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

Integrated Science Assessment for Oxides of Nitrogen – Health Criteria

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

List of Tables

List of Figures

Integrated Science Assessment Team For Oxides of Nitrogen

Executive Direction

Dr. Steven J. Dutton (Director)—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Anna Champlin (Associate Director)—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Scott Jenkins (Branch Chief)—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Jennifer Nichols (Branch Chief)—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Kate Schofield (Branch Chief)—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC

Dr. Andrew K. Hotchkiss (Branch Chief)—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Technical Support Staff

Ms. Christine Alvarez—former Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Martha Beazley—Research Planning and Implementation Staff, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC

Ms. Olivia Birkel—former Oak Ridge Associated Universities, Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC

Ms. Marieka Boyd—former Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. LaShonda Bunch—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Cheryl Itkin—former Research Planning and Implementation Staff, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC

Mr. Ryan Jones—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Ashley Martinez—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Nancy Perry—former Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Coral Reed—Research Planning and Implementation Staff, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC

Mr. Jack Rehmann—Research Assessment and Communications Outreach Staff, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC

Ms. Mira Sanderson—former Oak Ridge Associated Universities, Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Katherine Strozinski—former Oak Ridge Associated Universities, Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Allison Thompson— former Oak Ridge Associated Universities, Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Authors

Dr. Alison K. Krajewski* (Assessment Lead, Integrated Science Assessment for Oxides of Nitrogen)—Integrated Climate Sciences Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

*Denotes lead chapter authors

Ms. Claire M. Holesovsky* (Project Manager, Integrated Science Assessment for Oxides of Nitrogen)—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC

Dr. David M. Lehmann* (former Assessment Lead, Integrated Science Assessment for Oxides of Nitrogen)—former Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Anne Barkley—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. James Brown*—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Peter Byrley—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Catheryne Chiang—former Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Mr. Evan Coffman*—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. David Dillion—former Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Parker F. Duffney*—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Adiba Gaffar—former Oak Ridge Institute for Science and Education, Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Mr. Maxwell Hatala—former Oak Ridge Associated Universities, Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Amanda Haddock—former Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Kirstin Hester*—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Haesoo Kim—former Oak Ridge Institute for Science and Education, Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Ellen Kirrane—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Nichole Kulikowski—former Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC

Dr. Faith N. Lambert—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Jessica L. Levasseur—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Mengyuan Li—former Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Stephen McDow*—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Qingyu Meng*—former Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Nicole Olson*—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Russell D. Owen—former Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Adrien A. Wilkie*—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Lauren Wyatt—Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Contributors

Ms. Meredith Clemons—ICF International, Fairfax, VA

Dr. Sorina Eftim—ICF International, Fairfax, VA

Ms. Shanell Folger—ICF International, Fairfax, VA

Mr. Zhenyu Gan—ICF International, Fairfax, VA

Ms. Lindsey Green—ICF International, Fairfax, VA

Ms. Ali Goldstone—ICF International, Fairfax, VA

Mr. Anthony Hannani—ICF International, Fairfax, VA

Ms. Aishwarya Javali—ICF International, Fairfax, VA

Ms. Ila Kanneboyina—former Oak Ridge Associated Universities, Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Afroditi Katsigiannakis—ICF International, Fairfax, VA

Ms. Anna Kolanowski—ICF International, Fairfax, VA

Ms. Colleen Martin—ICF International, Fairfax, VA

Ms. Jaycee Mayer—ICF International, Fairfax, VA

Ms. Fatima Mumtaz—ICF International, Fairfax, VA

Dr. Natalia Neal-Walthall— ICF International, Fairfax, VA

Mr. Alexander Langsam—former Health and Environmental Effects Assessment Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC

Ms. Katie Lenae—ICF International, Fairfax, VA

Dr. Cynthia Lin—ICF International, Fairfax, VA

Mr. Alexander Lindahl—ICF International, Fairfax, VA

Ms. Mary Lundin—ICF International, Fairfax, VA

Dr. Pradeep Rajan—Pradeep Rajan LLC., Chapel Hill, NC

Ms. Sheerin Shirajan—ICF International, Fairfax, VA

Ms. Monique Slowly—ICF International, Fairfax, VA

Dr. Kate Weinberger—ICF International, Fairfax, VA

Mr. Harry Whately—ICF International, Fairfax, VA

Dr. Maricruz Zarco—ICF International, Fairfax, VA

Reviewers

Dr. Lisa Baxter—Public Health and Environmental Systems Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Tarik Benmarhnia—Scripps Institution, La Jolla, CA

Dr. Stephanie DeFlorio-Barker—Public Health and Environmental Systems Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Cassandra Elizalde—Health and Environmental Impacts Division, Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Jeffrey Geddes—Boston University, Boston, MA

Dr. Ian Gilmour—Public Health and Integrated Toxicology Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Janice Godrey—Air Quality Assessment Division, Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Daniel Goldberg—George Washington University, Washington D.C.

Ms. Elizabeth Good—Air Quality Assessment Division, Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Nicole Hagan—Health and Environmental Impacts Division, Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Iman Hassan—Health and Environmental Impacts Division, Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Julia Heck—University of California, Los Angeles, Los Angeles, CA

Dr. Barron Henderson—Atmospheric and Environmental Systems Modeling Division, Center for Environmental Measurement and Modeling, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Anthony Jones—Health and Environmental Impacts Division, Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Urmila Kodavanti—Public Health and Integrated Toxicology Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Erin Kulick—Temple University, Philadelphia, PA

Dr. Archana Lamichhane—Health and Environmental Impacts Division, Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Mr. John Langstaff—Health and Environmental Impacts Division, Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Mr. Zhen Liu—Air Quality Assessment Division, Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Danelle Lobdell—Public Health and Environmental Systems Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Thomas Long—Research Planning and Implementation Staff, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Tom Luben—Public Health and Environmental Systems Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Judit Marsillach—University of Washington, Seattle, WA

Dr. Colette Miller—Public Health and Integrated Toxicology Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Lars Perlmutt—Health and Environmental Impacts Division, Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Mr. Matthew Porter—Air Quality Assessment Division, Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Sandrah Proctor Eckel—University of Southern California, Los Angeles, CA

Mr. Henry Raab—Health and Environmental Impacts Division, Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Julia Rager—University of North Carolina, Chapel Hill, NC

Dr. Kristen Rappazzo—Public Health and Environmental Systems Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Jennifer Richmond-Bryant—North Carolina State University, Raleigh, NC

Dr. Beate Ritz—University of California, Los Angeles, Los Angeles, CA

Dr. Coralynn Sack—University of Washington, Seattle, WA

Ms. Jennifer Sellers—Health and Environmental Impacts Division, Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Heather Simon—Air Quality Assessment Division, Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Gregory Smith—University of North Carolina School of Medicine, Chapel Hill, NC

Dr. Marissa Sobolewski—University of Rochester, Rochester, NY

Dr. Jeanette Stingone—Columbia University, New York, NY

Dr. Haiyan Tong—Public Health and Integrated Toxicology Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Alan Vette—Atmospheric and Environmental Systems Modeling Division, Center for Environmental Measurement and Modeling, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Mr. Paul Van Rooy—Air Quality Assessment Division, Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Mr. Nealson Watkins—Air Quality Assessment Division, Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Anne Weaver—Public Health and Environmental Systems Division, Center for Public Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Dr. Scott Weichenthal—McGill University, Montreal, Quebec, Canada

Mr. Benjamin Wells—Air Quality Assessment Division, Office of Air Quality Planning and Standards, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Melina Williams—Air and Radiation Law Office, Office of General Counsel, U.S. Environmental Protection Agency, Research Triangle Park, NC

Ms. Margaret Zawacki—Assessment and Standards Division, Office of Transportation and Air Quality, Office of Air and Radiation, U.S. Environmental Protection Agency, Research Triangle Park, NC

Acronyms and Abbreviation

AMFs	air mass factors
AQCD	Air Quality Criteria Documents
BC	Black Carbon
BTEX	benzene, toluene, ethylbenzene, and xylenes
CASAC	Clean Air Scientific Advisory Committee
CMAQ	Community Multiscale Air Quality
CMT	chemical transport models
CO	carbon monoxide or Colorado, U.S.
COPD	Chronic Obstructive Pulmonary Disease
COVID-19	Coronavirus Disease 2019
CPHEA	Center for Public Health and Environmental Assessment
EC	Elemental carbon
ECRHS	European Community Research Health Survey
ED	Emergency Department
ES	Executive Summary
ESCAPE	European Study of Cohorts for Air Pollution Effects
eNO	Exhaled NO
ELF	epithelial lining fluid
EPA	U.S. Environmental Protection Agency
ES	Executive Summary
HERO	Health and Environmental Research Online
HISA	Highly Influential Scientific Assessments
HNO_3	nitric acid
HONO	nitrous acid
hr	hour(s)
Hz	hertz
IPW	inverse probability weighting
IRP	Integrated Review Plan
IS	Integrated Synthesis
ISA	Integrated Science Assessment
LCS	low-cost sensors
m	meter(s)
NAAQS	National Ambient Air Quality Standard
NO	nitric oxide
NASEM	National Academies of Science, Engineering and Medicine
NO_2	nitrogen dioxide
NO_X	the sum of NO_2 + NO
NO_Y	the sum of all oxides of nitrogen
NY	New York, U.S.
O_3	ozone
OH	hydroxyl radicals
ORD	Office of Research and Development
PECOS	population, exposure, comparison, outcome, study design
ppb	parts per billion
PQAPP	Program Quality Assurance Project Plan
$PM_{2.5}$	particulate matter with a nominal mean aerodynamic diameter less than or equal to 2.5 μm
$pNO3^-$	particulate nitrate
STEM	sources, transport and transformation, exposure/extent, and measurement and modeling
TEMPO	Tropospheric Emissions: Monitoring Pollution
TROPOMI	TROPOspheric Monitoring instrument
QA	quality assurance
UFP	ultra fine particles
U.S.	United States
VOCs	volatile organic compounds

Executive Summary

Since the 2016 Integrated Science Assessment for Oxides of Nitrogen – Health Criteria (hereafter referred to as the 2016 Oxides of Nitrogen ISA), there have been several important advances in the understanding of oxides of nitrogen emissions, atmospheric chemistry and ambient air concentrations, exposures, and health effects. The recent literature demonstrates a shift in important emission sources, improved understanding of chemistry and transport, and advancements in satellite measurement and modeling capabilities. Available data illustrates recent declines in anthropogenic oxides of nitrogen emissions and a broad trend of declining ambient air nitrogen dioxide (NO_2) concentrations across the United States. Exposures to NO_2 and other oxides of nitrogen in ambient air stem primarily from outdoor emission sources such as highway and off-road vehicles, utility power plants, and non-utility combustion sources. Correlations between ambient air NO_2 exposures and exposures to other traffic-related pollutants can be strong, particularly in urban areas near major roads, often complicating the interpretation of associations with health effects in epidemiologic studies. Recent health effects studies expand on findings from the 2016 Oxides of Nitrogen ISA and support a *causal relationship* between both short- and long-term oxides of nitrogen exposures and respiratory effects. Evidence for other health outcomes is more uncertain, and is either *suggestive of, but not sufficient to infer, a causal relationship* or *inadequate to infer the presence or absence of a causal relationship* with oxides of nitrogen exposure. People with pre-existing asthma are at increased risk of respiratory effects from short-term exposures, and children are at increased risk from either short- or long-term exposures. Compared to individuals with asthma and children, there is more uncertainty as to whether other lifestages or populations may also be at increased risk of short- and long-term NO_2 exposures. These conclusions are discussed more fully in the Integrated Synthesis with individual chapters of this ISA providing detailed study evaluations and in-depth characterization of conclusions for each topic area.

This Executive Summary (ES) provides an overview of this ISA for Oxides of Nitrogen, including the purpose and scope of the document (ES.1) and a summary of the important conclusions drawn in the areas of atmospheric science (ES.2), pathways of exposure and dosimetry (ES.3), and health effects (ES.4). Studies considered in developing this ISA are documented in the U.S Environmental Protection Agency (EPA) Health and Environmental Research Online (HERO) database.

ES.1 Purpose and Scope of this Integrated Science Assessment

This ISA, prepared by EPA, is a synthesis and evaluation of the most policy-relevant science and form the scientific foundation for the review of the primary (health-based) NAAQS for oxides of nitrogen, as described in the relevant provisions of the Clean Air Act. Sections 108 and 109 of the Clean Air Act, 42 U.S.C. 7408 and 7409, govern the periodic review and establishment of the NAAQS. Section 108 directs the Administrator to identify and list certain air pollutants and then to issue air quality criteria

for those pollutants. Under section 108(a)(2), air quality criteria are intended to "accurately reflect the latest scientific knowledge useful in indicating the kind and extent of all identifiable effects on public health or welfare which may be expected from the presence of [a] pollutant in the ambient air…". With respect to primary standards, section 109 directs the Administrator to propose and promulgate "primary" NAAQS for pollutants for which air quality criteria are issued, and section 109(b)(1) defines a primary standard as one "the attainment and maintenance of which in the judgment of the Administrator, based on such criteria and allowing an adequate margin of safety, are requisite to protect the public health."[1] Section 109(d)(1) of the Clean Air Act requires periodic review and, if appropriate, revision of existing air quality criteria to reflect advances in scientific knowledge on the effects of the pollutant on public health. Under the same provision, EPA is also to periodically review and, if appropriate, revise the NAAQS based on the revised air quality criteria.

The primary NAAQS for oxides of nitrogen are intended to provide the requisite public health protection from exposures to gaseous oxides of nitrogen. Nitrogen dioxide (NO_2) is the indicator of the current standards and the predominant oxide of nitrogen assessed in this ISA and in previous assessments. The evidence for human health effects associated with particulate oxides of nitrogen (e.g., nitrates) has been most recently evaluated in the ISA for Particulate Matter (U.S. EPA, 2019), and the evidence for ecological effects of oxides of nitrogen has been evaluated in the ISA for Oxides of Nitrogen, Oxides of Sulfur, and Particulate Matter – Ecological Criteria (U.S. EPA, 2020).

This ISA evaluates relevant studies published since the literature search cutoff-date for the 2016 Oxides of Nitrogen ISA (i.e., March 2014). The recent evidence is evaluated in the context of studies cited in previous assessments, including previous ISAs and Air Quality Criteria Documents (AQCD). To meet scoping criteria for this ISA, studies must present new information or analyses and must have undergone scientific peer review (see Chapter 14 for more details). Review articles that only summarize and interpret existing literature without presenting new information or analyses are outside the scope of this ISA. Beyond these general criteria, each chapter presents additional discipline-specific literature scoping criteria designed to focus the evidence assessment on the most relevant studies.

ES.2 Oxides of Nitrogen Emissions and Atmospheric Science

Current data and research advances on emissions sources, ambient air concentrations, and atmospheric chemistry inform this ISA's evaluation of oxides of nitrogen exposures and its assessment of the health effects evidence. Nitric oxide (NO) and NO_2 are the dominant forms of oxides of nitrogen emitted from major sources. Consistent with the convention in the atmospheric science research

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

community, this ISA uses the term NO_X when referring to the sum of NO and NO_2. Secondary reactions between NO_X and other pollutants result in the formation of other oxidized N-containing molecules such as nitric acid (HNO_3), nitrous acid (HONO), and organic nitrates. NO_Y is defined as the sum of NO_X and these secondary reaction products.

Major developments in the recent oxides of nitrogen literature include shifts in important emission sources, improved understanding of chemistry and transport, advances in satellite measurement and modeling capabilities, and decreasing trends in ambient concentrations. It is estimated that 70% of U.S. NO_X emissions result from motor vehicles (highway or non-road) and stationary fuel combustion (mainly for electricity or heat). Decreases in emissions from these sources have been the primary driver of the overall decrease in estimated nationwide anthropogenic NO_X emissions from 2002 to 2023. As U.S. NO_X emissions from motor vehicles and stationary fuel combustion have decreased, wildfires and agricultural soil have emerged as increasingly important contributors to total NO_X emissions.

Based on ambient outdoor air quality monitoring data from 2024, none of the over 400 NO_2 monitors in the United States measured concentrations exceeding the primary annual NO_2 NAAQS. Similarly, based on monitoring data from 2022-2023, no NO_2 monitors in the United States measured concentrations exceeding the primary 1-hour NO_2 NAAQS. In 2024, the maximum annual NO_2 design value was 26 parts per billion (ppb) and the maximum 1-hour design value was 68 ppb, well-below the annual and 1-hour standard levels of 53 and 100 ppb, respectively. Most of the highest NO_2 concentrations were measured at near-road monitoring sites.

The emergence of satellite-based remote sensing technology has been transformative in filling gaps in monitoring data that previously made characterizing small-scale concentration differences a greater challenge, and this technology continues to advance rapidly. Because they provide an aggregate measurement of NO_2 throughout the vertical column of the atmosphere, satellite-based measurements alone are currently less accurate than ground-level monitoring network data for estimating ground-level concentrations, but they are superior for characterizing urban and neighborhood scale spatial variability and identifying emissions hot spots far from monitors. Accurate characterization of differences in NO_2 concentrations within small areas, including near roads with heavy traffic, is one of the most important considerations for NO_2 exposure estimates. Beginning in 2010, the trend for satellite-based NO_2 estimates flattened out (i.e., not increasing or decreasing), and is likely a reflection of the increased relative contributions of lightning, soils, and wildfires to total NOx emissions as emissions from vehicle and fuel combustion sources have decreased.

ES.3 Exposure and Dosimetry of Inhaled Oxides of Nitrogen

ES.3.1 Exposure

Exposures to NO_2 and other oxides of nitrogen arise from a combination of ambient and nonambient air emissions sources. The ambient air portion of NO_2 exposure stems primarily from outdoor emission sources such as highway and off-road vehicles, utility power plants, and non-utility combustion sources. In the United States, highway vehicles account for approximately 26% of total national NO_X emissions, making them prominent contributors to population-level exposures, particularly in urban areas with large numbers of people living, working, and/or attending school near primary roads. Because personal exposures to ambient air NO_2 cannot be measured directly on a large scale, monitored or modeled ambient air NO_2 is often used as a surrogate for these exposures. The association between NO_2 in ambient air and personal exposure is complex and influenced by a number of factors, including proximity to traffic and other local sources, indoor/outdoor air exchange rates, time spent in different microenvironments (such as indoors and in vehicles), the presence of indoor combustion sources, seasonal variations in ventilation and source strengths, individual activity patterns, and study design (e.g., longitudinal vs. pooled studies).

A central aspect of human exposure to NO_2 from ambient air is proximity to major roadways. Research consistently identifies steep concentration gradients near roadways, with NO_2 concentrations sharply decreasing within approximately 150 to 300 meters from primary traffic routes. The high density of roads and populations in urban areas can result in large numbers of people with elevated NO_2 exposures. Sociodemographic and socioeconomic factors may also influence patterns of NO_2 and NO_X exposures, though these factors often intersect with roadway proximity. Neighborhood characteristics can further modify exposures. The presence of green spaces has been shown to mitigate NO_2 concentrations, with vegetated areas generally exhibiting lower pollutant levels.

In the literature, researchers employ various surrogate methods designed to estimate individual-level exposure to ambient air NO_2. These methods include measurements utilizing central site monitors, passive samplers, low-cost sensors, and remote sensing technologies, as well as sophisticated modeling techniques such as land use regression, dispersion models, chemical transport models, and hybrid modeling approaches. Each surrogate method has distinct strengths and limitations and may introduce different forms of exposure measurement error, including instrument inaccuracies, time-activity misclassification, contributions from nonambient sources to total exposure estimates, spatial misalignment, and model misspecification. Simulation studies assessing health outcomes associated with short-term or long-term exposures consistently indicate that exposure measurement errors tend to reduce precision and generally bias epidemiologic effect estimates toward the null. Such bias implies that actual health effects are likely larger than those reported by epidemiologic models in the presence of exposure error.

NO_2 has frequently been utilized as representative for traffic-related air pollution due to its prevalence in motor vehicle emissions. Its suitability as a surrogate pollutant is reinforced by moderate-to-strong correlations with other traffic-related pollutants including NO, carbon monoxide (CO), black

carbon (BC), ultra fine particles (UFPs), and volatile organic carbons (VOCs). These correlations are most pronounced within urban environments near roadways, where motor vehicle emissions are highest. Emerging research additionally highlights moderate correlations between NO_2 levels and nonchemical stressors such as traffic noise or the lack of green or blue spaces. These correlations underscore the complexity involved in disentangling the health effects identified in epidemiologic studies that are specifically attributable to NO_2 exposures from those that may result from exposures to correlated copollutants or from other environmental stressors. Consequently, careful consideration of these interrelated factors is essential when interpreting epidemiologic findings related to NO_2 exposure in ambient air.

ES.3.2 Dosimetry

Dosimetry of NO_X considers the properties of NO and NO_2 that affect their absorption, distribution, metabolism, and elimination. The absorption of inhaled NO_2 into the epithelial lining fluid of the lungs is governed by "reactive absorption" that involves dissolution followed by chemical reaction with substrates such as ascorbate, urate, and glutathione. The bioactive reaction products of NO_2 that reach cell surfaces may cause lipid oxidation and cellular injury. Although NO_2 is created endogenously, it acts primarily as a cytotoxic oxidant with products such as peroxynitrite within the respiratory tract.

The absorption of inhaled NO proceeds similarly to oxygen and CO, with diffusion through the lining fluid of the lung and into the vasculature, where it readily binds with hemoglobin. Within the respiratory tract, NO (whether inhaled or endogenous) acts as a pulmonary vasodilator that can reduce pulmonary hypertension, increase blood oxygenation, induce bronchodilation, and have antibacterial effects. Although NO is a radical species, it is more selective in its reactivity than NO_2; NO acts to inhibit oxidation of lipids, whereas NO_2 initiates lipid peroxidation. Exhaled NO from endogenous production increases with airway inflammation. NO can also negatively affect health when it contributes to excess oxidant burden (i.e., oxidative stress), as observed in some inflammatory disorders.

Nitrite and nitrate are metabolites of NO_2 and NO. Systemic amounts of nitrite and nitrate derived from NO_X are generally low relative to nitrite and nitrate from dietary intake. Nitrate is the primary stable NO_X product and is excreted in urine.

ES.4 Health Effects of Oxides of Nitrogen Exposure

This ISA evaluates health effects that occur following both short- and long-term exposures to oxides of nitrogen (predominantly NO_2) as examined in epidemiologic, controlled human exposure, and animal toxicological studies (see Chapters 3 through 13). The conclusions presented in Table ES-1 are informed by recent findings integrated with information from the 2016 Oxides of Nitrogen ISA (U.S.

EPA, 2016). Important considerations include the consistency and coherence of findings integrated across epidemiologic, controlled human exposure, and toxicological studies; support for biological plausibility; and uncertainty in the collective body of available studies.

Table ES-11 Summary of causality determinations by health outcome

Health Outcome		Causality Determination
Respiratory Effects – Short-term Exposure		
Respiratory Effects – Long-term Exposure		↑
Cardiovascular Effects – Short-term Exposure[a]		
Cardiovascular Effects – Long-term Exposure[a]		
Reproductive and Developmental Effects[b]	Male Reproductive Function	+
	Female Reproductive Function	+
	Pregnancy Outcomes	+
	Birth Outcomes	
	Postnatal Development	
Metabolic Syndrome and Diabetes – Short-term Exposure[a]		+
Metabolic Syndrome and Diabetes – Long-term Exposure[a]		+
Nervous System Effects – Short-term Exposure		+
Nervous System Effects – Long-term Exposure		+
Total Mortality – Short-term Exposure		
Total Mortality – Long-term Exposure		
Cancer		
Health Effects with Limited Evidence	Immune Effects	+
	Renal Effects	+
	Hepatic Effects	+
	Ocular Effects	+
	Gastrointestinal Effects	+
	Musculoskeletal Effects	+

Causal (2) Likely Causal (0) Suggestive (9) Inadequate (12)

↑ Denotes change in the causality determination from 2016 Oxides of Nitrogen ISA
+ Denotes a new causality determination
[a]The 2016 Oxides of Nitrogen ISA made a single causality determination for cardiovascular and metabolic effects (suggestive). For this draft ISA, separate causality determinations were made for cardiovascular effects and metabolic effects.
[b]The 2016 Oxides of Nitrogen ISA made three causality determinations with respect to reproductive and developmental effects: (1) Fertility, Reproduction, and Pregnancy (inadequate), (2) Birth Outcomes (suggestive), and (3) Postnatal Development (inadequate).

As in past Oxides of Nitrogen ISAs (U.S. EPA, 2016, 2008), the strongest evidence in this ISA is for respiratory effects, particularly effects related to asthma. Sections ES.4.1 and ES.4.2 summarize the evidence supporting causal relationships between short-term (ES.4.1) and long-term (ES.4.2) oxides of nitrogen exposures and respiratory effects. For more details regarding the evidence for short-term or long-term oxides of nitrogen exposures and respiratory effects see Chapters 3 and 4 in this ISA, respectively. Chapters 5 through 13 in this ISA evaluate evidence for other health outcomes with causality determinations that are *suggestive of, but not sufficient to infer, a causal relationship* or *inadequate to infer a causal relationship*. These other outcomes are not discussed in further detail in this Executive Summary.

ES.4.1 Short-Term Exposure and Respiratory Effects

The 2016 Oxides of Nitrogen ISA concluded that the available scientific evidence supported a *causal relationship* between short-term NO_2 exposure[2] and respiratory effects. This conclusion was based primarily on coherence along multiple lines of evidence and biological plausibility for effects on asthma exacerbation. Consistent with the evidence in the 2016 Oxides of Nitrogen ISA, recent studies continue to support the relationship between short-term oxides of nitrogen exposure, primarily NO_2, and asthma exacerbations. There is evidence for a continuum of effects ranging from the initial generation of reactive oxygen and nitrogen species and inflammatory changes in the respiratory tract to subclinical effects, including allergic inflammation in the respiratory system and increased airway responsiveness, to clinical events indicative of asthma exacerbation, such as respiratory symptoms in populations with asthma. This evidence is briefly summarized below and discussed in detail in Chapter 3 of this ISA.

Recent epidemiologic evidence includes multicity and nationwide studies conducted in the United States and Canada that observed NO_2-related increases in hospital admissions and emergency department (ED) visits for asthma, as well as smaller panel studies linking short-term NO_2 exposures to respiratory symptoms and medication use in children and adults with asthma. Across studies of hospital admissions and ED visits, there is consistent evidence demonstrating stronger associations among children compared to adults. Recent studies report positive associations based on previously unexamined exposure assignment techniques, and epidemiologic studies that compare results across exposure assignment methods report that exposure measurement error impacts the magnitude, but not the direction, of the observed associations. In the few available epidemiologic studies that examine the shape of the concentration-response relationship, the evidence does not indicate the presence of a threshold and there continues to be support for an association at the lower end of study concentration distributions.

[2]The criteria pollutant is oxides of nitrogen, although most health studies evaluated in the 2016 Oxides of Nitrogen ISA examined exposure to NO_2, resulting in the causality determination to be driven by the evidence of exposure to NO_2 and health effects.

Uncertainty remains regarding the degree to which observed epidemiologic associations represent an independent effect of NO_2 or serve as an indicator for effects of the traffic-related pollution mixture or components of that mixture. However, this uncertainty is reduced by epidemiologic studies reporting that associations with NO_2 exposures remain relatively unchanged in copollutant models and by experimental studies reporting asthma-related effects following exposures to NO_2 alone. In particular, controlled human exposure studies support an independent effect of short-term NO_2 exposures on the physiological processes that underlie asthma exacerbation. These studies provide evidence of NO_2-enhanced markers of allergic inflammation, which may promote asthma exacerbation through increases in airway reactivity, reductions in lung function, and/or increased airway resistance. Additional support comes from meta-analyses of controlled human exposure studies that indicate increased airway responsiveness in adults with asthma exposed to NO_2 at rest. Results of epidemiologic and controlled human exposure studies are additionally coherent with recent toxicological studies demonstrating NO_2-induced increases in markers of allergic inflammation in animal models.

When taken together, the evidence is sufficient to conclude that there is a *causal relationship* between short-term oxides of nitrogen exposure and respiratory effects. The strongest support for this determination comes from the coherence of findings integrated across controlled human exposure, epidemiologic, and animal toxicological studies supporting a relationship between short-term NO_2 exposure and asthma exacerbation. The evidence supporting conclusions on short-term oxides of nitrogen exposure and respiratory effects is discussed in detail in Chapter 3 of this ISA, and conclusions on biological plausibility, lifestages and populations at increased risk, and causality are summarized in Executive SummaryIS.5.1.1, Executive SummaryIS.5.1.2, and Executive SummaryIS.5.1.3, respectively.

ES.4.2 Long-Term Exposure and Respiratory Effects

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). This causality determination was driven by strong epidemiologic evidence demonstrating consistent associations between long-term NO_2 concentrations in ambient air and asthma development in children. The greatest uncertainty in the epidemiologic evidence at that time was a limited consideration of potential confounding by traffic-related copollutants. Because the number of supporting experimental studies was limited, there was remaining uncertainty regarding an independent effect of long-term NO_2 exposure on asthma development. However, animal toxicological and controlled human exposure studies supporting increased airway responsiveness and allergic inflammation, which are key events in the proposed mode of action for asthma development, were 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. Support for respiratory effects other than asthma development was more uncertain, lacking coherence across epidemiologic and experimental studies and, in some cases, lacking

consistency within lines of evidence. This evidence is briefly summarized below and discussed in detail in Chapter 4 of this ISA.

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. 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 and in smaller, recruitment-based cohorts that controlled for more individual-level covariates. These studies indicate 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. Compared to the evidence in adults evaluated in the 2016 Oxides of Nitrogen ISA, recent studies include larger sample sizes, greater geographic diversity, and a greater variety of exposure assessment methods. A limited number of studies that evaluated the shape of the concentration-response relationship noted an approximately linear relationship between NO_2 exposure and asthma incidence across the concentration distribution.

Potential copollutant confounding, particularly by traffic-related pollutants, remains an important uncertainty in the epidemiologic literature. Robust associations of NO_2 with asthma incidence in children and adults were observed in recent epidemiologic studies that used copollutant models to adjust for traffic-related pollutants, although high correlations between NO_2 and potential copollutant confounders is a key uncertainty of determining an independent effect of exposure to NO_2 on health effects in these studies. Uncertainty in the independent nature of the associations observed in the epidemiologic evidence base is mitigated by evidence from experimental animal studies that demonstrate respiratory effects following exposures to NO_2 alone. Consistency of recent animal studies, with the previously limited animal evidence related to increased allergic responses, reduces uncertainty of copollutant effects in the epidemiologic literature. Recent toxicological studies show consistent increases in pro-asthmatic phenotypes following prolonged or repeat NO_2 exposure and expand the support for key events that could drive asthma development. This includes increases in airway reactivity, inflammation, lung damage, and airway histologic changes in response to prolonged NO_2 exposure. Additionally, recent studies report that gestational exposure to NO_2 results in heightened inflammation and allergic phenotype in allergen-sensitized offspring, which is coherent with epidemiologic studies that observe associations between prenatal NO_2 exposure and asthma incidence in children. Animal toxicologic studies also report consistent support for worsening allergic responses in sensitized animals following prolonged or repeated NO_2 exposure. This recent animal toxicological studies supporting biological plausibility shows that repeat

exposure to NO_2 alone can increase inflammation and oxidative stress and response shows dose dependence.

Taken together, there is sufficient evidence to conclude that there is a *causal relationship* between long-term exposure to oxides of nitrogen and respiratory effects. This determination 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. 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. 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 evidence 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 supporting conclusions on long-term NO_2 exposure and respiratory effects is discussed in detail in Chapter 4 of this ISA, and conclusions on biological plausibility, lifestages and populations at increased risk, and causality are summarized in IS.5.2.1Executive SummaryIS.5.2.1, IS.5.2.2, and IS 5.2.3, respectively.

ES.5 References

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). (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). (2019). Integrated Science Assessment (ISA) for particulate matter (final report, Dec 2019). (EPA/600/R-19/188). Washington, DC. https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=347534

U.S. EPA (U.S. Environmental Protection Agency). (2020). Integrated science assessment for oxides of nitrogen, oxides of sulfur, and particulate matter—Ecological criteria (final report) [EPA Report]. (EPA/600/R-20/278). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development. https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=349473

Integrated Synthesis

Overall Conclusions of the Oxides of Nitrogen Integrated Science Assessment (ISA)

- Anthropogenic NO_X emissions and ambient NO_2 concentrations have decreased substantially across the United States since the early 2000s. Anthropogenic emissions continue to be dominated by mobile sources and power generation.
- Exposures to ambient NO_2 are elevated near busy roads; recent advances in modeling and remote sensing have improved NO_2 exposure estimates. Strong correlations between ambient NO_2 and other traffic-related pollutants can complicate interpretation of NO_2 health effect estimates in epidemiologic studies that use ambient NO_2 as an exposure surrogate.
- Recent epidemiologic, controlled human exposure, and animal toxicology studies reinforce evidence assessed in the 2016 Oxides of Nitrogen ISA and continue to support a *causal relationship* between short-term exposure to oxides of nitrogen and respiratory effects.
- Epidemiologic and experimental studies that have become available since the 2016 Oxides of Nitrogen ISA strengthen support for a *causal relationship* between long-term exposure to oxides of nitrogen and respiratory effects.
- Consistent with the evidence assessed in the 2016 Oxides of Nitrogen ISA, recent animal toxicological and epidemiologic studies are *suggestive of, but not sufficient to infer, a causal relationship* between short-term exposure to oxides of nitrogen and cardiovascular effects and total mortality, and long-term exposure to oxides of nitrogen and cardiovascular effects, birth outcomes (reproductive and developmental effects), cancer, and total mortality.
- The expanded recent evidence from animal toxicological and epidemiologic studies is *suggestive of, but not sufficient to infer, a causal relationship* between long-term exposure to oxides of nitrogen and metabolic syndrome and diabetes, nervous system effects, and pregnancy outcomes (reproductive and developmental effects).
- For all other health effect categories, uncertainties and limitations in available scientific studies contribute to evidence that is *inadequate to infer the presence or absence of a causal relationship*.

IS.1 Introduction

IS.1.1 Integrated Science Assessment Purpose

The Integrated Science Assessments (ISAs), prepared by the U.S. Environmental Protection Agency (EPA), serve as the scientific foundation for reviews of the National Ambient Air Quality Standards (NAAQS).[3] The ISAs provide a comprehensive evaluation and synthesis of the policy-relevant science "useful in indicating the kind and extent of all identifiable effects on public health or welfare

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

which may be expected from the presence of [the] pollutant in the ambient air," as described in Section 108 of the Clean Air Act (42 U.S. Code [U.S.C.] 7408). This ISA reviews and synthesizes scientific evidence related to the health effects of oxides of nitrogen exposures.[4] It draws on the existing body of evidence to evaluate and describe the state of scientific knowledge on the most relevant issues pertinent to the current review of the primary NAAQS for oxides of nitrogen; it identifies changes in the scientific evidence since the previous review (U.S. EPA, 2016); and it describes remaining or newly identified uncertainties and limitations in the evidence. This Integrated Synthesis (IS) provides a concise synopsis of the ISA conclusions and synthesizes the key findings considered in characterizing oxides of nitrogen exposure and relationships with health effects.

IS.1.2 Approach to Developing this Oxides of Nitrogen ISA

Each NAAQS review begins with a "Call for Information," published in the Federal Register, announcing the start of the review and inviting the public to assist in this process by identifying relevant research studies in the subject areas of concern. For this review of the primary NAAQS for oxides of nitrogen, the Call for Information was published in the Federal Register on December 9, 2022 (87 FR 75625). Following the Call for Information, the planning phase of the review included development of Volumes 1 and 2 of the Integrated Review Plan (IRP). Volume 1 provided background information on the health-based air quality criteria and primary standards for oxides of nitrogen. Volume 2 addressed the general approach for the review of the primary NAAQS for oxides of nitrogen and planning for the ISA and was made available for public comment and provided to the Clean Air Scientific Advisory Committee (CASAC) for consultation (U.S. EPA, 2024b).

The general process for developing an ISA, including the approaches to assessing the evidence and the frameworks for evaluating weight of evidence and populations potentially at increased risk, is discussed in Volume 2 to the IRP and described in detail in Appendix A to Volume 2 (U.S. EPA, 2024b). The ISA development process described in Volume 2 of the IRP builds on the approach described in the 2015 Preamble to the ISAs (U.S. EPA, 2015), with updates that reflect advances implemented in recent ISAs, recommendations on the ISA causality framework from an ad hoc committee of the National Academies of Science, Engineering and Medicine (NASEM) (NASEM, 2022), and consideration of comments on Volume 2 of the IRP provided by members of the CASAC NO_X Panel (CASAC, 2024). This approach is summarized below in Figure IS-1.

[4]Section 108(c) of the Clean Air Act, 42 U.S.C. § 7408(c) specifies that criteria for oxides of nitrogen include consideration of nitric and nitrous acids, nitrites, nitrates, nitrosamines, and other derivatives of oxides of nitrogen, including multiple gaseous and particulate species. Gaseous oxides of nitrogen include NO_2, nitric oxide (NO), and their various reaction products (see Chapter 1, Section 1.3.1; Figure 1-7).

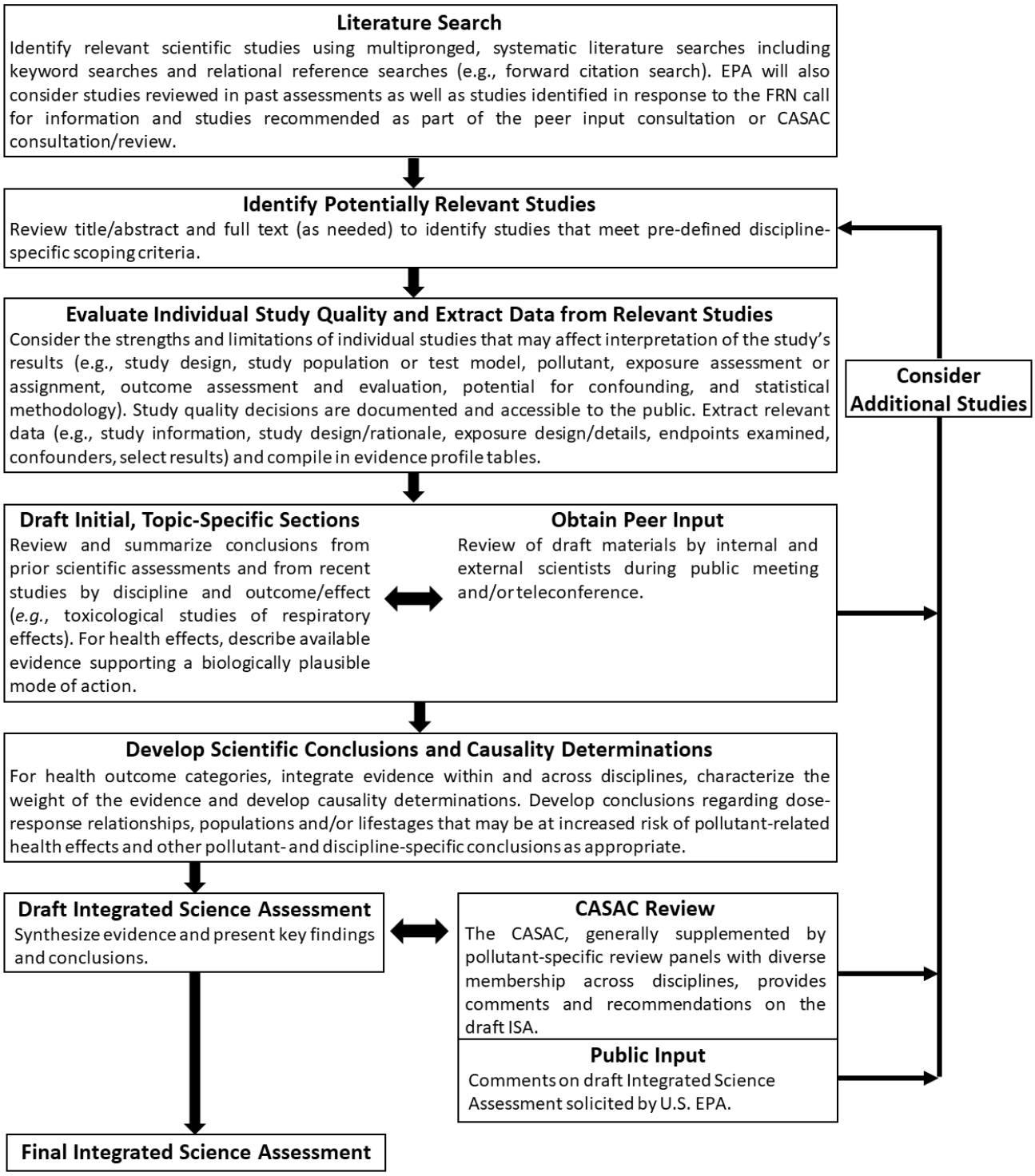


Figure IS-1 Process for developing the Oxides of Nitrogen Integrated Science Assessments.

Studies considered in this ISA are documented in the U.S. EPA Health and Environmental Research Online (HERO) database. The publicly accessible HERO project page for this ISA contains the references considered for inclusion and provides bibliographic information and abstracts. Within HERO, each reference has a unique HERO ID number. References can be viewed individually or filtered by chapter, discipline, or the versions of the document (e.g., peer input draft, final) in which they are referenced.

IS.1.3 Quality Assurance Summary

The EPA has an agency-wide quality assurance (QA) policy outlined in EPA's Quality Manual for Environmental Programs (see CIO 2105-P-01.1) that follows the specifications outlined in EPA Order CIO 2105.1. As required by CIO 2105.1, EPA's Office of Research and Development (ORD) maintains a Quality Management Program, which is documented in an internal Quality Management Plan. The ISAs are designated as Highly Influential Scientific Assessments (HISAs) and are classified as ORD QA Category A. As such, this ISA is subject to the EPA's Quality Management Program requirements for a Quality Management Plan and adheres to the Program Quality Assurance Project Plan (PQAPP) for the Integrated Science Assessment Program, (QAPP ID: L-HEEAD-0030253-QP-1-6). This ISA has been subjected to management and QA clearance review, and during this step the Center for Public Health and Environmental Assessment (CPHEA) QA Manager verifies that the EPA QA requirements are met (see Appendix A of Volume 2 of the IRP for the Oxides of Nitrogen ISA (U.S. EPA, 2024b). For more details, see Chapter 14, Section 14.8.

IS.1.4 ISA Scope

The primary NAAQS for oxides of nitrogen are intended to protect public health from exposures to gaseous oxides of nitrogen. Nitrogen dioxide (NO_2) is the indicator for the current standards, and most of the health effects evidence assessed in this ISA and in previous assessments examines NO_2 exposures. This ISA evaluates the atmospheric chemistry and ambient air concentrations of oxides of nitrogen, the evidence for human exposure and dosimetry of inhaled oxides of nitrogen, and the human health effects associated with exposure to the gaseous oxides of nitrogen. The evidence for human health effects associated with organic and inorganic nitrates was evaluated in the ISA for Particulate Matter (U.S. EPA, 2019) and considered in recent decisions on the particulate matter NAAQS (U.S. EPA, 2024c, d). The evidence for ecological effects of oxides of nitrogen was reviewed in conjunction with the evidence for ecological effects of sulfur oxides and particulate matter in the ISA for Oxides of Nitrogen, Oxides of Sulfur, and Particulate Matter – Ecological Effects (U.S. EPA, 2020) and considered in recent decisions (December 27, 2024, 89 FR 105692).

This ISA evaluates relevant studies that have become available since the literature search cutoff-date for the 2016 Oxides of Nitrogen ISA (i.e., March 2014) in the context of studies evaluated in previous assessments, including previous ISAs and Air Quality Criteria Documents (AQCDs) [i.e., U.S. EPA (2016, 2008, 1993, 1982)]. For topic areas in which research efforts have subsided and older studies remain the definitive works available in the literature, those older studies from previous assessments remain the primary focus of the evaluation. To meet ISA scoping criteria, studies must present new information or analyses and must have undergone scientific peer review (U.S. EPA, 2024b). Review articles (e.g., narrative, scoping, systematic, and meta-analyses of systematic review) that only summarize and interpret existing studies without presenting new information or analyses are outside the scope of this ISA. Beyond these general scoping criteria, each chapter presents discipline-specific literature scoping criteria to focus the evidence assessment on the most relevant studies.

IS.1.5 ISA Organization

The ISA consists of the Front Matter (list of authors, contributors, and reviewers), Executive Summary (ES), IS, and 14 chapters. This IS consolidates and integrates key findings from the chapters. Chapters are organized by subject area and include a detailed assessment and description of oxides of nitrogen atmospheric chemistry and ambient concentrations (Chapter 1), exposure and dosimetry (Chapter 2), and health effects evidence (Chapter 3 through Chapter 13). Chapter 14 describes the ISA development process.

Organization of this Oxides of Nitrogen ISA:

- 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

The remainder of this IS includes summaries of key information and conclusions for topic areas evaluated in detail in each of the chapters. Section IS.2 summarizes information and conclusions on atmospheric chemistry and ambient concentrations. Section IS.3 summarizes information and conclusions on exposure and dosimetry of inhaled oxides of nitrogen. Section IS.4 summarizes information and conclusions on the nature of health effects associated with oxides of nitrogen exposure, the lifestages and populations that may be at increased risk due to exposures, and the strength of evidence supporting those relationships. Section IS.5 provides a high-level summary of the major findings in this ISA.

IS.2 Atmospheric Chemistry and Ambient Concentration

The Atmospheric Chemistry and Ambient Concentration chapter (see Chapter 1) presents and evaluates the latest data related to emissions sources of oxides of nitrogen, emissions chemistry and concentration trends, spatial and temporal patterns for oxides of nitrogen in ambient air, and the spatial and temporal trends in oxides of nitrogen emissions and concentrations. This evidence evaluation provides essential context for understanding human exposure pathways and subsequent health effects of oxides of nitrogen.

IS.2.1 Evidence Overview

A multipronged approach was used to identify scientific studies that may be relevant for inclusion in this ISA. This approach employed both targeted and broad literature search strategies and considered studies identified by public commenters, studies recommended during the peer input workshop or the CASAC consultation on Volume 2 of the IRP, and studies identified by EPA scientists based on professional expertise. Recent studies identified during the literature search were evaluated for potential relevance using a two-step literature screening process. Initially, studies were evaluated by comparing their titles and abstracts to discipline-specific scoping criteria defined by sources, transport and transformation, exposure/extent, and measurement and modeling (STEM) statement (see Chapter 1). After title and abstract screening was complete, studies were evaluated at the full text level to confirm STEM relevance and to evaluate study quality (U.S. EPA, 2024b).

STEM-relevant studies published since March 2014 were identified for inclusion in the Atmospheric Chemistry and Ambient Concentration chapter (see Chapter 1) using the literature search,

screening, and study quality evaluation approaches described in the IRP (U.S. EPA, 2024b) and the scoping statement presented in Chapter 1 of this ISA. A breakdown of the topics covered by these studies and the number of studies addressing each topic are presented in the evidence map below (see Figure IS-1). For more detail on the literature search and screening process, see Chapter 14.

This ISA reports the latest scientific understanding of the sources, transport and transformation, concentration trends, and measurement techniques of oxides of nitrogen. As summarized in sections IS.2.2 to IS.2.5, and discussed in detail in Chapter 1, major developments in the recent literature include shifts in important emission sources, improved chemistry and transport understanding, progress in satellite measurement and modeling capabilities, and decreasing trends in ambient air concentrations.

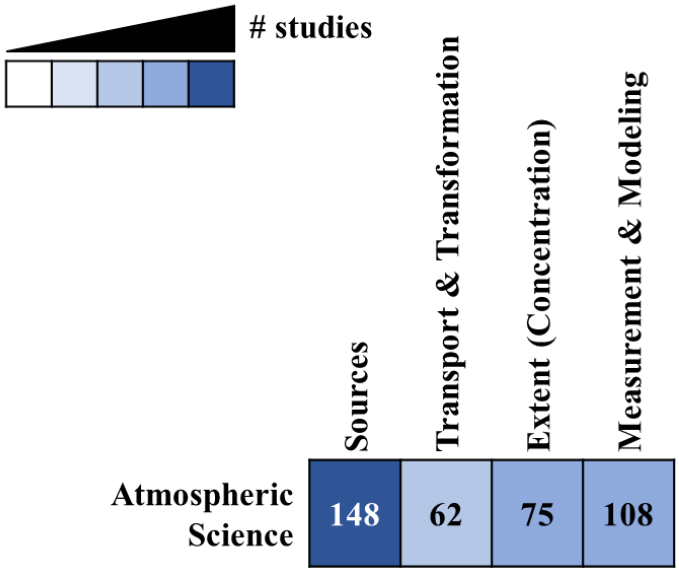


Figure IS-1 Evidence map depicting STEM relevant recent atmospheric science studies included in this ISA.

IS.2.2 Sources

Nitric oxide (NO) and NO_2 are the dominant forms of oxides of nitrogen emitted from major sources. Consistent with the convention in the atmospheric science research community, this ISA uses the term NO_X when referring to the sum of NO and NO_2. Secondary reactions between NO_X and other pollutants result in the formation of other oxidized forms of N-containing molecules such as nitric acid (HNO_3), nitrous acid (HONO), and organic nitrates. NO_Y is defined as the sum of NO_X and these secondary reaction products.

Major sources of NO_X emissions in the United States are highway vehicles (26%), non-road mobile sources (19%), stationary fuel combustion (25%), industrial processes (12%), biogenic emissions

(12%), and wildfires (5%) (see Chapter 1, Section 1.2.2). Urban areas generally have higher NOx emissions compared to rural areas, largely because they have a greater number of highway vehicles in operation, (see Chapter 1, Section 1.2.3). Non-road mobile source emissions are mostly from non-road construction and agricultural equipment and locomotives (see Chapter 1, Section 1.2.4). Most stationary fuel combustion emissions are from electricity generating units (see Chapter 1, Section 1.2.5), and the greatest contributors to industrial emissions are oil and gas production and cement manufacturing (see Chapter 1, Section 1.2.6).

Nationwide estimates based on the National Emissions Inventory indicate a 70% decrease in anthropogenic NO_X emissions from 2002 to 2023. This overall decrease in NO_X emissions has been driven primarily by decreases in the three largest anthropogenic emissions sources: an 84% reduction from highway vehicles, a 54% reduction from non-road mobile sources, and a 68% reduction from stationary fuel combustion (see Chapter 1, Section 1.2.2). Despite these reductions, these three sectors remain the largest contributors to total NO_X emissions, particularly vehicle emissions. Industrial sources are decreasing more slowly than emissions from other sources, falling by 7% from 2017 to 2020, compared to 21% for total U.S. NO_X emissions (see Chapter 1, Section 1.2.2).

As U.S. NO_X emissions from anthropogenic sources have decreased, wildfires and soil have emerged as proportionally more important contributors to overall NO_X emissions (see Chapter 1, Section 1.2.7). For example, biogenic soil sources accounted for 12% of total U.S. NO_X emissions in 2020, roughly double the 6% contribution reported in the 2016 Oxides of Nitrogen ISA. Wildfires and soil contrast with other major sources because their emissions are: (1) an increasing share of total U.S. NO_X emissions as motor vehicle and other source emissions decline; (2) highest in the warm season, when ozone formation from NO_2 is more efficient and NO_2 lifetimes are shorter; and (3) more difficult to estimate and more uncertain than anthropogenic emission sources. These differences have implications for the relationship between NO_2 emissions and exposure, the accuracy of NO_2 exposure estimates, and future research needs to better understand NO_2 exposure and health effects (see Chapter 1, Section 1.2.7). However, wildfires and soil together still only account for roughly one fifth of total U.S. emissions and even less in populated areas with the highest exposures.

IS.2.3 Atmospheric Chemistry and Fate

Major developments in the atmospheric chemistry and fate of NO_X center around several key topics, including improved understanding of urban and wintertime NO_X lifetimes; surfaces such as the ground and particulate nitrate (pNO_3^-) as sources of nitrous acid (HONO) and recycled NO_X; and NO_Y chemistry in wildfire smoke (see Chapter 1, Section 1.3). In urban areas, NO_X decreases have significantly changed urban NO_X lifetimes. A recent field campaign found that wintertime NO_X lifetimes were approximately six hours, longer than the ~three-hour summertime NO_X lifetime due to slowing of chemical reactions by reduced sunlight and/or hydroxyl radicals (OH). NO_X lifetimes can also be

lengthened through "renoxification," a process in which NO_X is temporarily transformed into intermediate forms, such as HONO, through either (1) homogeneous and heterogeneous reactions of NO_X with surfaces (i.e., aerosols or ground) or (2) photodecomposition of N-containing compounds and pNO_3^-. Over the past decades, burned area from wildfires has been increasing. Wildfires directly emit NO_X and are efficient environments for producing other NO_Y such as organic nitrates, which can also serve as intermediates that can increase NO_X concentrations far from fire sources (see Chapter 1, Section 1.3).

IS.2.4 Measurement and Modeling Methods

As the current NAAQS indicator, NO_2 is measured as part of a national NO_2 monitoring network using a Federal Reference Method based on chemiluminescence (see Chapter 1, Section 1.4.1). Monitors are operated by state, local, and tribal air agencies, and NO_2 measurements from the network are used in regulatory determinations. NO_2 accounts for less than 10% of freshly emitted NO_X near sources like highway vehicles. As that freshly emitted NO_X moves away from sources within seconds to minutes, more NO is oxidized to NO_2 (see Chapter 1, Section 1.3). For example, ambient NO_2/NO_X ratios have been measured to be >0.70 upwind and 100 meters downwind from an interstate (see Chapter 2, Section 2.3).

Because of the high spatial variability in NO_2 concentrations (see Chapter 1, Section 1.5) and the sparse coverage of ground-level NO_2 monitoring networks (see Chapter 1, Section 1.4.1), the emergence of satellite-based remote sensing technology has been transformative in interpolating gaps in NO_2 ground-level concentration monitoring data (see Chapter 1, Section 1.4.3). Unique spectral features in the visible blue wavelength range make NO_2 suitable for detection by satellite-based remote sensing (see Chapter 1, Section 1.4.5). The most recently developed instruments are the TROPOspheric Monitoring instrument (TROPOMI), launched in 2017, and the Tropospheric Emissions: Monitoring Pollution (TEMPO) ultraviolet and visible spectrometer, launched in April 2023 (see Chapter 1, Section 1.4.1). A prominent feature of the evolution of satellite NO_2 measurement technology is the steady improvement in spatial resolution (see Chapter 1, Section 1.4.3). However, inaccuracies in the vertical distribution of NO_2 and other properties can result in biases in remotely sensed NO_2 surface concentration estimates compared to ground-based monitoring data. As a result, satellite-based measurements alone are less accurate than monitoring network data but are superior for characterizing urban and neighborhood-scale spatial variability and identifying emission hot spots far from monitors. There has been recent progress in reducing satellite data retrieval uncertainty (see Chapter 1, Section 1.4.3), as well as integration of satellite data with land use and meteorological data, chemical transport modeling, and machine learning algorithms, to improve NO_2 surface concentration estimates (see Chapter 1, Section 1.4.4).

Like satellites, chemical transport models (CTMs) are used to predict NO_2 surface concentrations at finer spatial and temporal resolutions than monitoring networks. The Community Multiscale Air Quality (CMAQ) model, a CTM developed by EPA, is used for research and regulatory purposes. Recent

CMAQ improvements include treatment of vertical mixing and inclusion of nitrate photolysis, reducing both discrepancies between model results and ambient measurements and uncertainties in satellite retrieval data (see Section 1.4.6).

IS.2.5 Ambient Concentrations of NO_X and Copollutants

In 2023, none of the 410 NO_2 monitors in the United States recorded concentrations exceeding the annual or 1-hour NO_2 NAAQS. Overall, measurements of higher NO_2 concentrations match known NO_X emissions patterns, with highest concentrations measured in urban areas such as New York, NY; Denver, CO; and parts of Los Angeles and the Central Valley of California. Near-road monitors recorded some of the highest NO_2 concentrations due to motor vehicle emissions (see Chapter 1, Section 1.4), though the highest near road monitors still have concentrations below the standards. With a few exceptions, long-term NO_2 concentrations have decreased nationally since 2000. NO_2 concentrations have decreased the most in the eastern United States, a region that tends to have higher NO_X emissions due to vehicle traffic and industrial sources. Modest increases in NO_2 concentrations have been measured in rural areas with oil and natural gas operations, such as the Permian Basin in Texas and western North Dakota (see Chapter 1, Section 1.5). Multiple satellite products also show NO_2 measurements have decreased since 1996, consistent with decreasing NO_X emissions. Total column NO_2 densities measured by satellites show flattening trends beginning in 2010. The reason for this flattening is thought to be greater contributions of lightning, soils, and wildfires to total NO_X emissions, as described above. As the relative importance of natural versus anthropogenic sources has changed, seasonal and diurnal NO_X trends have changed as well (see Chapter 1, Section 1.5). Positive associations of NO_2 with copollutants such as particulate matter with a nominal mean aerodynamic diameter less than or equal to 2.5 μm ($PM_{2.5}$) frequently occur throughout the year. Positive associations between NO_2 and O_3 are common in summer, and negative associations frequently occur with O_3 in winter (see Chapter 1, Section 1.5).

IS.3 Exposure and Dosimetry of Inhaled Oxides of Nitrogen

This ISA characterizes the health effects associated with exposure to oxides of nitrogen, primarily NO and NO_2, in ambient air and the strengths and limitations of studies evaluating those associations. Characterizing the health effects evidence requires understanding the factors affecting inhalation exposures; the factors affecting the accurate estimation of those exposures; the relationships between oxides of nitrogen and other pollutants in ambient air or stressors; and oxides of nitrogen dosimetry. To facilitate this understanding, the Exposure and Dosimetry chapter (see Chapter 2) presents and evaluates evidence related to emissions sources contributing to exposure, populations with elevated exposures, copollutant exposures, exposure measurement error and implications for interpreting epidemiologic studies, and dosimetry. The recent evidence reviewed in Chapter 2 is summarized below.

IS.3.1 Evidence Overview

A multipronged approach was used to identify scientific studies that may be relevant for inclusion in this ISA. This approach employed both targeted and broad literature search strategies and considered studies identified by public commenters, studies recommended during the peer input workshop or the CASAC consultation on Volume 2 of the IRP, and studies identified by EPA scientists based on professional expertise. Recent studies identified during the literature search were evaluated for potential relevance using a two-step literature screening process. Initially, studies were evaluated by comparing their titles and abstracts to discipline-specific scoping criteria defined by a STEM statement. After title and abstract screening was complete, studies were evaluated at the full text level to confirm STEM relevance and to evaluate study quality (U.S. EPA, 2024b).

STEM relevant studies were identified for inclusion using the literature search, screening, and study quality evaluation approaches described in Volume 2 of the IRP (U.S. EPA, 2024b), and the scoping statement presented in Chapter 2 of this ISA. A breakdown of the topics covered by these studies and the number of studies addressing each topic are presented in the evidence map below (Figure IS-3). For more details on the literature search and screening process see Chapter 14.

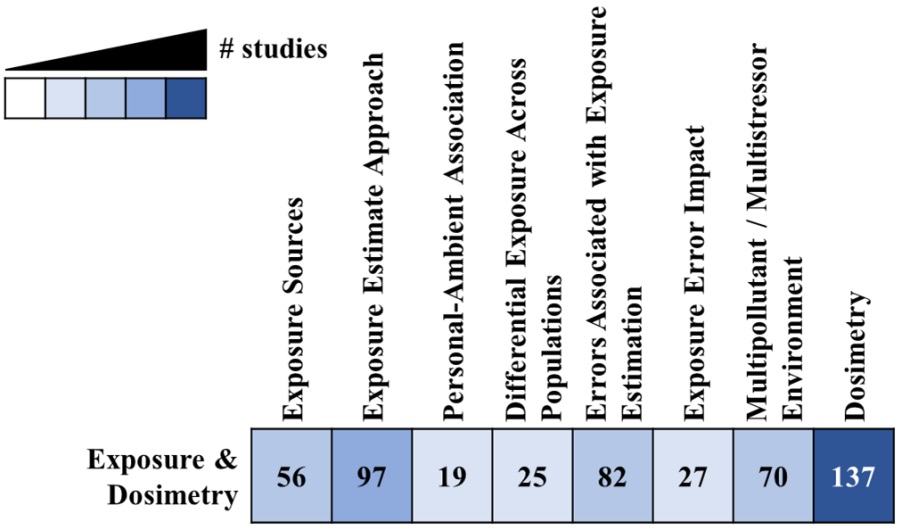


Figure IS-3 Evidence map depicting recent STEM-relevant exposure and dosimetry studies included in this ISA.

IS.3.1.1 Sources Contributing to Exposures

Exposures to NO_2 and other oxides of nitrogen arise from a combination of ambient and nonambient emission sources. Personal exposure represents the integration of pollutant concentrations

across all microenvironments encountered (e.g., residential spaces, workplaces, vehicle interiors) and the time spent within each environment. Mathematically, personal exposure can be partitioned into an ambient air component, derived from direct outdoor exposure and outdoor air pollutants entering indoor spaces through infiltration, and a nonambient air component reflecting pollutants emitted within indoor environments independently of outdoor air. This conceptual distinction facilitates clearer evaluation of each source's relative contribution to total personal exposure.

Ambient NO_2 exposure primarily stems from outdoor emission sources such as highway and off-road vehicles, utility power plants, non-utility combustion industries, and other anthropogenic sources, as well as biogenic emissions and wildfires. In the United States, highway vehicles account for approximately 26% of total national NO_X emissions and more than 75% in areas with populations over 2.5 million, making them prominent contributors to population-level exposures, especially in urbanized areas. The relationship between road traffic and NO_2 concentrations in ambient air is supported by multiple lines of evidence, such as emission profiles from motor vehicles, observed diurnal variability with peaks coinciding with rush hour traffic, pronounced microscale spatial gradients proximate to roads, and correlations between traffic volume or density metrics and elevated NO_2 concentrations. Furthermore, recent natural experiments—including traffic-reduction scenarios during Coronavirus Disease 2019 (COVID-19) lockdowns—demonstrate clear linkages between decreases in vehicle activity and substantial reductions in NO_2 concentrations in ambient air.

While ambient sources play a significant role, nonambient (indoor) sources are critical to understanding total personal exposure due to the substantial amount of time individuals spend indoors. Indoor-generated NO_X emissions primarily result from combustion processes involving common residential appliances and behaviors, including gas-fired stoves and ovens, oil or gas furnaces, kerosene space heaters, wood or coal-burning stoves, candles, and smoking. Numerous studies provide empirical evidence of significantly elevated concentrations of indoor NO_2 and HONO, a combustion-derived nitrogen species with less well-characterized health implications, associated with the presence and use of these combustion sources. For instance, peak NO_2 concentrations from gas stoves can exceed 100 parts per billion (ppb) during cooking events, while equipment such as kerosene heaters can elevate indoor HONO to levels several times higher than background conditions. Additionally, recent research indicates that increases in indoor NO_2 correlate directly and proportionally with the frequency of gas burner usage during cooking, highlighting a strong relationship between natural gas usage and indoor NO_X emissions.

Indoor chemical dynamics further complicate personal exposure assessments by modifying the concentrations and speciation of oxides of nitrogen. Indoor reactions include the oxidation of NO by ozone (O_3) to form NO_2, as well as heterogeneous reactions on indoor surfaces involving NO_2 that generate HONO. Indoor HONO concentrations can vary widely and persist at elevated levels after combustion events due to surface-mediated chemical reactions. Recent studies identify contributing factors like humidity, irradiance, surface material characteristics, and even household activities (e.g.,

cleaning, cooking, air conditioning operation) as significantly influencing indoor oxides of nitrogen chemistry and resulting exposures.

IS.3.1.2 Populations at Risk for Elevated Exposures

Human exposure to NO_2 and other oxides of nitrogen in ambient air is influenced by a complex interplay of factors, with proximity to major roadways playing a critical role. Vehicular emissions from highways constitute a significant source of NO_2 and NO_X, contributing substantially to elevated concentrations near roads. Studies consistently demonstrate steep concentration gradients, with NO_2 levels sharply declining within the first 150 to 300 meters away from major roadways.

The high density of roads and populations in urban areas can result in large numbers of people with elevated NO_2 exposures because they live, work, and/or attend school in close proximity to primary roads. The U.S. Census Bureau defines primary roads as generally divided, limited-access highways within the interstate highway system or under state management, and are distinguished by the presence of interchanges (U.S. Census Bureau, 2023). Analyses in Chapter 2 indicate that almost 17 million people across the 12 most populated U.S. metropolitan areas live near a primary road (see Chapter 2, Section 2.3.4.1). Children account for about four million people in these near-road communities, where they are potentially exposed to elevated concentrations of NO_2. Since children are at higher risk of NO_2-related asthma development and exacerbation compared to adults (see Sections IS.5.1.1 and IS.5.1.2), such near-road exposures represent an important public health concern for this at-risk population.

Sociodemographic and socioeconomic factors may also influence patterns of NO_2 and NO_X exposures, though these factors often intersect with roadway proximity. Recent research across the United States consistently reveals that lower-income populations typically experience higher long-term exposure to ambient NO_2 compared to higher-income groups, often by margins of approximately 2 to 5 ppb. Regarding sociodemographic impacts, recent national-scale studies indicate that minority populations—including those in Asian-dominant, Hispanic-dominant, and Black-dominant communities—experience higher average NO_2 exposures compared to white communities. Among school-age students nationwide, minority groups are exposed to approximately 2 ppb higher long-term NO_2 concentrations than non-Hispanic white students.

Neighborhood characteristics further modify these exposure differences. Urbanization consistently correlates with elevated NO_2 concentrations in ambient air; urban schools in the United States, for instance, typically experience median NO_2 concentrations nearly double those observed at suburban or rural schools. Additionally, U.S. and international studies consistently demonstrate inverse relationships between neighborhood greenspace and NO_2 concentrations in ambient air.

IS.3.1.3 Copollutant Exposures and Confounding in Epidemiologic Studies

Because NO_2 is a prominent component of traffic emissions, it is frequently used as an indicator for traffic-related air pollution. Its suitability as an indicator is supported by strong correlations with other traffic-related pollutants, such as carbon monoxide (CO), black carbon (BC), ultrafine particles (UFPs), and volatile organic compounds (VOCs), particularly benzene, toluene, ethylbenzene, and xylenes (BTEX). These correlations are most pronounced in urban environments near roadways, where vehicular emissions are highest. NO_2 concentrations typically exhibit clear diurnal patterns, peaking during morning and evening rush hours to reflect patterns of vehicular activity. Spatially, NO_2 concentrations are significantly elevated near major roadways and gradually decrease with increasing distance from roadways, though the gradient is generally less steep compared to other primary pollutants like CO and UFPs.

Recent studies employing advanced mobile monitoring technologies have further quantified real-time associations between NO_2 and other traffic-related air pollutants in near-road environments. These studies consistently report moderate-to-strong correlations (commonly used correlation coefficient, rho (ρ) >0.5) between NO_2 and copollutants such as BC, CO, and UFPs. In ambient settings beyond immediate roadway proximity, temporal correlations between NO_2 and other air pollutants such $PM_{2.5}$, CO, and O_3 are generally moderate to strong. Long-term studies utilizing land-use regression (LUR), or dispersion models report particularly robust associations between NO_2 and BC or EC (ρ typically ranging from 0.6 to 0.9), along with moderate correlations with $PM_{2.5}$ concentrations (ρ typically near 0.5). Correlations with VOCs typically range from 0.5 to 0.7. Thus, recent studies continue to support the importance of considering confounding by traffic-related copollutants when interpreting NO_2 associations in epidemiologic studies, particularly studies that estimate NO_2 exposures using LUR or dispersion models.

Beyond chemical copollutants, emerging evidence increasingly highlights the importance of traffic noise in epidemiologic studies involving NO_2 exposure. Recent U.S.-based studies have reported moderate correlations between annual average residential noise levels and NO_X concentrations (ρ typically 0.4 to 0.6). Similarly, European cohort studies consistently demonstrate moderate correlations between long-term residential noise exposure and NO_2 concentrations in ambient air (ρ typically 0.4–0.5). The evidence of the relationship between noise frequencies and NO_2 concentrations remains limited and appears inconsistent; notably, one recent study employing advanced modeling techniques identified significant associations between specific noise frequencies [i.e., 40 hertz (Hz), 500 Hz, and 800 Hz], and NO_2 concentrations in ambient air. The prominence of the 40 Hz frequency suggests that NO_2 is associated with lower frequency noise, typically associated with engine activity.

IS.3.1.4 Exposure Estimates and Impacts on Epidemiologic Inference

Personal exposures to oxides of nitrogen include contributions from both ambient and non-ambient (indoor) sources. While *total* personal exposure is directly measurable, personal exposure attributable to *ambient* oxides of nitrogen alone is not. Thus, epidemiologic studies use a variety of exposure estimation approaches as surrogates for personal exposure to oxides of nitrogen of ambient air origin, primarily NO_2. Analyses of the relationship between personal total NO_2 exposure and NO_2 concentration in ambient air typically report low to moderate correlations. These correlations can be influenced by factors like non-ambient sources, seasonal variations, ventilation, individual activities, and study designs. Among the different correlation metrics reported (i.e., pooled, longitudinal, and daily-average correlations), daily average correlations are most relevant to the exposure estimation methods commonly used in community time-series and long-term cohort epidemiologic research. These daily average correlation coefficients illustrate the relationship between daily ambient air NO_2 concentrations in a study area and daily NO_2 exposures averaged across study participants. They often indicate stronger relationships between ambient air and personal NO_2 exposure than the other commonly reported correlation metrics, providing support for the use of ambient air NO_2 concentrations in epidemiologic studies as a surrogate for personal exposure to NO_2 of ambient air origin. Epidemiologic studies use a variety of approaches to estimate exposures to NO_2 in ambient air. These approaches include measurement methods and modeling techniques that are intended to capture spatial and temporal patterns of NO_2 exposures. Improved exposure surrogates can reduce bias in epidemiologic health effect estimates, but evaluating the validity of particular exposure surrogates can be complex and should be based on a clear rationale supporting the intended purpose rather than being based solely on statistical criteria (e.g., model to monitor comparisons). Each surrogate has strengths and limitations, as discussed in Chapter 2 and summarized below.

Measurement techniques for estimating NO_2 exposure include central site and near-road monitors, personal and microenvironmental sampling methods, low-cost sensors (LCS), and satellite remote sensing. Central site monitors provide hourly data suitable for both short-term exposure time-series studies and long-term exposure comparisons across geographic areas; however, their representativeness can be compromised by spatial variability, activity patterns, and instrument biases from other oxidized nitrogen products. Personal monitoring methods (e.g., passive samplers such as Ogawa badges) capture individual-level variability well but are limited by implementation challenges and biases from internal reactions between NO and O_3. Newly developed LCSs offer improved spatial-temporal resolution at a lower cost but require careful field calibration due to sensor drift and environmental sensitivities. Satellite-based measurements (e.g., TROPOMI, TEMPO) provide extensive spatial coverage, which is particularly valuable in areas with sparse ground monitors. These satellites measure atmospheric column densities of NO_2; thus, scaling factors derived from CTMs or LUR models are needed to estimate ground-level concentrations. Errors associated with satellite measurements arise from inaccuracies in air mass factors (AMFs), indirect measurement approaches, and limited temporal resolution.

Modeling methods for NO_2 exposure estimation range from simpler statistical interpolation methods (e.g., kriging or inverse distance weighting) to more sophisticated approaches including LUR models, CTMs, dispersion models, microenvironmental models, and hybrid models that integrate multiple data sources. Statistical interpolation accuracy heavily depends on monitor density, and insufficient density can lead to substantial errors. LUR models predict pollutant concentrations based on land use characteristics and are commonly employed for long-term exposure assessment; however, errors can occur due to coarse spatial resolution grids or inappropriate model specification when applied outside original calibration areas. CTMs simulate pollutant transport at regional scales but typically have coarse spatial resolutions that limit precision within urban areas; they often overestimate NOx in urban areas and underestimate in rural regions. Dispersion models estimate pollutant spread from sources but may introduce errors due to simplifying assumptions about dispersion dynamics in complex urban environments. Hybrid models combine multiple measurement and modeling approaches to enhance accuracy, but their complexity makes generalizing errors challenging. The various measurement and modeling approaches used to estimate exposures in epidemiologic studies introduce different types of exposure measurement errors: instrument accuracy and precision issues; spatial misalignment between monitoring locations and actual human activities; temporal variability not captured by stationary measurements; indoor sources such as gas stoves contributing significantly to total personal exposure; infiltration rates varying by building characteristics; mechanical or natural ventilation practices while indoors; and time-activity patterns influencing individual-level exposures. Spatial misalignment is particularly important because it occurs when the time-activity patterns of study participants are not factored into the study design or when the location where NO_2 exposure is estimated does not coincide with the residential, school, or work location of interest.

Exposure measurement errors can be broadly categorized as classical or Berkson errors. Classical errors tend to bias effect estimates toward the null hypothesis (underestimating true effects) while reducing precision in epidemiologic studies. In contrast, Berkson errors generally reduce precision but do not introduce bias unless confounders are present. Simulation studies conducted for both short-term and long-term exposures generally indicate that exposure measurement error reduces precision and biases health effect estimates toward null in most situations. However, these simulations suggest that positive bias emerges under rare conditions when estimated exposures exhibit high correlation (e.g., $\rho = 0.9$) with true exposures while demonstrating substantially reduced variability (e.g., 50% reduction) compared to true values. This results in the health outcome variation being attributed to a smaller range of exposure differences causing the effect size per unit exposure to appear larger than it truly is.

For epidemiologic studies evaluating short-term exposures (e.g., time-series studies), using central site monitors often results in classical-like errors primarily arising from time-activity patterns not captured by stationary measurements. These errors generally bias effect estimates toward the null hypothesis and inflate standard errors around effect estimates. For epidemiologic studies examining long-term exposures (e.g., cohort studies), spatial misclassification arises from inadequate capture of local variability by central site monitors or modeling methods and typically leads to attenuation of observed

associations toward null or imprecise effect estimates. Bias towards the null implies that true health effects could be larger than what epidemiologic models indicate, and reduced precision makes it less likely that statistically significant associations will be detected. Consequently, observed health impacts could be important to consider even in the presence of this type of error.

IS.3.1.5 Dosimetry of Inhaled Oxides of Nitrogen

The dosimetry of NO_X considers the properties of inhaled NO_2 and NO that affect their absorption, distribution, metabolism, and elimination. The absorption of inhaled NO_2 into the epithelial lining fluid of the lung is governed by a process termed "reactive absorption" that involves NO_2 dissolution followed by chemical reaction with reactive substrates such as ascorbate, urate, and glutathione (see Chapter 2, Section 2.6.2.1). Most of the absorbed NO_2 diffuses less than a micron before reacting with substrates and does not reach underlying tissues. As such, inhaled NO_2 is not transported out of the respiratory tract. However, acute increases in plasma nitrite have been observed in humans following inhalation of high NO_2 concentrations (200 to 300 ppb) which could contribute to endogenous NO_2 production outside the lungs. In other words, inhaled NO_2 may be indirectly transferred from the lungs via reactive intermediates (namely nitrite/nitrate) which can form NO_2 outside the respiratory tract. The bioactive reaction products of NO_2 exposure (or in rare cases NO_2 itself) that reach cell surfaces may cause lipid oxidation and cellular injury. Secondary reactions of bioactive NO_2 products with antioxidants may quench or detoxify some of these bioactive reaction products. On a breath-by-breath basis, about 70 to 90% of inhaled NO_2 is absorbed in the respiratory tract (see Chapter 2, Section 2.6.2.2). With exercise, the amount of NO_2 absorbed in the respiratory tract is increased by 3- to 5-times relative to rest. Although NO_2 is created endogenously, it is created only through chemical reactions and acts primarily as a cytotoxic oxidant within the respiratory tract (see Chapter 2, Section 2.6.2.3). Nitrate is the primary stable NO_X product and is excreted in urine (see Chapter 2, Section 2.6.2.4).

The absorption of inhaled NO proceeds similarly to oxygen and CO with diffusion through the lung lining fluid and into the vasculature where it readily binds with hemoglobin (see Chapter 2, Section 2.6.3.1). Within the body, most endogenous NO is produced by three major isoforms of nitric oxide synthase (see Chapter 2, Section 2.6.3.2). Endogenous production of NO is variable between regions of the respiratory tract and affected by a variety of factors including disease state, diet, height, possibly sex (inconsistent results among studies) species, smoking history, and environmental exposures. Exhaled NO (eNO) from endogenous production increases with airway inflammation. With endogenous production, the respiratory uptake of inhaled exogenous NO is difficult to quantify (see Chapter 2, Section 2.6.3.3). Both inhaled and endogenous NO can have beneficial biological functions (see Chapter 2, Section 2.6.3.4). Within the respiratory tract, NO acts as a pulmonary vasodilator that can reduce pulmonary hypertension, increase blood oxygenation, induce bronchodilation, and have antibacterial effects. Although NO is a radical species, it is more selective in its reactivity than NO_2 and acts to inhibit oxidation of lipids, whereas NO_2 initiates lipid peroxidation. Despite its beneficial effects, NO can also

cause deleterious effects when it contributes to excess oxidant burden (i.e., oxidative stress) as observed in some inflammatory disorders. Inhaled NO at a concentration of 160 ppm has been used therapeutically to increase blood oxygenation but can lead to the formation of methemoglobin which can reduce oxygen release from the blood into tissues.

Nitrite and nitrate are metabolites of both NO_2 and NO. Although inhaled NO_2 may contribute to systemic amounts of nitrite/nitrate, these levels are generally considered low relative to nitrite/nitrate from dietary intake. Inhaled NO likely results in far less systemic nitrite/nitrate than endogenous NO production which even during inflammatory states is thought to be modest compared to nitrite/nitrate from dietary intake.

IS.4 Approach to Assessing the Health Effects Evidence

The health effects of short- and long-term exposures to oxides of nitrogen are evaluated in 11 chapters (Chapters 3 through 13) in this ISA. Depending on the specific evidence base for a particular health effect, chapters include epidemiologic, controlled human exposure, and/or animal toxicological studies. The health effects evidence has been integrated within and across disciplines with available information on exposure, dosimetry, and biological plausibility to inform the key ISA conclusions, including conclusions on populations that may be at increased risk and on the strength of evidence supporting causality (i.e., causality determinations). The approach in this ISA to evaluating and integrating the health effects evidence is described in detail in Volume 2 of the IRP (U.S. EPA, 2024b) and summarized below in Sections IS.4.1 to IS.4.4. Section IS.4.1 summarizes the general approach to identifying and evaluating the health effects evidence in this ISA and provides a breakdown of the number of studies included for each discipline and health outcome category. Section IS.4.2 summarizes the approach to evaluating biological plausibility, Section IS.4.3 summarizes the framework used to reach conclusions on lifestages and populations that may be at increased risk, and Section IS.4.4 summarizes the framework used to make causality determinations.

IS.4.1 Approach to Identifying and Evaluating the Health Effects Evidence

A multipronged approach was used to identify scientific studies that may be relevant for inclusion in this ISA. This approach employed both targeted and broad literature search strategies and considered studies identified by public commenters, studies recommended during the peer input workshop or the CASAC consultation on Volume 2 of the IRP, and studies identified by EPA scientists based on professional expertise. Recent studies identified during the literature search were evaluated for potential relevance using a two-step literature screening process. Initially, studies were evaluated by comparing their titles and abstracts to discipline-specific scoping criteria defined by specific population, exposure,

comparison, outcome, study design (PECOS) statements. After title and abstract screening was complete, studies were evaluated at the full text level to confirm PECOS relevance.

Studies determined to be relevant were then evaluated for individual study quality; only studies that satisfied study quality criteria were included in this ISA. Individual study quality was evaluated by considering the design, methods, and documentation of each study, but not study results. The approach to study quality evaluation considered the strengths and limitations of individual studies, including the possible role of chance, confounding, and other biases that may affect study interpretation and the strength of inference that can be drawn from study results. The specific attributes considered in evaluating study quality included (1) study design, (2) study population or test model, (3) pollutant, (4) exposure assessment or assignment, (5) outcome assignment evaluation, (6) potential confounding, and (7) statistical methodology.

Using the literature search and screening approaches described in Volume 2 of the IRP (U.S. EPA, 2024b) and the scoping statements presented in Volume 2 of the IRP and Chapters 3 through 13 of this draft ISA, PECOS relevant studies published since March 2014 and through October 2024 were identified for inclusion. A breakdown of the topics and number of recent studies included in the health chapters (see Chapter 3 through Chapter 13) is shown in the evidence map below (see Figures IS-4). For more details on the literature search and screening process see the Process Chapter (see Chapter 14) of this ISA and Volume 2 of the IRP (U.S. EPA, 2024b).

	Health Outcome										Health Effects with Limited Evidence						
	ST Respiratory Effects	LT Respiratory Effects	ST Cardiovascular Effects	LT Cardiovascular Effects	Reproductive & Developmental Effects	Metabolic Syndrome & Diabetes	Nervous System Effects	ST Mortality	LT Mortality	Cancer	Immune Effects	Renal Effects	Hepatic Effects	Ocular Effects	Gastrointestinal Effects	Musculoskeletal Effects	Other Health Effects
Epidemiology	59	70	76	116	120	50	127	14	50	67	13	7	2	6	3	7	4
Controlled Human Exposure	3	0	1	0	0	0	0				0	0	0	0	0	0	0
Animal Toxicology	8	10	4	4	5	3	7			0	0	0	1	0	0	0	0

Note: Numbers are approximate. Figure with final numbers updated for the release of the final Oxides of Nitrogen ISA.

Figure IS-4 Evidence map depicting recent health studies included in this ISA.

IS.4.2 Approach to Evaluating Biological Plausibility

Support for biological plausibility can strengthen the basis for causal inference (U.S. EPA, 2015). In this ISA, biological plausibility is part of the weight-of-evidence analysis that considers the totality of the health effects evidence, including consistency and coherence of effects described in experimental and observational studies. All health outcomes with a causality determination of *causal, likely to be causal,* or *suggestive of, but not sufficient to infer, a causal relationship* include an evaluation of biological plausibility (see Chapters 3 through 12). The biological plausibility sections summarize the evidence for potential pathways by which oxides of nitrogen exposures could result in adverse health outcomes at the population level. Although there is some overlap in the potential pathways between the chapters, each biological plausibility section is tailored to the specific health outcome category for which causality determinations are made.

Each biological plausibility section includes a figure illustrating possible pathways that connect oxides of nitrogen exposures with health outcomes. Pathways are based on evidence evaluated in previous assessments, both AQCDs and ISAs, as well as evidence from more recent studies. The accompanying text characterizes the evidence upon which the figures are based, including results of studies demonstrating specific effects related to oxides of nitrogen exposure and considerations of physiology and pathophysiology. Together, the figure and text portray the available evidence supporting the biological plausibility of oxides of nitrogen exposure leading to specific health outcomes. Gaps in the evidence base (e.g., health endpoints for which studies have not been conducted) are represented by corresponding gaps in the figures and are identified in the accompanying text.

In the model figure below (see Figure IS-5), each box represents evidence demonstrated in a study or group of studies for a particular effect related to oxides of nitrogen exposure. While most of the studies used to develop the figures are experimental studies (i.e., animal toxicological, controlled human exposure, and in vitro studies), some observational epidemiologic studies also contribute to the pathways. The boxes are arranged horizontally, with green boxes on the left side representing initial effects that reflect early biological responses, blue boxes representing intermediate effects, and orange boxes to the right representing key clinical and population-level effects, which are generally supported using epidemiologic evidence discussed throughout each chapter.

The arrows that connect the boxes indicate a progression of effects resulting from exposure to oxides of nitrogen. In most cases, the arrows are dotted, denoting a possible relationship between the effects. While most arrows point from left to right, some point from right to left, reflecting progression of effects in the opposite direction or a feedback loop. In a few cases, solid arrows are used to indicate evidence of essentiality, that is, evidence to show that if an upstream effect is prevented or fails to occur, progression to a subsequent downstream effect will not occur (OECD, 2018). This type of evidence is generally provided by experimental studies using pharmacologic agents (e.g., inhibitors) or genetic knockout animal models. The use of solid lines, rather than dotted lines, reflects the availability of data

showing that exposure to oxides of nitrogen results in an upstream effect that is necessary for progression to a downstream effect.

In these figures, an upstream effect may be linked to multiple downstream effects. To concisely illustrate this proposed relationship, related downstream effects can be grouped together within a larger grey shaded box, with a single arrow connecting the individual upstream effect to the edge of the downstream shaded box, indicating an association with all contained endpoints. Conversely, if multiple upstream effects converge into a single downstream effect, an arrow may originate from the edge of a shaded box and connect to an individual downstream box. Arrows may also connect individual upstream and downstream effects, even when one or both are contained within a larger shaded box. Thus, arrows may connect individual boxes, groupings of boxes, or single boxes nested within groupings depending on the proposed relationships between the represented effects.

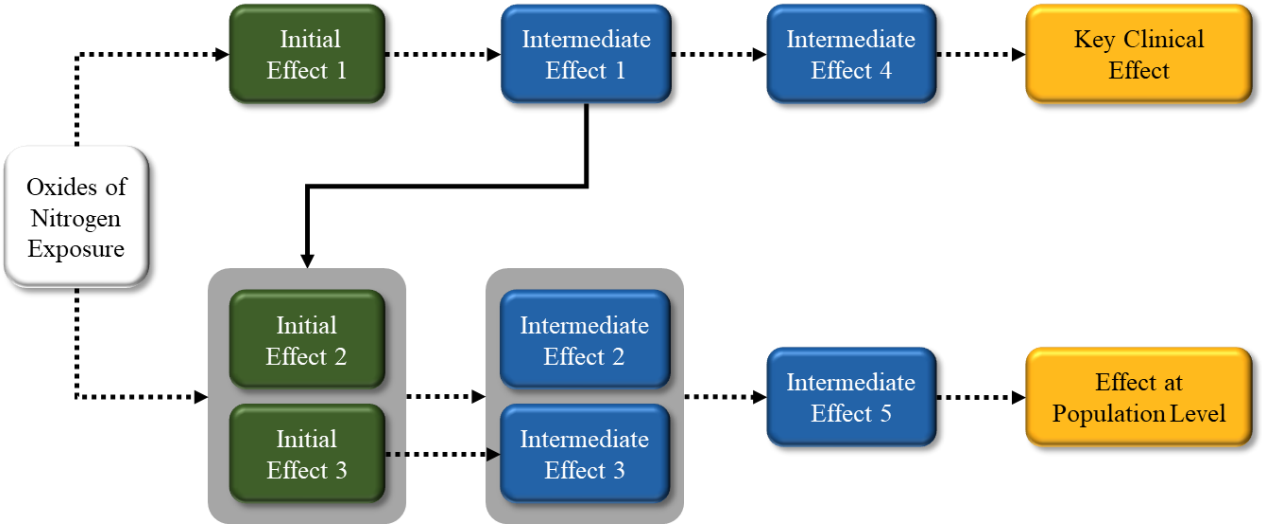


Figure IS-5 Illustrative figure for potential biological pathways for health effects following oxides of nitrogen exposure.

IS.4.3 Framework for Conclusions on Lifestages and Populations at Increased Risk

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

[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 lifestages and 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.

factors. The primary NAAQS are intended to protect public health with an adequate margin of safety. In so doing, protection is provided for the population as a whole and for groups at increased risk for health effects in response to exposure to a criteria pollutant (U.S. EPA, 2024b). The EPA has developed a framework to provide a consistent and transparent basis for informing the level of confidence for conclusions that specific lifestages or populations may be at increased risk of pollutant-related health effects according to one of four levels: adequate evidence, suggestive evidence, inadequate evidence, and evidence of no effect (see Table IS-1 and (U.S. EPA, 2024a, b) for additional details). All health outcomes with a causality determination of *causal, likely to be causal,* or *suggestive of, but not sufficient to infer, a causal relationship* include an evaluation of potentially at-risk lifestages and populations (see Chapters 3 through 12).

Table IS-1 At-risk determinations by health outcome

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

IS.4.4 Framework for Causality Determinations

The ISAs evaluate and integrate scientific evidence on health effects of criteria pollutant exposures and based on this integration and the weight of evidence, make judgments regarding the strength of support for causal relationships between pollutant exposure and particular effects.[7] In making these judgments, the ISAs consider various aspects of causality adapted from previous efforts (e.g., IOM, 2008; IARC, 2006; U.S. EPA, 2005; CDC, 2004), including consistency

[7]The 1964 Surgeon General's report on tobacco smoking defined "cause" as a "significant, effectual relationship between an agent and an associated disorder or disease in the host" (HEW, 1964). More generally, a cause is an agent that brings about an effect or a result.

of the evidence, coherence between different lines of evidence, experimental support and biological plausibility, as well as other aspects (U.S. EPA, 2024b). The ISA framework for reaching causality determinations is described in recent ISA planning documents (U.S. EPA, 2024a, b) and is summarized below in Table IS-2. This framework builds on the 2015 Preamble to the ISAs (U.S. EPA, 2015), with updates reflecting CASAC feedback over multiple ISAs and ISA planning documents and recent NASEM recommendations (U.S. EPA, 2024a; NASEM, 2022).

Table IS-2 Causality determinations for health outcomes

Descriptor	Evidence Characteristics
Causal relationship	Evidence is sufficient to conclude that there is a causal relationship with relevant pollutant exposures. That is, the pollutant exposures have been shown to result in health effects across studies in which chance, confounding, and other biases can be ruled out with reasonable confidence. A "causal" relationship is generally based on multiple high-quality studies conducted by different research groups. Evidence supporting this determination can include controlled human exposure studies that consistently demonstrate effects and/or observational studies reporting consistent health effect associations that, when considered in light of study quality and coherence with other lines of evidence (i.e., controlled human exposure studies, animal toxicological studies, and mode of action information), cannot be explained by plausible alternatives.
Likely to be a causal relationship	Evidence is sufficient to conclude that a causal relationship is likely to exist with relevant pollutant exposures. That is, the pollutant exposures have been shown to result in health effects in studies where chance, confounding, and other biases are minimized but uncertainties remain in the evidence overall. A "likely to be causal" relationship is generally based on multiple high-quality studies conducted by different research groups. Evidence supporting this determination can include 1) multiple high-quality observational studies consistently reporting health effect associations, but with uncertainty remaining related to potential confounding and/or limited coherence with other lines of evidence (i.e., controlled human exposure studies, animal toxicological studies, mode of action information) or 2) consistent evidence in animal models and/or *in vitro* models (e.g., for cancer-related effects) that can be reasonably extrapolated to human health, but limited availability of human data.
Suggestive of, but not sufficient to infer, a causal relationship	Evidence is suggestive of, but not sufficient to infer, a causal relationship with relevant pollutant exposures. That is, the pollutant exposures have been shown to result in health effects, but chance, confounding, and bias cannot be ruled out with confidence. Evidence supporting a "suggestive" relationship can be comprised of studies of varying quality that may be generally supportive of pollutant-related effects, but not entirely consistent and with limited coherence across lines of evidence. A suggestive determination can be reached with relatively small bodies of evidence or, in rare cases, one high quality study.
Inadequate to infer a causal relationship	Evidence is inadequate to determine that a causal relationship exists with relevant pollutant exposures. That is, the evidence supporting an "inadequate" relationship is limited and available studies are of insufficient quantity, quality, consistency, and/or statistical power to permit a conclusion regarding the presence or absence of an effect.

Not likely to be a causal relationship	Evidence indicates there is no causal relationship with relevant pollutant exposures. Several adequate studies, covering the full range of exposures that human beings are known to encounter and considering at-risk populations and lifestages, are consistent in not showing an effect at any level of exposure concentration.

IS.5 Health Effects Evidence for Oxides of Nitrogen

This ISA evaluates health effects that occur following both short- and long-term exposures to oxides of nitrogen (predominantly NO_2) as examined in epidemiologic, controlled human exposure, and animal toxicological studies (see Chapters 3 through 13 in this ISA). It presents causality determinations for 23 broad health effect categories. In addition to updated causality determinations for health outcomes evaluated in the 2016 Oxides of Nitrogen ISA, this ISA includes causality determinations for a number of additional health effect categories for which new evidence has become available. The causality determinations from this ISA and their relation to the conclusions from the 2016 Oxides of Nitrogen ISA are summarized in Table IS-3.

Table IS-3 Summary of causality determinations by health outcome

Health Outcome		Exposure Period	Causality Determination
Respiratory Effects		Short-term	
		Long-term	↑
Cardiovascular Effects[a]		Short-term	
		Long-term	
Reproductive and Developmental Effects[b]	Male Reproductive Funtion	Long-term	+
	Female Reproductive Function	Long-term	+
	Pregnancy Outcomes	Short-term and Long-term	+
	Birth Outcomes	Short-term and Long-term	
	Postnatal Development	Long-term	
Metabolic Syndrome and Diabetes[a]		Short-term	+
		Long-term	+
Nervous System Effects		Short-term	+
		Long-term	+
Total Mortality		Short-term	
		Long-term	
Cancer		Long-term	
Health Effects with Limited Evidence	Immune Effects	Short-term and Long-term	+
	Renal Effects	Long-term	+
	Hepatic Effects	Long-term	+
	Ocular Effects	Long-term	+
	Gastrointestinal Effects	Long-term	+
	Musculoskeletal Effects	Long-term	+

Causal (2) Likely Causal (0) Suggestive (9) Inadequate (12)

↑Denotes change in the causality determination from 2016 Oxides of Nitrogen ISA
+ Denotes a new causality determination
[a]The 2016 Oxides of Nitrogen ISA made a single causality determination for cardiovascular and metabolic effects (suggestive). For this draft ISA, separate causality determinations were made for cardiovascular effects and metabolic effects.
[b]The 2016 Oxides of Nitrogen ISA made three causality determinations with respect to reproductive and developmental effects: (1) Fertility, Reproduction, and Pregnancy (inadequate), (2) Birth Outcomes (suggestive), and (3) Postnatal Development (inadequate).

As in past Oxides of Nitrogen ISAs (U.S. EPA, 2016, 2008), the strongest evidence in this ISA is for respiratory effects, particularly effects related to asthma. Sections IS.5.1 and 0 below summarize the evidence supporting causal relationships between short-term (Section IS.5.1) and long-term (Section IS.5.2) oxides of nitrogen exposures and respiratory effects. For more details regarding the evidence for short-term or long-term oxides of nitrogen exposures and respiratory effects see Chapters 3 and 4 in this ISA, respectively. Chapters 5 through 13 in this ISA evaluate evidence for health outcomes with causality determinations that are *suggestive of, but not sufficient to infer, a causal relationship* or *inadequate to infer a causal relationship* and these outcomes are not discussed in further detail in this Integrated Synthesis.

IS.5.1 Short-Term Exposures and Respiratory Effects

IS.5.1.1 Evidence Overview

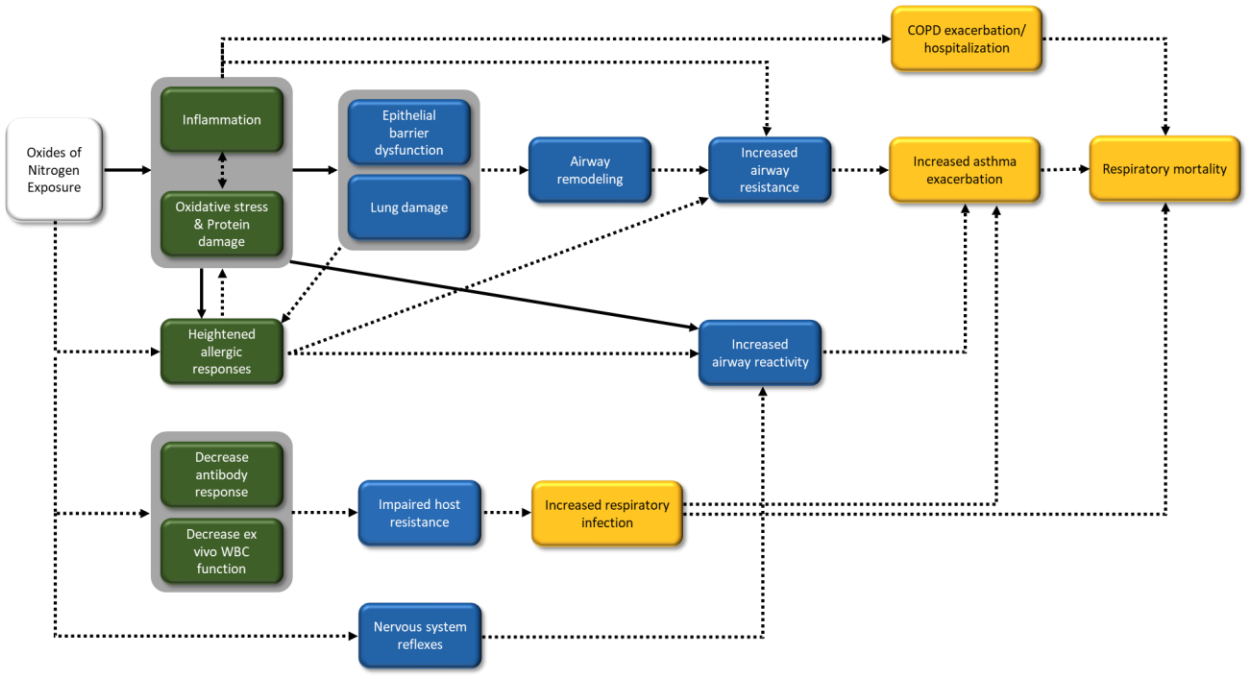
The 2016 Oxides of Nitrogen ISA concluded that the available scientific evidence supported a "causal relationship" between short-term NO_2 exposure[8] and respiratory effects. This conclusion was based primarily on coherence along multiple lines of evidence and biological plausibility for effects on asthma exacerbation. Consistent with the evidence in the 2016 Oxides of Nitrogen ISA, recent studies continue to support the relationship between short-term oxides of nitrogen exposure, primarily NO_2, and asthma exacerbations. There is evidence for a continuum of effects ranging from subclinical changes, including allergic inflammation in the respiratory system and increased airway responsiveness, to clinical events indicative of asthma exacerbation, such as respiratory symptoms in populations with asthma. In some cases, NO_2-associated asthma exacerbations are serious enough to result in emergency department (ED) visits and/or hospital admissions. The epidemiologic evidence provides strong support for an association between short-term NO_2 exposure and asthma exacerbation as indicated by asthma-related hospital admissions, asthma ED visits, and respiratory symptoms and medication use in children and adults with asthma. Epidemiologic studies are coherent with experimental evidence demonstrating effects of NO_2 exposure on allergic inflammation and airway responsiveness in adults with asthma, which provide strong support for an independent effect of short-term NO_2 exposures on the physiological processes that underly asthma exacerbation. Together, the evidence supports an effect of short-term NO_2 exposure from ambient air on asthma exacerbation independent of other traffic-related pollutants. The evidence supporting conclusions on short-term oxides of nitrogen exposure and respiratory effects is discussed in detail in Chapter 3 of this ISA, and conclusions on biological plausibility, lifestages and populations at increased risk, and causality are summarized below in Sections IS.5.1.1, IS.5.1.2, and IS.5.1.3, respectively.

IS.5.1.2 Biological Plausibility

As discussed in Chapter 3, the strongest support for a causal relationship between short-term oxides of nitrogen exposure and respiratory effects comes from studies examining endpoints related to asthma exacerbation. The biological plausibility of a causal relationship between short-term NO_2 exposures and asthma exacerbations is supported by a large of body of experimental data from animal toxicological and controlled human exposure studies. NO_2 is a highly reactive gas that occurs as a radical that, once inhaled, encounters the epithelial lining fluid (ELF) of the respiratory tract. In the ELF, NO_2 chemically interacts with antioxidants, unsaturated lipids, and other compounds generating reactive oxygen and nitrogen species and provoking inflammation. The strongest line of evidence for biological

[8]The criteria pollutant is oxides of nitrogen, though most the health studies evaluated in the 2016 Oxides of Nitrogen ISA examined exposure to NO_2.

plausibility comes from experimental studies demonstrating that short-term NO_2 exposure can activate pathways involved in inflammation and oxidative stress signaling and augmentation of allergic responses. Studies additionally provide some support for suppression of host defense mechanisms and changes to nervous system reflexes, though this evidence is subject to greater uncertainty. These potential pathways, including the pathways that could underly NO_2-associated Chronic Obstructive Pulmonary Disease (COPD) exacerbation/hospitalization and respiratory mortality, are discussed in detail in Chapter 3, Section 3.9 and are illustrated below in Figure IS-6Figure .



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. 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 Chapter 3, Section 3.9.

Figure IS-6 Biologically plausible pathways for respiratory effects associated with short-term exposure to oxides of nitrogen.

IS.5.1.3 Lifestages and Populations at Increased Risk

The populations at greatest risk of respiratory effects following short-term NO_2 exposures include children and individuals with pre-existing asthma. Evidence indicates that short-term NO_2 exposures can exacerbate asthma and that associations with indicators of asthma exacerbation are stronger in children than adults. Overall, this evidence is "adequate" to conclude that these populations are at greater risk of NO_2-related respiratory effects than the general population. For children in particular, epidemiologic evidence from high-quality studies across diverse locations (United States, Canada, Europe, Asia, Australia) consistently demonstrates that short-term increases in ambient NO_2 concentrations are associated with larger increases in asthma-related hospital admissions, ED visits, or outpatient visits among children than adults. Most results are based on comparisons between children ages 0 to14 years and people ages 15 to 64 years, and several studies show NO_2-associated increases in asthma hospital admissions that are 1.8 to 3.4-fold greater in children. These findings in epidemiologic studies are supported by recent animal evidence indicating increased NO_2-induced allergic asthma phenotype in mice

gestationally exposed to NO_2 when sensitized and challenged with OVA later in life (Yue et al., 2018; Yue et al., 2017). Compared to individuals with asthma and children, there is more uncertainty as to whether other lifestages or populations may also be at increased risk of short-term NO_2 exposures (see Table IS-4). The evidence supporting conclusions on lifestages and populations at increased risk from short-term oxides of nitrogen exposure and respiratory effects is discussed in detail in Chapter 3, Section 3.10 of this ISA.

Table IS-4 Summary of evidence for increased risk of respiratory effects from short-term exposure to oxides of nitrogen exposure

Determination	Key Evidence	Key References
Adequate evidence		
People with asthma	Consistent evidence for increased risk for NO_2-related asthma exacerbation.	Chapter 3, Section 3.3
	Consistent evidence of NO_2-induced increases in airway responsiveness in adults with asthma.	Brown (2015) Goodman et al. (2009) Kjaergaard and Rasmussen (1996) Folinsbee (1992)
Children	Consistent epidemiologic evidence that associations between short-term NO_2 exposure and asthma exacerbation are stronger in children relative to adults.	†Bi et al. (2023) †Wei et al. (2022) †Alhanti et al. (2016) Son et al. (2013) Sinclair et al. (2010) Ko et al. (2007) Hinwood et al. (2006) Peel et al. (2005) Atkinson et al. (1999) Anderson et al. (1998)
Suggestive evidence		
Older adults	Inconsistent epidemiologic evidence of age-specific differences in NO_2-related asthma exacerbations in adults, but consistently stronger NO_2-respiratory mortality associations observed among older adults compared to younger adults.	†Gariazzo et al. (2023) †Li et al. (2021) †Chen et al. (2018) Dimakopoulou et al. (2014)
Females	Inconsistent epidemiologic evidence of sex-specific differences in NO_2-related asthma exacerbations, but consistently stronger NO_2-respiratory mortality associations observed among females.	†Shin et al. (2022) †Chen et al. (2018) †Li et al. (2021) †Huang et al. (2021)

Determination	Key Evidence	Key References
Diet	Limited evidence from experimental studies for Vitamin C modification of NO_2-related increases in airway responsiveness. Additional evidence that changes in NO_2-related oxidant balance may be modified by vitamin intake, but results are not necessarily indicative of health effects.	Mohsenin (1987) Hatch et al. (1986) Mohsenin (1991) Elsayed and Mustafa (1982) Ayaz and Csallany (1978) Sevanian et al. (1982) Selgrade et al. (1981)
Inadequate evidence		
Obesity	A recent study reported larger NO_2-related increase in hospital admissions for asthma in neighborhoods with high average BMIs compared to neighborhoods with medium to low average BMIs. Notable uncertainty in spatial scale of BMI variable.	†Wei et al. (2022)
Race/ethnicity	Evidence of effect modification of associations between short-term NO_2 exposure and asthma exacerbation was inconsistent across racial and ethnic groups.	Chapter 3, Section 3.3.1.1.5
Socioeconomic status	Inconsistent results across epidemiologic studies of short-term NO_2 exposure indicate both smaller and larger risks of asthma exacerbation in low SES populations relative to high SES populations.	Chapter 3, Section 3.3.1.1.5

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

IS.5.1.4 Causality Determination

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

(see Chapter 3, Section 3.3.1.1.3). Recent studies also reported positive associations based on previously unexamined exposure assignment techniques, including CMAQ modeling estimates and LUR models (see Chapter 3, Section 3.3.1.1.2). Epidemiologic studies that compared results across exposure assignment methods reported that exposure measurement error resulting from the different approaches impacted the magnitude, but not the direction, of the observed associations, reducing the likelihood that the findings are due to exposure measurement error. There has also been limited evaluation of the shape of the C-R relationship. In the few available studies that did account for potential non-linearities in the C-R relationship, the evidence does not indicate the presence of a threshold and there continues to be support for an association at the lower end of those study's concentration distributions (see Chapter 3, Section 3.3.1.1.1).

Controlled human exposure studies add further support for an independent effect of short-term NO_2 exposures on the physiological processes that underly asthma exacerbation. Previously reviewed controlled human exposure studies provide evidence of NO_2-enhanced markers of allergic inflammation, including eosinophil activation, pro-allergic cytokine production, and increased infiltration of neutrophils in adults with asthma (see Chapter 3, Section 3.3.3.3.1). These effects were reported at slightly higher but still relevant concentrations (e.g., following exposures of 260 ppb NO_2 for 15to 30 minutes and 400 ppb NO_2 for six hours). As discussed in Chapter 3, Section 3.9, increased allergic inflammation may promote asthma exacerbation through increases in airway reactivity, reductions in lung function, and/or increased airway resistance. Additional support comes from several meta-analyses of controlled human exposure studies evaluated in the 2016 Oxides of Nitrogen ISA that indicated increased airway responsiveness in adults with asthma exposed to NO_2 at rest. Exposures as low as 100 ppb resulted in approximately 70% of study participants experiencing increased airway responsiveness to nonspecific stimuli (Brown, 2015; Goodman et al., 2009; Kjaergaard and Rasmussen, 1996; Folinsbee, 1992). A clinically relevant doubling reduction in provocative dose in response to NO_2 was also reported, with evidence for the reproducibility of response in individuals (Brown, 2015). The evidence for clinically relevant increases in airway responsiveness in adults with asthma following relatively low NO_2 exposures provides key support that exposure to ambient air concentrations of NO_2 can exacerbate asthma. These findings are coherent with epidemiologic studies reporting associations between short-term NO_2 exposures and endpoints related to asthma exacerbation. The lack of strong experimental evidence supporting direct NO_2-induced increases in asthma symptoms does not necessarily diminish this coherence given the ethical considerations that preclude controlled human exposure studies from enrolling the participants at greatest risk from asthma exacerbations.

Findings of allergic inflammation in controlled human exposure studies are also coherent with recent toxicological studies that demonstrated NO_2-induced increases in markers of allergic inflammation in animal models (see Chapter 3, Section 3.3.3.3.3), and with epidemiologic studies that reported ambient or personal NO_2-related increases in eNO (see Chapter 3, Section 3.3.3.3.1), most of which were evaluated in the 2016 Oxides of Nitrogen ISA. Further, the epidemiologic evidence provides support for NO_2-related decrements in lung function in children with asthma (see Chapter 3, Section 3.3.3.2.1). The

most consistent evidence for NO_2-related lung function decrements comes from well-designed, well-conducted studies that measured lung function under supervised conditions and assessed exposure in subjects' locations (Martins et al., 2012; Delfino et al., 2008; McCreanor et al., 2007). As discussed in Chapter 3, Section 3.9, the collective findings support biologically plausible pathways by which NO_2-induced inflammation and heightened allergic response may promote asthma exacerbation through increases in airway reactivity, reductions in lung function, and/or increased airway resistance.

There continues to be uncertainty regarding the degree to which observed epidemiologic associations between short-term NO_2 exposures and asthma exacerbation represent an independent effect of NO_2 or serve as an indicator for effects of the traffic-related pollution mixture or components of that mixture. In the limited number of studies that examined potential confounding by $PM_{2.5}$ or traffic-related pollutants (e.g., UFP, CO, or VOCs), associations between short-term NO_2 exposures and asthma hospital admissions and ED visits remained relatively unchanged in copollutant models (see Chapter 3, Section 3.3.1.1.6). Notably, many studies do not mutually adjust for highly correlated pollutants because resulting multicollinearity may produce unstable effect estimates. Although copollutant models have well-recognized limitations for distinguishing independent pollutant associations, particularly for highly correlated pollutants, the coherence of the epidemiologic evidence with results from experimental studies support an effect of NO_2 exposure from ambient air on asthma exacerbation independent of other traffic-related pollutants.

Overall, there is sufficient evidence to conclude that there is a *causal relationship* between short-term oxides of nitrogen exposure and respiratory effects. Coherence of findings integrated across controlled human exposure, epidemiologic, and animal toxicological studies supports a relationship between short-term NO_2 exposure and asthma exacerbation. NO_2-induced increases in allergic inflammation and airway responsiveness in controlled human exposure studies of adults with asthma comprise the key evidence that NO_2 exposure can independently exacerbate asthma and support the epidemiologic evidence for NO_2-related asthma hospital admissions and ED visits, as well as increased respiratory symptoms and pulmonary inflammation in populations with asthma. The evidence for the relationship between short-term exposure to NO_2 and respiratory effects is summarized in Table IS-5 using the framework for causality determinations described in Chapter 3, 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, 2024b).

Table IS-5 Summary of evidence for short-term oxides of nitrogen exposure and respiratory effects

Evidence from 2016 Oxides of Nitrogen ISA	Evidence from this Oxides of Nitrogen ISA
There is coherence among multiple lines of evidence and biological plausibility for effects on asthma exacerbation.	Continued support from epidemiologic studies, controlled human exposure studies, and animal toxicological studies for

Epidemiologic evidence indicates NO_2-associated asthma exacerbation, and biological plausibility is supported by NO_2-induced increases in airway responsiveness and allergic inflammation in adults with asthma. Biological plausibility continues to be provided by the NO_2-induced increases in airway responsiveness and allergic inflammation demonstrated in experimental studies. Some findings point to effects on allergy, COPD, respiratory infection, respiratory effects in healthy populations, and respiratory mortality, but there is inconsistency among disciplines and outcomes.	NO_2-associated asthma exacerbation, and biological plausibility is supported by NO_2-induced increases in airway responsiveness and allergic inflammation in adults with asthma. Evidence for non-asthma respiratory effects continues to be inconsistent within and across disciplines, but recent epidemiologic evidence supports and extends previous findings of NO_2-associated increases in risk of COPD exacerbation. A large number of recent epidemiologic studies provide consistent evidence of an association between short-term NO_2 exposure and respiratory mortality.

List of abbreviations in alphabetical order: COPD = Chronic Obstructive Pulmonary Disease; ISA = integrated science assessment; NO_2 = nitrogen dioxide.

IS.5.2 Long-Term Exposures and Respiratory Effects

IS.5.2.1 Evidence Overview

The 2016 Oxides of Nitrogen ISA concluded that the available scientific evidence supported a "likely to be causal" relationship between long-term NO_2[9] exposure and respiratory effects (U.S. EPA, 2016). The "likely to be causal relationship" with long-term exposure to 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 NO_2 concentrations in ambient air and asthma development in children. The greatest uncertainty in the epidemiologic evidence at that time was a limited 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 toxicological 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

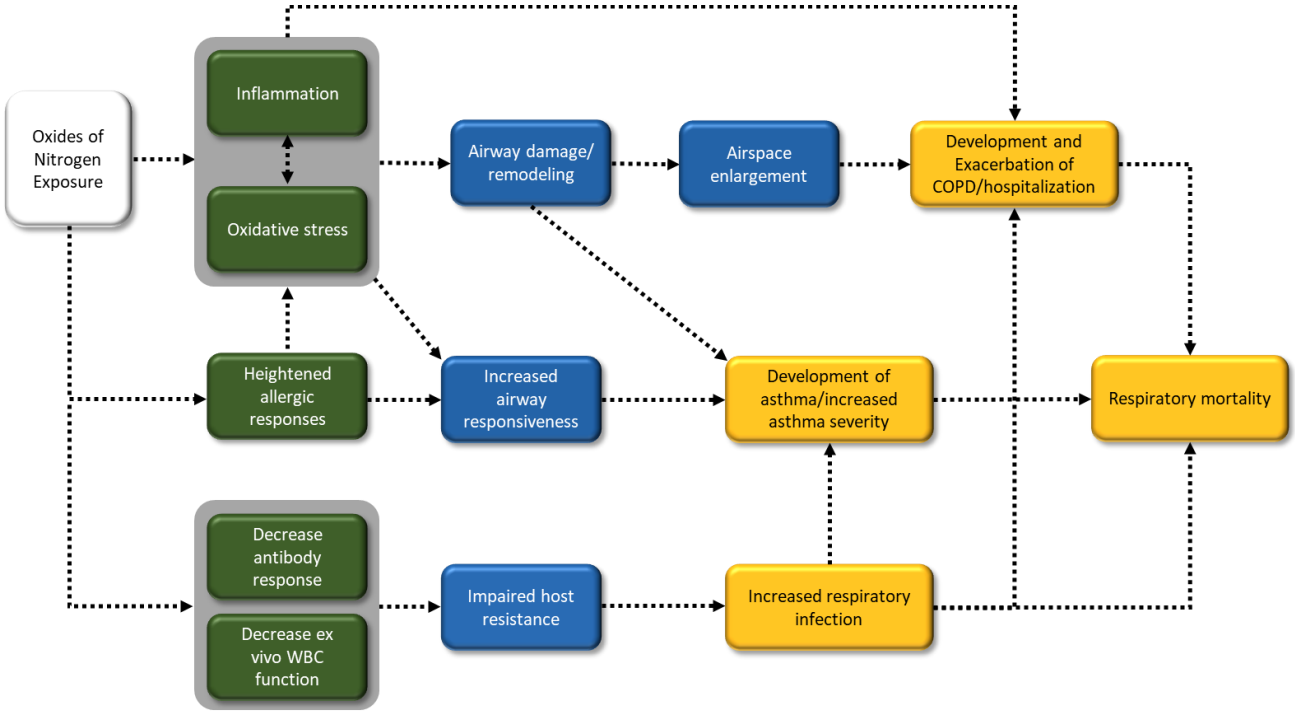
[9]The criteria pollutant is oxides of nitrogen, though most the health studies evaluated in the 2016 Oxides of Nitrogen ISA examined exposure to NO_2.

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.

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 exposure to oxides of nitrogen, primarily NO_2, and asthma development. Recent epidemiologic studies generally report positive associations with asthma incidence in children, and these associations are supported by recent experimental animal studies reporting markers of a pro-asthmatic phenotype following NO_2 exposures. The evidence supporting conclusions on long-term NO_2 exposure and respiratory effects is discussed in detail in Chapter 4 of this ISA, and conclusions on biological plausibility, lifestages and populations at increased risk, and causality are summarized below in Sections IS.5.2.1, IS.5.2.2, and IS.5.2.3, respectively.

IS.5.2.2 Biological Plausibility

NO_2 is a highly reactive gas that occurs as a radical that, once inhaled, encounters the 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, including the induction of inflammation and oxidative stress, the augmentation of allergic responses, and suppression of host resistance to pathogen infection. These pathways are discussed in detail in Chapter 4, Section 4.9, and illustrated in Figure IS-7.



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. The structure of the biological plausibility sections and the role of biological plausibility in contributing to the weight-of-evidence analysis used in the 2025 Oxides of Nitrogen ISA are discussed in Chapter 4, Section 4.9.

Figure IS-7 Biologically plausible pathways for respiratory effects associated with long-term exposure to oxides of nitrogen.

IS.5.2.3 Lifestages and Populations at Increased Risk

A large body of epidemiologic evidence supports associations between long-term NO_2 exposure and asthma incidence in children. 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 and reported consistent positive associations across varying levels of adjustment. Experimental studies of gestational NO_2 exposures in animals generally indicate an enhanced induction of asthma-related markers, including airway reactivity, inflammation, and airway histologic changes in response to allergen sensitization and challenge. Specifically, these studies support the biological plausibility of associations between long-term NO_2 exposure and asthma 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. This evidence is "adequate" to conclude that children are at increased risk of developing

asthma from long-term NO_2 exposures. There is more uncertainty whether other lifestages or populations may also be at increased risk of long-term NO_2 exposures (see Table IS-6).

Table IS-6 Summary of evidence for increased risk of respiratory effects from long-term exposure to 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.	Chapter 4, 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.

IS.5.2.4 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 not examined, contributing uncertainty regarding the independent nature of the observed associations. However, experimental evidence indicated airway hyperresponsiveness induced by long-term NO_2 exposure and development of an allergic phenotype with continuous or repeated NO_2 exposures over weeks to months. These experimental studies provided coherence with the epidemiologic evidence of associations between long-term NO_2 exposure and increased incidence of 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. Evidence for other respiratory effects 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. Recent evidence supports a change in the causality determination from a *likely to be causal relationship* in the 2016 Oxides of Nitrogen ISA to a *causal relationship* in this assessment. As detailed below, this change in the causality determination is driven by expanded, and consistent, animal toxicological evidence for NO_2-related impacts on markers of a pro-asthmatic phenotype, which supports expanded epidemiologic evidence for increases in asthma incidence associated with long-term NO_2 exposure and provides biological plausibility for NO_2-induced asthma development in humans.

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 U.S., 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 Chapter 4, 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., 2016) 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 European Community Research Health Survey (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 1 million adults in Toronto, Canada (Weichenthal et al., 2017) as well as a pooled analysis of three European Study of Cohorts for Air Pollution Effects (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), though, positive associations were generally 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 Chapter 4, Section 4.3.1.2.1).

In recent 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 Chapter 4, Sections 4.3.1.1.3 and 4.3.1.2.3). Although several recent studies additionally adjust for some copollutants, 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. Several studies in adults additionally 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., 2021b; Liu et al., 2021a; Weichenthal et al., 2017), though collinearity resulting from high correlations between NO_2 and potential copollutant confounders is also a key uncertainty in these studies.

Uncertainty in the independent nature of the associations observed in the epidemiologic evidence base is mitigated by evidence from experimental animal studies that demonstrate biological plausibility of NO_2-induced respiratory effects. Recent toxicological studies show consistent increases in pro-asthmatic phenotypes following prolonged or repeat NO_2 exposure and expand the support for key events that could drive asthma development. This includes 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 of asthma-relevant effects in unsensitized animals evaluated in the 2016 Oxides of Nitrogen ISA were limited in number but did report increases in asthma-related endpoints with long-term NO_2 exposures. Airway reactivity in guinea pigs was increased following exposure to 2,000 to 4,000 ppb NO_2 for six weeks 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). Lung morphologic changes were reported with exposure to 400 ppb NO_2 for at least 27 months Kubota et al. (1987), increases in markers of oxidative stress were reported with NO_2 exposures as low as 400 ppb for 18 months (Sagai et al., 1984), and signs of epithelial damage were reported following exposure to 500 ppb NO_2 for seven months (Yamamoto and Takahashi, 1984).

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., 2023; 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 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). Together, the recent studies provide additional consistency in the animal evidence for NO_2-driven development of asthma-related effects in unsensitized individuals and additional support for biological plausibility.

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 show that repeated NO_2 exposure increases neutrophils in healthy adults, and epidemiologic evidence also points to associations between ambient air NO_2 concentrations and increases in pulmonary inflammation in healthy children and adults (see Chapter 3, Section 3.6.4).

Toxicologic studies evaluated in the 2016 Oxides of Nitrogen ISA also 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. 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.

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., 2023; 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 a lack of coherence with toxicological studies, which do not provide strong evidence of NO_2-induced changes in lung function in animal models (see Chapter 4, 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 (>5 ppm) necessary to induce alveolar airspace enlargement in the available experimental animal studies may limit the relevance to human responses at ambient-relevant NO_2 concentrations.

Limited but consistent animal toxicological evidence indicates impaired host resistance to bacterial and viral respiratory 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 2 months resulted in reduced clearance of bacteria from the lungs and increased mortality when given an inhaled bacterial challenge (Henry et al., 1970). The host resistance assays used in these animal studies 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 Chapter 4, 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 inverse probability weighting (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., 2023; 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 Chapter 4, Figures 4-19 and 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 Chapter 4, 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, which reflects a change from a *likely to be causal relationship* in the 2016 Oxides of Nitrogen ISA, 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 evidence 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 IS-7, using the framework for causality determinations described in Chapter 3, 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, 2024b).

Table IS-7 Summary of evidence indicating a causal relationship between long-term oxides of nitrogen exposure and respiratory effects

Evidence from 2016 Oxides of Nitrogen ISA	Evidence from this Oxides of Nitrogen ISA
Epidemiologic evidence and experimental studies provided evidence for respiratory effects from long-term NO_2 exposure, strongly supported by evidence for asthma development. Evidence for other respiratory effects such as respiratory disease severity, lung function changes, and	Recent evidence supports strong additional support for an association between long-term NO_2 exposure and respiratory effects, based strongly on evidence integrated across disciplines for a relationship between long-term exposure to NO_2 and asthma development. Expanded animal

respiratory infection is more uncertain because the combined epidemiologic and/or experimental evidence does not clearly demonstrate an independent effect of long-term NO_2 exposure.	toxicological evidence for NO_2-related impacts on markers of a pro-asthmatic phenotype, which supports epidemiologic evidence for increases in asthma incidence associated with long-term NO_2 exposure and provides biological plausibility for NO_2-induced asthma development in humans, strengths the associations from epidemiologic studies. 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.

List of abbreviations in alphabetical order: COPD = Chronic Obstructive Pulmonary Disease; ISA = integrated science assessment; NO_2 = nitrogen dioxide.

IS.6 Summary of Major Findings

Since the 2016 Oxides of Nitrogen ISA, there have been several important advances in the understanding of oxides of nitrogen emissions, atmospheric chemistry and ambient concentrations, exposures, and health effects. This section briefly summarizes the major ISA findings on these topics. As in past reviews, most of the recent evidence is for NO_2, though NO and other oxides of nitrogen are also evaluated in some studies. Recent literature including shifts in important emission sources, improved chemistry and transport understanding, progress in satellite measurement and modeling capabilities. Available data illustrates recent declines in anthropogenic NO_X emissions, shifts in the relative importance of some NO_X emission sources, and a broad trend across the United States of declining ambient NO_2 concentrations. Overall, anthropogenic NO_X emissions in the United States have decreased by roughly 70% since 2002, from approximately 25,000 kTon/year to approximately 7,000 kTon/year in 2023. Though mobile vehicles remain dominant sources of NO_X emissions, recent decreases from these and other anthropogenic sources have been accompanied by the increasing relative importance of biogenic emissions such as wildfire smoke and soil emissions. Ambient NO_2 concentrations also have decreased substantially since 2000, with most sites reporting decreases of 0.5 ppb/year or greater. There were no monitoring stations with annual average NO_2 concentrations exceeding 53 ppb, the level of the annual NO_2 NAAQS, since the early 1990s. The maximum annual NO_2 design value in 2024 was 26 ppb, and the vast majority (74%) of monitoring stations had annual average NO_2 concentrations less than 10 ppb. Similarly, there were no monitoring stations exceeding 100 ppb, the level of the 1-hour NO_2 NAAQS. The maximum 1-hour NO_2 design value in 2024 was 68 ppb.

Exposures to NO_2 and other oxides of nitrogen arise from a combination of ambient and nonambient emissions sources. The ambient portion of NO_2 exposure stems primarily from outdoor emission sources such as highway and off-road vehicles, utility power plants, and non-utility combustion industries. In the United States, highway vehicles account for approximately 26% of total national NO_X emissions, making them prominent contributors to population-level exposures particularly in urban areas with large numbers of people living, working, and/or attending school near primary roads. Ambient NO_2

often exhibits strong correlations with other traffic-related pollutants, such as carbon monoxide (CO), black carbon (BC), ultrafine particles (UFPs), and volatile organic compounds (VOCs), particularly benzene, toluene, ethylbenzene, and xylenes (BTEX). These correlations are most pronounced in urban environments near roadways, where vehicular emissions are highest.

Because personal exposures to ambient NO_2 cannot be measured directly, monitored or modeled ambient NO_2 is often used as a surrogate for these exposures. The association between NO_2 in ambient air and personal exposure is complex and influenced by a number of factors, including proximity to traffic and other local sources, indoor/outdoor air exchange rates, time spent in different microenvironments (such as indoors and in vehicles), the presence of indoor sources, seasonal variations in ventilation and source strengths, individual activity patterns and study design (e.g., longitudinal vs. pooled studies). Various approaches have been used as surrogates for personal exposure to oxides of nitrogen in ambient air, primarily NO_2. Each surrogate has strengths and limitations and may be influenced by different types of exposure error (i.e., instrument error, time-activity error, contributions from nonambient sources to total exposure, spatial error, and model misspecification).

Compared to ambient monitors alone, exposure or air quality models have the potential to better capture the spatial and temporal variability of NO_2, therefore enhancing exposure assessment and reducing exposure measurement error in epidemiologic studies. However, the selection of an appropriate model is critically important. The structure of the chosen model must be well-justified, and its applicability to the specific study context should be clearly established. Otherwise, metrics such as cross-validation or goodness-of-fit cannot guarantee improved accuracy in exposure assignment. It is also essential to recognize that reduced precision in exposure assessment generally decreases the likelihood of detecting statistically significant associations. Such bias toward the null suggests that the true health effect may be larger than the effect estimate reported by the epidemiologic study. The association between NO_2 in ambient air and personal exposure is complex and influenced by a number of factors, including proximity to traffic and other local sources, indoor/outdoor air exchange rates, time spent in different microenvironments (such as indoors and in vehicles), the presence of indoor sources, seasonal variations in ventilation and source strengths, individual activity patterns and study design (e.g., longitudinal vs. pooled studies).

The available evidence supports a *causal relationship* between short-term exposure to oxides of nitrogen, primarily NO_2, and respiratory effects. This conclusion is consistent with that determined in the 2016 Oxides of Nitrogen ISA and is supported by the evidence for asthma exacerbation integrated from epidemiologic studies, controlled human exposure studies, and animal toxicological studies. The epidemiologic evidence provides strong support for an association between short-term NO_2 exposure and asthma exacerbation, including hospital admissions, ED visits, and respiratory symptoms and medication use in children and adults with asthma. Epidemiologic studies are coherent with experimental evidence demonstrating effects of ambient air NO_2 exposure on allergic inflammation and airway responsiveness in

adults with asthma. Together, the evidence supports an effect of short-term ambient air NO_2 exposure on asthma exacerbation independent of other traffic-related pollutants.

The available evidence supports a *causal relationship* between long-term oxides of nitrogen exposure, primarily NO_2, and respiratory effects. This determination is a change from the likely to be causal relationship from the 2016 Oxides of Nitrogen ISA. 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 and 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.

Evidence is suggestive of, but not sufficient to infer, a causal relationship between short-term exposure to oxides of nitrogen and cardiovascular effects and total mortality, and for long-term exposure to oxides of nitrogen and cardiovascular effects, pregnancy outcomes, birth outcomes, metabolic effects, nervous system effects, total mortality, and cancer. While there is some evidence of effects, there are remaining uncertainties regarding concentration-response, lifestages and population differences, and copollutant confounding in the epidemiologic studies. Furthermore, there is limited evidence from animal toxicological studies supporting these effects.

Evidence is inadequate to infer the presence or absence of a causal relationship between short-term exposure to oxides of nitrogen and metabolic effects, and nervous system effects, and exposure to oxides of nitrogen and male reproductive function, female reproductive function, postnatal development, immune effects, renal effects, hepatic effects, ocular effects, gastrointestinal effects, and musculoskeletal effects. The evidence base across epidemiologic studies, animal toxicological studies, and/or controlled human exposure studies is extremely limited for these endpoints, with remaining uncertainties regarding

biological plausibility, concentration-response, lifestages and population differences, and copollutant confounding.

IS.7 References

Alhanti, BA; Chang, HH; Winquist, A; Mulholland, JA; Darrow, LA; Sarnat, SE. (2016). Ambient air pollution and emergency department visits for asthma: A multi-city assessment of effect modification by age. J Expo Sci Environ Epidemiol 26: 180-188. http://dx.doi.org/10.1038/jes.2015.57

Anderson, HR; Ponce de Leon, A; Bland, JM; Bower, JS; Emberlin, J; Strachen, DP. (1998). Air pollution, pollens, and daily admissions for asthma in London 1987-92. Thorax 53: 842-848. http://dx.doi.org/10.1136/thx.53.10.842

Atkinson, RW; Anderson, HR; Strachan, DP; Bland, JM; Bremner, SA; Ponce de Leon, A. (1999). Short-term associations between outdoor air pollution and visits to accident and emergency departments in London for respiratory complaints. Eur Respir J 13: 257-265. http://dx.doi.org/10.1183/09031936.99.13225799

Ayaz, KL; Csallany, AS. (1978). Long-term NO2 exposure of mice in the presence and absence of vitamin E. II. Effect of glutathione peroxidase. Arch Environ Occup Health 33: 292-296. http://dx.doi.org/10.1080/00039896.1978.10667350

Bi, J, ianzhao; D'Souza, R, ohan R.; Moss, S, hannon; Senthilkumar, N, iru; Russell, A, rmistead G.; Scovronick, N, oah C.; Chang, H, oward H.; Ebelt, S, tefanie. (2023). Acute Effects of Ambient Air Pollution on Asthma Emergency Department Visits in Ten US States. Environ Health Perspect 131: 47003-. http://dx.doi.org/10.1289/ehp11661

Brown, JS. (2015). Nitrogen dioxide exposure and airway responsiveness in individuals with asthma. Inhal Toxicol 27: 1-14. http://dx.doi.org/10.3109/08958378.2014.979960

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 1041-5505 EISSN 2688-6855 Research Report No. 208). Boston, MA: Health Effects Institute.

CASAC (Clean Air Scientific Advisory Committee). (2024). Consultation on the EPA's 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 (March 2024). (EPA-CASAC-24-001).

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

CDC (Centers for Disease Control and Prevention). (2004). The health consequences of smoking: A report of the Surgeon General. Washington, DC: U.S. Department of Health and Human Services. http://www.cdc.gov/tobacco/data_statistics/sgr/2004/index.htm

Chen, R; Yin, P; Meng, X; Wang, L; Liu, C; Niu, Y; Lin, Z; Liu, Y; Liu, J; Qi, J; You, J; Kan, H; Zhou, M. (2018). Associations between ambient nitrogen dioxide and daily cause-specific mortality: Evidence from 272 Chinese cities. Epidemiology 29: 482-489. http://dx.doi.org/10.1097/EDE.0000000000000829

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

Delfino, RJ; Staimer, N; Tjoa, T; Gillen, D; Kleinman, MT; Sioutas, C; Cooper, D. (2008). Personal and ambient air pollution exposures and lung function decrements in children with asthma. Environ Health Perspect 116: 550-558. http://dx.doi.org/10.1289/ehp.10911

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

Elsayed, NM; Mustafa, MG. (1982). Dietary antioxidants and the biochemical response to oxidant inhalation: I: Influence of dietary vitamin E on the biochemical effects of nitrogen dioxide exposure in rat lung. Toxicol Appl Pharmacol 66: 319-328. http://dx.doi.org/10.1016/0041-008X(82)90298-8

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

Folinsbee, LJ. (1992). Does nitrogen dioxide exposure increase airways responsiveness? Toxicol Ind Health 8: 273-283.

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

Gariazzo, C; Renzi, M; Marinaccio, A; Michelozzi, P; Massari, S; Silibello, C; Carlino, G; Rossi, PG; Maio, S; Viegi, G; Stafoggia, M. (2023). Association between short-term exposure to air pollutants and cause-specific daily mortality in Italy. A nationwide analysis. Environ Res 216: 114676. http://dx.doi.org/10.1016/j.envres.2022.114676

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; 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

Goodman, JE; Chandalia, JK; Thakali, S; Seeley, M. (2009). Meta-analysis of nitrogen dioxide exposure and airway hyper-responsiveness in asthmatics. Crit Rev Toxicol 39: 719-742. http://dx.doi.org/10.3109/10408440903283641

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

Hatch, GE; Slade, R; Selgrade, MK; Stead, AG. (1986). Nitrogen dioxide exposure and lung antioxidants in ascorbic acid-deficient guinea pigs. Toxicol Appl Pharmacol 82: 351-359. http://dx.doi.org/10.1016/0041-008X(86)90212-7

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

HEW (U.S. Department of Health, Education and Welfare). (1964). Smoking and health: Report of the advisory committee to the surgeon general of the public health service (pp. 1-386). Washington, DC: U.S. Department of Health, Education, and Welfare. http://profiles.nlm.nih.gov/ps/retrieve/ResourceMetadata/NNBBMQ

Hinwood, AL; De Klerk, N; Rodriguez, C; Jacoby, P; Runnion, T; Rye, P; Landau, L; Murray, F; Feldwick, M; Spickett, J. (2006). The relationship between changes in daily air pollution and hospitalizations in Perth, Australia 1992-1998: A case-crossover study. Int J Environ Health Res 16: 27-46. http://dx.doi.org/10.1080/09603120500397680

Huang, J; Li, J; Yin, P; Wang, L; Pan, X; Zhou, M; Li, G. (2021). Ambient nitrogen dioxide and years of life lost from chronic obstructive pulmonary disease in the elderly: A multicity study in China. Chemosphere 275: 130041. http://dx.doi.org/10.1016/j.chemosphere.2021.130041

IARC (International Agency for Research on Cancer). (2006). IARC monographs on the evaluation of carcinogenic risks to humans: Preamble. Lyon, France: World Health Organization. http://monographs.iarc.fr/ENG/Preamble/

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

IOM (Institute of Medicine). (2008). Improving the presumptive disability decision-making process for veterans. In JM Samet; CC Bodurow (Eds.), Improving the Presumptive Disability Decision-Making Process for Veterans. Washington, DC: National Academies Press. http://dx.doi.org/10.17226/11908

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

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

Kjaergaard, SK; Rasmussen, TR. (1996). Clinical studies of effects of nitrogen oxides in healthy and asthmatic subjects. Cent Eur J Public Health 4: 23-26.

Ko, FWS; Tam, W; Wong, TW; Lai, CKW; Wong, GWK; Leung, TF; Ng, SSS; Hui, DSC. (2007). Effects of air pollution on asthma hospitalization rates in different age groups in Hong Kong. Clin Exp Allergy 37: 1312-1319. http://dx.doi.org/10.1111/j.1365-2222.2007.02791.x

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

Krall, JR; Chang, HH; Waller, LA; Mulholland, JA; Winquist, A; Talbott, EO; Rager, JR; Tolbert, PE; Sarnat, SE. (2018). A multicity study of air pollution and cardiorespiratory emergency department visits: Comparing approaches for combining estimates across cities. Environ Int 120: 312-320. http://dx.doi.org/10.1016/j.envint.2018.07.033

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

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, J; Zhang, X; Li, G; Wang, L; Yin, P; Zhou, M. (2021). Short-term effects of ambient nitrogen dioxide on years of life lost in 48 major Chinese cities, 2013–2017. Chemosphere 263: 127887. http://dx.doi.org/10.1016/j.chemosphere.2020.127887

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; 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. (2021b). 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; Wang, F; Liu, Q; Deng, M; Yang, X; Ma, P. (2023). 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

Martins, PC; Valente, J; Papoila, AL; Caires, I; Araújo-Martins, J; Mata, P; Lopes, M; Torres, S; Rosado-Pinto, J; Borrego, C; Annesi-Maesano, I; Neuparth, N. (2012). Airways changes related to air pollution exposure in wheezing children. Eur Respir J 39: 246-253. http://dx.doi.org/10.1183/09031936.00025111

McCreanor, J; Cullinan, P; Nieuwenhuijsen, MJ; Stewart-Evans, J; Malliarou, E; Jarup, L; Harrington, R; Svartengren, M; Han, IK; Ohman-Strickland, P; Chung, KF; Zhang, J. (2007). Respiratory effects of exposure to diesel traffic in persons with asthma. N Engl J Med 357: 2348-2358. http://dx.doi.org/10.1056/NEJMoa071535

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

Mohsenin, V. (1987). Effect of vitamin C on NO2-induced airway hyperresponsiveness in normal subjects: A randomized double-blind experiment. Am Rev Respir Dis 136: 1408-1411. http://dx.doi.org/10.1164/ajrccm/136.6.1408

Mohsenin, V. (1991). Lipid peroxidation and antielastase activity in the lung under oxidant stress: Role of antioxidant defenses. J Appl Physiol (1985) 70: 1456-1462.

NASEM (National Academies of Sciences, Engineering, and Medicine). (2022). Advancing the framework for assessing causality of health and welfare effects to inform National Ambient Air Quality Standards reviews. In Advancing the Framework for Assessing Causality of Health and Welfare Effects to Inform National Ambient Air Quality Standard Reviews. Washington, DC: National Academies Press. http://dx.doi.org/10.17226/26612

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

OECD (Organisation for Economic Co-operation and Development). (2018). Users' Handbook supplement to the Guidance Document for developing and assessing Adverse Outcome Pathways (AOPs). (ENV/JM/MONO(2016)12). Paris, France: OECD Publishing. http://dx.doi.org/10.1787/5jlv1m9d1g32-en

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

Peel, JL; Tolbert, PE; Klein, M; Metzger, KB; Flanders, WD; Todd, K; Mulholland, JA; Ryan, PB; Frumkin, H. (2005). Ambient air pollution and respiratory emergency department visits. Epidemiology 16: 164-174. http://dx.doi.org/10.1097/01.ede.0000152905.42113.db

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

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

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

Selgrade, MK; Mole, ML; Miller, FJ; Hatch, GE; Gardner, DE; Hu, PC. (1981). Effect of NO2 inhalation and vitamin C deficiency on protein and lipid accumulation in the lung. Environ Res 26: 422-437.

Sevanian, A; Hacker, AD; Elsayed, N. (1982). Influence of vitamin E and nitrogen dioxide on lipid peroxidation in rat lung and liver microsomes. Lipids 17: 269-277.

Shin, HH; Maquiling, A; Thomson, EM; Park, IW; Stieb, DM; Dehghani, P. (2022). Sex-difference in air pollution-related acute circulatory and respiratory mortality and hospitalization. Sci Total Environ 806: 150515. http://dx.doi.org/10.1016/j.scitotenv.2021.150515

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

Sinclair, AH; Edgerton, ES; Wyzga, R; Tolsma, D. (2010). A two-time-period comparison of the effects of ambient air pollution on outpatient visits for acute respiratory illnesses. J Air Waste Manag Assoc 60: 163-175. http://dx.doi.org/10.3155/1047-3289.60.2.163

Son, JY; Lee, JT; Park, YH; Bell, ML. (2013). Short-term effects of air pollution on hospital admissions in Korea. Epidemiology 24: 545-554. http://dx.doi.org/10.1097/EDE.0b013e3182953244

Su, JG; Barrett, MA; Combs, V; Henderson, K; Van Sickle, D; Hogg, C; Simrall, G; Moyer, SS; Tarini, P; Wojcik, O; Sublett, J; Smith, T; Renda, AM; Balmes, J; Gondalia, R; Kaye, L; Jerrett, M.

(2022). Identifying impacts of air pollution on subacute asthma symptoms using digital medication sensors. Int J Epidemiol 51: 213-224. http://dx.doi.org/10.1093/ije/dyab187

Su, JG; Vuong, V; Shahriary, E; Aslebagh, S; Yakutis, E; Sage, E; Haile, R; Balmes, J; Barrett, M. (2024). Health effects of air pollution on respiratory symptoms: A longitudinal study using digital health sensors. Environ Int 189: 108810. http://dx.doi.org/10.1016/j.envint.2024.108810

Tétreault, LF; Doucet, M; Gamache, P; Fournier, M; Brand, A; Kosatsky, T; Smargiassi, A. (2016). 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

U.S. Census Bureau. (2023). TIGER/Line Shapefile, 2023, Nation, U.S., Primary Roads [Database].

U.S. EPA (U.S. Environmental Protection Agency). (1971). Air quality criteria for nitrogen oxides [EPA Report]. (AP-84). Washington DC: U.S. Environmental Protection Agency, Air Pollution Control Office. http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=20013K3B.txt

U.S. EPA (U.S. Environmental Protection Agency). (1982). Air quality criteria for oxides of nitrogen [EPA Report]. (EPA/600/8-82-026). Research Triangle Park, NC: U.S. Environmental Protection Agency, Environmental Criteria and Assessment Office.

U.S. EPA (U.S. Environmental Protection Agency). (1993). Air quality criteria for oxides of nitrogen (final, 1993) [EPA Report]. (EPA/600/8-91/049aF-cF). Washington, DC: U.S. Environmental Protection Agency, Environmental Criteria and Assessment Office. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=40179

U.S. EPA (U.S. Environmental Protection Agency). (2005). Guidelines for carcinogen risk assessment [EPA Report]. (EPA630P03001F). Washington, DC. https://www.epa.gov/sites/production/files/2013-09/documents/cancer_guidelines_final_3-25-05.pdf

U.S. EPA (U.S. Environmental Protection Agency). (2008). Integrated science assessment for 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). (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). (2019). Integrated Science Assessment (ISA) for particulate matter (final report, Dec 2019). (EPA/600/R-19/188). Washington, DC. https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=347534

U.S. EPA (U.S. Environmental Protection Agency). (2020). Integrated science assessment for oxides of nitrogen, oxides of sulfur, and particulate matter—Ecological criteria (final report) [EPA Report]. (EPA/600/R-20/278). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development. https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=349473

U.S. EPA (U.S. Environmental Protection Agency). (2024a). Integrated Review Plan for the National Ambient Air Quality Standards for Ozone and Related Photochemical Oxidants. Volume 2: Planning for the Review and the Integrated Science Assessment. (EPA-452/R-24-001b).

U.S. EPA (U.S. Environmental Protection Agency). (2024b). 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.

U.S. EPA (U.S. Environmental Protection Agency). (2024c). Reconsideration of the National Ambient Air Quality Standards for Particulate Matter. (40 CFR Parts 50, 53, and 58 [EPA-HQ-OAR-2015-0072; FRL-8635-02-OAR] RIN 2060-AV52). https://www.federalregister.gov/documents/2024/03/06/2024-02637/reconsideration-of-the-national-ambient-air-quality-standards-for-particulate-matter

U.S. EPA (U.S. Environmental Protection Agency). (2024d). Reconsideration of the National Ambient Air Quality Standards for Particulate Matter; Correction. (40 CFR Parts 50 and 58 [EPA-HQ-OAR-2015-0072; FRL-8635.1-02-OAR] RIN 2060-AW48). https://www.federalregister.gov/documents/2024/12/19/2024-29223/reconsideration-of-the-national-ambient-air-quality-standards-for-particulate-matter-correction

Wang, J; Li, D; Ma, Y; Tang, L; Xie, J; Hu, Y; Tian, Y. (2023). 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

Wei, Y; Qiu, X; Sabath, MB; Yazdi, MD; Yin, K; Li, L; Peralta, AA; Wang, C; Koutrakis, P; Zanobetti, A; Dominici, F; Schwartz, JD. (2022). Air Pollutants and Asthma Hospitalization in the Medicaid Population. Am J Respir Crit Care Med 205: 1075-1083. http://dx.doi.org/10.1164/rccm.202107-1596OC

Weichenthal, S; Bai, L; Hatzopoulou, M; Van Ryswyk, K; Kwong, JC; Jerrett, M; van Donkelaar, A; Martin, RV; Burnett, RT; Lu, H; Chen, H. (2017). 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, SA; Lavigne, E; Evans, GJ; Godri Pollitt, KJ; Burnett, RT. (2016). PM2.5 and emergency room visits for respiratory illness: effect modification by oxidative potential. Am J Respir Crit Care Med 194: 577-586. http://dx.doi.org/10.1164/rccm.201512-2434OC

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; 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; 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. (2023). 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

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