Nitrogen ISA provide strong additional support for an association between long-term NO_2 exposure and asthma incidence in children. Evidence from cohort studies in the United States, Canada, and Europe is generally consistent across a variety of study designs that utilize a range of exposure assessment methods, including LUR models, dispersion models, hybrid prediction models, and fixed-site monitors (see Section 4.3.1.1.5). Consistent associations between long-term NO_2 exposure and asthma incidence in children were reported in large administrative cohorts with tens of thousands of incident asthma cases (Pedersen et al., 2023; Lavigne et al., 2018; Tétreault et al., 2016b) and smaller, recruitment-based cohorts with more individual-level covariates (Yu et al., 2024; Zanobetti et al., 2024; Garcia et al., 2019). Evidence suggests that long-term exposure to NO_2 during various lifestages or time periods throughout childhood, including prenatal, at-birth, or time-varying exposures, is consistently associated with asthma incidence.

Several recent epidemiologic studies extend the existing evidence base for asthma incidence in adults and similarly report positive associations with long-term exposure to NO_2. Relative to evidence evaluated in the 2016 Oxides of Nitrogen ISA, which assessed the association between NO_2 concentrations estimated from dispersion models and asthma incidence in small subsets of adults from the ECRHS cohort (Castro-Giner et al., 2009; Jacquemin et al., 2009; Modig et al., 2009), recent studies include larger sample sizes, greater geographic diversity, and a greater variety of exposure assessment methods, including LUR and hybrid prediction models. Strong evidence is provided by an administrative cohort study of over one million adults in Toronto, Canada (Weichenthal et al., 2017a) as well as a pooled analysis of three ESCAPE cohorts in Sweden and Denmark (Brunekreef et al., 2021; Liu et al., 2021a).

Notably, positive associations were not observed in all large, administrative cohort studies (Shin et al., 2021). In general, though, positive associations were observed across various averaging times and exposure time periods, including multi-year averages assigned at baseline and moving averages in time-varying regression models. Additionally, a limited number of studies that evaluated the shape of the C-R relationship noted an approximately linear relationship between NO_2 exposure and asthma incidence across the concentration distribution (see Section 4.3.1.2.1).

In studies of asthma incidence in children and adults, the available studies adjusted for a wide range of potential confounders using individual-level and area-level covariates and reported consistent positive associations across varying levels of adjustment (see Sections 4.3.1.1.3 and 4.3.1.2.3). Potential copollutant confounding, particularly by traffic-related pollutants, remains an important uncertainty. Robust associations of NO_2 with asthma incidence in children were observed in a few recent studies that adjusted for modeled near-road pollution or traffic-related particle pollution (Gehring et al., 2020; Lavigne et al., 2018). However, given the likelihood of moderate to high correlations between NO_2 and the modeled copollutants– which were not reported– there is uncertainty concerning the reliability of the copollutant model estimates. Collinearity resulting from high correlations between NO_2 concentrations and potential copollutant confounders is also a key uncertainty in several studies in adults that observed associations between long-term NO_2 exposure and asthma incidence that were relatively unchanged in models adjusted for $PM_{2.5}$, BC, O_3, distance from roadway, and traffic noise (Liu et al., 2021d; Liu et al., 2021a; Weichenthal et al., 2017a).

Recent rodent studies of long-term NO_2 exposures support and extend the evidence for NO_2-driven development of asthmatic phenotype in unsensitized animals. Exposure to approximately 1,000 to 5,000 ppb NO_2 for 27 days showed worsening airway histology and increased expression of mucus and inflammatory mediators (Lu et al., 2023b; Han et al., 2017). The severity of changes in airway histology and inflammatory mediator production showed a dose-dependent relationship (Han et al., 2017). Additionally, recent studies reported that gestational exposure to NO_2 result in heightened inflammation and allergic phenotype in allergen-sensitized offspring (Yue et al., 2018; Yue et al., 2017), which is coherent with epidemiologic studies that observed associations between prenatal NO_2 exposure and asthma incidence in children (Lavigne et al., 2018; Sbihi et al., 2017; Sbihi et al., 2016). Together, the recent studies provide additional consistency in the animal evidence for NO_2-driven development of asthma-relevant effects in unsensitized individuals and provide additional strength to support biological plausibility.

Toxicologic studies evaluated in the 2016 Oxides of Nitrogen ISA found consistent support for worsening allergic responses in sensitized animals following prolonged or repeated NO_2 exposure. Recent studies continue to support previous evidence of worsening asthma phenotype in sensitized animals is supported by recent studies. Several studies showed that animals exposed to long-term NO_2 had increased airway reactivity, heightened allergen-specific antibody response, increased skewing towards Th2

inflammation, and worsening of histologic and cellular measures of inflammation. The consistency in the animal evidence strengthens the biological plausibility of NO_2-associated worsening of existing asthma.

Uncertainty in the epidemiologic evidence base regarding the independent effect of NO_2 exposure is mitigated by evidence from experimental animal studies that demonstrate increases in markers of a pro-asthmatic phenotype, including airway reactivity, inflammation, lung damage, and airway histologic changes in response to prolonged NO_2 exposure. Studies evaluated in the 2016 Oxides of Nitrogen ISA reported increased airway reactivity in guinea pigs following exposure to 2,000 to 4,000 ppb NO_2 for six weeks (Kobayashi and Miura, 1995) and at lower NO_2 exposure concentrations (1,000 ppb to 2,000 ppb) with a longer exposure duration (12 weeks) (Kobayashi and Miura, 1995). Exposure concentrations below 1,000 ppb resulted in respiratory system effects when exposures were extended further, including lung morphologic changes with NO_2 exposures as low as low as 400 ppb for at least 27 months Kubota et al. (1987), increases in markers of oxidative stress with NO_2 exposures as low as 400 ppb for 18 months (Sagai et al., 1984), and signs of epithelial damage following exposure to 500 ppb NO_2 for seven months (Yamamoto and Takahashi, 1984). Experimental studies also indicate that short-term exposure repeated over several days and long-term NO_2 exposure can induce Th2 skewing/allergic sensitization, with observations of increased Th2 cytokines, airway eosinophils, and IgE-mediated responses following NO_2 exposure. Several recent experimental animal studies support and extend the previously limited evidence that repeated exposure to NO_2 alone can increase airway responsiveness, inflammation, and oxidative stress in a variety of non-primate animal species. Recent animal studies of long-term NO_2 exposures show increases in markers of airway inflammation and histologic signs of airway remodeling in unsensitized animals and increases in inflammatory and allergic responses in allergen-sensitized animals. Several studies suggest dose and exposure time-dependent effects on subclinical markers related to asthma development (see Section 4.3.1.3.1). Additionally, recent mouse studies reported that gestational exposure to NO_2 results in heightened inflammation and allergic phenotype in allergen-sensitized offspring (Yue et al., 2018; Yue et al., 2017), which is coherent with epidemiologic studies that observed associations between prenatal NO_2 exposure and asthma incidence in children (Lavigne et al., 2018; Sbihi et al., 2017; Sbihi et al., 2016). Findings for short-term NO_2 exposure also support an effect on asthma development by describing a potential role for repeated exposures to result in recurrent inflammation and allergic responses. Evidence from controlled human exposure studies shows that repeated NO_2 exposure increases neutrophils in healthy adults, and epidemiologic evidence also points to associations between ambient NO_2 concentrations and increases in pulmonary inflammation in healthy children and adults (see Chapter 3, Section 3.6.4).

Evidence for other respiratory effects– including lung function and development, development of COPD, respiratory infection, and respiratory mortality– is more uncertain due to a lack of coherence across epidemiologic and experimental studies. While several longitudinal epidemiologic studies provide generally consistent evidence of an association between long-term NO_2 exposure and reductions in lung development in children and young adults (Yu et al., 2023b; Zhang et al., 2021a; Ierodiakonou et al., 2015; Schultz et al., 2015; Gauderman et al., 2007; Gauderman et al., 2004), potential confounding of

long-term NO_2 exposure-related reductions in lung development by traffic-related copollutants has not been evaluated, and there is lack of coherence with toxicological studies, which do not provide strong evidence of NO_2-induced changes in lung function in animal models (see Section 4.6.2). Recent epidemiologic studies expand on limited and inconsistent evidence from the 2016 Oxides of Nitrogen ISA and, in contrast, provide generally consistent evidence of an association between long-term NO_2 exposure and COPD incidence in adults. Although several recent studies reported associations between long-term NO_2 exposure and COPD incidence that were robust to adjustment for long-term exposure to BC, high correlations add uncertainty to the observed results and potential confounding by other traffic-related copollutants was not thoroughly evaluated. Additionally, although animal toxicological studies provide potential biological plausibility for NO_2-induced development of COPD, the high concentrations necessary to induce alveolar airspace enlargement in the available experimental animal studies may limit the relevance to human responses at relevant NO_2 concentrations.

Limited but consistent animal toxicological evidence indicates impaired host resistance to bacterial and viral infection following long-term exposures to PECOS-relevant NO_2 concentrations in monkeys and mice. Exposure of mice to 200 ppb NO_2 with intermittent spikes of 800 ppb NO_2 for 16 to 52 weeks resulted in increased infectivity and mortality from a bacterial challenge which was not seen a parallel group exposed to 200 ppb NO_2 with no spikes (Miller et al., 1987). In squirrel monkeys, exposure to 5,000 ppb NO_2 for two months resulted in reduced clearance of bacteria from the lungs and increased mortality when given an inhaled bacterial challenge (Henry et al., 1970). Host resistance assays used in the available literature are considered strong indicators of immunotoxicity in humans (IPCS, 2012). Epidemiologic evidence, however, provides limited coherence. While a small number of studies reported evidence of an association between long-term NO_2 exposure and respiratory infections in infants or young children (e.g., pneumonia, otitis media, or bronchiolitis), results were mostly inconsistent (see Section 4.5.1). There were similarly inconsistent results for long-term NO_2 exposure and pneumonia incidence in adults within and across a limited number of studies. In a U.S. Medicare study that provides strong inference due to its representative study population, large sample size, and use of IPW to adjust for potential confounding, long-term NO_2 concentrations were not associated with pneumonia incidence across the entire concentration distribution, though associations were observed at low concentrations [(i.e., <20 ppb); (Danesh Yazdi et al., 2021)]. Additionally, while other studies of pneumonia incidence in adults reported positive associations with long-term NO_2 exposure (Wang et al., 2023a; Neupane et al., 2010), the entirety of the evidence for pneumonia incidence in adults comes from studies examining hospital admissions and does not address previous uncertainties regarding the role of short-term NO_2 exposures.

Epidemiologic evidence generally supports an increase in respiratory mortality associated with long-term NO_2 exposure, but important uncertainties concerning the nature of the relationship remain. In particular, it remains challenging to disentangle the potential independent effect of NO_2 on respiratory mortality from the effect of the traffic-related pollution mixture or components of that mixture. A large number of recent studies included copollutant models adjusting for $PM_{2.5}$, BC, or O_3 (see Figure 4-19 and

Figure 4-20). Several studies reported that associations between long-term NO_2 exposure and respiratory mortality were attenuated to the null after adjustment for BC. However, correlations between NO_2 and BC were high, which may produce unstable effect estimates and limits the confidence in copollutant model results. Additionally, there is a lack of studies controlling for other potentially important traffic-related copollutants such as UFPs and EC. There is also uncertainty in the continuum of effects that could lead from long-term NO_2 exposure to mortality. Specifically, there is not clear evidence that long-term NO_2 exposure has an independent effect on the respiratory morbidity outcomes that are major underlying causes of mortality, such as COPD or respiratory infection (see Sections 4.4 and 4.5).

Overall, there is sufficient evidence to conclude that there is a *causal relationship* between long-term oxides of nitrogen exposure and respiratory effects. Support for a causal relationship is based strongly on evidence integrated across disciplines for a relationship between long-term exposure to NO_2 and asthma development. Epidemiologic cohort studies consistently indicate a positive association between asthma incidence in children and long-term NO_2 exposures estimated at or near children's homes or schools. Epidemiologic evidence is generally consistent across a mix of study designs that utilize a range of exposure assessment methods. Available studies adjusted for a wide range of potential confounders and reported consistent positive associations across varying levels of adjustment. The consistent pattern of positive associations across various study designs and methods minimizes the likelihood that results are explained by chance, confounding, or bias due to exposure measurement error. While the available evidence evaluating potential copollutant confounding has expanded since the 2016 Oxides of Nitrogen ISA, potential confounding by traffic-related pollutants remains an uncertainty due to the limited evaluation of some traffic pollutants as well as the statistical limitations of mutual adjustment for highly correlated variables. However, expanded animal toxicological evidence provides further support for NO_2-induced pulmonary inflammation, allergic sensitization, and subsequent development of airway hyperresponsiveness. The demonstrated impacts on these markers of a pro-asthmatic phenotype are coherent with the epidemiologic evidence and support biological plausibility for an independent effect of NO_2 on asthma development in humans. Together with the full body of epidemiologic evidence, the expanded support from experimental animal studies strengthens support for an effect of long-term NO_2 exposure on asthma development independent of other traffic-related pollutants. The evidence for the relationship between long-term exposure to NO_2 and respiratory effects is summarized in Table 4-2, using the framework for causality determinations described in Section 3.3.4 of Volume 2 of the Integrated Review Plan for the Primary National Ambient Air Quality Standards for Oxides of Nitrogen (U.S. EPA, 2024).

Table 4-2 Summary of evidence indicating a causal relationship between long-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 Development			
Generally consistent epidemiologic evidence from multiple, high-quality studies with relevant NO_2 concentrations	Generally consistent evidence for increases in asthma incidence in diverse cohorts of children in U.S., Canada, and Europe. Asthma ascertainment by parental report of doctor diagnosis or first use of medical services.	†Garcia et al. (2019) †Zanobetti et al. (2024) Clark et al. (2010) Gehring et al. (2010) Jerrett et al. (2008) †Pedersen et al. (2023) †Lavigne et al. (2018) †Yu et al. (2024) Section 4.3.1.1	Means across studies: 5.4–21.2 ppb
	For adults, the epidemiologic evidence is more limited, but generally consistent positive associations are also observed between long-term NO_2 exposure and asthma onset.	†Weichenthal et al. (2017a) †Brunekreef et al. (2021) †Liu et al. (2021d) †Zhu et al. (2023)	Means across studies: 6.8–21.4 ppb
Generally consistent epidemiologic evidence for positive associations between asthma incidence and NO_2 exposure estimated using a variety of methods	Positive associations consistently observed across studies that used LUR models, dispersion models, hybrid prediction models, and fixed-site monitors	Section 4.3.1.1.5	
Uncertainty regarding potential confounding by $PM_{2.5}$ or traffic-related copollutants in epidemiologic studies	Limited evidence that associations between long-term NO_2 exposure and asthma incidence were robust to adjustment for modeled near-road pollution or traffic-related particle pollution. High correlations limit confidence in copollutant model results. Uncertainties reduced by support for an independent effect in animal studies.	Section 4.3.1.1.2 Section 4.3.1.2.2	

Rationale for Causality Determination[a]	Key Evidence[b]	Key References	Nitrogen Dioxide Concentrations Associated with Effects
Consistent animal evidence of NO_2-induced subclinical markers of asthma development in unsensitized animals	Long-term NO_2 exposures increase markers of airway inflammation and result in histologic signs of airway remodeling in unsensitized animals. Effects generally show a dose response with worsening effects at higher exposure concentrations or with lower concentrations for longer periods of time.	Section 4.3.1.3 †Han et al. (2017) Kobayashi and Miura (1995) Kubota et al. (1987) Yamamoto and Takahashi (1984) Sagai et al. (1984)	• Rat airway reactivity and inflammation: 1 ppm or 2.6 ppm for 5 hr/d for 28 days • Airway relativity in guinea pigs: continuous 2 or 4 ppm for 6 wk or 1–4 ppm for 12 wk • Rat lung cellular morphology: continuous 4ppm NO_2 for 9–27 mo or 0.4 ppm NO_2 for 18–27 mo • Rat lung ciliated cell changes: swelling – 0.5 ppm for 7 mo with loss of cilia at 1 and 4 ppm for 7 mo • Rat lung lipid peroxidation: 4 ppm for 9 mo or 0.4 and 4 ppm at 18 mo
NO_2 exposure enhances subclinical markers of asthma-like phenotype in models of allergen sensitization.	Long-term NO_2 exposures increase inflammatory and allergic responses in allergen-sensitized animals and corresponds to increased airway reactivity.	Section 4.3.2.2	
	Gestational exposure to NO_2 results in heightened inflammation and allergic phenotype in allergen-sensitized offspring.	Section 4.3.2.2 †Yue et al. (2018) †Yue et al. (2017)	Gestational exposures: 2.5 ppm 5 hr/d thought gestation (approximately 21 days)
Lung Function and Development			
Generally consistent epidemiologic evidence from multiple, high-quality studies with relevant NO_2 concentrations	Epidemiologic evidence for decrements in lung function reduced lung function development in children.	Gauderman et al. (2004) Gauderman et al. (2007) †Ierodiakonou et al. (2015) †Zhang et al. (2021a) Section 4.6.1	Range of means across studies: 11.9–42.6 ppb

Rationale for Causality Determination[a]	Key Evidence[b]	Key References	Nitrogen Dioxide Concentrations Associated with Effects
Uncertainty regarding potential copollutant confounding.	Evidence from cross-sectional studies of lung function in children indicate associations with long-term NO_2 exposure persist in models adjusting for $PM_{2.5}$ but not UFPs. High correlations limit confidence in copollutant model results.	Section 4.6.1.3	
Limited coherence from animal toxicological studies	Animal toxicological evidence for long-term NO_2 exposure and impacts on lung function remains limited and inconsistent.	Section 4.6.2	
Respiratory Infection			
Consistent evidence from animal toxicological studies at relevant NO_2 concentrations	Animal toxicological evidence suggests that host resistance to bacterial and viral infection is compromised following long-term exposures to PECOS-relevant NO_2 concentrations in monkeys and mice	Henry et al. (1970) Ehrlich and Henry (1968) Ehrlich (1980) Miller et al. (1987)	500 ppb for 3 mo, 5,000 ppb for 2 mo, 200 ppb base plus daily spike of 800 ppb for 16−52 weeks
Limited and inconsistent epidemiologic evidence	Some evidence of an association between long-term NO_2 exposure and respiratory infections in infants or young children (e.g., pneumonia, otitis media, or bronchiolitis), though results were mostly inconsistent.	Section 4.5.1	Range of means across studies: 7.2–14.2 ppb
	Inconsistent results for long-term NO_2 exposure and pneumonia incidence in adults within and across a limited number of studies. Limited but generally consistent evidence for aggregated respiratory infections.	†Danesh Yazdi et al. (2021) Section 4.5.1	Range of means across studies: 7.2–20.1 ppb
Development of Chronic Obstructive Pulmonary Disease			
Generally consistent epidemiologic evidence from multiple, high-quality studies with relevant NO_2 concentrations	Generally consistent evidence for COPD incidence. Positive associations were observed in relation to exposures assigned using LUR, dispersion, and hybrid prediction models.	†Brunekreef et al. (2021) †Shin et al. (2021) Section 4.4.1	Range of means across studies: 6.8–21.4 ppb

Rationale for Causality Determination[a]	Key Evidence[b]	Key References	Nitrogen Dioxide Concentrations Associated with Effects
Uncertainty regarding potential copollutant confounding.	Most studies reported that associations between long-term NO_2 exposure and respiratory mortality were robust to adjustment for long-term exposure to $PM_{2.5}$ or BC. Other important copollutants not considered (e.g., EC or UFPs). High correlations limit confidence in copollutant model results.	Section 4.4.1.3	
Uncertain relevance of toxicological evidence	Consistent animal toxicological evidence that long-term NO_2 exposures can lead to pulmonary inflammation, which is a common hallmark of COPD.	Section 4.3.1.3	
	Animal evidence for emphysema-like changes is more limited and inconsistent, with some studies showing airspace enlargement following long-term exposure to high NO_2 concentration	Section 4.4.2	
Respiratory Mortality			
Generally consistent epidemiologic evidence from multiple, high-quality studies with relevant NO_2 concentrations	Generally consistent evidence for increases in mortality risk in diverse cohorts in the U.S., Canada, and Europe that used a variety of exposure assessment methods, including central site monitors, LUR, dispersion models, and hybrid exposure models.	†Brunekreef et al. (2021) †Eum et al. (2022) †Weichenthal et al. (2017b) †Crouse et al. (2015) Section 4.8; Figure 4-15	Range of means across studies: 8.1–22.9 ppb
Some studies show no association	No association observed in several U.S. cohorts, including ACS, AHSMOG, and California Teachers Study.	Abbey et al. (1999) Jerrett et al. (2013) Lipsett et al. (2011) †Turner et al. (2016)	Range of means across studies: 11.6–36.7 ppb

Rationale for Causality Determination[a]	Key Evidence[b]	Key References	Nitrogen Dioxide Concentrations Associated with Effects
Uncertainty regarding potential copollutant confounding.	Most studies reported that associations between long-term NO_2 exposure and respiratory mortality were robust or attenuated but still positive after adjustment for $PM_{2.5}$ or O_3. Effect estimates adjusted for BC were less consistent and other important copollutants not considered (e.g., CO or UFPs). High correlations limit confidence in copollutant model results.	Section 4.8.3 Figure 4-19 and Figure 4-20	
Limited biological plausibility from studies of respiratory and cardiovascular morbidity	A lack of coherence between experimental and epidemiologic studies of long-term NO_2 exposure and respiratory and morbidity outcomes that support pathways for mortality, including COPD and respiratory infection.	Section 4.4 Section 4.5	

List of abbreviations in alphabetical order: ACS = American Cancer Society; AHSMOG = Adventist Health and Smog Study; BC = Black Carbon; CO = carbon monoxide; COPD = Chronic Obstructive Pulmonary Disease; d = day; hr = hour; LUR = Land Use Regression; mo = month; NO_2 = nitrogen dioxide; PECOS = Population, Exposure, Comparator, Outcome, Study Design; $PM_{2.5}$ = particulate matter with a nominal mean aerodynamic diameter less than 2.5 μm; PM_{10} = particulate matter with a nominal mean aerodynamic diameter less than 10 μm; ppb = parts per billion; ppm = parts per million; UFPs = ultrafine particles; U.S. = United States; wk = week.

[a]Based on application of the causality framework described in Section 3.3.4 of Volume 2 of the Integrated Review Plan for the Primary National Ambient Air Quality Standards for Oxides of Nitrogen (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.

4.12 References

Abbey, DE; Nishino, N; McDonnell, WF; Burchette, RJ; Knutsen, SF; Beeson, WL; Yang, JX. (1999). Long-term inhalable particles and other air pollutants related to mortality in nonsmokers. Am J Respir Crit Care Med 159: 373-382. http://dx.doi.org/10.1164/ajrccm.159.2.9806020

Ackermann-Liebrich, U; Leuenberger, P; Schwartz, J; Schindler, C; Monn, C; Bolognini, G; Bongard, JP; Brändli, O; Domenighetti, G; Elsasser, S; Grize, L; Karrer, W; Keller, R; Keller-Wossidlo, H; Künzli, N; Martin, BW; Medici, TC; Perruchoud, AP; Schöni, MH; Tschopp, JM; Villiger, B; Wüthrich, B; Zellweger, JP; Zemp, E. (1997). Lung function and long term exposure to air pollutants in Switzerland. Am J Respir Crit Care Med 155: 122-129. http://dx.doi.org/10.1164/ajrccm.155.1.9001300

Aguilera, I; Pedersen, M; Garcia-Esteban, R; Ballester, F; Basterrechea, M; Esplugues, A; Fernández-Somoano, A; Lertxundi, A; Tardón, A; Sunyer, J. (2013). Early-life exposure to outdoor air pollution and respiratory health, ear infections, and eczema in infants from the INMA study. Environ Health Perspect 121: 387-392. http://dx.doi.org/10.1289/ehp.1205281

Andersen, ZJ; Bønnelykke, K; Hvidberg, M; Jensen, SS; Ketzel, M; Loft, S; Sørensen, M; Tjønneland, A; Overvad, K; Raaschou-Nielsen, O. (2012). Long-term exposure to air pollution and asthma hospitalisations in older adults: A cohort study. Thorax 67: 6-11. http://dx.doi.org/10.1136/thoraxjnl-2011-200711

Andersen, ZJ; Hvidberg, M; Jensen, SS; Ketzel, M; Loft, S; Sørensen, M; Tjønneland, A; Overvad, K; Raaschou-Nielsen, O. (2011). Chronic obstructive pulmonary disease and long-term exposure to traffic-related air pollution: A cohort study. Am J Respir Crit Care Med 183: 455-461. http://dx.doi.org/10.1164/rccm.201006-0937OC

Arner, EC; Rhoades, RA. (1973). Long-term nitrogen dioxide exposure: Effects on lung lipids and mechanical properties. Arch Environ Health 26: 156-160. http://dx.doi.org/10.1080/00039896.1973.10666244

Belanger, K; Holford, TR; Gent, JF; Hill, ME; Kezik, JM; Leaderer, BP. (2013). Household levels of nitrogen dioxide and pediatric asthma severity. Epidemiology 24: 320-330. http://dx.doi.org/10.1097/EDE.0b013e318280e2ac

Benka-Coker, W; Hoskovec, L; Severson, R; Balmes, J; Wilson, A; Magzamen, S. (2020). The joint effect of ambient air pollution and agricultural pesticide exposures on lung function among children with asthma. Environ Res 190: 109903. http://dx.doi.org/10.1016/j.envres.2020.109903

Benmerad, M; Slama, R; Botturi, K; Claustre, J; Roux, A; Sage, E; Reynaud-Gaubert, M; Gomez, C; Kessler, R; Brugière, O; Mornex, JF; Mussot, S; Dahan, M; Boussaud, V; Danner-Boucher, I; Dromer, C; Knoop, C; Auffray, A; Lepeule, J; Malherbe, L; Meleux, F; Nicod, L; Magnan, A; Pison, C; Siroux, V; consortium, S. (2017). Chronic effects of air pollution on lung function after lung transplantation in the Systems prediction of Chronic Lung Allograft Dysfunction (SysCLAD) study. Eur Respir J 49. http://dx.doi.org/10.1183/13993003.00206-2016

Berhane, K; Chang, CC; McConnell, R; Gauderman, WJ; Avol, E; Rapapport, E; Urman, R; Lurmann, F; Gilliland, F. (2016). Association of changes in air quality with bronchitic symptoms in children in California, 1993-2012. JAMA 315: 1491-1501. http://dx.doi.org/10.1001/jama.2016.3444

Blackwell, DL; Lucas, JW; Clarke, TC. (2014). Summary health statistics for U.S. adults: National Health Interview Survey, 2012. (DHHA Publication No. 2014-1588). Hyattsville, MD: National Center for Health Statistics. http://www.cdc.gov/nchs/data/series/sr_10/sr10_260.pdf

Blair, WH; Henry, MC; Ehrlich, R. (1969). Chronic toxicity of nitrogen dioxide: II. Effect on histopathology of lung tissue. Arch Environ Health 18: 186-192. http://dx.doi.org/10.1080/00039896.1969.10665391

Bouma, F; Janssen, NA; Wesseling, J; van Ratingen, S; Strak, M; Kerckhoffs, J; Gehring, U; Hendricx, W; de Hoogh, K; Vermeulen, R; Hoek, G. (2023). Long-term exposure to ultrafine particles and

natural and cause-specific mortality. Environ Int 175: 107960. http://dx.doi.org/10.1016/j.envint.2023.107960

Bourbeau, J; Doiron, D; Biswas, S; Smith, BM; Benedetti, A; Brook, J. R.; Aaron, SD; Chapman, KR; Hernandez, P; Maltais, F; Marciniuk, DD; O'Donnell, D; Sin, DD; Walker, B; Dsilva, L; Nadeau, G; Coats, V; Compton, C; Miller, BE; Tan, WC. (2022). Ambient air pollution and dysanapsis: Associations with lung function and chronic obstructive pulmonary disease in the Canadian Cohort Obstructive Lung Disease study. Am J Respir Crit Care Med 206: 44-55. http://dx.doi.org/10.1164/rccm.202106-1439OC

Bourdrel, T; Annesi-Maesano, I; Alahmad, B; Maesano, CN; Bind, MA. (2021). The impact of outdoor air pollution on COVID-19: a review of evidence from in vitro, animal, and human studies [Review]. Eur Respir Rev 30. http://dx.doi.org/10.1183/16000617.0242-2020

Bozack, A; Pierre, S; DeFelice, N; Colicino, E; Jack, D; Chillrud, SN; Rundle, A; Astua, A; Quinn, JW; McGuinn, L; Yang, Q; Johnson, K; Masci, J; Lukban, L; Maru, D; Lee, AG. (2022). Long-term air pollution exposure and COVID-19 mortality: A patient-level analysis from New York City. Am J Respir Crit Care Med 205: 651-662. http://dx.doi.org/10.1164/rccm.202104-0845OC

Breton, CV; Salam, MT; Vora, H; Gauderman, WJ; Gilliland, FD. (2011). Genetic variation in the glutathione synthesis pathway, air pollution, and children's lung function growth. Am J Respir Crit Care Med 183: 243-248. http://dx.doi.org/10.1164/rccm.201006-0849OC

Brochu, P; Jimenez, MP; James, P; Kinney, PL; Lane, K. (2022). Benefits of Increasing Greenness on All-Cause Mortality in the Largest Metropolitan Areas of the United States Within the Past Two Decades. Front Public Health 10: 841936. http://dx.doi.org/10.3389/fpubh.2022.841936

Brunekreef, B; Strak, M; Chen, J; Andersen, ZJ; Atkinson, R; Bauwelinck, M; Bellander, T; Boutron, MC; Brandt, J; Carey, I; Cesaroni, G; Forastiere, F; Fecht, D; Gulliver, J; Hertel, O; Hoffmann, B; de Hoogh, K; Houthuijs, D; Hvidtfeldt, U; ... Hoek, G. (2021). Mortality and morbidity effects of long-term exposure to low-level PM2.5, BC, NO2, and O3: An analysis of European cohorts in the ELAPSE Project (pp. 1-127). (ISSN 1041-5505EISSN 2688-6855Research Report No. 208). Boston, MA: Health Effects Institute.

Buteau, S; Shekarrizfard, M; Hatzopolou, M; Gamache, P; Liu, L; Smargiassi, A. (2020). Air pollution from industries and asthma onset in childhood: A population-based birth cohort study using dispersion modeling. Environ Res 185: 109180. http://dx.doi.org/10.1016/j.envres.2020.109180

Cakmak, S; Hebbern, C; Cakmak, JD; Vanos, J. (2016). The modifying effect of socioeconomic status on the relationship between traffic, air pollution and respiratory health in elementary schoolchildren. J Environ Manage 177: 1-8. http://dx.doi.org/10.1016/j.jenvman.2016.03.051

Canning, BJ. (2003). Modeling asthma and COPD in animals: a pointless exercise? [Review]. Curr Opin Pharmacol 3: 244-250. http://dx.doi.org/10.1016/s1471-4892(03)00045-6

Carlsten, C; Brauer, M; Dimich-Ward, H; Dybuncio, A; Becker, AB; Chan-Yeung, M. (2011a). Combined exposure to dog and indoor pollution: Incident asthma in a high-risk birth cohort. Eur Respir J 37: 324-330. http://dx.doi.org/10.1183/09031936.00187609

Carlsten, C; Dybuncio, A; Becker, A; Chan-Yeung, M; Brauer, M. (2011b). Traffic-related air pollution and incident asthma in a high-risk birth cohort. Occup Environ Med 68: 291-295. http://dx.doi.org/10.1136/oem.2010.055152

Castro-Giner, F; Künzli, N; Jacquemin, B; Forsberg, B; de Cid, R; Sunyer, J; Jarvis, D; Briggs, D; Vienneau, D; Norback, D; González, JR; Guerra, S; Janson, C; Antó, JM; Wjst, M; Heinrich, J; Estivill, X; Kogevinas, M. (2009). Traffic-related air pollution, oxidative stress genes, and asthma (ECHRS). Environ Health Perspect 117: 1919-1924. http://dx.doi.org/10.1289/ehp.0900589

Chang, LY; Graham, JA; Miller, FJ; Ospital, JJ; Crapo, JD. (1986). Effects of subchronic inhalation of low concentrations of nitrogen dioxide: I. The proximal alveolar region of juvenile and adult rats. Toxicol Appl Pharmacol 83: 46-61. http://dx.doi.org/10.1016/0041-008X(86)90321-2

Chen, CH; Wu, CD; Lee, YL; Lee, KY; Lin, WY; Yeh, JI; Chen, HC; Guo, YL. (2022a). Air pollution enhance the progression of restrictive lung function impairment and diffusion capacity reduction: an elderly cohort study. Respir Res 23: 186. http://dx.doi.org/10.1186/s12931-022-02107-5

Chen, Z; Huang, BZ; Sidell, MA; Chow, T; Eckel, SP; Pavlovic, N; Martinez, MP; Lurmann, F; Thomas, DC; Gilliland, FD; Xiang, AH. (2021). Near-roadway air pollution associated with COVID-19 severity and mortality - Multiethnic cohort study in Southern California. Environ Int 157: 106862. http://dx.doi.org/10.1016/j.envint.2021.106862

Chen, Z; Sidell, MA; Huang, BZ; Chow, T; Eckel, SP; Martinez, MP; Gheissari, R; Lurmann, F; Thomas, DC; Gilliland, FD; Xiang, AH. (2022b). Ambient air pollutant exposures and COVID-19 severity and mortality in a cohort of patients with COVID-19 in Southern California. Am J Respir Crit Care Med 206: 440-448. http://dx.doi.org/10.1164/rccm.202108-1909OC

Clark, NA; Demers, PA; Karr, CJ; Koehoorn, M; Lencar, C; Tamburic, L; Brauer, M. (2010). Effect of early life exposure to air pollution on development of childhood asthma. Environ Health Perspect 118: 284-290. http://dx.doi.org/10.1289/ehp.0900916

Cockcroft, DW; Davis, BE. (2006). Mechanisms of airway hyperresponsiveness [Review]. J Allergy Clin Immunol 118: 551-559. http://dx.doi.org/10.1016/j.jaci.2006.07.012

Crapo, JD; Barry, BE; Chang, LY; Mercer, RR. (1984). Alterations in lung structure caused by inhalation of oxidants. J Toxicol Environ Health 13: 301-321. http://dx.doi.org/10.1080/15287398409530500

Crouse, DL; Peters, PA; Hystad, P; Brook, JR; van Donkelaar, A; Martin, RV; Villeneuve, PJ; Jerrett, M; Goldberg, MS; Pope, CA; Brauer, M; Brook, RD; Robichaud, A; Menard, R; Burnett, RT. (2015). Ambient PM 2.5, O 3, and NO 2 exposures and associations with mortality over 16 years of follow-up in the Canadian Census Health and Environment Cohort (CanCHEC). Environ Health Perspect 123: 1180-1186. http://dx.doi.org/10.1289/ehp.1409276

Cui, F; Sun, Y; Xie, J; Li, D; Wu, M; Song, L; Hu, Y; Tian, Y. (2023). Air pollutants, genetic susceptibility and risk of incident idiopathic pulmonary fibrosis. Eur Respir J 61. http://dx.doi.org/10.1183/13993003.00777-2022

Danesh Yazdi, M; Wang, Y; Di, Q; Wei, YG; Requia, WJ; Shi, LH; Sabath, MB; Dominici, F; Coull, BA; Evans, JS; Koutrakis, P; Schwartz, JD. (2021). Long-term association of air pollution and hospital admissions among Medicare participants using a doubly robust additive model. Circulation 143: 1584-1596. http://dx.doi.org/10.1161/CIRCULATIONAHA.120.050252

de Broucker, V; Hulo, S; Cherot-Kornobis, N; Sobaszek, A; Edme, JL. (2015). Increased Levels of 8-Isoprostane in EBC of NO2-Exposed Rats. J Toxicol Environ Health A 78: 666-670. http://dx.doi.org/10.1080/15287394.2015.1023915

de F C Lichtenfels, AJ; van der Plaat, DA; de Jong, K; van Diemen, CC; Postma, DS; Nedeljkovic, I; van Duijn, CM; Amin, N; la Bastide-Van Gemert, S; de Vries, M; Ward-Caviness, CK; Wolf, K; Waldenberger, M; Peters, A; Stolk, RP; Brunekreef, B; Boezen, HM; Vonk, JM. (2018). Long-term air pollution exposure, genome-wide DNA methylation and lung function in the LifeLines cohort study. Environ Health Perspect 126: 027004. http://dx.doi.org/10.1289/EHP2045

de Hoogh, K; Chen, J; Gulliver, J; Hoffmann, B; Hertel, O; Ketzel, M; Bauwelinck, M; van Donkelaar, A; Hvidtfeldt, UA; Katsouyanni, K; Klompmaker, J; Martin, RV; Samoli, P; Schwartz, PE; Stafoggia, M; Bellander, T; Strak, M; Wolf, K; Vienneau, D; Brunekreef, B; Hoek, G. (2018). Spatial PM2.5, NO2, O3 and BC models for Western Europe - Evaluation of spatiotemporal stability. Environ Int 120: 81-92. http://dx.doi.org/10.1016/j.envint.2018.07.036

Dearborn, LC; Hazlehurst, MF; Loftus, CT; Szpiro, AA; Carroll, KN; Moore, PE; Adgent, MA; Barrett, ES; Nguyen, RH; Sathyanarayana, S; Lewinn, KZ; Bush, NR; Kaufman, JD; Karr, CJ. (2023). Role of air pollution in the development of asthma among children with a history of bronchiolitis in infancy. Epidemiology 34: 554-564. http://dx.doi.org/10.1097/EDE.0000000000001613

Decrue, F; Gorlanova, O; Salem, Y; Vienneau, D; De Hoogh, K; Gisler, A; Usemann, J; Korten, I; Nahum, U; Sinues, P; Schulzke, S; Fuchs, O; Latzin, P; Roosli, M; Frey, U. (2022). Increased impact of air pollution on lung function in preterm versus term infants the BILD study. Am J Respir Crit Care Med 205: 99-+. http://dx.doi.org/10.1164/rccm.202102-0272OC

Diez Roux, AV; Mair, C. (2010). Neighborhoods and health. Ann N Y Acad Sci 1186: 125-145. http://dx.doi.org/10.1111/j.1749-6632.2009.05333.x

Dimakopoulou, K; Samoli, E; Beelen, R; Stafoggia, M; Andersen, ZJ; Hoffmann, B; Fischer, P; Nieuwenhuijsen, M; Vineis, P; Xun, W; Hoek, G; Raaschou-Nielsen, O; Oudin, A; Forsberg, B; Modig, L; Jousilahti, P; Lanki, T; Turunen, A; Oftedal, B; Nafstad, P; Schwarze, PE; Penell, J; Fratiglioni, L; Andersson, N; Pedersen, N; Korek, M; De Faire, U; Eriksen, KT; Tjønneland, A; Becker, T; Wang, M; Bueno-De-Mesquita, B; Tsai, MY; Eeftens, M; Peeters, PH; Meliefste, K; Marcon, A; Krämer, U; Kuhlbusch, TA; Vossoughi, M; Key, T; de Hoogh, K; Hampel, R; Peters, A; Heinrich, J; Weinmayr, G; Concin, H; Nagel, G; Ineichen, A; Jacquemin, B; Stempfelet, M; Vilier, A; Ricceri, F; Sacerdote, C; Pedeli, X; Katsoulis, M; Trichopoulou, A; Brunekreef, B; Katsouyanni, K. (2014). Air pollution and nonmalignant respiratory mortality in 16 cohorts within the ESCAPE project. Am J Respir Crit Care Med 189: 684-696. http://dx.doi.org/10.1164/rccm.201310-1777OC

Doiron, D; Bourbeau, J; de Hoogh, K; Hansell, AL. (2021). Ambient air pollution exposure and chronic bronchitis in the Lifelines cohort. Thorax 76: 772-779. http://dx.doi.org/10.1136/thoraxjnl-2020-216142

Doiron, D; De Hoogh, K; Probst-Hensch, N; Fortier, I; Cai, Y; De Matteis, S; Hansell, AL. (2019). Air pollution, lung function and COPD: results from the population-based UK Biobank study. Eur Respir J 54. http://dx.doi.org/10.1183/13993003.02140-2018

Dominici, F; Peng, RD; Barr, CD; Bell, ML. (2010). Protecting human health from air pollution: Shifting from a single-pollutant to a multipollutant approach. Epidemiology 21: 187-194. http://dx.doi.org/10.1097/EDE.0b013e3181cc86e8

Duong-Quy, S; Hua-Huy, T; Pham, H; Tang, XK; Mercier, JC; Baud, O; Dinh-Xuan, AT. (2014). Early inhaled nitric oxide at high dose enhances rat lung development after birth. Nitric Oxide 38: 8-16. http://dx.doi.org/10.1016/j.niox.2014.02.004

Eenhuizen, E; Gehring, U; Wijga, AH; Smit, HA; Fischer, PH; Brauer, M; Koppelman, GH; Kerkhof, M; de Jongste, JC; Brunekreef, B; Hoek, G. (2013). Traffic related air pollution is related to interrupter resistance in four-year old children. Eur Respir J 41: 1257-1263. http://dx.doi.org/10.1183/09031936.00020812

Ehrlich, R. (1980). Interaction between environmental pollutants and respiratory infections. Environ Health Perspect 35: 89-99.

Ehrlich, R; Henry, MC. (1968). Chronic toxicity of nitrogen dioxide: I: Effect on resistance to bacterial pneumonia. Arch Environ Health 17: 860-865. http://dx.doi.org/10.1080/00039896.1968.10665342

Eum, KD; Honda, TJ; Wang, B; Kazemiparkouhi, F; Manjourides, J; Pun, VC; Pavlu, V; Suh, H. (2022). Long-term nitrogen dioxide exposure and cause-specific mortality in the U.S. Medicare population. Environ Res 207: 112154. http://dx.doi.org/10.1016/j.envres.2021.112154

Eum, KD; Kazemiparkouhi, F; Wang, B; Manjourides, J; Pun, V; Pavlu, V; Suh, H. (2019). Long-term NO2 exposures and cause-specific mortality in American older adults. Environ Int 124: 10-15. http://dx.doi.org/10.1016/j.envint.2018.12.060

Forbes, LJL; Kapetanakis, V; Rudnicka, AR; Cook, DG; Bush, T; Stedman, JR; Whincup, PH; Strachan, DP; Anderson, HR. (2009). Chronic exposure to outdoor air pollution and lung function in adults. Thorax 64: 657-663. http://dx.doi.org/10.1136/thx.2008.109389

Franklin, M; Fruin, S. (2017). The role of traffic noise on the association between air pollution and children's lung function. Environ Res 157: 153-159. http://dx.doi.org/10.1016/j.envres.2017.05.024

Fuertes, E; Sunyer, J; Gehring, U; Porta, D; Forastiere, F; Cesaroni, G; Vrijheid, M; Guxens, M; Annesi-Maesano, I; Slama, R; Maier, D; Kogevinas, M; Bousquet, J; Chatzi, L; Lertxundi, A; Basterrechea, M; Esplugues, A; Ferrero, A; Wright, J; ... Jacquemin, B. (2020). Associations between air pollution and pediatric eczema, rhinoconjunctivitis and asthma: A meta-analysis of European birth cohorts. Environ Int 136: 105474. http://dx.doi.org/10.1016/j.envint.2020.105474

Furiosi, NJ; Crane, SC; Freeman, G. (1973). Mixed sodium chloride aerosol and nitrogen dioxide in air: Biological effects on monkeys and rats. Arch Environ Occup Health 27: 405-408. http://dx.doi.org/10.1080/00039896.1973.10666414

Gan, WQ; Fitzgerald, JM; Carlsten, C; Sadatsafavi, M; Brauer, M. (2013). Associations of ambient air pollution with chronic obstructive pulmonary disease hospitalization and mortality. Am J Respir Crit Care Med 187: 721-727. http://dx.doi.org/10.1164/rccm.201211-2004OC

Gandini, M; Scarinzi, C; Bande, S; Berti, G; Carnà, P; Ciancarella, L; Costa, G; Demaria, M; Ghigo, S; Piersanti, A; Rowinski, M; Spadea, T; Mueller, N; Rojas-Rueda, D; Khreis, H; Cirach, M; Milà, C; Espinosa, A; Foraster, M; Mceachan, RRC; Kelly, B; Wright, J; Nieuwenhuijsen, M. (2018a). Socioeconomic inequalities in urban and transport planning related exposures and mortality: A health impact assessment study for Bradford, UK. Environ Int 121: 931-941. http://dx.doi.org/10.1016/j.envint.2018.10.017

Gandini, M; Scarinzi, C; Bande, S; Berti, G; Carnà, P; Ciancarella, L; Costa, G; Demaria, M; Ghigo, S; Piersanti, A; Rowinski, M; Spadea, T; Stroscia; Cadum, E; Group, LMc. (2018b). Long term effect of air pollution on incident hospital admissions: Results from the Italian Longitudinal Study within LIFE MED HISS project. Environ Int 121: 1087-1097. http://dx.doi.org/10.1016/j.envint.2018.10.020

Garcia, E; Berhane, KT; Islam, T; McConnell, R; Urman, R; Chen, Z; Gilliland, FD. (2019). Association of changes in air quality with incident asthma in children in California, 1993-2014. JAMA 321: 1906-1915. http://dx.doi.org/10.1001/jama.2019.5357

Garcia, E; Birnhak, ZH; West, S; Howland, S; Lurmann, F; Pavlovic, NR; McConnell, R; Farzan, SF; Bastain, TM; Habre, R; Breton, CV. (2024). Childhood air pollution exposure associated with self-reported bronchitic symptoms in adulthood. Am J Respir Crit Care Med 210: 1025-1034. http://dx.doi.org/10.1164/rccm.202308-1484oc

Garcia, E; Marian, B; Chen, Z; Li, K; Lurmann, F; Gilliland, F; Eckel, SP. (2022). Long-term air pollution and COVID-19 mortality rates in California: Findings from the Spring/Summer and Winter surges of COVID-19. Environ Pollut 292: 118396. http://dx.doi.org/10.1016/j.envpol.2021.118396

Gariazzo, C; Carlino, G; Silibello, C; Tinarelli, G; Renzi, M; Finardi, S; Pepe, N; Barbero, D; Radice, P; Marinaccio, A; Forastiere, F; Michelozzi, P; Viegi, G; Stafoggia, M; Group, BC. (2021). Impact of different exposure models and spatial resolution on the long-term effects of air pollution. Environ Res 192: 110351. http://dx.doi.org/10.1016/j.envres.2020.110351

Gauderman, WJ; Avol, E; Gilliland, F; Vora, H; Thomas, D; Berhane, K; McConnell, R; Kuenzli, N; Lurmann, F; Rappaport, E; Margolis, H; Bates, D; Peters, J. (2004). The effect of air pollution on lung development from 10 to 18 years of age. N Engl J Med 351: 1057-1067. http://dx.doi.org/10.1056/NEJMoa040610

Gauderman, WJ; Vora, H; McConnell, R; Berhane, K; Gilliland, F; Thomas, D; Lurmann, F; Avol, E; Kunzli, N; Jerrett, M; Peters, J. (2007). Effect of exposure to traffic on lung development from 10 to 18 years of age: A cohort study. Lancet 369: 571-577. http://dx.doi.org/10.1016/S0140-6736(07)60037-3

Gehring, U; Gruzieva, O; Agius, RM; Beelen, R; Custovic, A; Cyrys, J; Eeftens, M; Flexeder, C; Fuertes, E; Heinrich, J; Hoffmann, B; de Jongste, JC; Kerkhof, M; Klümper, C; Korek, M; Mölter, A; Schultz, ES; Simpson, A; Sugiri, D; Svartengren, M; von Berg, A; Wijga, AH; Pershagen, G; Brunekreef, B. (2013). Air pollution exposure and lung function in children: the ESCAPE project. Environ Health Perspect 121: 1357-1364. http://dx.doi.org/10.1289/ehp.1306770

Gehring, U; Wijga, AH; Brauer, M; Fischer, P; de Jongste, JC; Kerkhof, M; Oldenwening, M; Smit, HA; Brunekreef, B. (2010). Traffic-related air pollution and the development of asthma and allergies during the first 8 years of life. Am J Respir Crit Care Med 181: 596-603. http://dx.doi.org/10.1164/rccm.200906-0858OC

Gehring, U; Wijga, AH; Hoek, G; Bellander, T; Berdel, D; Brueske, I; Fuertes, E; Gruzieva, O; Heinrich, J; Hoffmann, B; de Jongste, JC; Kluemper, C; Koppelman, GH; Korek, M; Kraemer, U; Maier,

D; Melen, E; Pershagen, G; Postma, DS; Standl, M; von Berg, A; Anto, JM; Bousquet, J; Keil, T; Smit, HA; Brunekreef, B. (2015). Exposure to air pollution and development of asthma and rhinoconjunctivitis throughout childhood and adolescence: a population-based birth cohort study [Supplemental Data]. Lancet Respir Med 3: 933-942. http://dx.doi.org/10.1016/S2213-2600(15)00426-9

Gehring, U; Wijga, AH; Koppelman, GH; Vonk, JM; Smit, HA; Brunekreef, B. (2020). Air pollution and the development of asthma from birth until young adulthood. Eur Respir J 56. http://dx.doi.org/10.1183/13993003.00147-2020

Götschi, T; Sunyer, J; Chinn, S; de Marco, R; Forsberg, B; Gauderman, JW; Garcia-Esteban, R; Heinrich, J; Jacquemin, B; Jarvis, D; Ponzio, M; Villani, S; Künzli, N. (2008). Air pollution and lung function in the European Community Respiratory Health Survey. Int J Epidemiol 37: 1349-1358. http://dx.doi.org/10.1093/ije/dyn136

Gregory, RE; Pickrell, JA; Hahn, FF; Hobbs, CH. (1983). Pulmonary effects of intermittent subacute exposure to low-level nitrogen dioxide. J Toxicol Environ Health 11: 405-414. http://dx.doi.org/10.1080/15287398309530354

Gruzieva, O; Bergström, A; Hulchiy, O; Kull, I; Lind, T; Melén, E; Moskalenko, V; Pershagen, G; Bellander, T. (2013). Exposure to air pollution from traffic and childhood asthma until 12 years of age. Epidemiology 24: 54-61. http://dx.doi.org/10.1097/EDE.0b013e318276c1ea

Han, M; Ji, X; Li, G; Sang, N. (2017). NO2 inhalation enhances asthma susceptibility in a rat model. Environ Sci Pollut Res Int 24: 27843-27854. http://dx.doi.org/10.1007/s11356-017-0402-7

Hayashi, Y; Kohno, T; Ohwada, H. (1987). Morphological effects of nitrogen dioxide on the rat lung. Environ Health Perspect 73: 135-145. http://dx.doi.org/10.1289/ehp.8773135

Henry, MC; Findlay, J; Spangler, J; Ehrlich, R. (1970). Chronic toxicity of NO2 in squirrel monkeys: III. Effect on resistance to bacterial and viral infection. Arch Environ Health 20: 566-570. http://dx.doi.org/10.1080/00039896.1970.10665663

Hooper, LG; Young, MT; Keller, JP; Szpiro, AA; O'Brien, KM; Sandler, DP; Vedal, S; Kaufman, JD; London, SJ. (2018). Ambient air pollution and chronic bronchitis in a cohort of U.S. women. Environ Health Perspect 126: 027005. http://dx.doi.org/10.1289/EHP2199

Howden, LM; Meyer, JA. (2011). Age and sex composition: 2010. (2010 Census Briefs, C2010BR-03). Washington, DC: U.S. Department of Commerce, Economics and Statistics Administration, U.S. Census Bureau. http://www.census.gov/prod/cen2010/briefs/c2010br-03.pdf

Hüls, A; Sugiri, D; Abramson, MJ; Hoffmann, B; Schwender, H; Ickstadt, K; Krämer, U; Schikowski, T. (2019). Benefits of improved air quality on ageing lungs: impacts of genetics and obesity. Eur Respir J 53: 1801780. http://dx.doi.org/10.1183/13993003.01780-2018

Hvidtfeldt, UA; Sørensen, M; Geels, C; Ketzel, M; Khan, J; Tjønneland, A; Overvad, K; Brandt, J; Raaschou-Nielsen, O. (2019). Long-term residential exposure to PM2.5, PM10, black carbon, NO2, and ozone and mortality in a Danish cohort. Environ Int 123: 265-272. http://dx.doi.org/10.1016/j.envint.2018.12.010

Ierodiakonou, D; Zanobetti, A; Coull, BA; Melly, S; Postma, DS; Boezen, HM; Vonk, JM; Williams, PV; Shapiro, GG; Mckone, EF; Hallstrand, TS; Koenig, JQ; Schildcrout, JS; Lumley, T; Fuhlbrigge, AN; Koutrakis, P; Schwartz, J; Weiss, ST; Gold, DR; Group, CAMPR. (2015). Ambient air pollution, lung function, and airway responsiveness in asthmatic children. J Allergy Clin Immunol 137: 390-399. http://dx.doi.org/10.1016/j.jaci.2015.05.028

IPCS (International Programme on Chemical Safety). (2012). Guidance for immunotoxicity risk assessment for chemicals. (Harmonization Project Document No. 10). Geneva, Switzerland: World Health Organization. https://apps.who.int/iris/handle/10665/330098

Jacquemin, B; Sunyer, J; Forsberg, B; Aguilera, I; Briggs, D; García-Esteban, R; Götschi, T; Heinrich, J; Järvholm, B; Jarvis, D; Vienneau, D; Künzli, N. (2009). Home outdoor NO2 and new onset of self-reported asthma in adults. Epidemiology 20: 119-126. http://dx.doi.org/10.1097/EDE.0b013e3181886e76

Jerrett, M; Burnett, RT; Beckerman, BS; Turner, MC; Krewski, D; Thurston, G; Martin, RV; van Donkelaar, A; Hughes, E; Shi, Y; Gapstur, SM; Thun, MJ; Pope, CA, III. (2013). Spatial analysis of air pollution and mortality in California. Am J Respir Crit Care Med 188: 593-599. http://dx.doi.org/10.1164/rccm.201303-0609OC

Jerrett, M; Nau, CL; Young, DR; Butler, RK; Batteate, CM; Su, J; Burnett, RT; Kleeman, MJ. (2023). Air pollution and meteorology as risk factors for COVID-19 death in a cohort from Southern California. Environ Int 171: 107675. http://dx.doi.org/10.1016/j.envint.2022.107675

Jerrett, M; Shankardass, K; Berhane, K; Gauderman, WJ; Künzli, N; Avol, E; Gilliland, F; Lurmann, F; Molitor, JN; Molitor, JT; Thomas, DC; Peters, J; McConnell, R. (2008). Traffic-related air pollution and asthma onset in children: A prospective cohort study with individual exposure measurement. Environ Health Perspect 116: 1433-1438. http://dx.doi.org/10.1289/ehp.10968

Kaspersen, KA; Antonsen, S; Horsdal, HT; Kjerulff, B; Brandt, J; Geels, C; Christensen, JH; Frohn, LM; Sabel, CE; Dinh, KM; Hertel, O; Sigsgaard, T; Pedersen, CB; Erikstrup, C. (2024). Exposure to air pollution and risk of respiratory tract infections in the adult Danish population-a nationwide study. Clin Microbiol Infect 30: 122-129. http://dx.doi.org/10.1016/j.cmi.2023.10.013

Katanoda, K; Sobue, T; Satoh, H; Tajima, K; Suzuki, T; Nakatsuka, H; Takezaki, T; Nakayama, T; Nitta, H; Tanabe, K; Tominaga, S. (2011). An association between long-term exposure to ambient air pollution and mortality from lung cancer and respiratory diseases in Japan. J Epidemiol 21: 132-143. http://dx.doi.org/10.2188/jea.JE20100098

Keidel, D; Maria Anto, J; Basagana, X; Bono, R; Burte, E; Carsin, AE; Forsberg, B; Fuertes, E; Galobardes, B; Heinrich, J; De Hoogh, K; Jarvis, D; Kunzli, N; Leynaert, B; Marcon, A; Le Moual, N; De Nazelle, A; Schindler, C; Siroux, V; Stempfelet, M; Sunyer, J; Temam, S; Tsai, MY; Varraso, R; Jacquemin, B; Probst-Hensch, N. (2019). The role of socioeconomic status in the association of lung function and air pollution - A pooled analysis of three adult ESCAPE cohorts. Int J Environ Res Public Health 16. http://dx.doi.org/10.3390/ijerph16111901

Keirsbulck, M; Savouré, M; Lequy, E; Chen, J; de Hoogh, K; Vienneau, D; Goldberg, M; Zins, M; Roche, N; Nadif, R; Jacquemin, B. (2023). Long-term exposure to ambient air pollution and asthma symptom score in the CONSTANCES cohort. Thorax 78: 9-15. http://dx.doi.org/10.1136/thoraxjnl-2021-218344

Kennedy, CM; Pennington, AF; Darrow, LA; Klein, M; Zhai, X; Bates, JT; Russell, AG; Hansen, C; Tolbert, PE; Strickland, MJ. (2018). Associations of mobile source air pollution during the first year of life with childhood pneumonia, bronchiolitis, and otitis media. Environ Epidemiol 2: e007. http://dx.doi.org/10.1097/EE9.0000000000000007

Kirwa, K; Eckert, CM; Vedal, S; Hajat, A; Kaufman, JD. (2021). Ambient air pollution and risk of respiratory infection among adults: Evidence from the multiethnic study of atherosclerosis (MESA). BMJ Open Resp Res 8: e000866. http://dx.doi.org/10.1136/bmjresp-2020-000866

Klompmaker, JO; Hoek, G; Bloemsma, LD; Marra, M; Wijga, AH; van den Brink, C; Brunekreef, B; Lebret, E; Gehring, U; Janssen, NAH. (2020). Surrounding green, air pollution, traffic noise exposure and non-accidental and cause-specific mortality. Environ Int 134: 105341. http://dx.doi.org/10.1016/j.envint.2019.105341

Klompmaker, JO; Janssen, N; Andersen, ZJ; Atkinson, R; Bauwelinck, M; Chen, J; de Hoogh, K; Houthuijs, D; Katsouyanni, K; Marra, M; Oftedal, B; Rodopoulou, S; Samoli, E; Stafoggia, M; Strak, M; Swart, W; Wesseling, J; Vienneau, D; Brunekreef, B; Hoek, G. (2021a). Comparison of associations between mortality and air pollution exposure estimated with a hybrid, a land-use regression and a dispersion model. Environ Int 146: 106306. http://dx.doi.org/10.1016/j.envint.2020.106306

Klompmaker, JO; Janssen, NAH; Bloemsma, LD; Marra, M; Lebret, E; Gehring, U; Hoek, G. (2021b). Effects of exposure to surrounding green, air pollution and traffic noise with non-accidental and cause-specific mortality in the Dutch national cohort. Environ Health 20: 82. http://dx.doi.org/10.1186/s12940-021-00769-0

Knibbs, LD; Cortés de Waterman, AM; Toelle, BG; Guo, Y; Denison, L; Jalaludin, B; Marks, GB; Williams, GM. (2018). The Australian Child Health and Air Pollution Study (ACHAPS): A national population-based cross-sectional study of long-term exposure to outdoor air pollution, asthma, and lung function. Environ Int 120: 394-403. http://dx.doi.org/10.1016/j.envint.2018.08.025

Kobayashi, T; Miura, T. (1995). Concentration- and time-dependent increase in specific airway resistance after induction of airway hyperresponsiveness by subchronic exposure of guinea pigs to nitrogen dioxide. Fundam Appl Toxicol 25: 154-158. http://dx.doi.org/10.1006/faat.1995.1049

Konstantinoudis, G; Padellini, T; Bennett, J; Davies, B; Ezzati, M; Blangiardo, M. (2021). Long-term exposure to air-pollution and COVID-19 mortality in England: A hierarchical spatial analysis. Environ Int 146: 106316. http://dx.doi.org/10.1016/j.envint.2020.106316

Kuang, Z; Wang, K, ai; Zhan, Y, u; Liu, R; Peng, M; Yang, J; Zhang, Y. (2023). Long-term air pollution exposure accelerates ageing-associated degradation of lung function. Atmos Pollut Res 14: 101899. http://dx.doi.org/10.1016/j.apr.2023.101899

Kubota, K; Murakami, M; Takenaka, S; Kawai, K; Kyono, H. (1987). Effects of long-term nitrogen dioxide exposure on rat lung: Morphological observations. Environ Health Perspect 73: 157-169. http://dx.doi.org/10.1289/ehp.8773157

Kwon, SO; Hong, SH; Han, YJ; Bak, SH; Kim, J; Lee, MK; London, SJ; Kim, WJ; Kim, SY. (2020). Long-term exposure to PM10 and NO2 in relation to lung function and imaging phenotypes in a COPD cohort. Respir Res 21: 247. http://dx.doi.org/10.1186/s12931-020-01514-w

Lafuma, C; Harf, A; Lange, F; Bozzi, L; Poncy, JL; Bignon, J. (1987). Effect of low-level NO2 chronic exposure on elastase-induced emphysema. Environ Res 43: 75-84. http://dx.doi.org/10.1016/S0013-9351(87)80059-2

Lavigne, É; Bélair, MA; Rodriguez Duque, D; Do, MT; Stieb, DM; Hystad, P; van Donkelaar, A; Martin, RV; Crouse, DL; Crighton, E; Chen, H; Burnett, RT; Weichenthal, S; Villeneuve, PJ; To, T; Brook, JR; Johnson, M; Cakmak, S; Yasseen, AS, III; Walker, M. (2018). Effect modification of perinatal exposure to air pollution and childhood asthma incidence. Eur Respir J 51: 1701884. http://dx.doi.org/10.1183/13993003.01884-2017

Li, Z, hi-Hao; Wang, X, iao-Meng; Liao, D, an-Qing; Zhang, Q, ian; Chen, Z, i-Ting; Qiu, C, heng-Shen; Tang, X, u-Lian; Li, H, ong -Min; Du, L, i-Ying; Zhang, P, ei-Dong; Shen, D, ong; Zhang, X, i-Ru; Gao, J, ian; Zhong, W, en -Fang; Chen, P, ei-Liang; Huang, Q, ing-Mei; Song, W, ei-Qi; Liu, D, an; Li, C, huan; Chen, H, uan; Mao, C, hen. (2024). Long-term air pollutants exposure and respiratory mortality: A large prospective cohort study. Ecotoxicol Environ Saf 274: 116176-. http://dx.doi.org/10.1016/j.ecoenv.2024.116176

Lim, CC; Hayes, RB; Ahn, J; Shao, Y; Silverman, DT; Jones, RR; Garcia, C; Bell, ML; Thurston, GD. (2019). Long-term exposure to ozone and cause-specific mortality risk in the United States. Am J Respir Crit Care Med 200: 1022-1031. http://dx.doi.org/10.1164/rccm.201806-1161OC

Lipsett, MJ; Ostro, BD; Reynolds, P; Goldberg, D; Hertz, A; Jerrett, M; Smith, DF; Garcia, C; Chang, ET; Bernstein, L. (2011). Long-term exposure to air pollution and cardiorespiratory disease in the California teachers study cohort. Am J Respir Crit Care Med 184: 828-835. http://dx.doi.org/10.1164/rccm.201012-2082OC

Lipsitt, J; Chan-Golston, AM; Liu, J; Su, J; Zhu, Y; Jerrett, M. (2021). Spatial analysis of COVID-19 and traffic-related air pollution in Los Angeles [Review]. Environ Int 153: 106531. http://dx.doi.org/10.1016/j.envint.2021.106531

LISS (Long Island Sound Study). (2020). Interactions between ambient air pollution and obesity on lung function in children: The Seven Northeastern Chinese Cities (SNEC) Study. Sci Total Environ 699: 134397. http://dx.doi.org/10.1016/j.scitotenv.2019.134397

Liu, S; Jørgensen, JT; Ljungman, P; Pershagen, G; Bellander, T; Leander, K; Magnusson, PKE; Rizzuto, D; Hvidtfeldt, UA; Raaschou-Nielsen, O; Wolf, K; Hoffmann, B; Brunekreef, B; Strak, M; Chen, J; Mehta, A; Atkinson, RW; Bauwelinck, M; Varraso, R; ... Andersen, ZJ. (2021a). Long-term

exposure to low-level air pollution and incidence of asthma: The ELAPSE project. Eur Respir J 57. http://dx.doi.org/10.1183/13993003.030992020

Liu, S; Jørgensen, JT; Ljungman, P; Pershagen, G; Bellander, T; Leander, K; Magnusson, PKE; Rizzuto, D; Hvidtfeldt, UA; Raaschou-Nielsen, O; Wolf, K; Hoffmann, B; Brunekreef, B; Strak, M; Chen, J; Mehta, A; Atkinson, RW; Bauwelinck, M; Varraso, R; Boutron-Ruault, MC; Brandt, J; Cesaroni, G; Forastiere, F; Fecht, D; Gulliver, J; Hertel, O; De Hoogh, K; Janssen, NAH; Katsouyanni, K; Ketzel, M; Klompmaker, JO; Nagel, G; Oftedal, B; Peters, A; Tjønneland, A; Rodopoulou, SP; Samoli, E; Bekkevold, T; Sigsgaard, T; Stafoggia, M; Vienneau, D; Weinmayr, G; Hoek, G; Andersen, ZJ. (2021b). Long-term exposure to low-level air pollution and incidence of chronic obstructive pulmonary disease: The ELAPSE project. Environ Int 146: 106267. http://dx.doi.org/10.1016/j.envint.2020.106267

Liu, S; Lim, YH; Pedersen, M; Jørgensen, JT; Amini, H; Cole-Hunter, T; Mehta, AJ; So, R; Mortensen, LH; Westendorp, RGJ; Loft, S; Bräuner, EV; Ketzel, M; Hertel, O; Brandt, J; Jensen, SS; Christensen, JH; Sigsgaard, T; Geels, C; ... Andersen, ZJ. (2021c). Long-term air pollution and road traffic noise exposure and COPD: The Danish Nurse Cohort. Eur Respir J 58. http://dx.doi.org/10.1183/13993003.04594-2020

Liu, S; Lim, YH; Pedersen, M; Jørgensen, JT; Amini, H; Cole-Hunter, T; Mehta, AJ; So, R; Mortensen, LH; Westendorp, RGJ; Loft, S; Bräuner, EV; Ketzel, M; Hertel, O; Brandt, J; Jensen, SS; Christensen, JH; Sigsgaard, T; Geels, C; Frohn, LM; Brborić, M; Radonić, J; Sekulic, MT; Bønnelykke, K; Backalarz, C; Simonsen, MK; Andersen, ZJ. (2021d). Long-term exposure to ambient air pollution and road traffic noise and asthma incidence in adults: The Danish Nurse cohort. Environ Int 152: 106464. http://dx.doi.org/10.1016/j.envint.2021.106464

Lu, C, han; Liu, Q, in; Qiao, Z, ipeng; Yang, X, u; Baghani, A, bbas Norouzian; Wang, F, aming. (2024). High humidity and NO 2 co-exposure exacerbates allergic asthma by increasing oxidative stress, inflammatory and TRP protein expressions in lung tissue. Environ Pollut 353: 124127-. http://dx.doi.org/10.1016/j.envpol.2024.124127

Lu, C; Liu, Q; Deng, M; Liao, H; Yang, X; Ma, P. (2023a). Interaction of high temperature and NO2 exposure on asthma risk: In vivo experimental evidence of inflammation and oxidative stress. Sci Total Environ 869: 161760. http://dx.doi.org/10.1016/j.scitotenv.2023.161760

Lu, C; Wang, F; Liu, Q; Deng, M; Yang, X; Ma, P. (2023b). Effect of NO2 exposure on airway inflammation and oxidative stress in asthmatic mice. J Hazard Mater 457: 131787. http://dx.doi.org/10.1016/j.jhazmat.2023.131787

MacIntyre, EA; Brauer, M; Melén, E; Bauer, CP; Bauer, M; Berdel, D; Bergström, A; Brunekreef, B; Chan-Yeung, M; Klümper, C; Fuertes, E; Gehring, U; Gref, A; Heinrich, J; Herbarth, O; Kerkhof, M; Koppelman, GH; Kozyrskyj, AL; Pershagen, G; Postma, DS; Thiering, E; Tiesler, CM; Carlsten, C. (2014a). GSTP1 and TNF gene variants and associations between air pollution and incident childhood asthma: The traffic, asthma and genetics (TAG) study. Environ Health Perspect 122: 418-424. http://dx.doi.org/10.1289/ehp.1307459

MacIntyre, EA; Gehring, U; Mölter, A; Fuertes, E; Klümper, C; Krämer, U; Quass, U; Hoffmann, B; Gascon, M; Brunekreef, B; Koppelman, GH; Beelen, R; Hoek, G; Birk, M; de Jongste, JC; Smit, HA; Cyrys, J; Gruzieva, O; Korek, M; Bergström, A; Agius, RM; de Vocht, F; Simpson, A; Porta, D; Forastiere, F; Badaloni, C; Cesaroni, G; Esplugues, A; Fernández-Somoano, A; Lerxundi, A; Sunyer, J; Cirach, M; Nieuwenhuijsen, MJ; Pershagen, G; Heinrich, J. (2014b). Air pollution and respiratory infections during early childhood: an analysis of 10 European birth cohorts within the ESCAPE Project. Environ Health Perspect 122: 107-113. http://dx.doi.org/10.1289/ehp.1306755

Madsen, C; Haberg, SE; Magnus, MC; Aamodt, G; Stigum, H; London, SJ; Nystad, W; Nafstad, P. (2017). Pregnancy exposure to air pollution and early childhood respiratory health in the Norwegian Mother and Child Cohort Study (MoBa). BMJ Open 7: e015796. http://dx.doi.org/10.1136/bmjopen-2016-015796

Marian, B; Yan, Y; Chen, Z; Lurmann, F; Li, K; Gilliland, F; Eckel, SP; Garcia, E. (2022). Independent associations of short- and long-term air pollution exposure with COVID-19 mortality among Californians. Environ Adv 9: 100280. http://dx.doi.org/10.1016/j.envadv.2022.100280
Martinez, FD; Wright, AL; Taussig, LM; Holberg, CJ; Halonen, M; Morgan, WJ. (1995). Asthma and wheezing in the first six years of life. N Engl J Med 332: 133-138. http://dx.doi.org/10.1056/NEJM199501193320301
McConnell, R; Berhane, K; Gilliland, F; Molitor, J; Thomas, D; Lurmann, F; Avol, E; Gauderman, WJ; Peters, JM. (2003). Prospective study of air pollution and bronchitic symptoms in children with asthma. Am J Respir Crit Care Med 168: 790-797. http://dx.doi.org/10.1164/rccm.200304-466OC
McConnell, R; Islam, T; Shankardass, K; Jerrett, M; Lurmann, F; Gilliland, F; Gauderman, J; Avol, E; Künzli, N; Yao, L; Peters, J; Berhane, K. (2010). Childhood incident asthma and traffic-related air pollution at home and school. Environ Health Perspect 118: 1021-1026. http://dx.doi.org/10.1289/ehp.0901232
Mendy, A; Wilkerson, J; Salo, PM; Weir, CH; Feinstein, L; Zeldin, DC; Thorne, PS. (2019). Synergistic association of house endotoxin exposure and ambient air pollution with asthma outcomes. Am J Respir Crit Care Med 200: 712-720. http://dx.doi.org/10.1164/rccm.201809-1733OC
Mercer, RR; Costa, DL; Crapo, JD. (1995). Effects of prolonged exposure to low doses of nitric oxide or nitrogen dioxide on the alveolar septa of the adult rat lung. Lab Invest 73: 20-28.
Miller, FJ; Graham, JA; Raub, JA; Illing, JW; Ménache, MG; House, DE; Gardner, DE. (1987). Evaluating the toxicity of urban patterns of oxidant gases. II. Effects in mice from chronic exposure to nitrogen dioxide. J Toxicol Environ Health 21: 99-112. http://dx.doi.org/10.1080/15287398709531005
Modig, L; Torén, K; Janson, C; Jarvholm, B; Forsberg, B. (2009). Vehicle exhaust outside the home and onset of asthma among adults. Eur Respir J 33: 1261-1267. http://dx.doi.org/10.1183/09031936.00101108
Nasari, MM; Szyszkowicz, M; Chen, H; Crouse, D; Turner, MC; Jerrett, M; Pope, CA; Hubbell, B; Fann, N; Cohen, A; Gapstur, SM; Diver, WR; Stieb, D; Forouzanfar, MH; Kim, SY; Olives, C; Krewski, D; Burnett, RT. (2016). A class of non-linear exposure-response models suitable for health impact assessment applicable to large cohort studies of ambient air pollution. Air Qual Atmos Health 9: 961-972. http://dx.doi.org/10.1007/s11869-016-0398-z
Neupane, B; Jerrett, M; Burnett, RT; Marrie, T; Arain, A; Loeb, M. (2010). Long-term exposure to ambient air pollution and risk of hospitalization with community-acquired pneumonia in older adults. Am J Respir Crit Care Med 181: 47-53. http://dx.doi.org/10.1164/rccm.200901-0160OC
O'Neill, MS; Jerrett, M; Kawachi, I; Levy, JI; Cohen, AJ; Gouveia, N; Wilkinson, P; Fletcher, T; Cifuentes, L; Schwartz, J. (2003). Health, wealth, and air pollution: Advancing theory and methods [Review]. Environ Health Perspect 111: 1861-1870. http://dx.doi.org/10.1289/ehp.6334
Oftedal, B; Brunekreef, B; Nystad, W; Madsen, C; Walker, SE; Nafstad, P. (2008). Residential outdoor air pollution and lung function in schoolchildren. Epidemiology 19: 129-137. http://dx.doi.org/10.1097/EDE.0b013e31815c0827
Oftedal, B; Nystad, W; Brunekreef, B; Nafstad, P. (2009). Long-term traffic-related exposures and asthma onset in schoolchildren in Oslo, Norway. Environ Health Perspect 117: 839-844. http://dx.doi.org/10.1289/ehp.11491
Ohyama, M; Horie, I; Isohama, Y; Azuma, K; Adachi, S; Minejima, C; Takenaka, N. (2018). Effects of nitrous acid exposure on baseline pulmonary resistance and Muc5ac in rats. Inhal Toxicol 30: 149-158. http://dx.doi.org/10.1080/08958378.2018.1476628
Ohyama, M; Nishimura, H; Azuma, K; Minejima, C; Takenaka, N; Adachi, S. (2020). Lowest observed adverse effect level of pulmonary pathological alterations due to nitrous acid exposure in guinea pigs. Environ Health Prev Med 25: 56. http://dx.doi.org/10.1186/s12199-020-00895-0
Oudin, A; Bråbäck, L; Oudin Åström, D; Forsberg, B. (2017). Air Pollution and Dispensed Medications for Asthma, and Possible Effect Modifiers Related to Mental Health and Socio-Economy: A

Longitudinal Cohort Study of Swedish Children and Adolescents. Int J Environ Res Public Health 14. http://dx.doi.org/10.3390/ijerph14111392

Pedersen, M; Liu, S; Zhang, J; Jovanovic Andersen, Z; Brandt, J; Budtz-Jørgensen, E; Bønnelykke, K; Frohn, LM; Nybo Andersen, AM; Ketzel, M; Khan, J; Stayner, L; Brunekreef, B; Loft, S. (2023). Early-Life Exposure to Ambient Air Pollution from Multiple Sources and Asthma Incidence in Children: A Nationwide Birth Cohort Study from Denmark. Environ Health Perspect 131: 57003. http://dx.doi.org/10.1289/EHP11539

Pujades-Rodriguez, M; Mckeever, T; Lewis, S; Whyatt, D; Britton, J; Venn, A. (2009). Effect of traffic pollution on respiratory and allergic disease in adults: Cross-sectional and longitudinal analyses. BMC Pulm Med 9: 42. http://dx.doi.org/10.1186/1471-2466-9-42

Raaschou-Nielsen, O; Thorsteinson, E; Antonsen, S; Holst, GJ; Sigsgaard, T; Geels, C; Frohn, LM; Christensen, JH; Brandt, J; Pedersen, CB; Hvidtfeldt, UA. (2020). Long-term exposure to air pollution and mortality in the Danish population a nationwide study. EClinicalMedicine 28: 100605. http://dx.doi.org/10.1016/j.eclinm.2020.100605

Ranzi, A; Porta, D; Badaloni, C; Cesaroni, G; Lauriola, P; Davoli, M; Forastiere, F. (2014). Exposure to air pollution and respiratory symptoms during the first 7 years of life in an Italian birth cohort. Occup Environ Med 71: 430-436. http://dx.doi.org/10.1136/oemed-2013-101867

Rojas-Martinez, R; Perez-Padilla, R; Olaiz-Fernandez, G; Mendoza-Alvarado, L; Moreno-Macias, H; Fortoul, T; McDonnell, W; Loomis, D; Romieu, I. (2007a). Lung function growth in children with long-term exposure to air pollutants in Mexico City. Am J Respir Crit Care Med 176: 377-384. http://dx.doi.org/10.1164/rccm.200510-1678OC

Rojas-Martinez, R; Perez-Padilla, R; Olaiz-Fernandez, G; Mendoza-Alvarado, L; Moreno-Macias, H; Fortoul, T; McDonnell, W; Loomis, D; Romieu, I. (2007b). Lung function growth in children with long-term exposure to air pollutants in Mexico City. Online data supplement [Supplemental Data]. Am J Respir Crit Care Med 176.

Rothman, KJ; Greenland, S. (1998). Modern epidemiology. In Modern Epidemiology (2 ed.). Philadelphia, PA: Lippincott-Raven.

Rousseau, MC; Conus, F; El-Zein, M; Benedetti, A; Parent, ME. (2023). Ascertaining asthma status in epidemiologic studies: a comparison between administrative health data and self-report. BMC Med Res Methodol 23: 201. http://dx.doi.org/10.1186/s12874-023-02011-6

Sack, C; Vedal, S; Sheppard, L; Raghu, G; Barr, RG; Podolanczuk, A; Doney, B; Hoffman, EA; Gassett, A; Hinckley-Stukovsky, K; Williams, K; Kawut, S; Lederer, DJ; Kaufman, JD. (2017). Air pollution and subclinical interstitial lung disease: the Multi-Ethnic Study of Atherosclerosis (MESA) air-lung study. Eur Respir J 50. http://dx.doi.org/10.1183/13993003.00559-2017

Sagai, M; Ichinose, T; Kubota, K. (1984). Studies on the biochemical effects of nitrogen dioxide. IV. Relation between the change of lipid peroxidation and the antioxidative protective system in rat lungs upon life span exposure to low levels of NO2. Toxicol Appl Pharmacol 73: 444-456. http://dx.doi.org/10.1016/0041-008X(84)90097-8

Sbihi, H; Koehoorn, M; Tamburic, L; Brauer, M. (2017). Asthma trajectories in a population-based birth cohort. Impacts of air pollution and greenness. Am J Respir Crit Care Med 195: 607-613. http://dx.doi.org/10.1164/rccm.201601-0164OC

Sbihi, H; Tamburic, L; Koehoorn, M; Brauer, M. (2016). Perinatal air pollution exposure and development of asthma from birth to age 10 years. Eur Respir J 47: 1062-1071. http://dx.doi.org/10.1183/13993003.00746-2015

Schikowski, T; Sugiri, D; Ranft, U; Gehring, U; Heinrich, J; Wichmann, HE; Krämer, U. (2005). Long-term air pollution exposure and living close to busy roads are associated with COPD in women. Respir Res 22: 152-161. http://dx.doi.org/10.1186/1465-9921-6-152

Schittny, JC. (2017). Development of the lung [Review]. Cell Tissue Res 367: 427-444. http://dx.doi.org/10.1007/s00441-016-2545-0

Schultz, ES; Gruzieva, O; Bellander, T; Bottai, M; Hallberg, J; Kull, I; Svartengren, M; Melén, E; Pershagen, G. (2012). Traffic-related air pollution and lung function in children at 8 years of age:

A birth cohort study. Am J Respir Crit Care Med 186: 1286-1291. http://dx.doi.org/10.1164/rccm.201206-1045OC

Schultz, ES; Hallberg, J; Bellander, T; Bergström, A; Bottai, M; Chiesa, F; Gustafsson, PM; Gruzieva, O; Thunqvist, P; Pershagen, G; Melén, E. (2015). Early Life Exposure to Traffic-related Air Pollution and Lung Function in Adolescence. Am J Respir Crit Care Med 193: 171-177. http://dx.doi.org/10.1164/rccm.201505-0928OC

Sekine, K; Shima, M; Nitta, Y; Adachi, M. (2004). Long term effects of exposure to automobile exhaust on the pulmonary function of female adults in Tokyo, Japan. Occup Environ Med 61: 350-357. http://dx.doi.org/10.1136/oem.2002.005934

Sherwin, RP; Richters, V. (1982). Hyperplasia of type 2 pneumocytes following 0.34 ppm nitrogen dioxide exposure: Quantitation by image analysis. Arch Environ Health 37: 306-315. http://dx.doi.org/10.1080/00039896.1982.10667584

Shin, S; Bai, L; Burnett, RT; Kwong, JC; Hystad, P; van Donkelaar, A; Lavigne, E; Weichenthal, S; Copes, R; Martin, RV; Kopp, A; Chen, H. (2021). Air pollution as a risk factor for incident chronic obstructive pulmonary disease and asthma. A 15-year population-based cohort study. Am J Respir Crit Care Med 203: 1138-1148. http://dx.doi.org/10.1164/rccm.201909-1744OC

Smith, GS; Van Den Eeden, SK; Garcia, C; Shan, J; Baxter, R; Herring, AH; Richardson, DB; Van Rie, A; Emch, M; Gammon, MD. (2016). Air pollution and pulmonary tuberculosis: A nested case-control study among members of a northern california health plan. Environ Health Perspect 124: 761-768. http://dx.doi.org/10.1289/ehp.1408166

So, R; Andersen, ZJ; Chen, J; Stafoggia, M; de Hoogh, K; Katsouyanni, K; Vienneau, D; Rodopoulou, S; Samoli, E; Lim, YH; Jørgensen, JT; Amini, H; Cole-Hunter, T; Mahmood Taghavi Shahri, S; Maric, M; Bergmann, M; Liu, S; Azam, S; Loft, S; ... Mehta, AJ. (2022). Long-term exposure to air pollution and mortality in a Danish nationwide administrative cohort study: Beyond mortality from cardiopulmonary disease and lung cancer. Environ Int 164: 107241. http://dx.doi.org/10.1016/j.envint.2022.107241

So, R; Jørgensen, JT; Lim, YH; Mehta, AJ; Amini, H; Mortensen, LH; Westendorp, R; Ketzel, M; Hertel, O; Brandt, J; Christensen, JH; Geels, C; Frohn, LM; Sisgaard, T; Bräuner, EV; Jensen, SS; Backalarz, C; Simonsen, MK; Loft, S; Cole-Hunter, T; Andersen, ZJ. (2020). Long-term exposure to low levels of air pollution and mortality adjusting for road traffic noise: A Danish Nurse Cohort study. Environ Int 143: 105983. http://dx.doi.org/10.1016/j.envint.2020.105983

Stanojevic, S; Wade, A; Stocks, J; Hankinson, J; Coates, AL; Pan, H; Rosenthal, M; Corey, M; Lebecque, P; Cole, TJ. (2008). Reference ranges for spirometry across all ages: A new approach. Am J Respir Crit Care Med 177: 253-260. http://dx.doi.org/10.1164/rccm.200708-1248OC

Stevens, MA; Menache, MG; Crapo, JD; Miller, FJ; Graham, JA. (1988). Pulmonary function in juvenile and young adult rats exposed to low-level NO2 with diurnal spikes. J Toxicol Environ Health 23: 229-240. http://dx.doi.org/10.1080/15287398809531109

Strak, M; Weinmayr, G; Rodopoulou, S; Chen, J; de Hoogh, K; Andersen, ZJ; Atkinson, R; Bauwelinck, M; Bekkevold, T; Bellander, T; Boutron-Ruault, MC; Brandt, J; Cesaroni, G; Concin, H; Fecht, D; Forastiere, F; Gulliver, J; Hertel, O; Hoffmann, B; ... Samoli, E. (2021). Long term exposure to low level air pollution and mortality in eight European cohorts within the ELAPSE project: Pooled analysis. BMJ 374: n1904. http://dx.doi.org/10.1136/bmj.n1904

Sunyer, J; Puig, C; Torrent, M; Garcia-Algar, O; Calico, I; Munoz-Ortiz, L; Barnes, M; Cullinan, P. (2004). Nitrogen dioxide is not associated with respiratory infection during the first year of life. Int J Epidemiol 33: 116-120. http://dx.doi.org/10.1093/ije/dyh037

Tao, C; Liu, Z; Fan, Y; Yuan, Y; Wang, X; Qiao, Z; Li, Z; Xu, Q; Lou, Z; Wang, H; Li, X; Li, R; Lu, C. (2024). Estimating neighborhood-based mortality risk associated with air pollution: A prospective study. J Hazard Mater 475: 134861. http://dx.doi.org/10.1016/j.jhazmat.2024.134861

Tepper, JS; Costa, DL; Winsett, DW; Stevens, MA; Doerfler, DL; Watkinson, WP. (1993). Near-lifetime exposure of the rat to a simulated urban profile of nitrogen dioxide: Pulmonary function evaluation. Fundam Appl Toxicol 20: 88-96. http://dx.doi.org/10.1093/toxsci/20.1.88

Tétreault, LF; Doucet, M; Gamache, P; Fournier, M; Brand, A; Kosatsky, T; Smargiassi, A. (2016a). Childhood exposure to ambient air pollutants and the onset of asthma: an administrative cohort study in Québec. Environ Health Perspect 124: 1276-1282. http://dx.doi.org/10.1289/ehp.1509838

Tétreault, LF; Doucet, M; Gamache, P; Fournier, M; Brand, A; Kosatsky, T; Smargiassi, A. (2016b). Severe and moderate asthma exacerbations in asthmatic children and exposure to ambient air pollutants. Int J Environ Res Public Health 13: 771. http://dx.doi.org/10.3390/ijerph13080771

Thurlbeck, WM. (1982). Postnatal human lung growth. Thorax 37: 564-571. http://dx.doi.org/10.1136/thx.37.8.564

Tomos, I; Dimakopoulou, K; Manali, ED; Papiris, SA; Karakatsani, A. (2021). Long-term personal air pollution exposure and risk for acute exacerbation of idiopathic pulmonary fibrosis. Environ Health 20: 99. http://dx.doi.org/10.1186/s12940-021-00786-z

Turner, MC; Jerrett, M; Pope, A, III; Krewski, D; Gapstur, SM; Diver, WR; Beckerman, BS; Marshall, JD; Su, J; Crouse, DL; Burnett, RT. (2016). Long-term ozone exposure and mortality in a large prospective study. Am J Respir Crit Care Med 193: 1134-1142. http://dx.doi.org/10.1164/rccm.201508-1633OC

U.S. EPA (U.S. Environmental Protection Agency). (2008). Integrated science assessment for oxides of nitrogen – Health criteria (Final report, 2008) [EPA Report]. (EPA/600/R-08/071). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment- RTP Division. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=194645

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

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

U.S. EPA (U.S. Environmental Protection Agency). (2016). Integrated science assessment for oxides of nitrogen: Health criteria (Final report, Jan 2016) [EPA Report]. (EPA/600/R-15/068). Washington, DC. https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=310879

U.S. EPA (U.S. Environmental Protection Agency). (2023). Overview of nitrogen dioxide (NO2) air quality in the United States, Updated: February 07, 2023. Washington, DC. https://www.epa.gov/system/files/documents/2022-08/NO2_2021.pdf

U.S. EPA (U.S. Environmental Protection Agency). (2024). Integrated Review Plan for the Primary National Ambient Air Quality Standards for Oxides of Nitrogen. Volume 2: Planning for the Review and the Integrated Science Assessment [EPA Report]. (EPA-452/R-24-010b). Washington, DC.

Urman, R; McConnell, R; Islam, T; Avol, EL; Lurmann, FW; Vora, H; Linn, WS; Rappaport, EB; Gilliland, FD; Gauderman, WJ. (2014). Associations of children's lung function with ambient air pollution: Joint effects of regional and near-roadway pollutants. Thorax 69: 540-547. http://dx.doi.org/10.1136/thoraxjnl-2012-203159

Usemann, J; Decrue, F; Korten, I; Proietti, E; Gorlanova, O; Vienneau, D; Fuchs, O; Latzin, P; Röösli, M; Frey, U. (2019). Exposure to moderate air pollution and associations with lung function at school-age: A birth cohort study. Environ Int 126: 682-689. http://dx.doi.org/10.1016/j.envint.2018.12.019

Villeneuve, PJ; Goldberg, MS. (2020). Methodological Considerations for Epidemiological Studies of Air Pollution and the SARS and COVID-19 Coronavirus Outbreaks [Review]. Environ Health Perspect 128: 95001. http://dx.doi.org/10.1289/EHP7411
Wagner, WD; Duncan, BR; Wright, PG; Stokinger, HE. (1965). Experimental study of threshold limit of NO2. Arch Environ Health 10: 455-466. http://dx.doi.org/10.1080/00039896.1965.10664029
Wang, J; Li, D; Ma, Y; Tang, L; Xie, J; Hu, Y; Tian, Y. (2023a). Long-term exposure to ambient air pollutants and increased risk of pneumonia in the UK Biobank. Chest 164: 39-52. http://dx.doi.org/10.1016/j.chest.2023.02.018
Wang, X; Chen, L; Cai, M; Tian, F; Zou, H; Qian, ZM; Zhang, Z; Li, H; Wang, C; Howard, SW; Peng, Y; Zhang, L; Bingheim, E; Lin, H; Zou, Y. (2023b). Air pollution associated with incidence and progression trajectory of chronic lung diseases: A population-based cohort study. Thorax 78: 698-705. http://dx.doi.org/10.1136/thorax-2022-219489
Weichenthal, S; Bai, L; Hatzopoulou, M; Van Ryswyk, K; Kwong, JC; Jerrett, M; van Donkelaar, A; Martin, RV; Burnett, RT; Lu, H; Chen, H. (2017a). Long-term exposure to ambient ultrafine particles and respiratory disease incidence in in Toronto, Canada: a cohort study. Environ Health 16: 64. http://dx.doi.org/10.1186/s12940-017-0276-7
Weichenthal, S; Pinault, LL; Burnett, RT. (2017b). Impact of oxidant gases on the relationship between outdoor fine particulate air pollution and nonaccidental, cardiovascular, and respiratory mortality. Sci Rep 7: 16401. http://dx.doi.org/10.1038/s41598-017-16770-y
Xu, S, hanshan; Marcon, A, lessandro; Bertelsen, R, andi Jacobsen; Benediktsdottir, B, ryndis; Brandt, J, orgen; Frohn, L, ise Marie; Geels, C, amilla; Gislason, T, horarinn; Heinrich, J, oachim; Holm, M, athias; Janson, C, hrister; Markevych, I, ana; Modig, L, ars; Orru, H, ans; Schlunssen, V, ivi; Sigsgaard, T, orben; Johannessen, A, ne. (2024). Associations of long-term exposure to air pollution and greenness with incidence of chronic obstructive pulmonary disease in Northern Europe: The Life-GAP project. Environ Res 257: 119240-. http://dx.doi.org/10.1016/j.envres.2024.119240
Yamamoto, I; Takahashi, M. (1984). Ultrastructural observations of rat lung exposed to nitrogen dioxide for 7 months. Kitasato Arch Exper Med 57: 57-65.
Yu, Z; Ekström, S; Bellander, T; Ljungman, P; Pershagen, G; Eneroth, K; Kull, I; Bergström, A; Georgelis, A; Stafoggia, M; Gruzieva, O; Melén, E. (2023a). Ambient air pollution exposure linked to long COVID among young adults: A nested survey in a population-based cohort in Sweden. Lancet Reg Health Eur 28: 100608. http://dx.doi.org/10.1016/j.lanepe.2023.100608
Yu, Z; Kebede Merid, S; Bellander, T; Bergström, A; Eneroth, K; Merritt, AS; Ödling, M; Kull, I; Ljungman, P; Klevebro, S; Stafoggia, M; Janson, C; Wang, G; Pershagen, G; Melén, E; Gruzieva, O. (2024). Improved Air Quality and Asthma Incidence from School Age to Young Adulthood: A Population-based Prospective Cohort Study. Ann Am Thorac Soc 21: 1432-1440. http://dx.doi.org/10.1513/annalsats.202402-200oc
Yu, Z; Koppelman, GH; Hoek, G; Kerckhoffs, J; Vonk, JM; Vermeulen, R; Gehring, U. (2021). Ultrafine particles, particle components and lung function at age 16 years: The PIAMA birth cohort study. Environ Int 157: 106792. http://dx.doi.org/10.1016/j.envint.2021.106792
Yu, Z; Merid, SK; Bellander, T; Bergström, A; Eneroth, K; Georgelis, A; Hallberg, J; Kull, I; Ljungman, P; Klevebro, S; Stafoggia, M; Wang, G; Pershagen, G; Gruzieva, O; Melén, E. (2023b). Associations of improved air quality with lung function growth from childhood to adulthood: The BAMSE study. Eur Respir J 61: 2201783. http://dx.doi.org/10.1183/13993003.01783-2022
Yue, H; Yan, W; Ji, X; Gao, R; Ma, J; Rao, Z; Li, G; Sang, N. (2017). Maternal exposure of BALB/c mice to indoor NO2 and allergic asthma syndrome in offspring at adulthood with evaluation of DNA methylation associated Th2 polarization. Environ Health Perspect 125: 097011. http://dx.doi.org/10.1289/EHP685
Yue, H; Yan, W; Ji, X; Zhang, Y; Li, G; Sang, N. (2018). Maternal exposure to NO2 enhances airway sensitivity to allergens in BALB/c mice through the JAK-STAT6 pathway. Chemosphere 200: 455-463. http://dx.doi.org/10.1016/j.chemosphere.2018.02.116

Zanobetti, A, ntonella; Ryan, P, atrick H.; Coull, B, rent A.; Luttmann-Gibson, H, eike; Datta, S, oma; Blossom, J, effrey; Brokamp, C, ole; Lothrop, N, athan; Miller, R, achel L.; Beamer, P, aloma I.; Visness, C, ynthia M.; Andrews, H, oward; Bacharier, L, eonard B.; Johnson, C, hristine C.; Hartert, T, ina; Ownby, D, ennis R.; Hershey, G, urjit K. Khurana; Joseph, C, hristine L. M.; Mendonca, E, neida A.; Jackson, D, aniel J.; Zoratti, E, dward M.; Wright, A, nne L.; Martinez, F, ernando D.; Seroogy, C, hristine M.; Ramratnam, S, ima K.; Calatroni, A, gustin; Gern, J, ames E.; Gold, D, iane R. (2024). Early-Life Exposure to Air Pollution and Childhood Asthma Cumulative Incidence in the ECHO CREW Consortium. JAMA Netw Open 7: e240535-. http://dx.doi.org/10.1001/jamanetworkopen.2024.0535

Zeman, KL; Bennett, WD. (2006). Growth of the small airways and alveoli from childhood to the adult lung measured by aerosol-derived airway morphometry. J Appl Physiol (1985) 100: 965-971. http://dx.doi.org/10.1152/japplphysiol.00409.2005

Zhang, J; Lim, YH; So, R; Jørgensen, JT; Mortensen, LH; Napolitano, GM; Cole-Hunter, T; Loft, S; Bhatt, S; Hoek, G; Brunekreef, B; Westendorp, R; Ketzel, M; Brandt, J; Lange, T; Kølsen-Fisher, T; Andersen, ZJ. (2023). Long-term exposure to air pollution and risk of SARS-CoV-2 infection and COVID-19 hospitalisation or death: Danish nationwide cohort study. Eur Respir J 62: 2300280. http://dx.doi.org/10.1183/13993003.00280-2023

Zhang, J; Lim, YH; So, R; Mortensen, LH; Napolitano, GM; Cole-Hunter, T; Tuffier, S; Bergmann, M; Maric, M; Shahri, SMT; Brandt, J; Ketzel, M; Loft, S; Andersen, ZJ. (2024). Long-term exposure to air pollution and risk of acute lower respiratory infections in the Danish nurse cohort. Ann Am Thorac Soc 21: 1129-1138. http://dx.doi.org/10.1513/annalsats.202401-074oc

Zhang, Y; Eckel, SP; Berhane, K; Garcia, E; Muchmore, P; Molshatzki, NB; Rappaport, EB; Linn, WS; Habre, R; Gilliland, FD. (2021a). Long-term exposures to air pollutants affect F eNO in children: A longitudinal study [Letter]. Eur Respir J 58: 2100705. http://dx.doi.org/10.1183/13993003.00705-2021

Zhang, Z; Wang, J; Kwong, JC; Burnett, RT; van Donkelaar, A; Hystad, P; Martin, RV; Bai, L; Mclaughlin, J; Chen, H. (2021b). Long-term exposure to air pollution and mortality in a prospective cohort: The Ontario Health Study. Environ Int 154: 106570. http://dx.doi.org/10.1016/j.envint.2021.106570

Zhao, Q; Kress, S; Markevych, I; Berdel, D; von Berg, A; Gappa, M; Koletzko, S; Bauer, CP; Schulz, H; Standl, M; Heinrich, J; Schikowski, T. (2021). Long-term air pollution exposure under European Union limits and adolescents' lung function: Modifying effect of abnormal weight in the GINIplus and LISA birth cohorts. Chest 160: 249-258. http://dx.doi.org/10.1016/j.chest.2021.02.007

Zhu, Y, iqun; Wu, Y, ao; Cheng, J, un; Liang, H, uaying; Chang, Q, inyu; Lin, F, engyu; Li, D, ianwu; Zhou, X, in; Chen, X, iang; Pan, P, inhua; Liu, H, ong; Guo, Y, uming; Zhang, Y, an. (2024). Ambient air pollution, lifestyle, and genetic predisposition on all-cause and cause-specific mortality: A prospective cohort study. Sci Total Environ 933: 173120-. http://dx.doi.org/10.1016/j.scitotenv.2024.173120

Zhu, Y; Pan, Z; Jing, D; Liang, H; Cheng, J; Li, D; Zhou, X; Lin, F; Liu, H; Pan, P; Zhang, Y. (2023). Association of air pollution, genetic risk, and lifestyle with incident adult-onset asthma: A prospective cohort study. Ecotoxicol Environ Saf 257: 114922. http://dx.doi.org/10.1016/j.ecoenv.2023.114922