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Integrated Science Assessment for Oxides of Nitrogen–Health Criteria

Chapter 5: Cardiovascular Effects–Short-Term Exposure

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Document Guide

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

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Acronyms and Abbreviation

8-OhdG	8-hydroxy-29-deoxyguanosine
AA	ascorbate
ACS	acute coronary syndrome
Afib	Atrial Fibrillation
AMI	acute myocardial infarction
APPROACH	Alberta Provincial Project for Outcome Assessment in Coronary Heart Disease
AQHI	air quality hazard index
AQS	Air Quality System
avg	average
β	beta
BC	Black Carbon
BIC	Bayesian information criteria
BL	bronchial lavage
BMI	body mass index
BP	Blood Pressure
CAD	coronary artery disease
CanCHEC	Canadian Census Health and Environment Cohort
CBVD	cerebrovascular disease
CCHS	Canadian Community Health Survey
CHAPS	Children's Health and Air Pollution Study
CHD	Congenital Heart Disease
CHF	congestive heart failure
CI	confidence interval
CMAQ	Community Muti-Scale Air Quality
CO	carbon monoxide
COPD	chronic obstructive pulmonary disease
CRP	high-sensitivity C-reactive protein
CSCA	Chinese Stroke Center Alliance
CVD	cardiovascular disease
C-R	concentration-response
d	square distance
DBP	diastolic blood pressure
df	degrees of freedom
DNA	Deoxyribonucleic acid
DNC	deoxyribonucleic acid
Dutch PHM	Dutch Public Health Monitor
DVT	deep vein thrombosis
Dx	diagnosis
ED	emergency department
EDEN	´Etude des D´eterminants pr´e- et postnatals du d´eveloppement et de la sant´e de l'Enfant
EE	effect estimate
EC	elemental carbon
ECG	Electrocardiograms
e.g.	exempli gratia (for example)
ELF	epithelial lining fluid
ERR	excess relative risk
ESCAPE	European Study of Cohorts for Air Pollution Effects
ETS	environmental tobacco smoke
F	female(s)
FDMA	Fire and Disaster Management Agency
Fe	Iron
g	gram(s)
h	hour
HDL	high density lipid
HELIX	Human Early-life Exposome project
HES	Hospital Episode Statistics
HF	high frequency
HFn	normalized high frequency
HNR	Heinz Nixdorf Recall
HR	hazard ratio
HR	Heart rate
hr	hour(s)
HRV	heart rate variability
HS	Hemorrhagic Stroke
ICAM-1	intercellular adhesion molecule 1
ICC	immunocytochemistry
ICD	Implantable Cardioverter Defibrillator
ICD-(10)	International Classification of Diseases-(Clinical Modification)
ICH	Intracerebral Hemorrhage
ICU	Intensive care unit
IDW	Inverse distance weighting
IHD	ischemic heart disease
IL-6	interleukin 6
IL-8	interleukin 8
IQR	interquartile range
IRR	incidence rate ratio
IS	Ischemic Stroke
ISA	Integrated Science Assessment
L	liter(s)
LDL	low density lipid
LF	low frequency
LOX-1	lectin-like oxLDL
Lp-PLA2	lipoprotein-associated phospholipase A2
LUR	Land use regression
m^2	Meters squared
M	male
m	milligram
M/F	male/female
m/s	meter per second
m	meter(s)
MAP	Mean Arterial Pressure
Max	Maximum
MBDS	Minimum Basic Data Set
MCM	Multi-City outcome Model
MDA	malondialdehyde
MECU	Mobile Emergency Care Unit

MESA	Multi-Ethnic Study of Atherosclerosis
mg/dL	milligrams per deciliter
MI	myocardial infarction
min	minute(s)
MINAP	Myocardial Ischemia National Audit Project
mL	milliliter
mmHg	millimeters of mercury
mo	month(s)
MRI	magnetic resonance imaging
ms	milliseconds
MS	multiple sclerosis
msec	millisecond
N	Population size
NAPS	National Air Pollution Surveillance
NHI	National Health Institute
NMMAPS	National Morbidity, Mortality, and Air Pollution Study
NO	nitric oxide
NO_2	nitrogen dioxide
NO_X	NO_2 + NO
NR	Not reported
NSTEMI	non-ST-elevation myocardial infarction
NYCDOHMH	New York City Department of Health and Mental Hygiene
O_3	ozone
OHCA	out-of-hospital cardiac arrests
OP^{GSH}	oxidative potential
OR	odds ratio
ρ	rho
PBMC	peripheral blood mononuclear cell
PE	pulmonary embolism
PE	Prediction Error
PECOS	Population, Exposure, Comparison, Outcome, and Study Design
PI	posterior interval
PM	particulate matter
$PM_{2.5}$	particulate matter with a nominal mean aerodynamic diameter less than or equal to 2.5 µm
PM_{10}	particulate matter with a nominal mean aerodynamic diameter less than or equal to 10 µm
ppb	parts per billion
ppm	parts per million
PUFA	polyunsaturated fatty acids
PWV	pulse wave velocity
Q	quantile (could be quartile or quintile)
R	Organic radical
r^2	quality assurance
RA	Right Atrial
RD	Risk Difference
Ref	Reference
RHI	reactive hyperemia index
ROSC	Return of Spontaneous Circulation
RR	relative risk or risk ratio
SD	standard deviation
SDQ	Strengths and Difficulties Questionnaire
SE	standard error
SES	socioeconomic status
sLOX-1	soluble LOX-1
SO_2	sulfur dioxide
SPB	Systolic Blood Pressure
SPE	standardized prediction error
STEMI	ST-elevation myocardial infarction
T	tertile
t	time
UA	unstable angina
UEBMI	Urban Employee Basic Medical Insurance
UFP	Ultrafine particle
µg	microgram
U.K.	United Kingdom
µm	Micrometer
U.S.	United States
VCAM-1	vascular cell adhesion molecule-1
VLF	very-low frequency
vs.	versus
WHI	Women's Health Initiative
wk	week(s)
YLL	years of life lost
yr	year(s)

CHAPTER 5 Cardiovascular Effects–Short-Term Exposure

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

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

Outcome	Causality Determination
Cardiovascular Effects	Suggestive of, but not sufficient to infer, a causal relationship

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

5.1 Introduction

5.1.1 Summary of Previous Integrated Science Assessment

The 2016 Integrated Science Assessment for Oxides of Nitrogen–Health Criteria (hereafter referred to as 2016 Oxides of Nitrogen ISA) issued a causality determination for short-term oxides of nitrogen exposure and cardiovascular effects (U.S. EPA, 2016). The evidence underpinning this causality determination is briefly summarized below.

The consistent epidemiologic evidence from multiple studies at relevant nitrogen dioxide (NO_2) concentrations that was assessed in the 2016 Oxides of Nitrogen ISA was determined to be suggestive of, but not sufficient to infer, a causal relationship between short-term NO_2 exposure and cardiovascular

health effects. The strongest evidence supporting this causality determination comprised outcomes related to triggering of a myocardial infarction (MI). Studies of adults consistently demonstrated NO_2-associated hospital admissions and emergency department (ED) visits for ischemic heart disease (IHD), MI, and angina, as well as all cardiovascular diseases. These findings were coherent with evidence for NO_2-related ST-segment decrements and mortality from cardiovascular disease. Findings were replicated by different researchers in different locations and have adjusted for numerous potential confounding factors including meteorological factors and time trends. However, uncertainty remained regarding exposure measurement error and potential confounding by traffic-related copollutants. Although experimental studies demonstrated non-specific effects (i.e., inflammation and oxidative stress) following NO_2 exposure, these studies did not provide strong coherence for the epidemiologic studies that would help rule out chance, confounding, and other biases. Evidence for other cardiovascular effects (i.e., cardiac arrest and arrhythmia, cerebrovascular disease and stroke, increased blood pressure and hypertension, and decompensation of heart failure) was inconclusive. Largely due to limited analysis of potentially correlated pollutants and recognized limitations of copollutant models, uncertainty remained regarding the extent to which NO_2 exposure was independently associated with cardiovascular effects or if NO_2 serves primarily as a marker for the effects of another traffic-related pollutant or mix of pollutants. Thus, the 2016 Oxides of Nitrogen ISA determined that the combined evidence from epidemiologic and experimental studies was suggestive of, but not sufficient to infer, a causal relationship between short-term NO_2 exposure and cardiovascular effects.

5.1.2 Chapter Organization and Conventions

This chapter evaluates recent evidence examining the relationship between short-term oxides of nitrogen exposure and cardiovascular effects. Its conclusions are drawn from integration of the full body of evidence available in the 2016 Oxides of Nitrogen ISA and evidence that has become available since. The following sections provide an overview of study inclusion criteria for this chapter (see Section 5.2), summaries of recent health effects evidence (see Sections 5.3 to 5.11), a discussion of biological plausibility (see Section 5.12), summary of evidence for lifestages and populations (see Section 5.13, Table 5-1), and a discussion of the causality determination for short-term oxides of nitrogen exposure and cardiovascular effects (see Section 5.14, Table 5-2). Evidence inventories include study-specific details for the epidemiologic and toxicological studies including, for example, study population characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), potential confounders, and select results are located in Appendix A – Appendix Tables. For outcomes supported by evidence from multiple disciplines, subsections titled “Synthesis Across Lines of Evidence” are included.

5.1.2.1 Epidemiologic Studies

In sections assessing the epidemiologic evidence, this chapter summarizes the evidence from previous ISAs and from recent studies that have become available since the last ISA. When relevant information is available from older or more recent epidemiologic studies, these sections additionally include targeted discussion of available analyses examining concentration-response relationships, effects in particular lifestages and populations, and the potential for confounding by copollutants. For apical endpoints with no or limited evidence on these topics, the subsections addressing them are not included.

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

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

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

To increase the comparability of results across epidemiologic studies, this oxides of nitrogen ISA presents effect estimates for associations with health outcomes scaled to the same increment of oxide of nitrogen concentration.[1] The increments for standardization for short-term exposure vary by averaging time (e.g., 24-hours average, 8-hour maximum, 1-hour maximum) and oxide of nitrogen. For 24-hour average, effect estimates were scaled to a 20 parts per billion (ppb) increase for NO_2 or nitric oxide (NO) and a 30 ppb increase for NO_2 and NO (NO_X). For a 1-hour maximum, effect estimates were scaled to a

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

25 ppb increase for NO_2, a 60 ppb increase for NO, and an 80 ppb increase for NO_X. For an 8-hour maximum, the increments for standardization are 25 ppb increase for NO_2, 40 ppb increase for NO, and 55 ppb increase for NO_X. These increments were derived by calculating the U.S.-wide percentile distributions for a given averaging time and then calculating the approximate difference between the median (a typical pollution day) and the 95th percentile (a more polluted day) for a given averaging time (U.S. EPA, 2015c). There were common exceptions to this standardization method. Averaging times other than 24-hour average or 1-hour maximum were examined, for example, 2- to 15-hour averages. Effect estimates based on these averaging times were not standardized but are presented in the ISA as reported in their respective studies.

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

5.1.2.2 Animal Toxicological Studies

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

5.2 Scope

The evidence that has become available since the 2016 Oxides of Nitrogen ISA has been evaluated for relevance using scoping criteria defined by Population, Exposure, Comparison, Outcome, and Study Design (PECOS) statements. The PECOS statements help to define the scope of the assessment and establish study inclusion criteria, thereby facilitating identification of the relevant literature for this oxides of nitrogen ISA.[2]In order to identify the most relevant literature, the body of evidence from the

[2]The following types of publications are generally considered to fall outside the scope and are not included in the ISA: review (narrative, scoping, systematic, and meta-analyses of systematic reviews) articles (which typically present summaries or interpretations of existing studies rather than bringing forward recent information in the form of original research or new analyses), risk or benefits analyses (e.g., that apply concentration-response functions or effect estimates to exposure estimates for differing cases), and health impact assessments (an assessment tool used to

2016 Oxides of Nitrogen ISA was considered in the development of the PECOS statements for this chapter. Specifically, well-established areas of research; gaps in the literature; and inherent uncertainties in specific populations, exposure metrics, comparison groups, and study designs identified in the 2016 Oxides of Nitrogen ISA inform the scope of this chapter. Studies that were included in the 2016 Oxides of Nitrogen ISA, including studies that do not meet the current PECOS criteria, are discussed in this chapter to establish the state of the evidence prior to this assessment. Except for supporting evidence used to examine the biological plausibility of oxides of nitrogen associated cardiovascular effects, recent studies were only included if they met all components of the discipline-specific PECOS statements (see below) and satisfied discipline-specific study quality criteria (see Chapter 14).

Epidemiologic Studies:

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

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

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

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

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

Controlled Human Exposure Studies:

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

designed to investigate how a proposed program, project, policy, or plan may impact health and well-being and inform decision-makers of these potential outcomes before the decision is made).

[3]Epidemiologic studies examining associations with NO_2 that report an overall average of NO_2 exposures (i.e., averaged across study populations/locations and over study periods) above 22 ppb were flagged for additional evaluation of relevance and study quality. This concentration cutoff reflects the 98th percentile of annual average NO_2 concentrations measured at ambient monitors in the United States during the most recent 15 years of available data (i.e., 2008 to 2022) (U.S. EPA, 2023). Studies reporting average NO_2 exposures greater than 22 ppb are considered for inclusion in the ISA if they meet additional study quality criteria described in Section 14.4.3.2.

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

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

Outcome: Cardiovascular health effects.

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

Animal Toxicological Studies:

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

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

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

Outcome: Cardiovascular health effects.

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

5.3 Ischemic Heart Disease

Myocardial infarction (MI), commonly known as a heart attack, is an acute event caused by insufficient blood flow to a portion of the heart, leading to cardiac necrosis. This often occurs secondary to atherosclerosis, where coronary arteries become progressively narrow or blocked due to plaque buildup. Individuals who have had an MI or are at higher risk of MI due to atherosclerotic changes may be diagnosed with underlying IHD, also referred to as coronary heart disease or coronary artery disease. Epidemiologic studies of short-term oxides of nitrogen exposure have assessed ED visits and hospital

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

admissions for MI, IHD, and related symptoms such as angina pectoris (i.e., chest pain). Additionally, electrocardiographic signs of MI, such as ST-segment depression, have been investigated.

5.3.1 Epidemiologic Studies of Ischemic Heart Disease

The 2016 Oxides of Nitrogen ISA concluded that data from epidemiologic studies conducted in Asian, Australian/New Zealand, European, and United States populations supported an association between short-term increases in ambient NO_2 concentrations and increased risk of triggering an MI [see Table 5-40 of U.S. EPA (2016) and Wang et al. (2015); Bhaskaran et al. (2011) Rich et al. (2010); Stieb et al. (2009); Larrieu et al. (2007); Peel et al. (2007); Szyszkowicz (2007); Barnett et al. (2006); Simpson et al. (2005); von Klot et al. (2005); Mann et al. (2002); Linn et al. (2000)]. However, adjustment for potential copollutant confounding was not consistently performed across studies, and the results for those studies that adjusted for another traffic-related pollutant were inconsistent. All of the studies of MI-related health effects used central site monitors, and none of the studies reported information on the extent to which concentrations at central site monitors captured the temporal variation in NO_2 across the study area. Recent studies examining populations in the United Kingdom, China, Japan, Canada, and the United States have been published since the 2016 Oxides of Nitrogen ISA (Chen et al., 2022c; Shin et al., 2022; Liu et al., 2020; Krall et al., 2018; Ha et al., 2017; Yamaji et al., 2017; Butland et al., 2016; Weichenthal et al., 2016; Wang et al., 2015; Milojevic et al., 2014). These studies, mostly case-crossover in design, were conducted among large patient populations across multiple locations and typically employed central site monitors or modeling as exposure surrogates. Overall, these studies indicate associations between short-term increases in ambient NO_2 concentrations and increased risk of triggering an MI, including ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI), angina, and IHD. Evidence from recent studies is summarized in the bullets below, followed by an additional discussion of the shapes of concentration-response relationships in these studies (see Section 5.3.1.1), the lifestages and populations examined (see Section 5.3.1.2), the impact of copollutant confounding on reported associations (see Section 5.3.1.3), approaches to exposure assignment (see Section 5.3.1.4), lag structure (see Section 5.3.1.5), and the impact of season and temperature on associations (see Section 5.3.1.6). Section 5.3.1.7 presents conclusions from the epidemiologic evidence for IHD and summarizes the remaining uncertainties. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are summarized in Appendix A – Appendix Table 5-1.

- A case-crossover study of the England and Wales Myocardial Ischemia National Audit Project (MINAP) database reported a 20 ppb increase in NO_2 concentrations (measurements within 50 km of the residential address) from an unconstrained distributed lag of 0 to 4 days was associated with a 2.01% increase (95% Confidence Interval [CI]: 0.25, 3.76%) in risk of emergency hospital admission for overall MI (Milojevic et al., 2014). The authors also reported a 2.59% increase (95% CI: 0.21, 4.98%) in risk of hospital admission for NSTEMI for a 10^{th} to 90^{th} percentile increase in NO_2 concentrations at lags 0 to 4 days. A subsequent

case-crossover study of the MINAP database that relied on exposure data derived from a validated chemical transport model, Butland et al. (2016) reported a 1.02% (95% CI: 0.02, 2.01%) increase in the risk of NSTEMI hospitalizations per increase in the daily maximum 1-hour NO_2 concentrations at lags 0 to 2 days. Butland et al. (2016) did not observe an association between daily maximum 1-hour NO_2 concentrations at lags 0 to 2 days and risk of MI or STEMI hospitalizations, and Milojevic et al. (2014) did not observe an association between NO_2 concentrations at lags 0 to 4 days and risk of emergency hospital admission for IHD and STEMI.

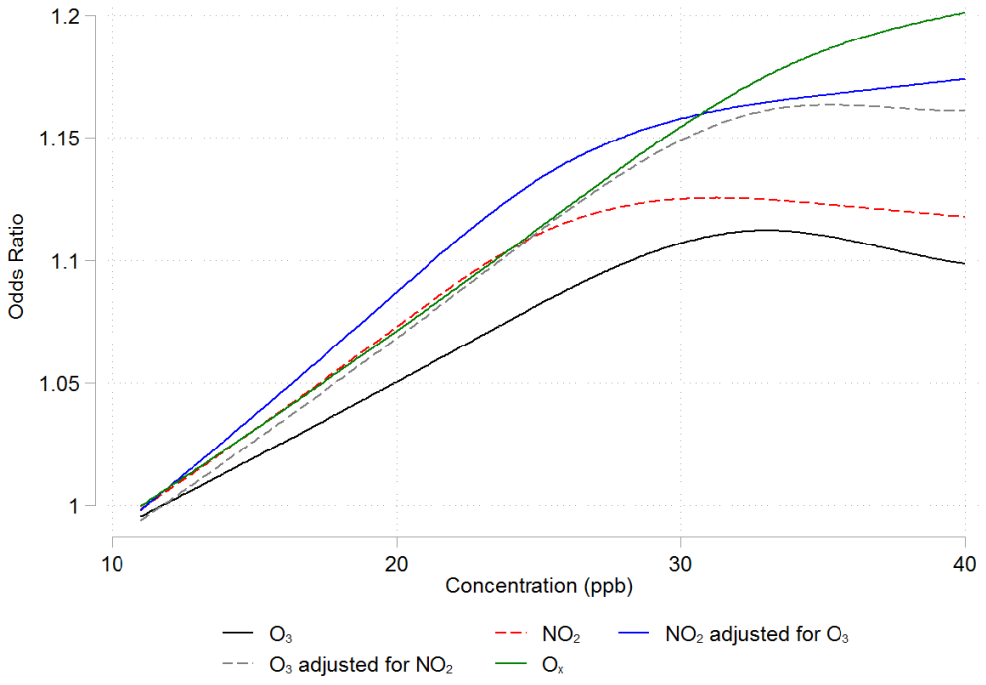
- A case-crossover multi-city study in China of patients who visited a Pain Center reported a 20 ppb increase in NO_2 concentrations (0- to 24-hour prior to symptom onset) was associated with a 5.67% (95% CI: 4.72, 6.62%), 5.05% (95% CI: 4.43, 5.67%), 4.72% (95% CI: 3.68, 5.77%), and a 4.63% (95% CI: 3.26, 6.01%) increase in risk of unstable angina, acute coronary syndrome, STEMI, and NSTEMI, respectively (Chen et al., 2022c).
- Among patients in the Alberta Provincial Project for Outcome Assessment in Coronary Heart Disease registry, Liu et al. (2020) used a time-stratified case-crossover study design to evaluate associations between short-term exposure to NO_2, estimated from a land use regression (LUR) model previously demonstrated as remaining stable over a 5-year period, and hospital admissions for MI. Among participants living in areas with higher NO_2 concentrations, there was a 20% increase in the odds of MI hospital admissions (OR: 0.98 [95% CI: 0.87, 1.09]) for the 0- to 4-day average.
- (Weichenthal et al., 2016) conducted a case-crossover study in 16 cities in Ontario, Canada to evaluate the impact of $PM_{2.5}$ oxidative potential on the relationship between $PM_{2.5}$ and ED visits for MI in the main analysis. Oxidative burden metrics were derived by multiplying ambient $PM_{2.5}$ mass by regional estimates of glutathione- (GSH) and ascorbate- (AA) related oxidative potential (i.e., the potential for filter extracts to deplete GSH and AA in an in vitro assay-based on a synthetic respiratory tract lining fluid). The association between 3-day mean NO_2 and ED visits for MI in single (5.60% increase [95% CI: -3.3, 14.50%]) and copollutant models (see Section 5.3.1.3) were reported as a supplemental analysis.
- A case-crossover analysis of air pollution and cardiovascular events at labor and delivery in 12 clinical sites in the United States. Although (Ha et al., 2017) observed positive associations with single day lag exposures there was no association between 7-day average exposure and IHD event (OR: 0.99 [95% CI: 0.48, 2.01]). NO_X exposure, which was based on Community Muti-Scale Air Quality (CMAQ) model estimates fused with monitoring data, was estimated for the period during the week prior to labor/delivery (i.e., single day lags and the 7-day average.
- A time series analysis of five U.S. cities reported positive associations between 1-hour maximum NO_2 and NO_X concentrations based on population weighted average estimates that used fused monitor concentrations with CMAQ model estimates) at lag 0 and ED visits for IHD (NO_2 RR:1.04 [95% CI: 1.02, 1.07]; NO_X RR: 1.01 [95% CI: 1.00, 1.02]) (Krall et al., 2018).
- In a cohort study in Japan, Yamaji et al. (2017) reported a near null association (OR: 1.01 [95% CI: 0.99, 1.03]) between NO_X, estimated from monitoring data, and risk of STEMI.
- A large exploratory case-crossover study conducted in Alberta, Canada reported the most robust association using a bootstrap (resampling) analysis to account for model uncertainty occurred between a 17.2 ppb increase in 6-hour average NO_2 concentrations at lag 1 and a corresponding 9.2% (95% CI: 3.9, 14.8%) increase in the risk of hospitalizations for acute MI

(Wang et al., 2015). Other lags evaluated included a 12-hour average (lag 1) and a 24-hour average (lag 1).

5.3.1.1 Concentration-Response

The shape of the concentration-response (C-R) relationship for health effects related to short-term NO_2 exposure and cardiovascular effects was not examined in the epidemiologic studies assessed in the 2016 Oxides of Nitrogen ISA. Recent studies establish the evaluation of the shape of the C-R relationship by explicitly examining its shape, and whether the relationship is linear across the full range of NO_2 concentrations. The recent evidence is summarized below.

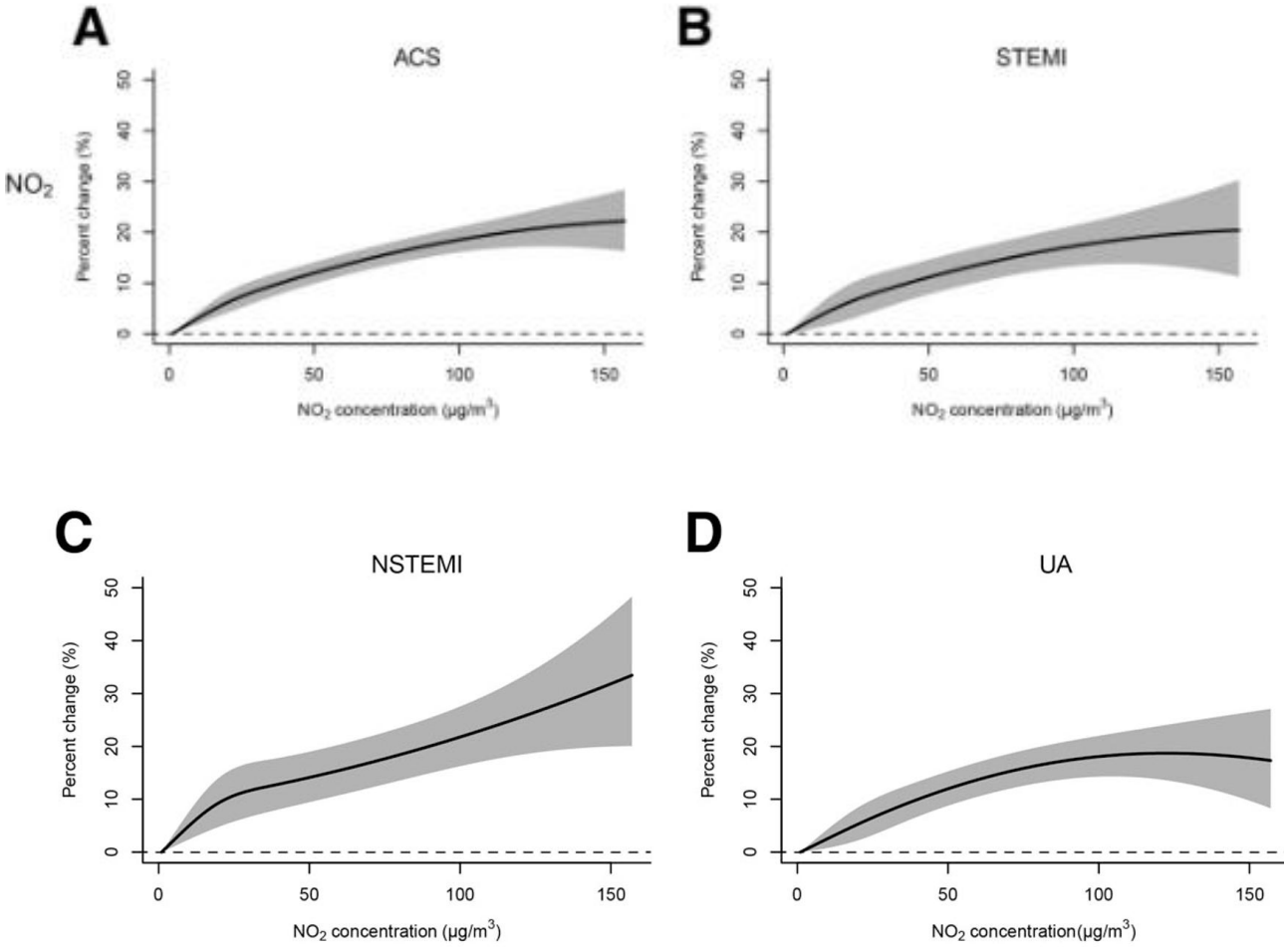
- Weichenthal et al. (2016) conducted a case-crossover study in 16 cities in Ontario, Canada. A C-R curve was obtained using cubic splines with four knots (see Figure 5-1). The dotted red line depicts the relationship between NO_2 and ED visits for MI in a model that is adjusted for $PM_{2.5}$ oxidative burden (i.e. lag 0 $PM_{2.5}$*oxidative potential $[OP]^{GSH}$), 3-day mean ambient temperature, and relative humidity. The solid blue line depicts the relationship between NO_2 and ED visits for MI in a model that is adjusted for both OP^{GSH} and ozone. The slope of the relationship between 3-day mean NO_2 and ED visits for MI was larger in the copollutant model that was adjusted for ozone. The NO_2 curve is approximately linear until NO_2 concentrations reach approximately 25 ppb when it appears to attenuate.



List of abbreviations in alphabetical order: NO_2 = nitrogen dioxide, O_3 = ozone, O_X = combined oxidant capacity of nitrogen dioxide and ozone.
Note: All models used to derive the plots were adjusted for the glutathione-related oxidative burden, 3-day mean ambient temperature and relative humidity.
Source: Weichenthal et al. (2016). Copyright permission pending.

Figure 5-1 Concentration-response plots using cubic splines with 4 knots for nitrogen dioxide (other pollutants are included on the figure).

- In a case-crossover study conducted in more than 300 Chinese cities (2015 to 2020), Chen et al. (2022c) examined the shape of the C-R function for the association of lag 0- to 23-hour NO_2 exposure with acute coronary syndrome (ACS), which is a group of conditions that can severely reduce blood flow to the heart including MI and unstable angina. Associations for MI and STEMI, non-STEMI, and unstable angina were also estimated separately in the study. The cross-basis function for NO_2 (natural cubic spline with 2 knots) was derived using a distributed lag model, which allowed nonlinear associations to vary across lag periods. Separate models were refitted for NO_2 to plot the cumulative C-R curves that are shown in Figure 5-2. No threshold was observed across the range of concentrations in the study (0.1th percentile 1 µg/m^3 to 99.9th percentile 157 µg/m^3). The approximately linear relationships appeared to attenuate at NO_2 concentrations approaching approximately 30 µg/m^3 (15.9 ppb).



List of abbreviations in alphabetical order: ACS = Acute Coronary Syndrome; NO_2 = Nitrogen Dioxide; NSTEMI = non-ST elevation myocardial infarction; STEMI = ST segment elevation myocardial infarction; UA = unstable angina.
Note: The solid black lines are the average percent change in the risk of onset and the gray areas are the 95% confidence intervals.
Source: Chen et al. (2022c). Copyright permission pending.

Figure 5-2 Concentration-response curves for the association between lag 0 to 24 nitrogen dioxide concentration and acute coronary syndrome, ST segment elevation myocardial infarction, non-ST segment elevation myocardial infarction and unstable angina.

5.3.1.2 Lifestages and Populations

This section focuses on the evaluation and characterization of evidence determining whether there are populations or lifestages potentially at increased risk of short-term NO_2 exposure-related IHD, with specific emphasis placed on studies that compare responses to a reference population. The strongest evidence for a relationship between short-term NO_2 exposure and cardiovascular effects that was characterized in the 2016 Oxides of Nitrogen ISA was for MI [see Section 5.4.8, (U.S. EPA, 2016)]; however, most studies showed no difference in the association in groups with hypertension, arrhythmia, or congestive heart failure. A small number of studies also examined obesity or body mass index (BMI) as a potential effect measure modifier, but overall, the evidence was inconsistent as to whether obesity leads to increased risk of NO_2-related cardiovascular effects.

Recent studies expand the evidence that potentially informs whether there are populations or lifestages at increased risk of short-term NO_2-exposure-related cardiovascular effects. Results from recent studies are presented in greater detail below. Overall, the findings did not indicate consistent differences in the risk of MI or related outcomes in age- or sex-stratified analysis or for those with pre-existing conditions.

Lifestage

- Inverse associations were observed among those <70 years old, while positive associations were observed among older participants in age-stratified analysis for lag 0- to 4-day NO_2-associated emergency hospital admissions for both MI (-2.7% change [95% CI: -6.7, 1.2] vs. 3.11% change [95% CI: -0.58, 6.81]) and IHD (-1.69% change [95% CI: -4.15, 0.78] vs. 3.5% change [95% CI: 0.97, 6.03]), respectively (Milojevic et al., 2014).
- Chen et al. (2022c) reported similar increases in risk for 0- to 24-hour average NO_2 concentration and acute coronary syndrome onset in those less than 65 years old (5.39% change [95% CI: 4.5, 6.27]) and those greater or equal to 65 years old (4.89% change [95% CI: 4.01, 5.77]).
- In a study of hospitalization data in Alberta Canada, Wang et al. (2015) reported that 1-day lag NO_2 concentration (and 6-, 12-, and 24-hour average concentrations) was associated with acute MI (the association was driven primarily by non-STEMI as opposed to STEMI). Further, the association with 24-hour average NO_2 concentration and NSTEMI was only present among older adults ≥65 years old compared to adults who were <65 years old (OR: 1.15 [95% CI: 1.05, 1.27] vs. 1.00 [95% CI: 1.00, 1.00]). The association was similar among those ≥65-years old with hypertension.

- In age-stratified analysis (>69 years or ≤69 years), a cohort study in Japan, Yamaji et al. (2017) reported the association between NO_X and risk of STEMI were relatively unchanged (>69 years OR: 1.02 [95% CI: 0. 0.99, 1.05] and ≤69 years OR: 1.01 [95% CI: 0.98, 1.04]).

Sex

- Lag 0- to 4-day NO_2 exposure was associated with an increase in emergency hospital admissions for MI among males (2.73% change [95% CI: -0.78, 6.23]) and a decrease among females (-3.11% change [95% CI: -7.33, 1.1]) (Milojevic et al., 2014).
- Chen et al. (2022c) reported a similar increase in risk for 0- to 24-hour average NO_2 concentration and acute coronary syndrome onset among males (4.85% change [95% CI: 4.11, 5.602]) and among females (5.67% change [95% CI: 4.54, 6.81]).
- A multi-city Canadian time series analysis Shin et al. (2022) reported males were at higher risk for lag 0 NO_2-associated hospitalization due to IHD (3.20% change, [95% CI 1.40, 5.00]) compared to females (-0.60 % change, [95% CI: -3.40, 2.20]).

Smoking

- Chen et al. (2022c) reported a higher risk for 0- to 24-hour average NO_2 concentration and acute coronary syndrome onset among non-smokers (7.16% change [95% CI: 5.68, 8.64]) compared to smokers (4.43% change [95% CI: 2.16, 6.69]).

Pre-existing conditions

- Milojevic et al. (2014) reported that an increase in lag 0- to 4-day NO_2 concentrations was associated with a decrease in NO_2-associated hospital admissions for MI among those with chronic renal failure, those who use the antiplatelet medication clopidogrel on a regular basis, and those with a previous percutaneous coronary intervention.
- Chen et al. (2022c) reported similar increases in the risk for 0- to 24-hour average NO_2 concentration and acute coronary syndrome onset among individuals with hypertension, diabetes, coronary heart disease, a family history of cardiovascular disease (CVD), valvular heart disease, atrial fibrillation, chronic obstructive pulmonary disease (COPD), and thyroid disorders compared to those without these conditions.

5.3.1.3 Copollutants

There were a limited number of epidemiologic studies reviewed in the 2016 Oxides of Nitrogen ISA that evaluated $PM_{2.5}$ or other traffic-related pollutants (i.e., black carbon [BC], elemental carbon [EC], ultra fine particles [UFP], organic carbon [OC], volatile organic carbons [VOCs]) in copollutant models with NO_2. In some studies, associations of short-term exposure to NO_2 and MI were attenuated with adjustment for $PM_{2.5}$ or UFP [see (U.S. EPA, 2015b)]. Therefore, uncertainties remained about whether NO_2 served as an indicator for other traffic-related pollutants or mixtures. Recent studies expand the evidence pertaining to the independent effect of short-term NO_2 exposure on MI (see Table 5-3).

These results, although limited, suggest the association between short-term NO_2 exposure and MI remains robust to copollutant adjustment.

- In sensitivity analyses conducted in the large case-crossover study in China, the results for copollutant models that adjusted for $PM_{2.5}$, $PM_{10-2.5}$, SO_2, carbon monoxide (CO), and O_3 persisted and were essentially the same in magnitude and precision as the results from to the single pollutant model (Chen et al., 2022c).
- Similar findings of robust associations between NO_2 and risk of MI with adjustment for $PM_{2.5}$ or O_3 were also observed in a couple of U.K. based case-crossover studies of the same database (Butland et al., 2016; Milojevic et al., 2014).
- Sensitivity analyses in a case-crossover study in Canada found the association between 3-day mean NO_2 concentrations and emergency room visits for MI was larger in magnitude in copollutant models that adjusted for O_3 and lag 0 $PM_{2.5}$, than in single pollutant models or copollutant models that adjusted for O_3 and lag 0 $PM_{2.5}$*GSH (Weichenthal et al., 2016). Similarly, Butland et al. (2016) reported that the association with non-STEMI was larger after adjustment for $PM_{2.5}$.
- In a hierarchical time-series analysis of five U.S. cities fitted single pollutant models that included multiple outcomes simultaneously. To evaluate multipollutant exposures, the study used hierarchical principal components analysis to identify related groups of pollutants. Consistent with findings from the single pollutant model, a positive association was reported between the principal component that included NO_2 and IHD (Krall et al., 2018).

Table 5-3 Summary of results from studies that estimate associations between short-term oxides of nitrogen exposure and myocardial infarction in models that adjust for copollutant exposure

Study	Pollutant Averaging time, Lag	Outcome(s)	Single Pollutant	Copollutants
†Weichenthal et al. (2016) Ontario, Canada 2004–2011	NO_2, 3-d mean	MI	4.4% (-0.30, 9.2) Positive	3.7% (-0.70, 8.4), + Lag 0 $PM_{2.5}$ 2.8% (-1.6, 7.3), + GSH oxidative burden Positive but attenuated 6.0% (1.1, 11), + O_3 Positive (larger)
†Chen et al. (2022c) 318 Cities, China 2015–2020	NO_2 0–24-hr average	ACS onset	3.89% (3.41, 4.37) Positive	Figure (+$PM_{2.5}$, $PM_{10-2.5}$, SO_2, CO, O_3) Positive
†Milojevic et al. (2014) England and Wales MINAP 2003–2009	NO_2, Lag 0–4 d	MI	2.4% (0.3, 4.5) Positive	Robust (no quantitative values provided)
		STEMI	1.4% (-1.8, 4.6) Positive	Robust (no quantitative values provided)

Study	Pollutant Averaging time, Lag	Outcome(s)	Single Pollutant	Copollutants
		Non-STEMI	3.1% (0.3, 6.0) Positive	Robust (no quantitative values provided)
†Butland et al. (2016) MINAP 2003–2010	NO_2, unconstrained distributed lags 0–2 d	All MI	0.09 (-0.10, 0.28) Approximately null	0.07 (-0.13, 0.27) + O_3 Null 0.14 (-0.08, 0.37) + $PM_{2.5}$ Positive, lost precision
		STEMI	-0.16% (-0.49, 0.18) Negative	-0.23% (-0.57, 0.12) + O_3 Negative -0.07% (-0.46, 0.32) + $PM_{2.5}$ Null
		Non-STEMI	0.27% (0.01, 0.54) Positive	0.28% (0.00, 0.57) + O_3 Positive 0.43% (0.11, 0.74) + $PM_{2.5}$ Positive (larger)
		ED visits acute MI	NR	RR: 0.98 (0.98, 0.98), + $PM_{2.5}$ and O_3 Negative
		Hospitalizations acute MI	NR	RR: 1.06 (1.06, 1.06), + $PM_{2.5}$ and O_3 Positive

List of abbreviations in alphabetical order: ACS = acute coronary syndrome, CO = carbon monoxide; d = day; ED = emergency departments, GHS = glutathione; hr = hour; MI = myocardial infarction; MINAP = Myocardial Ischaemia National Audit Project, NO_2 = nitrogen dioxide; NR = no response; O_3 = ozone; $PM_{\#}$ = particulate matter with a nominal mean aerodynamic diameter less than or equal to # μm; RR = Relative Risk; STEMI = ST elevation myocardial infarction; SO_2 = sulfer dioxide.
†Studies published since the 2016 Oxides of Nitrogen ISA.

5.3.1.4 Exposure Assignment

In the 2016 Oxides of Nitrogen ISA all the studies of MI-related health effects used central site monitors, and none of the studies of MI reported information on the extent to which concentrations at central site monitors captured the temporal variation in NO_2 across the study area. Utilization of exposure surrogates such as single monitors and unweighted or population-weighted averages of multiple monitors contributes to uncertainty in the measurement of exposure in epidemiologic studies that may result in biased health effect estimates. Exposure measurement error that does not adequately capture temporal variability in short-term NO_2 concentrations may underestimate the magnitude, but not the directionality of the association. Chapter 2 (see Section 2.5.1) provides a thorough discussion of the bias resulting from

exposure assessment methods common to the epidemiologic literature and its impact on the inferences that can be drawn about health effects. Most studies of short-term NO_2 exposure and MI are case-crossover and time series analyses that continue to assign daily NO_2 concentrations using fixed and central site monitors to capture temporal variability in exposure. A few recent studies used modeled exposure estimates based on inputs from several sources (e.g., emissions, weather, pollutant properties) fused with monitoring data (Krall et al., 2018; Ha et al., 2017; Butland et al., 2016), or utilized a LUR model to assess spatial variability (Liu et al., 2020). Positive associations were observed between short-term exposures based on modeled exposures and MI (Liu et al., 2020; Krall et al., 2018) and IHD (Ha et al., 2017), respectively. However, one study (Butland et al., 2016) using modeling data to assign exposure compared monitor data from a prior study (Milojevic et al., 2014) to assess model performance, reporting a predicted 45% attenuation in the NO_2 effect estimate due to measurement error in the chemistry transport model. Overall, the evidence is too limited to determine whether modeled exposures introduce systematic measurement error that can bias the association between short-term exposure to NO_2 and IHD.

5.3.1.5 Lag Structure

As discussed in the 2016 Oxides of Nitrogen ISA, many studies reported that MI was associated with shorter lags of NO_2 exposure. Most typically, associations were reported within the first few days of the event, such as lags of 0, 0 to 1 or 0 to 2 days, which are indicative of more immediate effects. Studies published since the 2016 Oxides of Nitrogen ISA continue to report associations between distributed lags of 0 to 1 and 0- to 2- and single-day lags 0 and 1 with MI and related outcomes.

- The association between NO_2 exposure over a longer duration of lag 0 to 4 days with MI and NSTEMI was stronger than at lag 0 to 1 days (Milojevic et al., 2014). However, a subsequent analysis of the same database Butland et al. (2016) reported stronger associations with more recent exposures (lag 0) days compared to lag 1 or 2 days. Differences across these few studies may be due to variations in exposure assessment methodologies described in Section 5.3.1.4.

Associations with concurrent day exposures were observed in multiple studies.

- A multicity study in Canada reported a positive association between lag 0 NO_2 exposure and hospital admissions for IHD, but not lag 1 or 2 (Shin et al., 2022).
- A case-crossover multi-city study examining the association between sub-daily (0- to 23-hour) NO_2 exposure and ACS onset reported the strongest effect (risk of ACS onset) occurred in the concurrent hour of NO_2 exposure, with attenuation afterwards and a nonsignificant association after 15 to 29 hours (Chen et al., 2022c).
- A large exploratory study of acute MI in Canada observed positive associations with NSTEMI at 6, 12, and 24 hours prior to event (Wang et al., 2015).

Some studies compared associations using different lag structures ranging from 0 to 14 days.

- A multi-city Canadian time series analysis by Shin et al. (2022) and nationwide analysis of 272 cities in China by Chen et al. (2018) reported stronger positive associations between NO_2 exposure and cardiovascular mortality at more proximal lags (e.g., lag 0, lag 1, and lag 0 to 1) and attenuated estimates at more distal lags (e.g., lag 2 and lag 3).
- A case-crossover analysis of air pollution and cardiovascular events at labor and delivery in 12 clinical sites in the United States. Ha et al. (2017) reported positive, but imprecise associations between exposure to NO_X at individual lag days of 4 to 6 and an IHD event.
- A cohort study in Japan by Yamaji et al. (2017) assessed the association between NO, NO_2, and NO_X and risk of STEMI reported no associations over a 14-day exposure period in analyses adjusted for meteorological and livelihood variables. In univariate analysis, positive associations were reported between exposures at 14, 7, and 0 days prior to the event for all multipollutants.

5.3.1.6 Seasonal and Temperature Differences

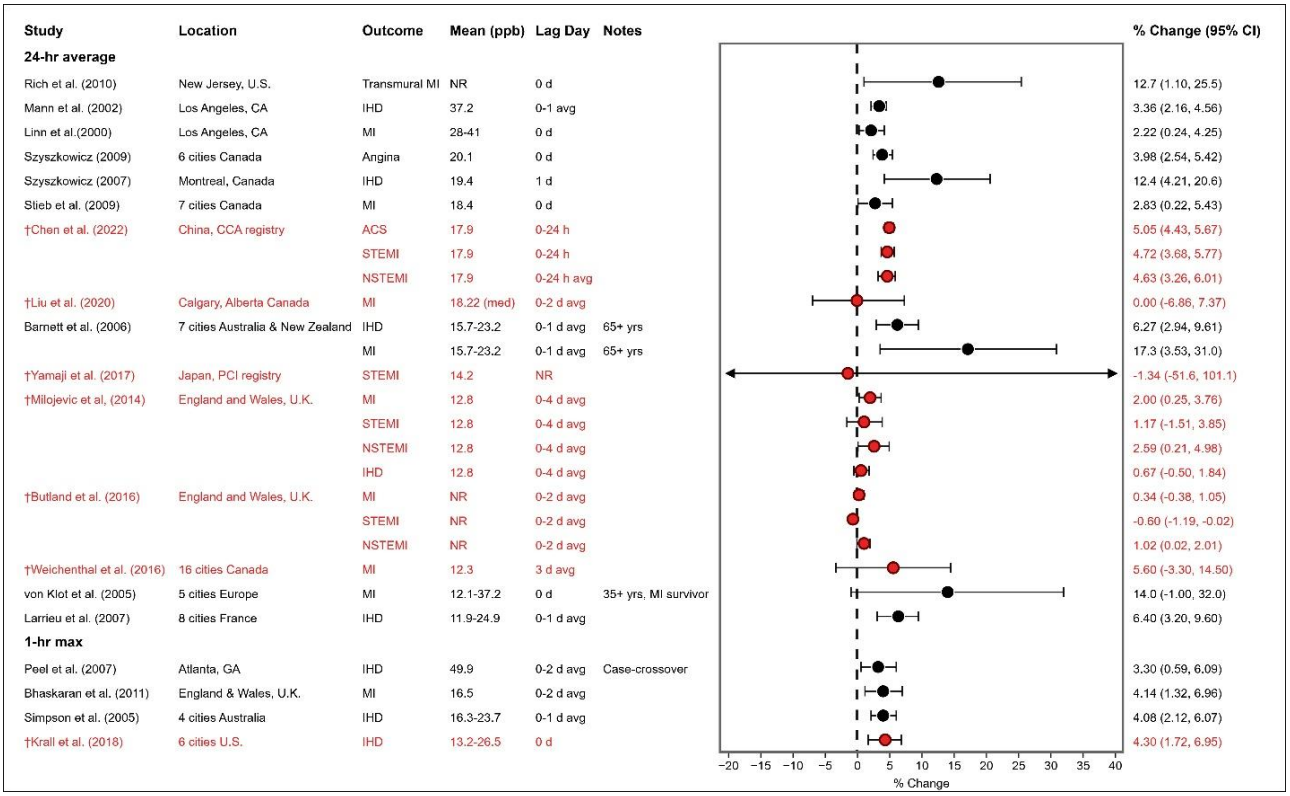
A limited number of studies evaluated in the 2016 Oxides of Nitrogen ISA reported on potential seasonal modification of the NO_2-IHD association. These studies suggested a stronger association in the cold season (Qiu et al., 2013; Nuvolone et al., 2011). In contrast, a stronger association was observed in the warm season for NO_2 and angina (Szyszkowicz, 2009).

Recent multicity studies conducted in Canada (Shin et al., 2022; Wang et al., 2015), Japan (Yamaji et al., 2017), China (Chen et al., 2022c; Huang et al., 2021; Li et al., 2021), and England/Wales (Butland et al., 2016) that assessed the influence of seasonality and temperature reported inconsistent results.

- A case-crossover multi-city study in China reported a larger increase in the risk for 0- to 24-hour average NO_2 concentration and acute coronary syndrome onset in the cold season compared to the warm seasons (Chen et al., 2022c).
- A multi-city Canadian time series analysis reported males were at higher risk for lag 0 NO_2-associated hospitalization due to IHD during the warm season (April to September) (5.80% change [95% CI: 2.60, 9.00]) compared to null associations in the cold season (October to March) (Shin et al., 2022). Null associations were observed during both the warm and cold seasons for females.
- A study from England and Wales reported that seasonality did not influence the association between NO_2 and MI or STEMI (p-value for interaction) (Butland et al., 2016).
- A single-city Canadian study observed higher NO and NO_2 concentrations during the winter, but found the monthly frequency of acute myocardial infarction (AMI) hospitalizations averaged over the study period did not vary in comparison to monthly concentrations of NO and NO_2 (Wang et al., 2015).
- A registry study in Japan, Yamaji et al. (2017) reported the association between NO_X and STEMI was unchanged in seasonally stratified analysis (OR: 0.94 [95% CI: 0.87, 1.01] for summer months and 1.01 [95% CI: 0.98, 1.05] for winter months).

5.3.1.7 Conclusions and Remaining Uncertainties

Overall, the epidemiologic data available continues to support associations between short-term exposures to ambient NO_2 and increased risk of triggering an MI, including STEMI and NSTEMI, as well as associations with angina and IHD (see Figure 5-3). The few studies examining the shape of the C-R relationship observed the curves were approximately linear across the range of concentrations until NO_2 concentrations reached approximately 16 ppb (Chen et al., 2022c) to 25 ppb (Weichenthal et al., 2016) when they appear to attenuate. Recent studies (Chen et al., 2022c; Butland et al., 2016; Weichenthal et al., 2016; Milojevic et al., 2014) expand the evidence pertaining to the independent effect of short-term NO_2 exposure on MI. These results, although limited, suggest the association between short-term NO_2 exposure and MI remains robust to copollutant adjustment. Additionally, the results from prior and recent studies did not indicate consistent differences in the risk of MI or related outcomes in age- or sex-stratified analysis or for those with pre-existing conditions. The evidence continues to indicate MI is associated with shorter lags of NO_2 exposure, with associations reported within the first few days of the event, such as lags of 0, 0 to 1 or 0 to 2 days, which are indicative of more immediate effects. The evidence from studies that assessed the influence of seasonality and temperature remains inconsistent; few studies have compared associations across exposure estimation approaches, or extended lag periods, and no clear patterns are evident from the studies that have made these comparisons. Taken together, the available epidemiologic evidence provides consistent support for associations between short-term NO_2 exposures and MI and IHD.



List of abbreviations in alphabetical order: ACS = Acute Coronary Symptom; Avg = average; CA = California, U.S.; CI = confidence interval; d = day; h = hour; IHD = ischemic heart disease; Max = maximum; MI = myocardial infarction; NO_2 = nitrogen dioxide; NR = no response; NSTEMI = Non ST segment Myocardial Infarction; STEMI = ST segment Elevation Myocardial Infarction; U.K. = United Kingdom; U.S. = United States; yrs = years.
Note: Effect estimates for associations with NO_2 are indicated by a circle.
†Studies published since the 2016 Oxides of Nitrogen ISA.

Figure 5-3 Percentage increase in emergency department visits and hospital admissions for ischemic heart disease, acute coronary syndrome, angina or myocardial infarction in relation to nitrogen dioxide exposure.

5.4 Arrhythmia and Cardiac Arrest

The typical rhythm of the heart relies on tightly coordinated electrical impulses to stimulate muscle contraction in its upper (atria) and lower (ventricles) chambers. Dysfunctions in this process are called arrhythmias. Arrhythmias can originate from any chamber of the heart and may manifest as altered heart rate or irregular rhythm. While some arrhythmias are benign, others are life-threatening and may result in cardiac arrest–a sudden and complete loss of heart function. In epidemiologic studies, the impact of short-term oxides of nitrogen exposure on arrhythmias is typically assessed using International Classification of Disease (ICD) codes for ED visits, hospital admissions, and out-of-hospital cardiac arrests (OHCA). There is also a body of epidemiologic evidence focusing on arrhythmias recorded by ambulatory electrocardiograms (ECGs) or implantable cardioverter-defibrillators.

5.4.1 Epidemiologic Studies of Arrhythmia and Cardiac Arrest

The 2016 Oxides of Nitrogen ISA concluded that data from epidemiologic studies was inconsistent for an association between 24-hour average NO_2, NO, or NO_X and risk of cardiac arrhythmias. Mixed results were observed across several endpoints, including implantable cardioverter-defibrillators (Link et al. (2013); Metzger et al. (2007)), continuous ECG recordings (Bartell et al. (2013)), out-of-hospital cardiac arrest (Silverman et al. (2010); Ensor et al. (2013)), and hospital admissions and ED visits Tsai et al. (2009). Studies had several sources of uncertainty, including potential copollutant confounding by traffic pollutants (adjustment was rarely performed across studies) and reliance on central site monitors. Recent, primarily case-crossover studies published since the 2016 Oxides of Nitrogen ISA from North America (Dales et al., 2020; Krall et al., 2018; Ha et al., 2017), Europe (Raza et al., 2019; Santurtún et al., 2016; Milojevic et al., 2014), and Asia (Zhao et al., 2023; Kranc et al., 2021; Zhao et al., 2020; Lee et al., 2019) which analyzed implantable cardioverter-defibrillators, ED visits for cardiac dysrhythmia, hospital admissions for arrhythmias and atrial fibrillation, and OHCA reported inconsistent findings, similar to earlier studies. These studies were conducted among large patient populations across multiple locations and typically employed central site monitors or modelling as exposure surrogates. Evidence from recent studies is summarized in the bullets below, followed by additional discussion of the lifestages and populations evaluated in these studies

lifestages (see Section 5.4.1.1), potential copollutant confounding (see Section 5.4.1.2), exposure assignment (see Section 5.4.1.3), the lag structure of association (see Section 5.4.1.4), and potential seasonal and temperature differences (see Section 5.4.1.5). Section 5.4.1.6 presents conclusions from the epidemiologic evidence and summarizes remaining uncertainties. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 5-2.

- A few studies reported positive associations between short-term exposure to oxides of nitrogen (primarily NO_2) and risk of arrhythmia admissions (Krall et al., 2018; Santurtún et al., 2016; Milojevic et al., 2014). A case-crossover study in England and Wales reported an increase in NO_2 concentrations at distributed lags of 0 to 4 days was associated with a 2.43% increase (95% CI: 0.5, 4.35%) in risk of emergency hospitalization for arrythmias and a 2.34% increase (95% CI: 0.21, 4.47%) in risk of atrial fibrillation (Milojevic et al., 2014). A time series analysis conducted across multiple regions in Spain reported a higher risk of cardiac arrhythmia admissions (26.34% [95% CI: 22.39, 30.29] per 5.3 ppb increment in lag 0 NO_2 concentrations) (Santurtún et al., 2016). A time series analysis of five U.S. cities using a Bayesian multicity multi-outcome model (MCM) reported positive associations between exposure to 1-hour maximum NO_2 and NO_X (based on population weighted average estimates that used fused monitor concentrations with Community Muti-Scale Air Quality (CMAQ) model estimates) at lag 0 and ED visits for cardiac dysrhythmia (NO_2 RR: 1.04 [95% CI: 1.01, 1.08] per 9.4 ppb increase in NO_2; NO_X RR: 1.03 [95% CI: 1.01, 1.05] per 34.54 ppb increase in NO_X) (Krall et al., 2018). Similar results were reported for traditional multi-city models.
- A case-crossover nationwide study from Taiwan reported mixed findings for associations between NO, NO_X and NO_2 exposures and hospital admissions for atrial fibrillation (e.g., NO [Lag 1] OR: 0.83 [95% CI: 0.5, 1.38], NO_X [Lag 1] OR: 0.82 [95% CI: 0.57, 1.19] and NO [Lag 1 to 5] OR: 1.43 [95% CI: 0.65, 3.12], NO_2 [Lag 1 to 5] OR: 0.89 [95% CI: 0.61, 1.28]) (Lee et al., 2019).
- Positive associations were not observed between short-term NO_2 exposure (lag 0 to 3 days) and implantable cardioverter-defibrillators discharge in response to ventricular fibrillation or ventricular tachycardia in a case-crossover study from Canada (Dales et al., 2020). For example, the ORs for the associations between defibrillator discharge and same-day lag NO_2 exposure and 4-day average NO_2 exposure were 1.00 (95% CI: 0.78, 1.28) and 0.78 (95% CI: 0.54, 1.12), respectively.
- Available studies have not observed consistently positive associations between short-term exposure to oxides of nitrogen and cardiac arrest. A case-crossover analysis of air pollution and cardiovascular events at labor and delivery in 12 clinical sites in the United States. Ha et al. (2017) observed average NO_X concentration (based on CMAQ model estimates fused with monitoring data) during the week prior to labor/delivery (lag 0 to 7) was positively associated with cardiac arrest (OR: 1.42 [95% CI: 1.08, 1.86]). A nationwide case-crossover study from Japan reported an inverse association (lag 0-3 OR: 0.97 [95% CI: 0.93, 1.00]) for OHCAs among those listed in the country's emergency response registry (Zhao et al., 2020). Inverse associations with 2-hour moving average exposure and 24-hour moving average NO_2 exposures (OR: 0.97 [95% CI: 0.94, 1.00] per 5.3 ppb increase in NO_2 OR: 0.89 [95% CI: 0.79, 1.00]) were reported for OHCAs in a case-crossover study of three cities in Sweden (Raza et al., 2019), and a nationwide registry study from Israel (Kranc et al., 2021) observed the association between lag 0 NO_2 (OR: 1.11 [95% CI: 0.95, 1.29]) with OHCA included the null value.

5.4.1.1 Lifestages and Populations

This section focuses on the evaluation and characterization of evidence determining whether there are populations or lifestages potentially at increased risk of a short-term NO_2 exposure-related health effect, with specific emphasis placed on studies that compare responses to a reference population. The evidence presented in the 2016 Oxides of Nitrogen ISA on potential effect modification was limited to a single epidemiologic study (Wichmann et al., 2013) that reported ambient NO_X concentration was associated with a larger percent increase in OHCA in females (23.67% [95% CI 3.72, 43.62] compared to males (1.33% [95% CI: -13.16, 15.82]) for a 30 ppb increase in 24-hour average NO_X at lag 3, although there were slightly under two thirds the number of cases observed in females compared to males. Recent evidence to inform whether there are populations or lifestages at increased risk of NO_2-related effects remain limited. The results from recent studies that reported on the potential influence of age and sex on NO_2-associated arrhythmia and OHCA are described below.

Lifestage

- For OHCA, a larger point estimate was reported among patients older than 70 compared to younger patients in the nationwide registry study from Israel (OR: 1.02 [95% CI: 0.77, 1.34] vs. 1.15 [95% CI: 0.96, 1.38] per 36 ppb increase in NO_2) (Kranc et al., 2021).
- (Milojevic et al., 2014) reported positive associations between lag 0- to 4-day NO_2 and emergency hospital admissions for arrhythmia among participants ≥70 years (3.09% change [95% CI: 0.54, 5.98]) and participants <70 years (1.51% change [95% CI: -1.21, 4.221]).

Sex

- In the United Kingdom based study, women exhibited a higher risk of NO_2-associated emergency hospital admissions for arrythmias compared to men (1.76% [95% CI: -0.84, 4.35] increase vs. 3.01% [95% CI: 0.38, 5.64]) (Milojevic et al., 2014).
- For OHCA, a stronger association was reported among men compared to women in the nationwide registry study from Israel (OR: 1.2 [95% CI: 0.98, 1.47] vs. 1.0 [95% CI: 0.79, 1.26]) (Kranc et al., 2021).

5.4.1.2 Copollutants

A small number of studies assessed in the 2016 Oxides of Nitrogen ISA examined confounding by copollutants. The studies that did present results from multipollutant models did not typically evaluate traffic-related copollutants (i.e., BC/EC, UFP, OC, VOCs). A few recent studies add to the limited evidence that informs the independent association of oxides of nitrogen exposure with cardiac arrhythmia. These results, although limited, suggest the association between NO_2 exposure and arrhythmia remains robust to copollutant adjustment.

- In a hierarchical time-series analysis of five U.S. cities fitted single pollutant models that included multiple outcomes simultaneously (Krall et al., 2018). To evaluate multipollutant

exposures, the study used hierarchical principal components analysis to identify related groups of pollutants. A positive association between the principal component that included NO_2 and dysrhythmia ED visits was positive, and consistent with the association between NO_2 and dysrhythmia in the single pollutant model (Krall et al., 2018).

- Similar findings of a robust association between NO_2 and risk of arrhythmia and cardiac arrest with adjustment for a second pollutant (CO, O_3, PM_{10}, $PM_{2.5}$, SO_2) were also observed in a U.K. study (Milojevic et al., 2014).
- Similar to findings from single pollutant analysis, the association between oxides of nitrogen concentrations and OHCA remained inconsistent following copollutant adjustment. A nationwide case-crossover study by Zhao et al. (2020) reported a negative association between exposure to single day lag periods (0, 1, 2, 3) and distributed lags (0 to 1, 0 to 3) of NO_2 concentrations and OHCAs following adjustment for $PM_{2.5}$ (e.g., OR (Lag 0 to 3): 0.86 [95% CI: 0.82, 0.91]). The inverse association between 2-hour moving average NO_2 concentrations and OHCA in a case-crossover study of three cities in Sweden remained in multipollutant models that adjusted for O_3 (OR: 0.97 [95% CI: 0.94, 1.00] per 5.3 ppb) (Raza et al., 2019).

5.4.1.3 Exposure Assignment

In the 2016 Oxides of Nitrogen ISA all the studies of arrhythmia and cardiac arrest used central or fixed site monitors, and none of the studies reported information on the extent to which concentrations at central site monitors captured the temporal variation in NO_2 concentrations in ambient air across the study area. Utilization of exposure surrogates such as single monitors and unweighted or population-weighted averages of multiple monitors contributes to uncertainty in the measurement of exposure in epidemiologic studies that may result in biased health effect estimates. Exposure measurement error that does not adequately capture temporal variability in short-term NO_2 concentrations may underestimate the magnitude but not the directionality of the association. Chapter 2 (see Section 2.5.1) provides a thorough discussion of the bias resulting from exposure assessment methods common to the epidemiologic literature and its impact on the inferences that can be drawn about health effects. Except for a single registry study by Kranc et al. (2021), studies of short-term NO_2 and cardiac arrhythmia and cardiac arrest published since the 2016 Oxides of Nitrogen ISA were case-crossover and time series analyses. Most studies continue to assign daily NO_2 concentrations using fixed and central site monitors and none of the recent studies reported on the accuracy or sensitivity of these measurements in capturing temporal variability in NO_2 across the study area. Krall et al. (2018) reported positive associations between cardiac dysrhythmia and exposure to 1-hour maximum NO_2 and NO_X based on population weighted average estimates that used fused monitor concentrations with CMAQ model estimates. Ha et al. (2017), also utilizing a fused approach with CMAQ and monitor concentration to estimate daily NO_X exposure reported a positive association with cardiac arrest. These findings suggest the association with arrhythmia and cardiac arrest was similar across modelled and central site monitor-based exposure estimation approaches. However, direct comparisons are complicated by differences in study populations, lag periods, analysis methods, and the limited data available on alternative exposure approaches.

5.4.1.4 Lag Structure

As discussed in the 2016 Oxides of Nitrogen ISA, the limited studies available reported that cardiac arrhythmia was associated with shorter lags of NO_2 exposure. Most typically, associations with NO_2 were reported within the first few hours or the day of the event which are indicative of more immediate effects. Studies published since the 2016 Oxides of Nitrogen ISA continue to report associations with arrhythmia and cardiac arrest occurring in close proximity to the exposure period, such as single day lags of 0 and 1. With regard to associations with sub-daily exposure, Kranc et al. (2021) observed patients were more likely to be exposed to the 95th percentile of NO_2 5- to 10-hours prior to an OHCA event although the association was included the null value.

The impact of longer lag periods on these outcomes is equivocal. The association between NO_2 exposure over a lag 0 to 4 days with arrythmias and atrial fibrillation was stronger than at lag 0 to 1 days (Milojevic et al., 2014). A case-crossover analysis of NO_X and cardiovascular events at labor and delivery in 12 clinical sites in the United States. Ha et al. (2017) explored multiple lag days the week before delivery, reporting positive associations between a cardiac arrest event and individual lag days of 0 and 1, lag days 5 and 6, and the weekly average. However, a nationwide case-crossover study from Japan of multiple NO_2 lag periods (single day lag exposures [0, 1, 2, 3] and distributed lags [lag 0 to 1, lag 0 to 3]) with OHCA reported negative associations across all lag periods in analysis that adjusted for $PM_{2.5}$ (lag 0-3 OR: 0.931 [95% CI: 0.912, 0.951] per 10-unit change in NO_2) (Zhao et al., 2020). No association was observed for lag 0, lag 1, and lag 0 to 1. Another assessment of the impact of multiple lag days of NO exposure, did not observe associations across all lag periods (0 to 4) (Lee et al., 2019). Similarly, no association was reported between NO_2 exposure at multiple lag periods (lag 0, lag 1, lag 2, lag 3, lag 0 to 3 days) and implantable cardioverter-defibrillators discharge in response to ventricular fibrillation or ventricular tachycardia in a case-crossover study from Canada (Dales et al., 2020). Finally, Raza et al. (2019) did not report associations between second- and third-day exposures to NO_2 and OHCA.

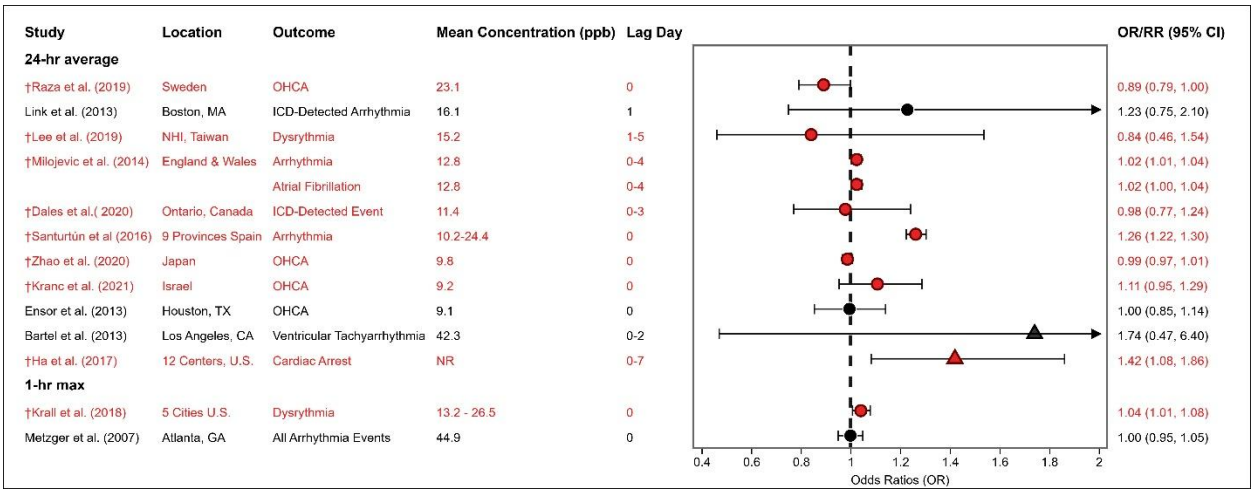
5.4.1.5 Seasonal and Temperature Differences

The 2016 Oxides of Nitrogen ISA reported limited, conflicting data on the influence of season and temperature on arrhythmia and cardiac arrest. A study of OHCA described in the 2016 Oxides of Nitrogen ISA reported generally null associations with NO_2 concentrations in all year and cold season analyses, and a positive association in the warm season analysis (Silverman et al., 2010). A study of arrhythmias by Tsai et al. (2009) reported a positive association in Taipei, Taiwan that was stronger on cool days (OR: 1.34 [95% CI: 1.25, 1.44] in 24-hour average NO_2) than warm days (OR: 1.19 [95% CI: 1.10, 1.28] in 24-hour average NO_2). The evidence from studies published since the 2016 Oxides of Nitrogen ISA remains limited. A time series analysis conducted across multiple regions in Spain by Santurtún et al. (2016) reported findings consistent with Tsai et al. (2009). Seasonal models indicated a stronger incremental effect of NO_2 exposure during cooler periods, with an increase of 35.75% (95% CI:

27.66, 43.84%) and 31.61% (95% CI: 23.52, 39.7%) in cardiac arrhythmia hospital admissions in the winter and autumn respectively, compared to 18.06% (95% CI: 12.04, 24.08%) and 21.45% (95% CI: 15.43, 27.47%) in the spring and summer, respectively. In a seasonally-stratified case-crossover analysis in a Canadian population, a negative, but imprecise association was reported between 4-day mean 24-hour NO_2 exposure (per an interquartile range (IQR) increase of 9.29 ppb) and implantable cardioverter-defibrillators discharge in response to ventricular fibrillation or ventricular tachycardia for both warm (OR: 0.65 [95% CI: 0.33, 1.29]) and cold seasons (OR: 0.82 [95% CI: 0.53, 1.26]) (Dales et al., 2020). However, point estimates were generally positive during the cold season (e.g., OR: [lag 0] 1.16 [95% CI: 0.87, 1.54]) and negative (OR [lag 0] 0.64: [95% CI: 0.40, 1.01]) in the warm season across multiple single day lag periods in this study.

5.4.1.6 Conclusions and Remaining Uncertainties

The overall evidence for an association between short-term exposures to ambient oxides of nitrogen and ICDs, ED visits for cardiac dysrhythmia, hospital admissions for arrhythmias and atrial fibrillation, and OHCA, is limited and associations are inconsistent in both direction and precision. Additionally, adjustment for potential copollutant confounding was inconsistently implemented across studies, and the extent to which the reported observations are attributable to other traffic-related pollutants remains unknown. Some studies report associations that remained positive in copollutant models (Zhao et al., 2023; Santurtún et al., 2016; Milojevic et al., 2014), while others report negative associations (Zhao et al., 2020). Few studies have compared associations across lifestages or populations, exposure estimation approaches, lags, or seasons, and no clear patterns are evident from the studies that have made these comparisons. Taken together, the available epidemiologic evidence does not provide consistent support for associations between exposures to oxides of nitrogen and arrhythmia and cardiac arrest (see Figure 5-4).



List of abbreviations in alphabetical order: CA = California, U.S.; CI = confidence interval; GA = Georgia, U.S.; hr = hour; ICD = Implantable Cardioverter Defibrillator; MA = Massachusetts, U.S.; OHCA = Out of Hospital Cardiac Arrest; OR = Odds Ratio; ppb = parts per billion; RR = Relative Risk; TX = Texas, U.S.; U.S. = United States.
Note: Effect estimates for associations with NO_2 are indicated by a circle and effect estimates for associations with NO_X are indicated by a triangle.
†Studies published since the 2016 Oxides of Nitrogen ISA.

Figure 5-4 Associations of short-term exposure to oxides of nitrogen with arrythmia and cardiac arrest.

5.4.2 Animal Toxicological Studies of Arrhythmia and Cardiac Arrest

The 2016 Oxides of Nitrogen ISA did not evaluate any animal toxicological studies that measured the effects of NO_2 exposure on cardiac arrest. A recent study looked at the impact of NO_2 exposure on arrhythmia and cardiac arrest. Study specific details, exposure conditions, and endpoints examined are highlighted in the text below and in Appendix A – Appendix Table 5-3.

- (Farraj et al., 2016) reported that exposure of spontaneously hypertensive rats to 500 ppb NO_2 for three hours in the morning followed by filtered air for three hours in the afternoon reduced the threshold of aconitine needed to induce cardiac arrest. This corresponded to decreases in the dose of aconitine needed to induce a ventricular premature beat and ventricular tachycardia. The dose of aconitine needed to induce ventricular fibrillation was reduced but not statistically significant compared to air exposed controls.

This study provides evidence that NO_2 exposure can promote electrical alterations that can lead to cardiac arrest following subsequent stimulation.

5.4.2.1 Conclusions and Remaining Uncertainties

Animal toxicologic evidence of short-term NO_2 exposure impacting cardiac arrest or arrhythmia is very limited in number although one recently identified study does provide evidence of reduced thresholds for induction of arrythmias and cardiac arrest with a specific arrythmia-causing stimuli. The ability of NO_2 to induce these effects in the absence of external stimuli or with other more general stressors is unknown.

5.4.3 Synthesis Across Lines of Evidence

Similar to the 2016 Oxides of Nitrogen ISA, the epidemiology results for associations between short-term NO_2 exposure and hospitalizations or ED visits for arrythmias, atrial fibrillation, or for procedures for implanting internal defibrillators that would result from serious arrythmia conditions

continue to be mixed. Results of epidemiology studies of OHCA are similarly inconsistent. Few studies have controlled for copollutants effects, particularly traffic-related copollutants. However, in studies that did account for copollutants, observed associations were not greatly impacted. The associations between NO_2 exposure lag and arrythmia or cardiac arrest events still supports a stronger association with shorter (same day or sub day NO_2 concentrations) compared to longer averaging times or lag periods. Of note, most of the epidemiologic studies of arrythmia and cardiac arrest utilize central monitoring data and the ability of these data to capture spatial and temporal differences within study areas is unknown. The association with arrhythmia was similar across modelled and central site monitor-based exposure estimation approaches. The animal toxicologic evidence is extremely limited, but a recently evaluated study suggests that NO_2 exposure can make animals more sensitive to a future arrythmia-inducing stimulus.

5.5 Cerebrovascular Disease and Stroke

Cerebrovascular disease (CBVD) encompasses a range of conditions affecting blood vessels in the brain and cerebral circulation. Stroke, a type of CBVD, comes in two forms: ischemic and hemorrhagic. Ischemic stroke, also called cerebral infarction, occurs when a blood vessel becomes occluded, cutting off oxygen flow to the brain. Hemorrhagic stroke results from an arterial rupture and can be classified by the affected brain region (e.g., intracerebral or subarachnoid). While ischemic strokes are more common, hemorrhagic strokes account for a disproportionate number of fatalities. In epidemiologic studies, the effect of short-term oxides of nitrogen exposure on CBVD and stroke is typically assessed using ICD codes for ED visits and hospital admissions.

5.5.1 Epidemiologic Studies of Cerebrovascular Disease and Stroke

The 2016 Oxides of Nitrogen ISA concluded that data from epidemiologic studies, which used clinical registries and administrative databases, provided inconsistent evidence for a potential association between ambient NO_2 concentrations and risk of cerebrovascular disease and stroke overall. U.S. and Canadian studies, however, generally reported positive associations (Chen et al., 2014; Villeneuve et al., 2012; Wellenius et al., 2012; Peel et al., 2007; Villeneuve et al., 2006; Wellenius et al., 2005; Linn et al., 2000). Areas of uncertainty included the utilization of central site monitors to estimate NO_2 exposures and potential confounding due to copollutants. Adjustment for potential copollutant confounding was not consistently performed across studies, and for the limited number of studies with copollutant adjustment, NO_2 associated effects were inconsistently observed. Recent studies from North America (Shin et al., 2022; Sun et al., 2019; Krall et al., 2018; Wilker et al., 2018; Ha et al., 2017), Europe (Milojevic et al., 2014), Asia (Li et al., 2023; Liu et al., 2023; Hasegawa et al., 2022; Ho et al., 2022b; Ho et al., 2022a; Kim et al., 2022; Tian et al., 2018), and Australia (Groves et al., 2020), many of which analyzed large numbers of participants or events in multiple cities, found inconsistent evidence for an association

between oxides of nitrogen and risk of cerebrovascular disease including stroke. These studies, primarily time series and case-crossover in design, typically utilized central site monitors or modelling to estimate exposure.

Evidence from recent studies is summarized in the bullets below, followed by discussion of the concentration-response relationships in these studies (see Section 5.5.1.1), the lifestages and populations examined (see Section 5.5.1.2), copollutant confounding (see Section 5.5.1.3), exposure assignment (see Section 5.6.1.4), the lag structure of association (see Section 5.5.1.5), and an examination of potential seasonal and temperature differences (see Section 5.5.1.6). Section 5.5.1.7 discusses conclusions on the epidemiologic evidence and summarizes remaining uncertainties. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 5-4.

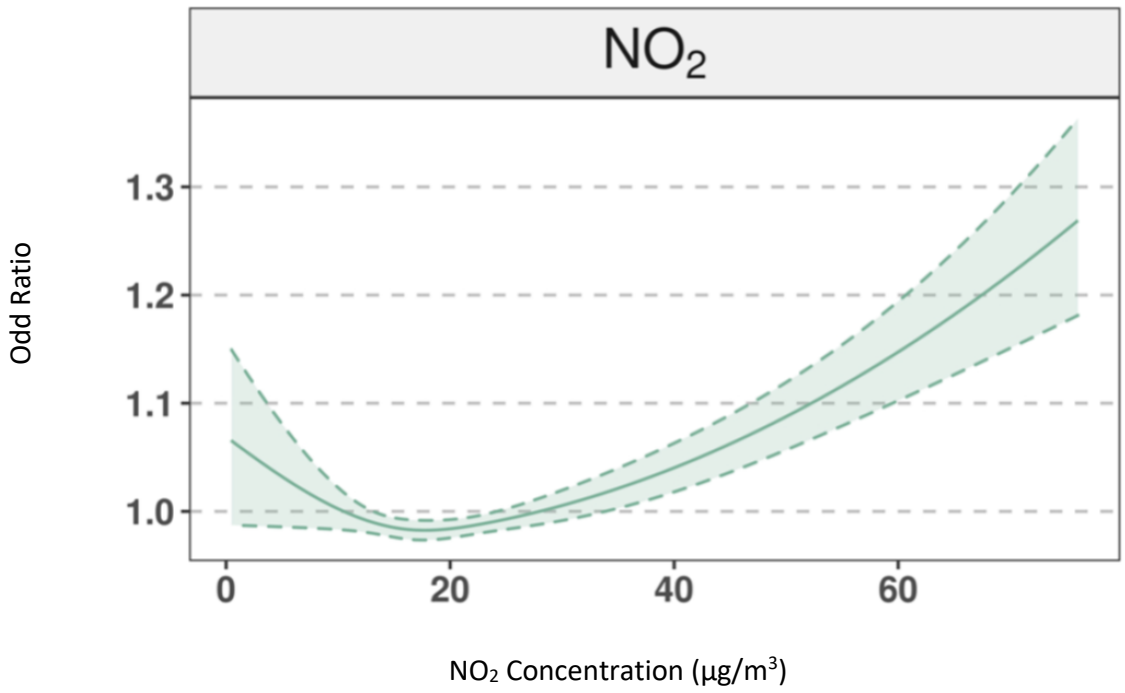
- A time-series analysis of five U.S. cities reported an increase in NO_2 (RR: 1.06 [95% posterior interval (PI): 1.02, 1.09]) and NO_X (RR: 1.04 [95% PI: 1.01, 1.06]) concentrations at lag 0 was associated with a higher risk of ED visits for stroke (Krall et al., 2018). Exposure estimates were based on population weighted average estimates that used fused monitor concentrations with CMAQ model estimates. Two time-series analyses, one of 172 cities in China (Tian et al., 2018) and another of 97 cities in Japan (Hasegawa et al., 2022), both reported an increase in ischemic stroke related hospital admissions at lag 0 NO_2 exposure (6.91% [95% CI: 5.47, 8.35] and 3.78% [95% CI: 2.1, 5.46], respectively). A time-series analysis in Australia reported a positive, but imprecise association between lag 0- to 2-day NO_2 concentrations and risk of stroke-related ICU admissions (RR: 1.38 [95% CI: 0.83, 2.31]) (Groves et al., 2020). These results were robust to adjustment for seasonality.
- Case-crossover studies from England and Wales (Milojevic et al., 2014), the United States (Sun et al., 2019), and China (Li et al., 2023; Liu et al., 2023) reported positive associations between increasing NO_2 concentrations at varying exposure periods (lag 0 to 2, 0 to 4, and 0- to 1-day) and the risk of stroke (all or cause-specific). The U.S. case-crossover study by Sun et al. (2019), which modeled daily NO_2 and NO_X concentrations at each participant address based on a weighted average of measurements from nearby monitors, examined the associations of 1- to 3-day moving average NO_2 exposure with stroke, ischemic stroke and hemorrhagic stroke. The associations with stroke and ischemic stroke were imprecise and overlapped substantially with the null value (i.e., OR (3-day moving average for stroke): 1.08 [95% CI: 0.78, 1.49] and OR (3-day moving average for ischemic stroke): 0.93 [95% CI: 0.64, 1.35]). Positive associations between 3-day moving average of NO_2 (OR: 1.24 [95% CI: 1.01, 1.52] per 10.2 ppb NO_2) and NO_X (OR: 1.18 [95% CI: 1.03, 1.34] per 21.8 ppb NO_X) and risk of hemorrhagic stroke was reported (Sun et al., 2019). A case-crossover study of the England and Wales MINAP database reported an association of NO_2 exposure estimated using an unconstrained distributed lag model (0 to 4 days) was not associated (0.17% [95% CI: -1.55, 1.88]) with an increase emergency hospital admission for stroke (Milojevic et al., 2014). A case-crossover study from China, which utilized bilinear interpolation to estimate NO_2 concentration at a participant's residential location observed that the 2-day average NO_2 was associated with a higher risk of stroke hospital admissions (OR: 1.14 [95% CI: 1.09, 1.19]) (Li et al., 2023). In contrast to these findings, a case-crossover study from Singapore that analyzed lag periods up to five days did not report an association between higher lag 0 NO_2 concentrations and increased risk of acute ischemic stroke (incidence rate ratio (IRR): 0.99 [95% CI: 0.96, 1.02] highest vs. lowest NO_2 quartile) (Ho et al., 2022b).

- Recent studies observed inverse associations between NO_2 and NO_X exposures and the risk of stroke. Kim et al. (2022) reported an inverse risk in stroke hospitalization was observed in association with lag 7-day (OR: 0.87 [95% CI: 0.84, 0.9]) and 30-day (OR: 0.85 [95% CI: 0.81, 0.88]) NO_2 concentrations in a retrospective cohort study from South Korea. A case-crossover analysis of air pollution and cardiovascular events at labor and delivery in 12 clinical sites in the United States. Ha et al. (2017) reported average NO_X concentration (based on CMAQ model estimates fused with monitoring data) prior to labor/delivery were inversely associated with the odds of stroke (7-day average NO_X OR: 0.64 [95% CI: 0.32, 1.29]). A decrease in risk of hemorrhagic stroke was observed for up to three days following NO_2 exposure in a case-crossover study conducted in Singapore (Ho et al., 2022a). A U.S. case-crossover analysis of a single hospital in the United States reported a negative, but imprecise association between NO_2 and spontaneous intracerebral hemorrhage (Wilker et al., 2018). The inverse associations observed in these studies may have been influenced to highly correlated co-occurring pollutants (e.g., O_3 and NO_X) or low concentrations.

5.5.1.1 Concentration-Response

The shape of the concentration-response (C-R) relationship for health effects related to short-term NO_2 exposure and ED or hospital admissions for cerebrovascular disease and stroke was not examined in the epidemiologic studies assessed in the 2016 Oxides of Nitrogen ISA. The limited number of recent studies that examine the C-R relationship are summarized below.

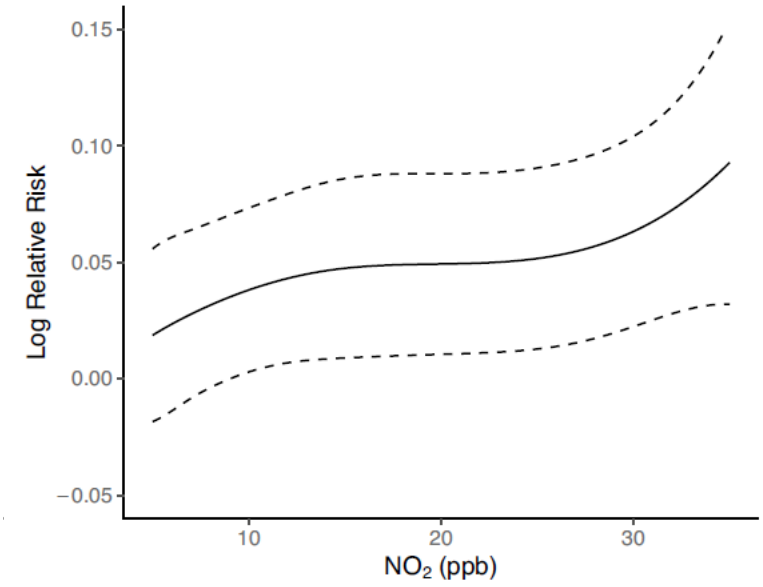
- A multi-city case-crossover study conducted in China used a natural cubic spline function with 3 degrees of freedom to plot the C-R curve for the association (OR) between 2-day moving average (lag 0 to 1) NO_2 exposure and hospital admissions for stroke (see Figure 5-5) (Li et al., 2023). The pollutant was trimmed at the 0.1^{th} percentile to prevent excessively large CIs due to the small number of observations at very low concentrations. The curve for this relationship decreased and flattened between 0 and 20 $\mu g/m^3$ (approximately 10.6 ppb) (Li et al., 2023). At higher concentrations the slope increased markedly and appeared nearly linear as NO_2 concentrations approached approximately 30 $\mu g/m^3$ (15.9 ppb) and continuing to 60 $\mu g/m^3$ with widening confidence intervals. The IQR for NO_2 in this study was 14.4 $\mu g/m^3$ to 25.6 $\mu g/m^3$ (approximately 7.7 to 13.6 ppb). Although the full range is not reported, the data appears to be sparse below the 25th percentile based on the width of the confidence interval.



List of abbreviations in alphabetical order: NO_2 = nitrogen dioxide; $\mu g/m^3$ = micrograms per cubic meter.
Note: The solid lines with shaded bands represent the OR of hospital admissions and their 95% confidence interval.
Source: Li et al. (2023). Copyright permission pending.

Figure 5-5 Concentration-response curve for the association (i.e., odds ratio) between nitrogen dioxide concentrations and hospital admissions for stroke.

- A multi-city time series study conducted in Japan used a cubic spline function with knots at 7.0 and 14.0 ppb for NO_2 to plot the C-R curve for the association between lag 0 NO_2 exposure and hospital admissions for ischemic stroke (see Figure 5-6) (Hasegawa et al., 2022). To mitigate the impact of extreme values, the analysis was restricted to days with concentrations above the 0.5th and below the 99.5th quantile. The curve for this relationship increased up to approximately 15 ppb, flattened until 25 ppb, where the slope increased and appeared linear as NO_2 concentrations exceeded 30 ppb. The IQR for annual mean NO_2 concentrations in this study was 7.26 to 13.08 ppb.



List of abbreviations in alphabetical order: NO_2 = nitrogen dioxide; ppb = parts per billion.
Note: The x-axis shows the concentrations on the same day of admission for ischemic stroke. The y-axis is the log relative risk. The solid lines represent the mean estimate, and the dotted lines show the 95% confidence interval.
Source: Hasegawa et al. (2022). Copyright permission pending.

Figure 5-6 Concentration-response relationship for nitrogen dioxide associated with hospital admissions for ischemic stroke in 97 cities in Japan (2016 to 2019).

5.5.1.2 Lifestages and Populations

This section focuses on the evaluation and characterization of evidence determining whether there are populations or lifestages potentially at increased risk of a short-term NO_2 exposure-related health effect, with specific emphasis placed on studies that compare responses to a reference population. The 2016 Oxides of Nitrogen ISA did not report evidence to indicate differences in the association with cerebrovascular disease and stroke by populations or lifestages. Recent studies expand the evidence that potentially informs whether there are populations or lifestages at increased risk of NO_2 related cerebrovascular effects. Results from recent studies are presented in greater detail below. Overall, the findings did not indicate consistent differences in the risk of cerebrovascular disease or stroke outcomes in age- or sex-stratified analysis or for those with pre-existing conditions.

Lifestage

- In an age-stratified analysis, the study of 172 cities in China reported larger percentage increase in ischemic stroke admissions associated with NO_2 exposures for older ages compared to younger ages at lag 0 (4.93% [18 to 64 years]; 6.21% [65 to 74 years] and 7.64% [≥75 years]) (Tian et al., 2018).

- Lag 0- to 4-day NO_2 exposure was associated with higher emergency hospital admissions for stroke among those ≥70 years old (1.51% change [95% CI: -0.5, 3.51] per 23.9 ppb) compared to those <70 years old (-2.59% change [95% CI: -5.39, 0.21]) (Milojevic et al., 2014).
- The lag 0 to 1 NO_2-associated risk of ischemic stroke was similar in those <65 and ≥65 years old in a case-crossover study of stroke hospital admissions in China (Liu et al., 2023). The increase in ischemic stroke admissions was also similar among participants ≥75, 65 to 74, and 20 to 64 years old in a multi-city time-series analysis in Japan at lag 0 NO_2 (Hasegawa et al., 2022).
- Case-crossover studies from Singapore (Ho et al., 2022b; Ho et al., 2022a), China (Li et al., 2023), and the United States (Sun et al., 2019) also observed no difference in NO_2-associated risk of stroke hospital admissions in older adults (≥65 years) compared to those <65 years of age.

Sex

- In a sex-stratified analysis, although confidence intervals were overlapping, the study of 172 cities in China reported a slightly larger percentage increase in ischemic stroke admissions associated with lag 0 NO_2 exposures for males compared to females at lag 0 (7.04% vs. 5.80%) (Tian et al., 2018). Similar findings of a slightly larger percentage increase in ischemic stroke admissions associated with lag 0 NO_2 exposures for males compared to females were also observed in a multi-city time-series analysis in Japan (Hasegawa et al., 2022).
- (Liu et al., 2023) reported that the increased risk of hospitalization (lag 0- to 1-day) for stroke was similar for males and females. In contrast, a multi-city Canadian time series analysis by Shin et al. (2022) did not observe an association between lag 0 NO_2 and hospitalization due to cerebrovascular disease in either males or females in all-year sex-stratified analysis. In an analysis stratified by season, a higher risk was observed among males (see Section 5.5.1.6).
- Lag 0- to 4-day NO_2 exposure was associated with an increase in emergency hospital admissions for stroke among males (0.75% change [95% CI: -0.96, 2.47]) and a decrease among females (-0.33% change [95% CI: -2.59, 1.92]) (Milojevic et al., 2014). However, the estimates for both males and females were imprecise.

Pre-existing conditions

Recent studies examined the extent to which atrial fibrillation or hypertension modified the association between short-term exposure to oxides of nitrogen and stroke.

- A study from Singapore reported a lower risk of hospital admissions for hemorrhagic stroke associated with the 2nd (IRR: 0.88 [95% CI: 0.66, 0.99] and 3rd tertile (IRR: 0.82 [95% CI: 0.67, 1.02] of NO_2 concentrations compared to the first tertile among those with atrial fibrillation compared to those without atrial fibrillation (2nd tertile IRR: 0.97 [95% CI: 0.93, 1.02] and 3rd tertile IRR: 0.98 [95% CI: 0.93, 1.03] (Ho et al., 2022a).
- In an analysis of the Women's Health Initiative study, (Sun et al., 2019) observed similar risk of total stroke associated with an IQR increase in 3-day moving average NO_2 or NO_X by history of atrial fibrillation or hypertension.
- Sun et al. (2019) reported positive but imprecise associations between the risk of hemorrhagic stroke and an IQR increase in 3-day moving averages for NO_2 (IQR 10.2 ppb) and NO_X (IQR

21.8 ppb) among non-obese participants. In contrast, an inverse, but imprecise association with NO_2 and NO_X was observed among obese participants (BMI ≥30).

- A higher percentage increase in ischemic stroke admissions associated with lag 0 NO_2 exposures was reported for patients taking antihypertensive medications and for patients not taking anti-platelet drugs in a multi-city time-series analysis in Japan (Hasegawa et al., 2022).

5.5.1.3 Copollutants

Results from studies of short-term exposure to oxides of nitrogen and cerebrovascular disease assessed in the 2016 Oxides of Nitrogen ISA were inconsistent in both magnitude and direction. A subset of the studies evaluated copollutant confounding in multipollutant models that included PM_{10}, SO_2, or ozone with mixed results. The relatively few studies that controlled for other pollutants (e.g., $PM_{2.5}$, UFP, CO) also yielded mixed results. Recent studies published since the 2016 Oxides of Nitrogen ISA add to the evidence that informs the independent association of oxides of nitrogen with cerebrovascular disease. The results from these studies, which are detailed below, continue to yield inconsistencies between oxides of nitrogen and stroke following copollutant adjustment.

- A multi-city case-crossover study from China reported the association between lag 0 to 1 NO_2 concentration and risk of total stroke remained positive following adjustment for 24-hour average PM_{10}, $PM_{2.5}$, SO_2, CO, and maximum 8-hour average O_3 (Liu et al., 2023). Similar findings following copollutant adjustment were also observed at a longer lag period. Milojevic et al. (2014) reported NO_2 concentrations at lags 0 to 4 days were associated with a higher risk of emergency hospital admissions stroke in a U.K. study that were robust to adjustment for the copollutants examined, i.e., CO, O_3, PM_{10}, $PM_{2.5}$ and SO_2.
- Recent studies have observed the association between NO_2 exposure and ischemic stroke remains robust following copollutant adjustment. A time-series of 97 cities in Japan reported an increase in ischemic stroke admissions in association with lag 0 NO_2 (Hasegawa et al., 2022). This positive association strengthened following simultaneous adjustment for SO_2, photochemical oxidants (surrogate measure for O_3), and $PM_{2.5}$. A nationwide case-crossover study in China reported the association between lag 0- to 1-day NO_2 exposure and ischemic stroke remained positive but was attenuated in a multipollutant model adjusted for $PM_{2.5}$, O_3, SO_2, and CO (Liu et al., 2023). The associations between NO_2 and ischemic stroke were also robust to adjustment for PM_{10}, $PM_{2.5}$, SO_2, CO, and O_3 at lag 0 to 1 (Li et al., 2023) and $PM_{2.5}$, SO_2, and CO at lag 0 (Tian et al., 2018), respectively, in two multi-city studies from China.
- In contrast to these findings, a case-crossover study of ischemic stroke from Singapore reported null associations between same-day NO_2 exposure and ischemic stroke in multipollutant models that adjusted for $PM_{2.5}$, PM_{10}, O_3, SO_2, and CO (Ho et al., 2022b). A retrospective cohort study from South Korea continued to observe a negative (inverse) association between 7-, 30-, 90-, and 365-day average NO_2 concentrations and stroke following adjustment for SO_2, O_3, CO, and PM_{10} (Kim et al., 2022).

5.5.1.4 Exposure Assignment

In the 2016 Oxides of Nitrogen ISA most studies of cerebrovascular disease and stroke used central or fixed-site monitors, and none of the studies reported information on the extent to which concentrations at central site monitors captured the temporal variation in NO_2 across the study area. Utilization of exposure surrogates such as single monitors and unweighted or population-weighted averages of multiple monitors contributes to uncertainty in the measurement of exposure in epidemiologic studies that may result in biased health effect estimates. Exposure measurement error that does not adequately capture temporal variability in short-term NO_2 concentrations may underestimate the magnitude, but not the directionality, of the association. Additionally, for studies that assessed the association between oxides of nitrogen and stroke there is the potential for misclassification of the exposure due to differences in the timing of stroke symptoms and the corresponding ED visit or hospital admission. Chapter 2 (see Section 2.5.1) provides a thorough discussion of the bias resulting from exposure assessment methods common to the epidemiologic literature and its impact on the inferences that can be drawn about health effects. Most studies that examined the association between short-term NO_2 exposure and cerebrovascular disease and stroke published since the 2016 Oxides of Nitrogen ISA continue to assign daily NO_2 concentrations using fixed and central site monitors without reporting on the accuracy or sensitivity of these measurements in capturing temporal variability in NO_2 across the study area. (Krall et al., 2018) reported positive associations between ED visits for stroke and exposure to 1-hour maximum NO_2 and NO_X based on population weighted average estimates that used fused monitor concentrations with CMAQ model estimates. Ha et al. (2017) also utilizing a fused approach with CMAQ and monitor concentration to estimate daily NO_X exposure reported a positive association with stroke. Recent studies modeled exposures on the basis of residence (Li et al., 2023; Sun et al., 2019). The approach by Li et al. (2023) utilized bilinear interpolation to estimate NO_2 concentration at a participant's residential location. Sun et al. (2019) modeled daily NO_2 and NO_X concentrations at each participant address based on a weighted average of measurements from nearby monitors. Prior assessment of model performance in (Sun et al., 2019) using cross-validation parameters indicated a low propensity for error. Both Li et al. (2023) and Sun et al. (2019) reported positive associations between short-term NO_2 exposure and stroke. These findings suggest the association with cerebrovascular disease and stroke was similar across modelled and central site monitor-based exposure estimation approaches. However, direct comparisons are complicated by differences in study populations, lag periods, analysis methods, and the limited data available on alternative exposure approaches.

5.5.1.5 Lag Structure

As discussed in the 2016 Oxides of Nitrogen ISA, the available evidence, although inconsistent, reported that stroke was associated with shorter lags of NO_2 exposure. Most typically, associations with NO_2 exposure were reported within a few days (lag days 0 to 3) of the event which is indicative of more immediate effects. Studies published since the 2016 Oxides of Nitrogen ISA continue to report

associations between exposure to NO_2 and cerebrovascular disease and stroke occurring in close proximity to the exposure period, typically as single day lag 0 with inconsistent findings at one to two days prior to the event. The impact of longer lag periods was mixed in studies published since the prior ISA.

- A multi-city study from China assessed the exposure to NO_2 from single-day lags from lag 0 to lag 7 and the moving average of multi-day lags of lag 0 to 1 to lag 0 to 7 (Li et al., 2023). For total and ischemic stroke the odds of stroke decreased with increasing lag days, but the odds of hemorrhagic stroke did not follow a consistent pattern.
- A U.S. case-crossover study of participants from the Women's Health Initiative reported a positive association with a similar magnitude (imprecise and inverse) of association between 1-, 2- and 3-day moving average exposures of NO_2 and ischemic stroke (Sun et al., 2019). By contrast, the positive association with hemorrhagic stroke strengthened with increasing averaging time (OR for 1-day moving average: 1.47 [95% CI: 0.84, 2.57], OR for 2-day moving average: 1.84 [95% CI: 0.94, 3.60] and OR for 3-day moving average: 2.21 [95% CI: 1.04, 4.70]).
- The association between NO_2 exposure over a lag of 0 to 4 days with stroke-related hospital admissions in a U.K.-based study was similar to lag of 0 to 1 days (Milojevic et al., 2014).
- A decrease in risk of hemorrhagic stroke was observed for single day lags 0 to 5 NO_2 exposure in a study conducted in Singapore (Ho et al., 2022a). Ho et al. (2022b) reported null findings for ischemic stroke in another study conducted in Singapore (OR for lag 0: 0.99 [95% CI: 0.96, 1.02]).
- An inverse risk in stroke was also observed in association with seven days (OR: 0.40 [95% CI: 0.31, 0.52] per 0.1 ppm NO_2), 30 days (OR: 0.41 [95% CI: 0.33, 0.51] per 0.1 ppm NO_2), 90 days, and 365 days of exposure to NO_2 in a retrospective cohort study from South Korea (Kim et al., 2022).
- A case-crossover analysis of NO_X and cardiovascular events at labor and delivery in 12 clinical sites in the United States explored multiple lag days the week before delivery, reporting inverse associations between a stroke event and exposure to NO_X at individual lag days 0 (OR: 0.83 [95% CI: 0.41, 1.67]), lag 2 (OR: 0.26 [95% CI 0.09, 0.80]), lag 3 (OR: 0.57 [95% CI: 0.27, 1.21]), lag 4 (OR: 0.92 [95% CI: 0.51, 1.66]), lag 5 (OR: 0.70 [95% CI: 0.36, 1.36]), and the weekly average (OR: 0.64 [95% CI: 0.32, 1.29]) (Ha et al., 2017).
- A single city U.S.-based study did not observe a clear association between NO_2 exposures estimated at moving averages of one day to seven days and spontaneous intracerebral hemorrhage (ICH) (Wilker et al., 2018).

5.5.1.6 Season and Temperature Differences

The 2016 Oxides of Nitrogen ISA reported limited and conflicting data on the influence of season and temperature on the association between NO_2 exposure and cerebrovascular disease and stroke. A stronger association between total and ischemic stroke and NO_2 was observed during the cold season in a study from China (Xiang et al., 2013), but another study from Canada found a stronger association for ischemic stroke during the warm season (Chen et al., 2014). Recent evidence for a seasonal and

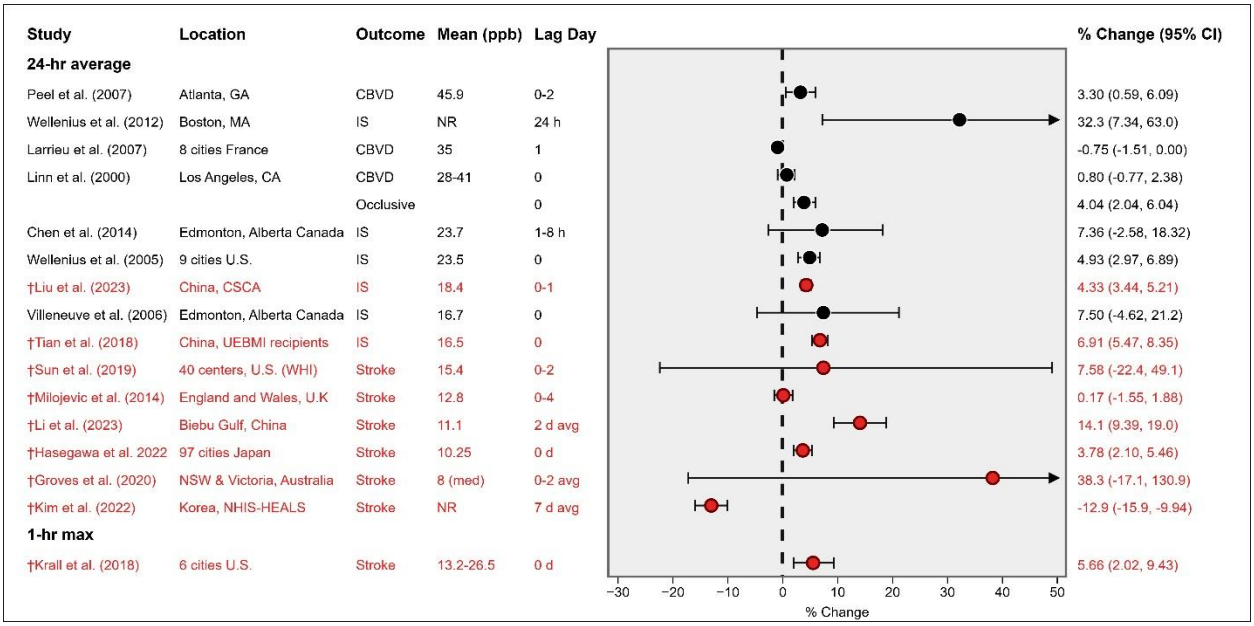
temperature influence on the oxides of nitrogen-cerebrovascular disease association are similarly inconsistent.

- A multi-city Canadian time-series analysis reported males (6.2% change [95% CI: 1.8, 10.6]) and females (3.0% change [95% CI: -3.0, 9.1]) were at higher risk for lag 0 NO_2-associated hospitalization due to cerebrovascular diseases during the warm season (April to September) compared to the cold season (October to March; null associations) (Shin et al., 2022).
- In a nationwide time-series analysis from China, Tian et al. (2018) reported the association between NO_2 and hospital admissions for ischemic stroke were slightly stronger in the warm season (9.45% change, [95% CI: 6.04, 12.85]) compared to the cool season (8.54% change, [95% CI: 6.12, 10.97]). The study also found warmer temperatures (>50th [16.4°C]) may enhance the effects of NO_2 on ischemic stroke hospital admissions in both high-polluted (concentrations >50th) (7.79% change, [95% CI: 5.80, 9.78]) and low-polluted (15.8% change, [95% CI: 9.33, 22.3]) areas compared to high-polluted (5.19% change, [95% CI: 3.42, 6.96]) and low-polluted areas (-3.46% change, [95% CI: 0.04, -6.96]) with cooler temperatures.
- In analyses that stratified by season, a case-crossover study from China reported an overall positive association (OR: 1.14 [95% CI: 1.09, 1.19]) with stroke hospitalization, and the positive association with stroke hospitalization remained in the cool season for lag 0 to 1 NO_2 (OR: 1.21 [95% CI: 1.14, 1.28]) with no association observed in the warm season (OR: 0.92 [95% CI: 0.82, 1.04]) (Li et al., 2023).
- Recent studies in Australia (Groves et al., 2020), China (Liu et al., 2023), and Japan (Hasegawa et al., 2022) did not observe a modifying effect of season on NO_2-associated stroke admission.

5.5.1.7 Conclusions and Remaining Uncertainties

The overall epidemiologic data available is inconsistent for an association between short-term exposure to oxides of nitrogen and cerebrovascular disease including stroke (see Figure 5-7). The few studies examining the shape of the C-R relationship observed the curves decreased and flattened between 0 and 20 μg/m^3 (approximately 10.6 ppb) (Li et al., 2023) or increased up to approximately 15 ppb and then flattened up 25 ppb (Hasegawa et al., 2022) with the slope increasing and appearing linear as levels approached 16 ppb (Li et al., 2023) to 30 ppb (Hasegawa et al., 2022). Adjustment for potential copollutant confounding was inconsistently implemented across studies, and the extent to which the reported observations are attributable to other traffic-related pollutants remains unknown. Some studies report associations that remained positive in copollutant models (Li et al., 2023; Liu et al., 2023; Hasegawa et al., 2022; Tian et al., 2018; Milojevic et al., 2014) while others report null (Ho et al., 2022b) or negative associations (Kim et al., 2022). Additionally, the results from prior and recent studies did not indicate consistent differences in the risk of cerebrovascular disease or stroke in age- or sex-stratified analysis or for those with pre-existing conditions. The evidence continues to indicate cerebrovascular disease or stroke is associated with shorter lags of NO_2 exposure, with associations reported within the first few days of the event, such as lags of zero, zero to one, or zero to two days, which are indicative of

more immediate effects. The evidence from studies that assessed the influence of seasonality and temperature remains inconsistent; few studies have compared associations across different exposure estimation approaches, or extended lag periods, and no clear patterns are evident from the studies that have made these comparisons. Taken together, the available epidemiologic evidence does not provide consistent support for associations between short-term NO_2 exposures and cerebrovascular disease including stroke.



List of abbreviations in alphabetical order: avg = averge; CA = California, U.S.; CBVD = cerebrovascular disease; CI = confidence interval; CSCA = Chinese Stroke Center Alliance; d = day; GA = Georgia, U.S.; h = hour; IS = ischemic stroke; MA = Massachusetts, U.S.; med = median; NHIS-HEALS = National Health Insurance Service-National Health Screening Cohort; NR = not reported; NSW = New South Whales; ppb = parts per billion; UEBMI = Urban Employee Basic Medical Insurance; U.K. = United Kingdom; U.S. = United States; WHI = Women's Health Initiative.
Note: Effect estimates for associations with NO_2 are indicated by a circle.
†Studies published since the 2016 Oxides of Nitrogen ISA.

Figure 5-7 Percentage increase in emergency department visits and hospital admissions for cerebrovascular disease including stroke in relation to nitrogen dioxide exposure.

5.6 Heart Failure

Heart failure encompasses a range of conditions where the heart's pumping ability is compromised. In congestive heart failure (CHF), blood flow from the heart slows down, leading to an inadequate supply of oxygenated blood to the body. The effect of short-term oxides of nitrogen exposure on individuals with CHF, a chronic condition, is typically assessed through ICD codes recorded when a patient is admitted or discharged from the hospital or ED.

5.6.1 Epidemiologic Studies of Heart Failure

The 2016 Oxides of Nitrogen ISA described limited evidence in support of an association between short-term NO_2 exposure and heart failure. A multi-city study from Canada (Stieb et al., 2009) and a single-city Taiwanese study (Yang, 2008) both reported associations between NO_2 concentrations and hospital admissions or ED visits for heart failure. The study conducted in Taiwan found the risk of hospital admissions for heart failure were only associated with NO_2 concentrations on warmer days, but the results were robust to copollutant adjustment. The limited evidence from studies published since the 2016 Oxides of Nitrogen ISA is consistent with prior studies. These studies were mostly case-crossover in design, conducted in North America (Krall et al., 2018; Ha et al., 2017), Europe (Milojevic et al., 2014), and Asia (Zhao et al., 2023) among large patient populations across multiple locations and typically employed central site monitors or modelling as exposure surrogates.

Evidence from recent studies is summarized in the bullets below, followed by additional discussion of the lifestages and populations evaluated in these studies (see Section 5.6.1.1), copollutant analyses (see Section 5.6.1.2), exposure assignment (see Section 5.6.1.3), the lag structure of association (see Section 5.6.1.4), and an examination of potential seasonal differences (see Section 5.6.1.5). Section 5.6.1.6 discusses conclusions from these studies and summarizes remaining uncertainties. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 5-5.

- A time-series analysis of five U.S. cities reported an increased risk of ED visits for CHF associated with same day increase in 1-hour max NO_2 concentration (RR: 1.05 [95% CI: 1.01, 1.08]) and in 1-hour max NO_X concentration (RR: 1.04 [95% CI: 1.02, 1.06]), both estimated using used fused monitor concentrations with CMAQ model estimates (Krall et al., 2018).
- A case-crossover study in England and Wales reported an increase in 24-hour average NO_2 concentrations at lags 0 to 4 days was associated with a 3.68% increase (95% CI: 1.67, 5.69) in risk of emergency heart failure hospital admissions (Milojevic et al., 2014).
- A case-crossover analysis of air pollution and cardiovascular events at labor and delivery in 12 clinical sites in the United States. Ha et al. (2017) observed average NO_X concentration (based on CMAQ model estimates fused with monitoring data) during the week prior to labor/delivery (lag 0 to 7) was associated with a higher odds of heart failure (OR: 2.46 [95% CI: 1.15, 5.28]). Positive associations were also observed for NO_X exposures occurring at single day lag 0 (OR: 1.74 (95% CI: 0.95, 3.17]), lag 1 (OR: 1.68 [95% CI: 1.08, 2.6]) and lag 6 (OR: 3.10 [95% CI: 1.26, 7.64]). An inverse, imprecise association was observed at single day lag 7 (OR: 0.68 [95% CI: 0.31, 1.48]).
- A case-crossover study of a single region from Japan reported positive, but imprecise associations for NO and the risk of heart failure for lag 1 (OR: 1.64 [95% CI: 0.76, 3.54]) and lag 0-1 (OR: 1.49 [95% CI: 0. 0.52, 4.28]) (Zhao et al., 2023). Associations that were much closer to the null value were observed for same day (OR: 0.96 [95% CI: 0.44, 2.12]) and single day lag 2 exposures (OR: 1.06 [95% CI: 0.46, 2.45]). Additionally, associations with NO_2 and NO_X concentrations, respectively, with an increased risk of heart failure close to the

null value (OR for lag 0 to 1: 0.92 [95% CI: 0.50, 1.70] and OR for lag 0 to 1: 1.03 [95% CI: 0.541, 1.962]).

5.6.1.1 Lifestages and Populations

This section focuses on the evaluation and characterization of evidence determining whether there are populations or lifestages potentially at increased risk of a NO_2-related heart failure, with specific emphasis placed on studies that compare responses to a reference population. Evidence on populations potentially at increased risk for short-term NO_2 exposure-associated heart failure was not reported in the 2016 Oxides of Nitrogen ISA. Recent evidence to inform whether there are populations or lifestages at increased risk of heart failure remains limited.

Lifestage

- No differences in age-stratified analysis (<70 years compared to ≥70 years) were observed for lag 0- to 4-day NO_2-associated emergency hospital admissions for heart failure (3.76% change [95% CI: 0.96, 6.56] vs. 3.51% change [95% CI: 0.75, 6.27] per 23.9 ppb) in the U.K.-based study (Milojevic et al., 2014).

Sex

- In the U.K.-based study (Milojevic et al., 2014) women exhibited a higher lag 0- to 4-day NO_2-associated risk of emergency hospital admissions for heart failure compared to men (3.35 [95% CI: 1.13, 5.56] increase vs. 4.93% [95% CI: 0.59, 9.28] increase per 23.9 ppb) (p-value for interaction = 0.04).

5.6.1.2 Copollutants

The 2016 Oxides of Nitrogen ISA described a single study that examined confounding by copollutants. The authors reported the association between NO_2 and hospital admission for heart failure occurring on warm days remained robust following copollutant adjustment for PM_{10}, SO_2, CO, and O_3 (Yang, 2008). A few recent studies add to the limited evidence that informs the independent association of oxides of nitrogen with heart failure. These results, although limited, suggest the association between NO_2 and heart failure remains robust to copollutant adjustment.

- In a hierarchical time-series analysis of five U.S. cities fitted single pollutant models that included multiple outcomes simultaneously (Krall et al., 2018). To evaluate multipollutant exposures, the study used hierarchical principal components analysis to identify related groups of pollutants. Consistent with findings from the single pollutant model, a positive association was reported between the principal component that included NO_2 and CHF (Krall et al., 2018).
- Similar findings of a robust association between oxides of nitrogen and risk of heart failure with adjustment for a second pollutant (CO, O_3, PM_{10}, $PM_{2.5}$, SO_2) were also observed in a U.K. study (Milojevic et al., 2014).

5.6.1.3 Exposure Assignment

In the 2016 Oxides of Nitrogen ISA studies of heart failure used central or fixed site monitors, and none of the studies reported information on the extent to which concentrations at central site monitors captured the temporal variation in NO_2 across the study area. Utilization of exposure surrogates such as single monitors and unweighted or population-weighted averages of multiple monitors contributes to uncertainty in the measurement of exposure in epidemiologic studies that may result in biased health effect estimates. Exposure measurement error that does not adequately capture temporal variability in short-term NO_2 concentrations in ambient air may underestimate the magnitude, but not the directionality, of the association. Chapter 2 (see Section 2.5.1) provides a thorough discussion of the bias resulting from exposure assessment methods common to the epidemiologic literature and its impact on the inferences that can be drawn about health effects. All studies that examined the association between short-term NO_2 and heart failure published since the 2016 Oxides of Nitrogen ISA were case-crossover and time-series analyses. Most studies continue to assign daily NO_2 concentrations using fixed and central site monitors and none of the recent studies reported on the accuracy or sensitivity of these measurements in capturing temporal variability in NO_2 across the study area. Krall et al. (2018) reported positive associations between CHF and exposure to 1-hour maximum NO_2 and NO_X based on population weighted average estimates that used fused monitor concentrations with CMAQ model estimates. Ha et al. (2017) also utilizing a fused approach with CMAQ and monitor concentration to estimate daily NO_X exposure reported a positive association with heart failure. These findings suggest the association with heart failure was similar across modelled and central site monitor-based exposure estimation approaches. However, direct comparisons are complicated by differences in study populations, lag periods, analysis methods, and the limited data available on alternative exposure approaches.

5.6.1.4 Lag Structure

As discussed in the 2016 Oxides of Nitrogen ISA, the limited studies available at the time reported that heart failure was associated with shorter lags of NO_2 exposure. Most typically, associations with NO_2 concentrations were reported for the day of event (lag 0) and up to two days (lag 0 to 2) preceding the event, which are indicative of more immediate effects. Studies published since the 2016 Oxides of Nitrogen ISA continue to report associations with heart failure occurring in close proximity to the NO_2 exposure period, such as lag 0 (Krall et al., 2018). However, findings were not consistent over short exposure periods. A case-crossover study of a single region from Japan that assessed multiple lag periods with heart failure reported lag 0, lag 1, lag 2, and lag 0 to 1 NO, NO_2, or NO_X concentrations were not associated with an increased risk of heart failure (Zhao et al., 2023). The impact of longer lag periods was also explored in recent studies. The association between NO_2 exposure over a lag 0 to 4 days with heart failure hospital admissions was similar to lag 0- to 1-day (Milojevic et al., 2014). A case-crossover analysis of NO_X and cardiovascular events at labor and delivery in 12 clinical sites in the United States. (Ha et al., 2017) explored multiple lag days the week before delivery, reporting associations between

heart failure and exposure to NO_X at individual lag days of 1 (OR: 1.68 [95% CI: 1.08, 2.60]) and 6 (OR: 3.10 (95% CI: 1.26, 7.64]), where the strongest effect was observed, as well as the weekly average (OR: 2.46 [95% CI: 1.15, 5.28]).

5.6.1.5 Seasonal and Temperature Differences

The 2016 Oxides of Nitrogen ISA reported that a single study conducted in Taiwan found the risk of hospital admissions for heart failure were only associated with NO_2 concentrations on warmer days, but the results were robust to copollutant adjustment (Yang, 2008). No studies published since the 2016 Oxides of Nitrogen ISA reported on potential seasonal and temperature differences in the association between oxides of nitrogen and heart failure.

5.6.1.6 Conclusions and Remaining Uncertainties

The overall evidence base for evaluating the association between short-term exposure to oxides of nitrogen and heart failure is limited, but results of a couple of multi-city studies published since the 2016 Oxides of Nitrogen ISA are consistent with the older studies in reporting associations between short-term exposures to oxides of nitrogen, including NO_2, and increased risk of ED visits for heart failure. Additionally, adjustment for potential copollutant confounding was inconsistently implemented across studies, and the extent to which the reported observations are attributable to other traffic-related pollutants remains unknown. A single study reported associations that remained positive in copollutant models (Milojevic et al., 2014). A limited number of studies have compared associations across lifestages or populations, exposure estimation approaches, lags, or seasons, and no clear patterns are evident from the studies that have made these comparisons. Taken together, the available epidemiologic evidence provides limited evidence to support an association between exposures to oxides of nitrogen and heart failure.

5.7 Increased Blood Pressure and Hypertension

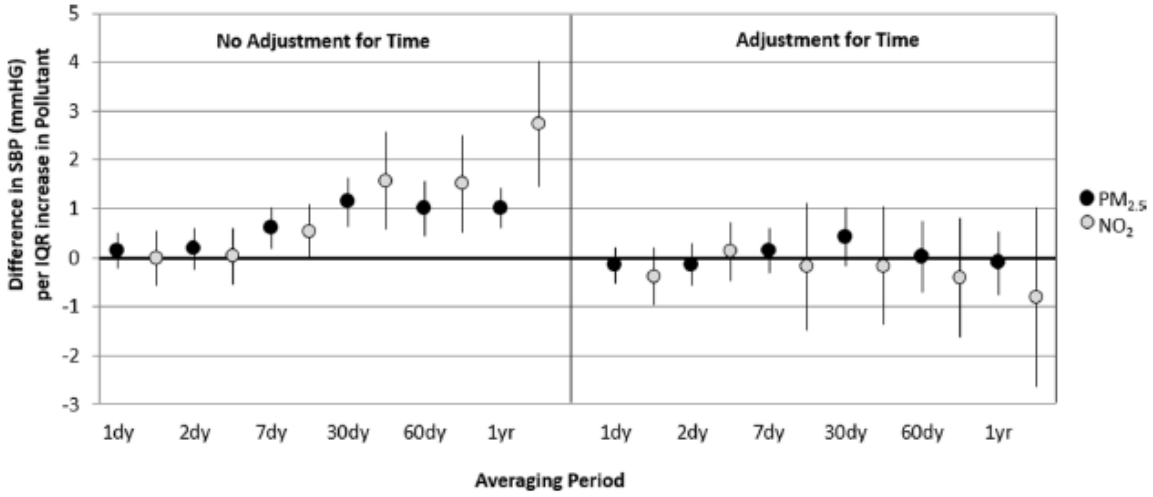
Blood pressure (BP) refers to the pressure exerted by circulating blood on the walls of blood vessels. High blood pressure is typically defined as a systolic blood pressure (SPB) above 130 mm Hg or a diastolic blood pressure (DBP) above 80 mm Hg. SBP reflects arterial pressure during heart contraction, and DBP reflects arterial pressure when the heart is relaxed and is filling with blood. Prolonged high BP, known as hypertension, can cause the ventricular wall to thicken resulting in diminished filling during diastole. Hypertension can ultimately contribute to the development of cardiovascular outcomes such as ischemic heart disease, arrythmia, and heart failure. Epidemiologic, controlled human exposure, and animal toxicologic studies have evaluated the impact of short-term oxides of nitrogen exposure on blood pressure and hypertension.

5.7.1 Epidemiologic Studies of Increased Blood Pressure and Hypertension

The 2016 Oxides of Nitrogen ISA concluded that there was limited evidence to support an association between short-term increases in ambient NO_2 concentrations and increased blood pressure. No association was observed in longitudinal studies blood pressure and inconsistent results were observed in cross-sectional studies. Earlier studies have reported a positive association between NO_2 and ED visits for hypertension, but the findings were mixed in multipollutant models (Szyszkowicz et al., 2012). Recent studies published since the 2016 Oxides of Nitrogen ISA, include analyses of several established U.S. cohorts of adults and children (Wen et al., 2023; Mann et al., 2021; Adar et al., 2018). In additon, studies from Europe (de Prado-Bert et al., 2022; Stieb et al., 2018) and Canada (Biel et al., 2020; Scheers et al., 2018) add to the evidence. These studies were conducted among large patient populations across multiple locations and typically employed central site monitors or modelling as exposure surrogates. Overall, these studies provide limited evidence of an association between short-term increases in NO_2 concentrations and blood pressure and hypertension.

Evidence from recent studies is summarized in the bullets below, followed by discussion of the lifestages and populations evaluated in these studies (see Section 5.7.1.1), copollutant analyses in recent studies (see Section 5.7.1.2), exposure assignment (see Section 5.7.1.3), the lag structure of associations (see Section 5.7.1.4), and an examination of potential seasonal and temperature differences (see Section 5.7.1.5). Section 5.7.1.6 discusses conclusions from this evidence and summarizes remaining uncertainties. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 5-6.

- In an analysis by Adar et al. (2018) of the Multi-Ethnic Study of Atherosclerosis (MESA) cohort, 1- and 2-day average NO_2 exposure estimated using spatiotemporal models based on area, community, and residential monitoring data was not associated with increased SBP. The observed associations strengthened with increasing averaging time in the model with no adjustment for calendar time; however, when calendar time was included in the model to account for a potential period effect, the associations were substantially attenuated (see Figure 5-8). In another national cohort analysis, post-menopausal women enrolled in the Women's Health Initiative (WHI), Wen et al. (2023) reported that an increase in lag 3- to 5-day NO_2 concentrations (estimated using lognormal ordinal kriging models based on monitoring data) was associated with a 0.45 mm Hg decrease (95% CI: -0.63, -0.27) in SBP and a 0.28 mm Hg increase (95% CI: 0.18, 0.38) in DBP after adjustment for calendar time. The estimate for DPB was attenuated in models that adjusted for calendar time, i.e. in models that did not include this adjustment, the increase in DBP was larger in magnitude (0.92 mm Hg [95% CI: 0.83, 1.02]) and the decrease in SPB changed direction becoming positive, and increased in magnitude (1.29 mm Hg [95% CI: 1.11, 1.47]). In addition, the NO_2-DBP association varied by U.S. Census region. The largest increase in DBP was observed in the West (0.73 mm Hg [95% CI: 0.56, 0.9]) while the associations in other regions ranged from -0.09 to 0.19 mm Hg with all estimates including the null value.



List of abbreviations in alphabetical order: BMI = body mass index; CI = confidence interval; dy = day; IQR = interquartile range; NO_2 = nitrogen dioxide; $PM_{2.5}$ = particulate matter with a nominal mean aerodynamic diameter less than or equal to 2.5 µm; SBP = systolic blood pressure; SES = socioeconomic status; yr = year.
Note: Models were adjusted for age at exam, sex, race/ethnicity, education, study site, neighborhood SES and its interaction with study site, season and its interaction with study site, BMI, waist-to-hip ratio, tobacco smoke exposure, physical activity, and calendar time (as noted) as well as random slopes and intercepts for subject.
Source: Adar et al. (2018). Copyright permission pending.

Figure 5-8 Associations (95% CI) of systolic blood pressure with $PM_{2.5}$ and NO_2 concentrations with and without adjustment for calendar time.

- Mann et al. (2021) examined the association of several short-term NO_2 exposure averaging times (i.e., 1-day lag, 1-week, and 1-month) with blood pressure in children residing in Fresno California who were enrolled in the Children's Health and Air Pollution Study CHAPS. The exposure metrics were derived using daily fixed site monitoring data and Inverse distance weighting (IDW) to develop spatial-temporal models such that exposure estimates were assigned to reflect the child's residential history. Short-term NO_2 exposure, e.g., 1-day lag, was associated with imprecise increases in SBP (2.88 mm Hg [95% CI: -5.30, 11.07]) and DBP (1.21 mm Hg [95% CI: -3.84, 6.26]). Exposures of 1-month were associated with larger and more precise increases in blood pressure (5.61 [95% CI: 0.22, 11.20]) and 1.65 [95% CI: -0.43, 3.74] for SBP and DBP, respectively). The study also examined longer term metrics and glucose dysregulation, which are discussed in Chapter 6, Section 6.7.1 and Chapter 8, Section 8.5 and Section 8.7, respectively.
- Biel et al. (2020) conducted a panel study of 46 non-smoking adults in Toronto, Canada with the aim of distinguishing the effects of noise and air pollution on subclinical cardiovascular endpoints including blood pressure. No discernable pattern of association was evident of 24-hour regional NO_2 exposure (estimated from fixed site monitors) with SBP (4.67 [95% CI: -7.59, 16.9]) or DBP (-11.9 [95% CI: -20.1, -3.63]), regardless of adjustment for traffic-related noise (β: 4.30 [95% CI: -6.41, 15.0] and β: -1.19 [95% CI: -9.70, 7.33], respectively). In addition, a panel study of 70 older adults (≥55 years of age) from Canada reported lag 1-day NO_2 concentrations were inversely associated with diastolic (e.g., pre-exercise control: -0.10% change [95% CI: -0.52, 0.31]; pre-exercise intervention: -0.36% change [95% CI: -0.74, 0.03]) and systolic blood pressure (e.g., pre-exercise control: -0.36% change [95% CI: -0.74, 0.03]; pre-exercise intervention: -0.29% change [95% CI: -0.67, 0.09]) (Stieb et al., 2018).

- A panel study of 20 participants from Europe assessed 5-day average concentrations of NO_2 using personal monitors and estimating area exposure using monitoring stations. From observations with personal monitors, a 10 $\mu g/m^3$ (5.3 ppb) increase in 5-day average NO_2 was observed to have negative, associations (confidence intervals included the null value) with systolic (-0.14 mm Hg [95% CI: -1.17, 0.88]) and diastolic blood pressure (-0.28 mm Hg [95% CI: -1.00, 0.43]). From area-concentration exposure estimates, a 10 $\mu g/m^3$ (5.3 ppb) increase in 5-day average NO_2 was also observed to have negative, but imprecise associations) with systolic (-3.69 [95% CI: -8.37, 1.00]) and diastolic blood pressure (-2.48 [95% CI: -5.70, 0.73]) (Scheers et al., 2018).
- In another European study, de Prado-Bert et al. (2022) examined the association of short-term (i.e., assessed 1-day and 1-week before blood pressure was assessed) NO_2 exposure (area exposure estimated using LUR) and blood pressure in children enrolled in the European Human Early-life Exposome project (HELIX) cohort. Daily and week-long NO_2 exposure at home and at school were associated with similar magnitude increases in SBP (e.g., β: 1.21 [95% CI: 0.92, 3.73] week-long home exposure per $\mu g/m^3$) but smaller less precise increases in DBP (e.g., β: 0.351 [95% CI: -0.99, 1.69] week-long home per $\mu g/m^3$). See also Chapter 8, Section 8.5.1 for results pertaining to glucose regulation.

5.7.1.1 Lifestages and Populations

This section focuses on the evaluation and characterization of evidence determining whether there are populations or lifestages potentially at increased risk of a short-term NO_2 exposure-related blood pressure and hypertension with specific emphasis placed on studies that compare responses to a reference population. Evidence on populations potentially at increased risk for short-term NO_2-exposure-associated changes in blood pressure was not reported in the 2016 Oxides of Nitrogen ISA. Recent evidence to inform whether there are populations or lifestages at increased risk of blood pressure and hypertension remains limited.

Lifestage

- Adar et al. (2018) did not observe a modifying influence for the association between blood pressure and NO_2 exposure by age (data NR).

Sex

- No differences in blood pressure associated with short-term exposure to NO_2 by sex were observed in Stieb et al. (2018) (data NR).

Pre-existing conditions

- Wen et al. (2023) observed effect modification by BMI, diabetes, dietary sodium intake, and SEP, and long-term $PM_{2.5}$ respectively, for the association between NO_2 and blood pressure. The directionality was mixed, with a negative NO_2 interaction for SBP and a positive NO_2 interaction for DBP [e.g., high BMI β = 0.52 mm Hg (95% CI: 0.33, 0.70); diabetics β = 0.77 mm Hg (95% CI: 0.35, 1.19); tertile 3 sodium intake β = 0.24 mm Hg (95% CI: 0.02, 0.45)].

- Adar et al. (2018) did not observe a modifying influence for the association between blood pressure and NO_2 exposure by diabetes and obesity (data NR).
- No differences in blood pressure associated with short-term exposure to NO_2 by statin usage were observed in Stieb et al. (2018) (data NR).
- In another Canadian study, Dales and Cakmak (2016) evaluated whether children with mental health conditions are at higher risk of NO_2-associated increases in blood pressure and heart rate compared to children without mental health conditions. Children were assigned NO_2 concentrations on the day of the exam based on the closest monitor to their neighborhood/clinic. There was little evidence (i.e., overlapping CIs for percent changes across groups) to indicate that NO_2-related cardiovascular effects were different depending on reported happiness, emotional symptoms on the Strengths and Difficulties Questionnaire (SDQ) or among those with reported mood disorders.

5.7.1.2 Copollutants

The 2016 short-term Oxides of Nitrogen ISA findings for hypertension were mixed in multipollutant models. Guo et al. (2010) reported the association between NO_2 and ED visits for hypertension remained robust following copollutant adjustment for PM_{10} or SO_2. However, Szyszkowicz et al. (2012) reported the association between NO_2 and ED visits for hypertension were attenuated in models adjusting for PM_{10} or SO_2. Neither of the studies evaluated confounding by traffic-related pollutants. A few recent studies add to the limited evidence that informs the independent association of short-term increased exposure to oxides of nitrogen with blood pressure. These results, although limited, suggest the association between NO_2 and blood pressure remains robust to copollutant adjustment.

- In a U.S. national cohort study, Wen et al. (2023) reported the positive association between lag 3- to 5-day NO_2 and DBP persisted in a multipollutant model following adjustment for $PM_{2.5}$ (β:1.01 mm Hg [95% CI: 0.82, 1.20]). The NO_2-DBP association following adjustment for $PM_{2.5}$ continued to vary by U.S. Census region, with a positive association observed in the South (β:2.41 mm Hg [95% CI: 1.90, 2.91]) and West (β:1.38 mm Hg [95% CI: 0.00, 2.75]).
- In an European study, de Prado-Bert et al. (2022) reported the association between unit increases in daily and week-long NO_2 exposure at home and school with blood pressure, observed increases in SBP (β: 2.32 [95% CI: 0.38, 2.70] with week-long home exposure per $\mu g/m^3$) and in DBP (β: 0.63 [95% CI: -1.22, 2.49] with week-long home per $\mu g/m^3$) persisted in models adjusted for $PM_{2.5}$, with imprecise estimates reported for DBP.

5.7.1.3 Exposure Assignment

In the 2016 Oxides of Nitrogen ISA studies of blood pressure largely used central or fixed site monitors, and none of these studies reported information on the extent to which concentrations at central site monitors captured the temporal variation in NO_2 concentrations in ambient air across the study area. Utilization of exposure surrogates such as single monitors and unweighted or population-weighted averages of multiple monitors contributes to uncertainty in the measurement of exposure in epidemiologic

studies that may result in biased health effect estimates. Exposure measurement error that does not adequately capture temporal variability in short-term NO_2 concentrations may underestimate the magnitude, but not the directionality, of the association. A single study included in the 2016 Oxides of Nitrogen ISA utilized personal exposure measurements, which are less susceptible to exposure error due to the variability in NO_2 concentrations and variation in time-activity patterns than central site monitoring (Williams et al., 2012). The authors did not observe an association between blood pressure and either personal or ambient NO_2 concentrations. Chapter 2 (see Section 2.5.1) provides a thorough discussion of the bias resulting from particular exposure assessment methods common to the epidemiologic literature and its impact on the inferences that can be drawn about health effects**.** Most studies published since the 2016 Oxides of Nitrogen ISA that examined the association between short-term NO_2 exposure and blood pressure assigned daily NO_2 concentrations using fixed and central site monitors and did not report on the accuracy or sensitivity of these measurements in capturing temporal variability in NO_2 across the study area.

Personal exposure measurements were conducted in a single study (Scheers et al., 2018). Scheers et al. (2018) collected NO_2 data from monitoring stations and personal exposure samplers from three locations in Belgium, Italy, and Sweden. These studies found substantial differences in the NO_2 concentrations between the locations, likely due to spatial variability arising from traffic-related sources. However, the results for NO_2 measurements from monitoring stations were consistent with those obtained from personal sampling for the respective location where measurements were collected. Scheers et al. (2018) reported negative, imprecise associations. Scheers et al. (2018) also reported negative, imprecise associations in analyses using an LUR model to estimate NO_2 exposures, suggesting that differential exposure error between personal monitoring and ambient monitoring-based approaches was not a major driver of the direction or lack of precision in this study. A few recent studies used modeled exposure estimates based on spatiotemporal models that included area, community, and residential monitoring data (Adar et al., 2018) or daily fixed site monitoring data and IDW (Mann et al., 2021), applied a LUR model (de Prado-Bert et al., 2022), or estimated daily exposures with improved spatial resolution using log-normal ordinal kriging models that incorporated monitoring data (Wen et al., 2023). Across these studies, the association between short-term NO_2 exposure and blood pressure was mixed, with positive (Wen et al., 2023; de Prado-Bert et al., 2022; Mann et al., 2021), negative (Wen et al., 2023), and no associations (Adar et al., 2018) reported. The precision of the estimates also varied depending on covariate adjustment (Wen et al., 2023; de Prado-Bert et al., 2022; Mann et al., 2021). These findings suggest the association with blood pressure was similar across modelled and central site monitor-based exposure estimation approaches. However, direct comparisons are complicated by differences in study populations, exposure periods, analysis methods, and the limited data available on alternative exposure approaches.

5.7.1.4 Lag Structure

As discussed in the 2016 Oxides of Nitrogen ISA, results of cross-sectional studies (Bai et al., 2018; Chen et al., 2012b; Cakmak et al., 2011) of the association between NO_2 and BP measured on the same day or with the NO_2 measurement lagged one to three days before the BP measurement were mixed. Studies published since the 2016 Oxides of Nitrogen ISA continue to report associations with blood pressure and hypertension occurring in close proximity to the NO_2 exposure period. A few studies found blood pressure was associated with NO_2 concentrations reported for the day of event (Stieb et al., 2018) and the prior day preceding the event, which are indicative of more immediate effects. The impact of longer lag periods on blood pressure observed in recent studies was mixed. Wen et al. (2023) assessed the association between multiple NO_2 lag periods (single day lag exposures [0, 1, 2, 3, 4, 5, 6]) and DBP and SBP. The study found lag days 0 to 5 were negatively associated with SBP, and lag days 0, 3, 4, 5, and 6 were positively associated with DBP. The strongest NO_2 associated effect for DBP was observed at lag 6. Scheers et al. (2018) reported an increase in 5-day average NO_2 concentrations (estimated from both personal and area monitors) was observed to have negative, but imprecise associations (i.e., wide confidence intervals) with SBP and DBP, respectively.

5.7.1.5 Seasonal and Temperature Differences

An older study reviewed in the 2016 Oxides of Nitrogen ISA did not observe differences in the association between NO_2 and SBP by season (Dales et al., 2010). A recent panel study by Stieb et al. (2018) was conducted to assess the cardiorespiratory effects of air pollution on winter outdoor physical activity among adults 55-years of age and older. The study reported lag 1-day NO_2 concentrations were negatively associated with diastolic and systolic blood pressure. No other studies published since the 2016 Oxides of Nitrogen ISA reported seasonally stratified results for NO_2 in association with blood pressure or hypertension.

5.7.1.6 Conclusion and Remaining Uncertainties

The overall evidence for an association between short-term increased exposures to ambient oxides of nitrogen and blood pressure and hypertension remains limited, and associations are inconsistent in direction and precision. Additionally, adjustment for potential copollutant confounding was inconsistently implemented across studies, and the extent to which the reported observations are attributable to other traffic-related pollutants remains unknown. Some studies report associations that remained positive in copollutant models (Wen et al., 2023; de Prado-Bert et al., 2022; Guo et al., 2010) while others report negative associations (Stieb et al., 2018; Szyszkowicz et al., 2012). Few studies have compared associations across lifestages or populations, exposure estimation approaches, lags, or seasons, and no clear patterns are evident from the studies that have made these comparisons. Taken together, the

available epidemiologic evidence does not provide consistent support for associations between exposures to oxides of nitrogen and blood pressure and hypertension.

5.7.2 Controlled Human Exposure Studies of Increased Blood Pressure and Hypertension

The 2016 and 2008 Oxides of Nitrogen ISAs evaluated several controlled human exposure studies that examined the effects of NO_2 exposure on BP and cardiac output. Cardiac output refers to the volume of blood that is pumped out by the heart ventricles per minute. The cardiac output is thus affected by changes to the heart rate (beats/min) and/or the stroke volume (mL of blood/beat). BP is the product of cardiac output and vascular resistance, and these three variables interact to ensure that circulatory demands of the system are met. Controlled human exposures described in the 2008 Oxides of Nitrogen ISA and 2016 Oxides of Nitrogen ISA generally showed null or little impact of NO_2 exposure on cardiac output or BP (U.S. EPA, 2016, 2008). No recent controlled human exposure studies have examined NO_2-induced effects on blood pressure or cardiac output. Therefore, a summary of key findings from the older studies is provided below:

- Exposure of young healthy adult males to 620 ppb NO_2 for two hours did not alter cardiac output or BP (Folinsbee et al., 1978). This experiment utilized three different experimental arms with NO_2 exposure taking place with exercise schemes varying from 15 to 60 minutes. There were no changes to cardiac output or BP in any of the exercise paradigms.
- Exposure of eight older healthy adults to 600 ppb NO_2 for two hours with intermittent exercise did not result in changes in cardiac output or stroke volume (Drechsler-Parks, 1995). This study also looked at co-exposure to O_3 and found that while neither pollutant exposure alone resulted in changes in cardiac output, exposure to 600 ppb NO_2 + 450 ppb O_3 significantly decreases in cardiac output.
- Similar to Folinsbee et al. (1978), Gong et al. (2005) did not see changes in BP after exposure to two hours of 400 ppb NO_2 with exercise in study participants with or without COPD, although there was a small but non-significant increase in heart rate. NO_2 exposure in conjunction with concentrated particulate matter had little effect on BP or heart rate regardless of disease state.
- Exposure of study participants with or without asthma to a higher concentration of NO_2 (4,000 ppb) for 75 minutes with exercise resulted in a small (~5 mm Hg) but statistically significant decrease in mean SBP with no change to heart rate (Linn et al., 1985b).

5.7.2.1 Conclusions and Remaining Uncertainties

The controlled human exposure literature examining blood pressure and cardiac output is limited. Available studies show little or no effect at exposure concentrations between 420 ppb and 620 ppb and only small changes at higher concentrations (4,000 ppb). These studies have failed to find effects in both younger and older subjects, as well as those with asthma and COPD. Together, the controlled human exposure evidence does not support effects on blood pressure or cardiac output following short-term NO_2

exposures. However, all of the existing studies utilize exercise during exposure and, like with respiratory effects, exercise alone causes baseline changes in cardiovascular measures. Studies in resting subjects may show different results. Also, repeated exposure studies could give insight into the cumulative effects of longer-term exposures to NO_2.

5.7.3 Animal Toxicological Studies of Increased Blood Pressure and Hypertension

There were no animal toxicological studies evaluated in the 2016 Oxides of Nitrogen ISA investigating the effect of short-term NO_2 exposure on blood pressure or hypertension. Recent studies looked at the effect of NO_2 exposure on BP. Study specific details, exposure conditions, and endpoints examined are highlighted in the text below and described in more detail in Appendix A – Appendix Table 5-7.

- Exposure of C57BL/6J mice to 2.5 ppm NO_2 5-hour/day for 28-days did not result in changes to blood pressure (Ji et al., 2022).
- Similarly, a study in spontaneously hypertensive rats showed no impact of 3-hour exposure to 500 ppb NO_2 on pulse pressure, SBP or DBP (Farraj et al., 2016). This study exposed animals to NO_2 or filtered air in the morning followed by either filtered air or 300 ppb O_3 in the afternoon. While NO_2 followed by air exposure did not alter blood pressure measurements, NO_2 exposure followed by ozone exposure resulted in exacerbation of ozone-induced decrease in blood pressure. It is also worth noting that the BP measurement data was obtained in the 6-hour period starting immediately after the completion of the afternoon ozone or filtered air exposure which means that the blood pressure values for the NO_2-air group were taken five to 11 hours after the end of the NO_2 exposure. This delay may impact the ability to see transient effects on blood pressure.

5.7.3.1 Conclusions and Remaining Uncertainties

The animal toxicologic evidence is very limited, and the available studies do not support an effect of short-term NO_2 exposure, by itself, on blood pressure. Studies of repeated exposure, co-exposures with other pollutants, and studies examining the dose-response relationship could further elucidate the potential for NO_2 exposure to impact blood pressure.

5.7.4 Synthesis Across Lines of Evidence

Epidemiologic evidence continues to provide mixed results for associations of short-term NO_2 exposures with changes in blood pressure. No evidence of association was seen in previously reviewed prospective studies, and mixed results were seen in cross-sectional studies which are limited in the ability to establish temporality and are prone to individual level confounding. Evaluation of potential copollutant confounding has been limited, contributing to uncertainty regarding the independent effect of NO_2 on

observed associations. In addition, no recent controlled human exposure studies have been conducted that look at effects on blood pressure. Controlled human exposure studies reviewed in the 2016 Oxides of Nitrogen ISA did not provide compelling evidence of NO_2-induced changes in blood pressure. Animal toxicologic studies of NO_2 impacts on blood pressure are limited in number and do not provide evidence of NO_2-induced changes in blood pressure. One study found that prior NO_2 exposure led to exacerbation of the decreased blood pressure in response to ozone exposure, suggesting that NO_2 may modulate the response to other BP-impacting stimuli.

5.8 Venous Thromboembolism

Venous thromboembolism encompasses deep vein thrombosis (DVT) and pulmonary embolism (PE). DVT occurs when a blood clot (thrombus) develops in deep veins, most commonly in the lower extremities. If untreated, DVT can lead to PE, where an embolus (i.e., a fragment of the clot) travels to the lungs and occludes blood flow. The effect of short-term oxides of nitrogen exposure on venous thromboembolism is generally evaluated using ICD codes recorded during hospital or ED admissions or discharges.

5.8.1 Epidemiologic Studies of Venous Thromboembolism

The 2016 Oxides of Nitrogen ISA evaluated a few epidemiologic studies that reported higher short-term NO_2 concentrations were associated with an increased risk of hospital admissions for venous thrombosis and/or pulmonary embolism (Spiezia et al., 2014; Dales et al., 2010). A study conducted in Santiago, Chile, Dales et al. (2010) reported a 9.7% (95% CI: 4.1, 15.4) and 8.4% (95% CI: 5.0, 11.8) increase in hospital admissions for venous thrombosis and pulmonary embolism, respectively, in association with 24-hour average NO_2 exposure. A small case-control study from Italy reported an increase in the risk of unprovoked pulmonary embolism (OR: 2.35 [95% CI: 0.76, 7.25]) among participants exposed to the upper tertile of NO_X concentration (average exposure for the month leading up to hospitalization ≥124 μg/m^3) compared to those in the bottom two exposure tertiles (Spiezia et al., 2014).

Recent studies evaluating oxides of nitrogen exposure and venous thromboembolism, including pulmonary embolism were identified since the 2016 Oxides of Nitrogen ISA (Li et al., 2022a; de Miguel-Diez et al., 2016; Milojevic et al., 2014). These studies were conducted among large patient populations across multiple locations and typically employed central site monitors or modelling as exposure surrogates. Evidence from recent studies is summarized in the bullets below, followed by discussion of the lifestages and populations evaluated in these studies (see Section 5.8.1.1), copollutant analyses (see Section 5.8.1.2), exposure assignment (see Section 5.8.1.3), the lag structure of association (see Section 5.8.1.4), and potential seasonal and temperature differences (see Section 5.8.1.5). Section 5.8.1.6

discusses conclusions based on the epidemiologic evidence and summarizes remaining uncertainties. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 5-8.

- The association between short-term NO_2 exposures and pulmonary thromboembolism was inconsistent across recent studies. A nationwide case-crossover in Spain reported lag 0 NO_2 concentrations were not associated with hospital admissions for pulmonary thromboembolism with a two-week control period prior to admission (OR: 0.99 [95% CI: 0.98, 1.01] per $\mu g/m^3$ increase in NO_2) (de Miguel-Diez et al., 2016). Whereas a case-crossover study in England and Wales reported NO_2 exposure (lag 0 to 4 days) was associated with a decreased risk of emergency hospital admission for pulmonary embolism (-2.93% [95% CI: -6.69, 0.84]) (Milojevic et al., 2014).
- In a U.K. cohort study, Li et al. (2022a) observed an increase in daily cumulative exposure to NO_2 and NO (estimated from monitoring data that accounted for spatial variability using a LUR model) was associated with a 5% and 4% higher risk of hospital admissions for venous thromboembolism, respectively (NO_2 HR: 1.04 [95% CI: 1.02, 1.06] and NO HR: 1.02 [95% CI: 1.01, 1.03]).

5.8.1.1 Lifestages and Populations

This section focuses on the evaluation and characterization of evidence determining whether there are populations or lifestages potentially at increased risk of short-term NO_2 exposure-related venous thromboembolism, with specific emphasis placed on studies that compare responses to a reference population. An older study found higher risk of venous thrombosis and pulmonary embolism among older patients (>64 years), and no difference in risk based on gender. Recent evidence to inform whether there are populations or lifestages at increased risk of venous thromboembolism remains limited.

Age:

- Milojevic et al. (2014) reported a difference in NO_2-associated risk of emergency hospital admissions for pulmonary embolism by age. Lag 0- to 4-day NO_2 exposure was associated with a decrease in emergency hospital admissions for pulmonary embolism among those <70 years old (β: -6.19% change [95% CI: -10.87, -1.51]). Whereas for those ≥70 years old, lag 0- to 4-day NO_2 exposure was associated with an imprecise increase (β: 1.34% change [95% CI: -4.35, 7.02]) in emergency hospital admissions for pulmonary embolism (Milojevic et al., 2014).

Sex:

- Although the estimates were imprecise, lag 0- to 4-day NO_2 exposure was associated with a larger decrease in emergency hospital admissions for pulmonary embolism among males (β: -4.43% [95% CI: -9.66, 0.79]) compared to females (β: -1.7% [95% CI: -6.6, 3.3]) (Milojevic et al., 2014).

5.8.1.2 Copollutants

A study by (Dales et al., 2010) reviewed in the 2016 Oxides of Nitrogen ISA reported that associations between short-term NO_2 exposure and venous thrombosis (RR: 1.05 [95% CI: 1.00, 1.10]) and pulmonary embolism (RR: 1.05 [95% CI: 1.02, 1.06]) were attenuated following adjustment for multiple pollutants (CO, O_3, SO_2, PM_{10}, $PM_{2.5}$). None of the studies identified since the 2016 Oxides of Nitrogen ISA assessed the independent association of oxides of nitrogen with venous thromboembolism following adjustment for copollutant(s).

5.8.1.3 Exposure Assignment

In the 2016 Oxides of Nitrogen ISA studies of venous thromboembolism used central or fixed site monitors, and none of the studies reported information on the extent to which concentrations at central site monitors captured the temporal variation in NO_2 concentrations in ambient air across the study area. Utilization of exposure surrogates such as single monitors and unweighted or population-weighted averages of multiple monitors contributes to uncertainty in the measurement of exposure in epidemiologic studies that may result in biased health effect estimates. In particular, exposure measurement error that does not adequately capture temporal variability in short-term NO_2 concentrations may underestimate the magnitude and not the directionality of the association. Chapter 2 (see Section 2.5.1) provides a thorough discussion of the bias resulting from particular exposure assessment methods common to the epidemiologic literature and its impact on the inferences that can be drawn about health effects. All studies that examined the association between short-term NO_2 exposure and venous thromboembolism published since the 2016 Oxides of Nitrogen ISA were case-crossover and time series analyses. These studies typically assigned daily NO_2 concentrations using fixed and central site monitors and did not report on the accuracy or sensitivity of these measurements in capturing temporal variability in NO_2 across the study area. One study by Li et al. (2022a) utilized modeled exposures fused with monitoring data that accounted for spatial variability in annual average NO_2 and NO_X concentrations based on a LUR model that incorporated spatial predictors from several sources (e.g., traffic, land use, topography). The authors reported a positive association between exposure to NO_2 and NO_X, respectively, and hospital admissions for venous thromboembolism. A comparison of modelled air pollution exposures among study participants with spatial distributions using public data indicated a similar distribution pattern of air pollution exposure across both data sources (Wang et al., 2021). These findings suggest modeled exposures are unlikely to introduce differential measurement error that can bias the association between short-term exposure to NO_2 and venous thromboembolism. However, direct comparisons are complicated by differences in study populations, lag periods, analysis methods, and the limited data available on alternative exposure approaches.

5.8.1.4 Lag Structure

There were a couple of studies reviewed in the 2016 Oxides of Nitrogen ISA that reported NO_2 utilizing a distributed lag of zero to 14 days was associated with venous thromboembolism (Dales et al., 2010), and the average NO_X exposure for the month leading up to hospitalization was associated with an increased risk of pulmonary embolism (Spiezia et al., 2014). No statistical differences were observed between 2-, 7-, and 14-day unconstrained lags and venous thrombosis and pulmonary embolism in (Dales et al., 2010). Studies published since the 2016 Oxides of Nitrogen ISA reported associations with venous thromboembolism occurring in close proximity to the exposure period, such as lag 0 for NO_2 associated pulmonary thromboembolism (de Miguel-Diez et al., 2016) and a measure of daily cumulative exposure for both NO and NO_2 associated venous thromboembolism (Li et al., 2022a). However, the overall findings were mixed, with a null association observed between NO_2 and pulmonary embolism at both lag 0 to 1 and 0 to 4 days in a U.K.-based study of hospital admissions (Milojevic et al., 2014).

5.8.1.5 Seasonal and Temperature Differences

Dales et al. (2010), which was reviewed in the 2016 Oxides of Nitrogen ISA, did not observe differences in the NO_2-venous thromboembolism association by season. Among the studies published since the 2016 Oxides of Nitrogen ISA, the nationwide case-crossover in Spain by de Miguel-Diez et al. 2016) reported a seasonal effect for pulmonary embolism, with most hospital admissions occurring in the colder seasons (autumn to winter). The authors did not present seasonally stratified results, but noted higher concentrations of NO_2 were observed during the colder season, and both factors were identified as risk factors for hospital admissions with pulmonary embolism.

5.8.1.6 Conclusions and Remaining Uncertainties

The overall evidence for an association between short-term exposures to ambient oxides of nitrogen and venous thromboembolism remains limited and associations are inconsistent in direction and precision. Except for a single study reporting attenuated NO_2 associated effects in multipollutant models, adjustment for potential copollutant confounding was not implemented across studies, and the extent to which the reported observations are attributable to other traffic-related pollutants remains unknown (Dales et al., 2010). Few studies have compared associations across lifestages or populations, exposure estimation approaches, lags, or seasons, and no clear patterns are evident from the studies that have made these comparisons. Taken together, the existing epidemiologic evidence of effects of NO_2 on venous thromboembolism is limited to a few studies and is not sufficient to draw broader conclusions on the potential effect of NO_2 exposure.

5.9 Aggregated Cardiovascular Effects

Recent epidemiologic studies have examined the impact of short-term oxides of nitrogen exposure on hospital admissions or ED visits for aggregated CVD, which encompasses all circulatory system diseases (e.g., heart diseases and cerebrovascular diseases). These studies generally include hospital admissions or ED visits where the ICD codes identify a cardiovascular health outcome as the primary reason for the visit. Thus, this measure is not specific to any particular cardiovascular outcome but represents a composite of all cardiovascular-related diseases.

5.9.1 Epidemiologic Studies of Aggregated Cardiovascular Effects

The 2016 Oxides of Nitrogen ISA concluded that data from epidemiologic studies demonstrated consistent associations between ambient NO_2 concentrations and risk of hospital admissions for cardiovascular diseases. Recent studies reported positive associations between short-term NO_2 exposure and cardiovascular effects in Asia (Son et al., 2013), Europe (Ballester et al., 2006 Larrieu, 2007, 93031; von Klot et al., 2005), North America (Sarnat et al., 2013; Ito et al., 2011; Peel et al., 2007; Tolbert et al., 2007; Fung et al., 2005; Linn et al., 2000), and Australia (Simpson et al., 2005). However, adjustment for potential copollutant confounding was not consistently performed across studies, and studies generally estimated exposures based on central site monitors which may not have captured spatial and temporal variations in NO_2 concentrations across study areas. Recent studies from South America (Rodríguez-Villamizar et al., 2019), North America (Ebelt et al., 2022; Shin et al., 2022; Krall et al., 2018; Yoo et al., 2018; Ha et al., 2017; Ye et al., 2016), Europe (Milojevic et al., 2014), Australia (Groves et al., 2020), and Asia (Chen et al., 2022b; Seposo et al., 2020) published since the 2016 Oxides of Nitrogen ISA, several of which analyzed large numbers of events in multiple cities and typically employed central site monitors or modelling as exposure surrogates, generally reported positive, although not entirely consistent, associations with aggregated cardiovascular effects. Heterogeneity in the outcomes assessed in relation to short-term oxides of nitrogen exposure across these studies, which included cardiorespiratory diseases, circulatory diseases, CVD related ED visits/hospital admissions, and cardiovascular emergency ambulance calls may have contributed to the mixed results. Evidence from recent studies is summarized in the bullets below, followed by additional discussion of the lifestages and populations evaluated in these studies (see Section 5.9.1.1), copollutant analyses (see Section 5.9.1.2), exposure assignment (see Section 5.9.1.3), the lag structure of associations (see Section 5.9.1.4), and an examination of potential seasonal and temperature differences (see Section 5.9.1.5). Section 5.9.1.6 discusses conclusions from epidemiologic studies examining cardiovascular effects and summarizes remaining uncertainties in the evidence. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 5-9.

- A number of time-series analyses published since the 2016 Oxides of Nitrogen ISA yielded findings that supported a positive association with aggregated cardiovascular effects. In a

Canadian time-series analysis, Shin et al. (2022) reported both males (β: 3.60% [95% CI: 2.30, 4.90]) and females (β: 1.4% [95% CI: 0.2, 2.6]) were at higher risk for lag 0 NO_2 associated hospitalization due to circulatory diseases in year-round analysis. A multi-city time series analysis from Columbia reported lag 0 to 1-hour maximum NO_2 concentrations were associated with a 11.89% (95% CI: 0.75, 23.02) per 6 $\mu g/m^3$ increase in NO_2 increase in circulatory ED visits (Rodríguez-Villamizar et al., 2019).

- A multi-city time series analysis from China reported lag 0 to 1 NO_2 was associated with a higher excess risk of cardiovascular emergency ambulance calls (NO_2 excess relative risk (ERR): 2.75 [95% CI: 0.68, 4.82] per 10 $\mu g/m^3$ increase in NO_2) (Chen et al., 2022b).
- A time-series analysis of five U.S. cities reported a positive, but imprecise association between NO_2 (RR: 1.03 [95% PI: 1.00, 1.005]) and NO_X (RR: 1.02 [95% PI: 1.00, 1.04]) concentrations at lag 0 and the risk of ED visits for CVD ED visits (Krall et al., 2018). Exposure estimates were based on population weighted average estimates that used fused monitor concentrations with CMAQ model estimates.
- A time-series analysis conducted in a single U.S. city compared monitoring and modeled exposures of NO_2 (using CMAQ, LUR, and satellite-based measures) in relation to cardiovascular ED visits (Ebelt et al., 2022). Imprecise associations ranging from approximately null to positive were observed between cardiovascular ED visits and lag 0 to 7 NO_2 exposure, modeled using a central monitor (RR: 1.02 [95% CI: 1.0, 1.04]), a full spatial model using LUR (RR: 0.98 [95% CI: 0.95, 1.02]), and a full spatial with Kriging (RR: 1.04 [95% CI: 1.01, 1.07]). Additionally, no association was observed between lag 0 NO_2 concentrations and cardiovascular ED visits in a U.S. time series analysis (RR: 1.00 [95% CI: 0.99, 1.02]) (Ye et al., 2016).
- A time-series analysis in Australia reported a positive, but imprecise association between lag 0- to 2-day NO_2 concentrations and risk of CVD-related ICU admissions (RR: 1.04 [95% CI: 0.09, 12.50]) in models adjusted for temperature and humidity, influenza-like illness, and public holidays (Groves et al., 2020).
- Recent case-crossover studies reported positive associations between NO_2 and aggregated cardiovascular effects. A case-crossover study in U.K. patients reported a 10^{th} to 90^{th} percentile increase in NO_2 concentrations at lags 0 to 4 days was associated with a 1.42% increase (95% CI: 0.71, 2.13%) in risk of CVD emergency hospital admissions (Milojevic et al., 2014).
- A case-crossover analysis of air pollution and cardiovascular events at labor and delivery in 12 clinical sites in the United States, Ha et al. (2017) observed that an IQR increase in average NO_X concentration (based on CMAQ model estimates fused with monitoring data) during the week prior to labor/delivery (lag 0 to 7) was associated with a 21% increase in the odds of any cardiovascular event (OR: 1.29 [95% CI: 1.05, 1.59]).
- In a multi-city case-crossover study from Japan, Seposo et al. (2020) reported lag 0 NO_2 concentrations were associated with an increase (β: 5.52% [95% CI: 4.29, 6.75%]) in outpatient cardiovascular visits.
- A U.S. cross-sectional study observed an association between NO_2 (based on CMAQ modelling at 12 km x 12 km resolution) estimates fused with monitoring data) and ED visits for CVD in both single (data NR) and multipollutant models (reported in Section 5.9.1.2) that accounted for spatial variability and autocorrelation in ED utilization (Yoo et al., 2018).

5.9.1.1 Lifestages and Populations

This section focuses on the evaluation and characterization of evidence determining whether there are populations or lifestages potentially at increased risk of a NO_2-related health effect, with specific emphasis placed on studies that compare responses to a reference population. The 2016 Oxides of Nitrogen ISA did not report evidence to indicate differences in the association with cardiovascular effects by populations or lifestages.

Recent studies expand the evidence that potentially informs whether there are populations or lifestages at increased risk of NO_2-related effects. Results from recent studies are presented in greater detail below. Overall, the findings did not indicate consistent differences in the risk of aggregated cardiovascular effects in age- or sex-stratified analysis or for those with pre-existing conditions.

Age

- Participants 70 years and older (2.43% [95% CI: 1.51, 3.35] per 23.9 ppb NO_2) were at heightened risk of CVD emergency hospital admissions compared to participants <70 years old (0.8% [95% CI: -0.92, 1.09]) in the U.K. based study (Milojevic et al., 2014). The difference in risk of emergency hospital admissions for all CVD was significant between the two age groups (p-value for the interaction term).
- In contrast, the multi-city time-series analysis from China reported participants 65 years and older (2.60% [95% CI: 0.77, 4.42]) exhibited a lower excess risk of cardiovascular emergency ambulance calls with increasing lag 0 to 1 NO_2 concentrations compared to participants less than 65years (3.65% [95% CI: 0.85, 6.452]) (Chen et al., 2022b).

Sex

- A multi-city time-series analysis from China reported a positive association between lag 0 to 1 NO_2 and risk of cardiovascular emergency ambulance calls among both females (3.58% [95% CI: 0.98, 6.17]) and males (2.15% [95% CI: -0.62, 4.91]) (Chen et al., 2022b). However, the association was imprecise among males.
- Shin et al. (2022) observed both males (3.60% [95% CI: 2.30, 4.90]) and females (1.40%, [95% CI: 0.20, 2.60]) exhibited a higher risk of lag 0 NO_2 associated hospitalization due to circulatory diseases in all-year sex-stratified analysis. In analysis stratified by season, a higher risk was observed among males (reported in Section 5.9.1.5).
- Milojevic et al. (2014) reported lag 0- to 4-day NO_2 exposure was associated with a similar increase in emergency hospital admissions for all CVD among females (1.42% change [95% CI: 0.42, 2.43]) and males (1.42% change [95% CI: 0.46, 2.38]).

5.9.1.2 Copollutants

There were a limited number of epidemiologic studies reviewed in the 2016 Oxides of Nitrogen ISA that evaluated potential confounding of the association between ED visits and hospital admissions for

all cardiovascular diseases and oxides of nitrogen by correlated copollutants. In some studies, associations with NO_2 were attenuated after adjustment for $PM_{2.5}$ or SO_2. Therefore, uncertainties remained about whether NO_2 served as marker for other traffic-related pollutants or mixtures. Recent studies published since the 2016 Oxides of Nitrogen ISA add to the evidence that informs the independent association of oxides of nitrogen with aggregated cardiovascular effects. The results from these studies, which are highlighted below, continue to yield inconsistencies following copollutant adjustment.

- A multi-city time series analysis from Columbia reported lag 0 1-hour maximum NO_2 concentrations were associated with a 11.89% increase (95% CI: 0.75, 23.02) in circulatory ED visits (Rodríguez-Villamizar et al., 2019). The magnitude of the association between NO_2 and circulatory disease emergency department visits increased in copollutant models that adjusted for $PM_{2.5}$ (13.38% [95% CI: 0.03, 26.72]) and did not change when adjusted for CO but was attenuated in multipollutant models that included O_3, PM_{10}, and SO_2 (Rodríguez-Villamizar et al., 2019). Models assessing the joint effects of mixtures indicated "traffic-related pollutants" exhibited the highest increase in percentage of circulatory ED visits (13.38% [95% CI: 0.03, 26.72]). The effect was similar in magnitude to that of NO_2 from single pollutant models which the authors noted may indicate NO_2 acts as a potential mixture indicator for circulatory diseases. The association between oxides of nitrogen and risk of CVD hospital admissions did not change following adjustment for a second pollutant (CO, O_3, PM_{10}, $PM_{2.5}$, SO_2) in the case-crossover analysis of the MINAP database (Milojevic et al., 2014). The magnitude of the short-term NO_2 effect on cardiovascular outpatient visits increased in copollutant models in a multi-city case-crossover study from Japan (Seposo et al., 2020). The highest percentage change in lag 0 NO_2-associated cardiovascular visits occurred with adjustment for $PM_{2.5}$ (9.62% [95% CI: 8.22, 11.02%]), followed by a 5.98% increase (95% CI: 4.73, 7.23%) with adjustment for O_3.
- The association between lag 0 to 1 NO_2 and excess risk of cardiovascular emergency ambulance calls did not persist in copollutant models that controlled for $PM_{2.5}$ (2.52% [95% CI: -0.75, 5.8]) and PM_{10} (2.26% [95% CI: -0.32, 4.84]) in the multi-city time series analysis from China (Chen et al., 2022b).
- A U.S. cross-sectional study using spatially and temporally resolved multipollutant exposures reported NO_2 was associated with an increased risk of ED visits for CVD (RR: 1.15 [95% CI: 1.01, 1.30] per 10-ppmv increase in NO_2) in an analysis that accounted for spatial variability and autocorrelation in ED utilization (Yoo et al., 2018).

5.9.1.3 Exposure Assignment

In the 2016 Oxides of Nitrogen ISA studies of aggregated cardiovascular effects, exposures were represented as ambient concentrations at central site monitors, and none of these studies reported information on the extent to which central site monitors captured the temporal variation in NO_2 concentrations in ambient air across the study area. Utilization of exposure surrogates such as single monitors and unweighted or population-weighted averages of multiple monitors contributes to uncertainty in the measurement of exposure in epidemiologic studies that may result in biased health effect estimates. Exposure measurement error that does not adequately capture temporal variability in short-term NO_2 concentrations may underestimate the magnitude but not the directionality of the association. Chapter 2

(see Section 2.5.1) provides a thorough discussion of the bias resulting from exposure assessment methods common to the epidemiologic literature and its impact on the inferences that can be drawn about health effects.

Most studies that examined the association between short-term NO_2 and aggregated cardiovascular effects published since the 2016 Oxides of Nitrogen ISA assigned daily NO_2 concentrations using fixed and central site monitors and did not compare across multiple exposure assignment approaches. A few recent studies used modeled exposure estimates based on output from CMAQ fused with monitoring data (Krall et al., 2018; Yoo et al., 2018; Ha et al., 2017). Other studies used a hybrid approach that included monitor-based, model-based, LUR, and satellite-based measures (Ebelt et al., 2022).

Positive associations were observed between modeled short-term NO_2 exposures that fused CMAQ output with monitoring data and aggregated CVD effects (Krall et al., 2018; Yoo et al., 2018; Ha et al., 2017). A recent time-series analysis conducted in a single U.S. city compared monitoring and modeled-based daily exposures to 1-hour maximum concentrations of NO_2 in relation to cardiovascular ED visits (Ebelt et al., 2022). The authors utilized air pollution models fused with monitoring data that incorporated spatial interpolation along with land use regression. The authors reported associations with cardiovascular ED visits and NO_2 exposure using three approaches: a central monitoring approach, assigning the daily value of each pollutant from non-central monitor exposure metrics at the ZIP code where the central monitor was located, and incorporating spatiotemporal information (i.e., assigning to each non-central monitor exposure metric the daily ZIP code-specific pollution value to each study area ZIP code). A positive association was observed between cardiovascular ED visits and lag 0 to 7 NO_2 exposure modeled using a central monitor or modeled concentrations using central ZIP codes. However, the association was attenuated in models that utilized spatiotemporal information for some exposure metrics compared to the central ZIP code approach. The authors suggested lower pollution concentrations with limited variability at the outskirts of the city as well as a non-linear C-R may have contributed to the weaker associations in models that accounted for spatiotemporal information.

Overall, the evidence is too limited to determine whether modeled exposures introduce systematic measurement error that can bias the association between short-term exposure to NO_2 and aggregated CVD effects. Inconsistencies in findings among studies that assigned exposure using model predictions are similar to the inconsistent results reported across studies using central site monitors, though direct comparisons are complicated by differences in study populations, lag periods, analysis methods, and the limited data available on alternative exposure approaches.

5.9.1.4 Lag Structure

As discussed in the 2016 Oxides of Nitrogen ISA, the available evidence, although inconsistent, reported that ED visits and hospital admissions due to cardiovascular diseases were associated with

shorter lags of NO_2 exposure. Most typically, associations with NO_2 were reported within a few days of exposure (lags 0 to 2) of the event which are indicative of more immediate effects. Studies published since the 2016 Oxides of Nitrogen ISA continue to report associations with aggregated cardiovascular effects occurring in close proximity to the exposure period, typically as single day lag 0 and distributed lags of 0 to 1 and 0 to 2.

Studies examining alternate lag structures provided equivocal results. A case-crossover analysis of NO_X and cardiovascular events at labor and delivery in 12 clinical sites in the United States. Ha et al. (2017) explored multiple lag days the week before delivery, reporting positive associations between all cardiovascular events and exposure to NO_X at individual lag days 0, 1, 5, 6 (where the strongest effect was observed), and the weekly average. Ebelt et al. (2022) reported the directionality of the association between NO_2 and cardiovascular ED visits in a single U.S. city was similar across multiple lag periods assessed, although the magnitude for lag days 0 to 7 was larger compared to alternative lag periods (0, 0 to 2, 0 to 5 days) for several of the exposure modelling approaches. In contrast, a case-crossover study from the United Kingdom found the association between NO_2 exposure over a lag 0 to 4 days with all CVD emergency hospital admissions was similar to lag 0 to 1 days (Milojevic et al., 2014).

Recent studies observed that the magnitude of the association between NO_2 and aggregated cardiovascular effects was strongest in relation to exposures occurring on the same day as the event, and diminished over longer lag periods.

- Chen et al. (2022b) reported associations between NO_2 and emergency ambulance calls due to cardiovascular diseases decreased in strength from lag 0 to lag 5 in single day lag analyses. The strongest associations were observed at average lag periods of 0 to 1 and 0 to 2 days.
- Similar findings were observed by Shin et al. (2022), which observed the association between short-term exposure to NO_2 and hospitalization due to circulatory diseases was strongest at lag 0 and weakened over subsequent days (lag 1 and 2 days). A multi-city time series analysis from Columbia observed a stronger effect on the change in circulatory ED visits in relation to lag 0 NO_2 concentrations than for cumulative 7 days exposure (lag 1 to 7) (Rodríguez-Villamizar et al., 2019).
- A multi-city case-crossover study from Japan reported positive associations between outpatient cardiovascular visits and NO_2 with concurrent day exposure (lag 0) and for cumulative lag 0 to 3 days following adjustment for $PM_{2.5}$ and O_3, respectively (Seposo et al., 2020). A positive association was observed at lag 0 to 14 following adjustment for $PM_{2.5}$, but the association was attenuated with adjustment for O_3.
- Ebelt et al. (2022) reported the directionality of the association between NO_2 and cardiovascular ED visits was similar across multiple lag periods assessed, although the magnitude for lag days 0 to 7 was larger compared to alternative lag periods assessed (0, 0 to 2, 0 to 5 days) for several of the exposure modelling approaches.

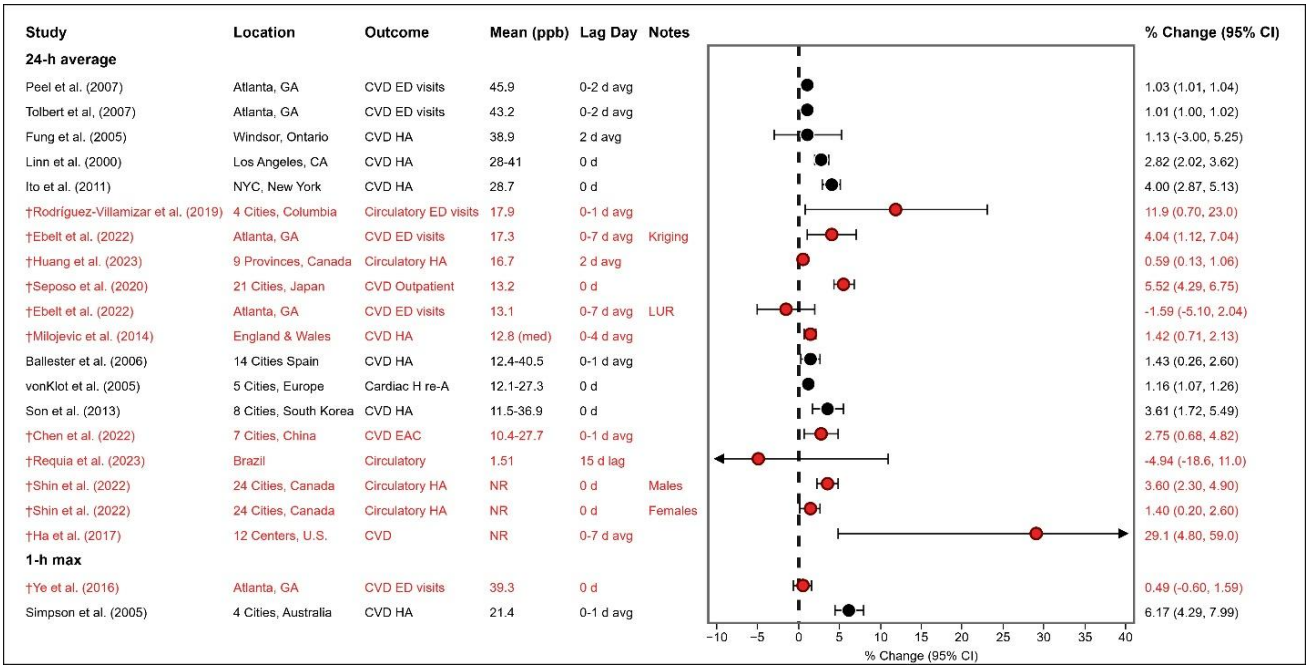
5.9.1.5 Seasonal and Temperature Differences

The 2016 Oxides of Nitrogen ISA reported limited and conflicting data on the influence of season and temperature on the association between NO_2 and aggregated cardiovascular effects. A stronger association between cardiovascular hospital admissions and NO_2 was observed during the warm and cold season in a few of studies from China (Zheng et al. (2013) and Son et al. (2013), respectively). Another study from the United States did not find any seasonal differences (Ito et al., 2011). Overall, recent evidence for a seasonal and temperature influence on the oxides of nitrogen-aggregated cardiovascular effects association is equivocal.

- A Canadian time series analysis reported among males a larger lag 0 NO_2 association with hospitalization due to circulatory diseases in the warm season (5.2% [95% CI: 3.5, 6.9]) compared to the cold season (2.4% [95% CI: 0.3, 4.5]) (Shin et al., 2022). In females, the opposite was observed, with a larger association reported in the cold season (2.4% [95% CI: 0.9, 3.9] per 10 ppb) compared to the warm season (0.4% [95% CI: -3.4, 4.2]).
- Although the associations were null, excess risk of emergency ambulance calls due to cardiovascular diseases was higher for lag 0 to 1 NO_2 concentrations in the warm season (1.66% [95% CI: -0.38, 3.69]) compared to the cold season (-0.34% [95% CI: -2.77, 2.09] per 10 $\mu g/m^3$ increase in NO_2) in a multi-city time series study in China (Chen et al., 2022b).
- A time-series analysis in Australia did not observe a modifying effect of season on NO_2-associated ICU admissions for CVD (data NR) (Groves et al., 2020).

5.9.1.6 Conclusions and Remaining Uncertainties

Overall, the epidemiologic data available continues to support associations between short-term exposures to ambient NO_2 and CVD outcomes (see Figure 5-9). However, the extent to which the reported observations are attributable to other traffic-related pollutants remains unknown. Some studies report associations that remained positive in copollutant models (Seposo et al., 2020; Rodríguez-Villamizar et al., 2019; Milojevic et al., 2014), while another reports a negative association (Chen et al., 2022b). Additionally, the results from prior and recent studies did not indicate consistent differences in the risk of CVD outcomes in age- or sex-stratified analysis or for those with pre-existing conditions. The evidence continues to indicate CVD outcomes are associated with shorter lags of NO_2 exposure, with associations reported within the first few days of the event, such as lags of 0, 0 to 1 or 0 to 2 days, which are indicative of more immediate effects. The evidence from studies that assessed the influence of seasonality and temperature remains inconsistent; few studies have compared associations across exposure estimation approaches, or extended lag periods, and no clear patterns are evident from the studies that have made these comparisons. Taken together, the available epidemiologic evidence provides consistent support for associations between short-term NO_2 exposures and aggregated CVD effects.



List of abbreviations in alphabetical order: avg = averge; CA = California, U.S.; CVD = cardiovascular disease; CI = confidence interval; d = day; EAC = exercise-associated collapse; ED = emergency department; GA = Georgia, U.S.; h = hour; HA = hospital admission ; H re-A = hospital re-admission; med = median; NR = not reported; NYC = New York City, N.Y, U.S.; ppb = parts per billion; U.S. = United States.
Note: Effect estimates for associations with NO_2 are indicated by a circle.
†Studie published since the 2016 Oxides of Nitrogen ISA.

Figure 5-9 Percentage increase in emergency department visits and hospital admissions for cardiovascular disease in relation to nitrogen dioxide exposure.

5.10 Subclinical Effects Underlying Cardiovascular Effects

The following subsections review studies on subclinical effects that serve as measures of physiological and biochemical responses that could provide mechanistic evidence to explain the role of oxides of nitrogen in cardiovascular disease. While these subclinical effects are not widely validated as markers for specific clinical cardiovascular outcomes, they could potentially underlie the development, progression, or indication of various clinical events.

5.10.1 Heart Rate and Heart Rate Variability

Heart rate (HR) and heart rate variability (HRV) are non-invasive indicators of cardiac autonomic nervous system function. HR indicates how many times the heart beats in a given timeframe (e.g., per minute). HR is controlled at the sinoatrial node by both the parasympathetic and sympathetic branches of the autonomic nervous system. Generally, increased sympathetic activation raises HR, while heightened parasympathetic activation decreases it (Lahiri et al., 2008). HRV measures the difference in inter-beat

intervals of successive heart beats, and changes are reflective of the balance between sympathetic and parasympathetic tone to the heart and their interaction (Rowan et al., 2007). HRV can be quantified using either time domain or frequency domain metrics. Common time-domain measures of HRV include the standard deviation of all normal-to-normal intervals (SDNN, an index of total HRV) and the root-mean-square of successive differences (rMSSD, an index reflecting parasympathetic influence on the heart). In the frequency domain, HRV is usually divided into the high frequency (HF) domain, thought to reflect parasympathetic tone, and the low frequency (LF) domain, which is posited as an indicator of the interaction between sympathetic and parasympathetic nervous systems (Billman, 2013), although its linkage with sympathetic tone is controversial and uncertain (Notarius et al., 1999). The ratio of LF to HF measures (LF/HF) is often used as another measure of sympathetic to parasympathetic autonomic balance, however, as stated, this can be controversial. Epidemiologic, controlled human exposure, and animal toxicological studies have evaluated the impact of short-term oxides of nitrogen exposure on HR and HRV.

5.10.1.1 Epidemiologic Studies of Heart Rate and Heart Rate Variability

The 2016 Oxides of Nitrogen ISA reported evidence for an association between short-term NO_2 exposure and HR or HRV in populations with pre-existing disease but not in healthy individuals [see Table 5-47 in U.S. EPA (2016)]. Recent studies evaluating these outcomes are summarized below, followed by additional discussions of the lifestages and populations evaluated in these studies (see Section 5.10.1.1.2) and the potential impacts of copollutants (see Section 5.10.1.1.3). Section 5.10.1.1.4 discusses conclusions from the epidemiologic evidence and summarizes remaining uncertainties. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 5-10. A summary of the key findings is as follows:

Heart Rate

In the 2016 Oxides of Nitrogen ISA, there were recent studies evaluating associations between short-term exposure to NO_2 and HR (Zhang et al., 2013; Rich et al., 2012; Williams et al., 2012; Goldberg et al., 2008). Multiple studies reported associations in the same direction. A positive association between short-term NO_2 exposure and HR were reported in studies examining associations during a period of NO_2 reduction related to the 2008 Olympics in Beijing (Zhang et al., 2013; Rich et al., 2012). While Williams et al. (2012) reported an inverse association between short-term exposure to NO_2 and HR, where decreased HR was reported among those with underlying cardiovascular health issues, another study reported no associations (Goldberg et al., 2008).

There were a few recent studies that add to the support for associations between short-term NO_2 exposure and increased HR.

- In a randomized 60 study of healthy adults (mean age = 24 years old), Shutt et al. (2017) used exposure of five consecutive 8-hour days outdoors either near a steel plant or on a college campus several kilometers away in a crossover design (nine to 16 days washout in between). Mixed effects linear regression models reported positive associations between NO, NO_2, and NO_X and HR. Where a 60 ppb increase in NO was associated with a 16.64 bpm increase in HR (95%: 4.16, 29.11), a 25 ppb increase in NO_2 was associated with an 8.14 bpm increase in HR (95%: 2.79, 13.49), and an 80 ppb increase in NO_X was associated with a 14.46 bpm increase in HR (95%: 5.45, 23.47).
- A Canadian study of older adults (>55 years of age) investigated the impact of indoor exercise during periods of diminished air quality (Air Quality Hazard Index [AQHI] >5) on HR (Stieb et al., 2019). Participants were asked to participate in light exercise (i.e., walking) outside every day for 10 weeks with one group directed to walk indoors on days when the AQHI was >5 (the intervention group). Measures of HRV were measured weekly both pre and post exercise. Using linear mixed effect regression models the authors reported that NO_2 exposures were associated with increased HR at lag day 1 in the outdoor only group but not in the intervention group.
- A similar study conducted in the winter by the same group examined the effect of air pollution on HR in older adults (>55 years old) who exercised outdoors (Stieb et al., 2018). NO_2 was measured using fixed site monitors. The authors used linear mixed effect regression models and reported a positive association of HR and NO_2 concentrations at lag day 1. The association between NO_2 and HR was reduced when accounting for $PM_{2.5}$, O_3, and SO_2.

Heart Rate Variability

The epidemiologic studies in the 2016 Oxides of Nitrogen ISA suggested that associations between ambient NO_2 concentrations and HRV indices indicative of altered autonomic control were more consistent in populations with pre-existing or elevated risk for cardiovascular disease. This included studies of those with stable coronary artery disease (Zanobetti et al., 2010; Timonen et al., 2006), pre-existing cardiovascular disease (Zhang et al., 2013; Huang et al., 2012a), recent MI or COPD (Suh and Zanobetti, 2010). A study of diabetics did not show association with HRV measures (Laumbach et al., 2010). Studies in generally healthy populations did not commonly find associations with HRV changes (Shields et al., 2013; Jia et al., 2011; Weichenthal et al., 2011; Park et al., 2010; Min et al., 2008; Chuang et al., 2007).

There were a few recent studies that add to the support for associations between short-term NO_2 exposures and decreases in HRV measures.

- In a Canadian study of 60 study of healthy adults (mean age = 24 years old), participants spent five consecutive 8-hour days outdoors either near a steel plant or on a college campus several kilometers away in a crossover design (nine to 16 days washout in between) (Shutt et al., 2017). The authors reported decreases in HRV measures when subjects spent time outdoors near the steel plant compared to the college campus. Mixed effects linear regression models with HR, age, sex, BMI, study site, and relative humidity as covariates reported that increases in NO, NO_2, and NO_X were associated with decreased SDNN (NO β: -6,368.18 ms [95% CI: -6,425.8, -6,310.57] per 60 ppb NO, NO_2 β: -37.27 ms [95% CI: -61.919, -12.62] per 25 ppb NO_2, and NO_X β: -63.61 ms [95% CI: -105.11, -22.12] per 80 ppb NO_X), and

rMSSD (NO_X β: -56.32 ms [95% CI: -117.48, 4.84] per 60 ppb NO, NO_2 β: -35.00 ms [95% CI: -61.19, -8.81] per 25 ppb NO_2 and NO_X β: -57.93 ms [95% CI: -101.98, -13.88] per 80 ppb NO_X). There were no associations with frequency domain measures despite a general trend towards decreases in these measures being reported. In a similar manner, NO concentrations were associated with decreased pNN50 (β: -24.14% [95% CI: -48, -0.27] per 60 ppb NO) and LF power measurements (β: -20.59 ms^2 [95% CI: -41.05, -0.14] per 60 ppb NO).

- A Canadian study of older adults (>55 years old) investigated the impact of indoor exercise during periods of diminished air quality (AQHI >5) on HRV measures (Stieb et al., 2019). Participants were asked to participate in light exercise (i.e. walking) outside every day for 10-weeks with one group directed to walk indoors on days when the AQHI was >5 (the intervention group). Measures of HRV were measured weekly both pre and post exercise. Using linear mixed effect regression models the authors reported an inverse association between daily maximum 3-hour average NO_2 and pre-workout LF, pNN50, rMSSD, and reactive hyperemia index (RHI) in the outdoor only exposure group and not in the intervention group. Similar trends were seen post exercise though not always reaching statistical significance. In a study by the same authors that examined the effect of short-term NO_2 exposure on HRV measures reported null associations with HRV measures (Stieb et al., 2018).
- A U.S. panel study of 62 healthy participants assessed the potential modulating effect of supplementation with omega-3 polyunsaturated fatty acids (PUFA) on the association between short-term NO_2 exposure and HRV measures. Chen et al. (2021) reported that an increase in lag 3 24-hour NO_2 exposure by 20 ppb was negatively associated with an index of HRV, normalized high frequency (HFn) (-37.89% [95% CI: -71.58, -4.21]), and positively associated with low-to-high frequency (LF/HF) ratio (70.5% [95% CI: -3.4, 144.5]) (indicative of sympathetic dominance and stress response) in participants with high omega-3 intake. This association was not reported in participants with low omega-3 intake. Lag 0 NO_2 exposure was negatively associated with very-low frequency (VLF) measures (-113.2% [95% CI: -190.00, -36.30]) (associated with arrhythmic death and inflammation) in participants with low omega-3 intake. This association was not reported in participants with high omega-3 intake. NO_2 was measured using a fixed site monitor.
- Biel et al. (2020) conducted a panel study of 46 non-smoking adults in Toronto Canada with the aim of distinguishing the effects of noise and air pollution on subclinical cardiovascular endpoints including HRV. No discernable pattern of association was reported of 24-hour regional NO_2 exposure (estimated from fixed site monitors) with SDNN, RMSSD, LF, HF or LF:HF ratio, regardless of noise adjustment.

5.10.1.1.2 Lifestages and Populations

The 2016 Oxides of Nitrogen ISA concluded that the epidemiologic evidence reported associations between ambient NO_2 concentrations and decreases in indices of HRV and changes in ventricular repolarization among populations with pre-existing cardiovascular disease or at elevated risk for cardiovascular disease, but not in individuals without pre-existing disease or elevated risk. Recent studies provide some support for an association between short-term exposures to NO_2 and increased HR and decreased HRV among healthy older adults, though results were not always consistent across various measures of HRV. Recent studies did not examine individuals with underlying comorbidities, and thus

they do not provide additional evidence as to whether members of these groups are at enhanced risk for NO_2-associated decreases in HRV or changes in ventricular repolarization.

5.10.1.1.3 Copollutants

The associations between short-term exposure to NO_2 and markers of HRV were robust in copollutant models after adjustment for simultaneous exposure to $PM_{2.5}$ or O_3 (Chen et al., 2021). However, the magnitude of the association between NO_2 and HR was attenuated in copollutant and multipollutant models adjusted with O_3, $PM_{2.5}$, SO_2 (Stieb et al., 2018).

5.10.1.1.4 Conclusions and Remaining Uncertainties

Together, recent studies provide some additional support for an association between increased short-term exposure to NO_2 and increased HR as well as decreased HRV. However, the overall evidence base for evaluating the association between oxides of nitrogen exposure and HR and HRV remains limited. Studies to assess the NO_2-HRV association and the potential influence of traffic-related copollutants are lacking. Exposure in most studies was assessed using data from fixed site monitors. While some earlier studies in the 2016 Oxides of Nitrogen ISA used individual level monitoring, this exposure assessment method was not used in recent studies. Fixed site monitors offer improved spatial and temporal resolution compared to city or region assessments; however, they may still introduce uncertainty in estimating individual level ambient exposure given the high spatial and temporal variability of traffic-related pollutants.

5.10.1.2 Controlled Human Exposure Studies of Heart Rate and Heart Rate Variability

The 2016 Oxides of Nitrogen ISA evaluated two controlled human exposures that investigated the impact of short-term NO_2 exposure on HRV and five others that looked at HR. No recent controlled human exposure studies looking at HRV or HR were identified. The results of the older studies are summarized below, followed by additional discussion of the lifestages and populations evaluated in these studies (see Section 5.10.1.2.1). Section 5.10.1.2.2 discusses conclusions from these studies and summarizes remaining uncertainties.

Heart Rate

- Recent studies that exposed healthy volunteers to 600 to 620 ppb NO_2 for two hours showed no change in HR (Drechsler-Parks, 1995; Folinsbee et al., 1978). These studies utilized young healthy adult males (Folinsbee et al., 1978) and older healthy adults (Drechsler-Parks, 1995).

- In study subjects with underlying coronary heart disease, who may be more prone to cardiovascular effects, exposure to 400 ppb NO_2 for 1-hour while sedated did not impact HR (Scaife et al., 2012).
- Exposure to 400 ppb NO_2 led to nonsignificant increases in HR in healthy or COPD subjects (Gong et al., 2005).
- Exposure of healthy volunteers and those with asthma to 500, 1,000, or 2,000 ppb NO_2 during intermittent exercise did not result in changes to HR (Linn et al., 1985a).

Heart Rate Variability

- Huang et al. (2012b) reported that exposure of healthy adults to 500 ppb NO_2 with intermittent exercise did not affect HRV time domain intervals but the authors reported an 11.6% and 13% decrease in the HF domain normalized for HR at one and 18 hours post exposure, respectively. This resulted in a slight increase in LF/HF although the increase did not reach statistical significance. Combined exposure to 500 ppb NO_2 and $PM_{2.5}$ CAPs increased LF/HF at 1-hour post exposure, which corresponded to a decreased normalized LF domain measure and cardiac T wave amplitude that were not seen with CAPs alone suggesting some level of interaction of NO_2 and CAPs on HRV.
- A study in resting adults with stable coronary heart disease and impaired left ventricular systolic function did not report statistically significant changes in HRV after exposure to 400 ppb NO_2 (Scaife et al., 2012). The 2016 Oxides of Nitrogen ISA noted that this study only had 75% power to detect significant differences in the HF domain of 50% or less.

In summary, the existing controlled human exposure evidence does not provide strong support for short-term NO_2-induced changes to HR or HRV.

5.10.1.2.1 Lifestages and Populations

Studies of NO_2-induced changes to HR have been conducted in subjects with underlying health conditions like COPD (Gong et al., 2005), asthma (Linn et al., 1985a), and coronary artery disease (Scaife et al., 2012) and reported little evidence to support changes in HR by NO_2 exposure. This is mirrored by a lack of effect on HR seen in subjects without underlying disease including studies that looked at older individuals (Drechsler-Parks, 1995). For HRV, studies have reported changes to HRV parameters following NO_2 exposure in both subjects without underlying disease (Huang et al., 2012b) and those with coronary heart disease (Scaife et al., 2012) although comparison of relative effect threshold or magnitude between disease states is not possible from these studies.

5.10.1.2.2 Conclusions and Remaining Uncertainties

The controlled human exposure evidence for effects of NO_2 on HR and HRV are limited in number, and among available studies there is little evidence to suggest impacts of NO_2 exposure on HR. The limited evidence available for HRV does indicate that NO_2 exposure can reduce HRV, which could be an underlying indicator of reduced cardiovascular health.

5.10.1.3 Animal Toxicological Studies of Heart Rate and Heart Rate Variability

The 2016 Oxides of Nitrogen ISA described one mouse study that investigated the effect of exposure to filtered or unfiltered ambient air consisting of PM, CO, and NO_2 over 40-weekdays which reported that decreased HR was associated with NO_2 at lag 3- and 7-day cumulative concentration with adjustment for PM and CO but changes in HRV were independent of NO_2 (Ramos-Bonilla et al., 2010). The interpretation of the reported effects with regard to NO_2 specifically is unclear because of the multicollinearity among pollutants in the multipollutant models. Recent studies investigated the impact of NO_2 exposure on HR and HRV. The results of the recent studies are summarized below. Study specific details, exposure conditions, and endpoints examined are highlighted in the text below. Studies are described in more detail in Appendix A – Appendix Table 5-11.

- A study in spontaneously hypertensive rats showed no impact of 3-hour exposure to 500 ppb NO_2 on HR or time domain (rMSSD, SDNN) and frequency domain (HF) measures of HRV (Farraj et al., 2016). This study exposed animals to NO_2 or filtered air in the morning followed by either filtered air or 300 ppb O_3 in the afternoon. NO_2 or O_3 alone did not affect HR but NO_2 exposure followed by O_3 exposure resulted in significant decrease in HR. O_3 exposure alone caused decreases in rMSDD, SDNN and HF measurements which was either attenuated or increased with prior NO_2 exposure. These results suggest that NO_2 exposure may alter cardiac responses to other pollutants in a mixture. It is also worth noting that the HR and HRV measurement data was obtained in the 6-hours period starting immediately after the completion of the afternoon ozone or filtered air exposure which means that the HR and HRV values for the NO_2-air group were taken five to 11 hours after the end of the NO_2 exposure. This delay may impact the ability to see transient effects.
- Exposure of C57BL/6J mice to 2.5 ppm NO_2 five hours per a day for 28 days did not result in changes to HR (Ji et al., 2022).

5.10.1.3.1 Conclusions and Remaining Uncertainties

The animal toxicologic database for effects of NO_2 exposure on HR and HRV remains very limited. Available data suggests that NO_2 exposure has little effect on HR and HRV. Some results suggest a possible interaction between NO_2 and O_3 exposure, though only a single study has evaluated this issue.

5.10.2 QT Interval Duration

Electrocardiograms (ECGs) record the heart's electrical activity over time using surface electrodes. ECG readings consist of three main components: the P wave, QRS complex, and the T wave, each representing different steps the cardiac cycle. The QT interval measures the total time from ventricular depolarization to repolarization. Prolongation and increased variability of the QT interval are associated with a higher risk of life-threatening arrhythmias. Epidemiologic, controlled human exposure,

and animal toxicologic studies have evaluated the impact of short-term oxides of nitrogen exposure on QT interval duration.

5.10.2.1 Epidemiologic Studies of QT Interval Duration

The evidence from the 2016 Oxides of Nitrogen ISA did not clearly indicate an association between short-term NO_2 exposure and changes to markers of ventricular repolarization. In an analysis of the Normative Aging Study, which includes predominantly older generally White male participants, Baja et al. (2010) reported a positive, but imprecise, association between the 10-hour moving average NO_2 concentration and heart-rate-corrected QT interval (QTc) (2.56 millisecond [msec] increase in mean QTc [95% CI: -0.88, 6.00] per 13 ppb increase in NO_2). Another study reported that NO_2 concentrations six to 11 hours prior to measurement were positively associated with QTc duration, although the confidence intervals were wide (4.47 msec [95% CI: 1.02, 7.93] per 17.2 μg/m^3) (Henneberger et al., 2005). When 6-hour moving average NO_2 concentrations were considered as the exposure, there was no association with QTc duration. The evidence base remains unchanged, with the 2016 Oxides of Nitrogen ISA continues to reflect the latest available evidence. No recent studies were identified indicating an association between short-term NO_2 concentrations with QTc interval changes. Section 5.10.2.1.1 below provides additional discussion of the lifestages and populations evaluated by Baja et al. (2010), and Section 5.10.2.1.2 discusses conclusions and remaining uncertainties from the available evidence.

5.10.2.1.1 Lifestages and Populations

In 580 participants from the Veterans Affairs Normative Aging Study, Baja et al. (2010) examined effect modification of associations between short-term NO_2 concentrations and changes to QTc interval by presence of diabetes, obesity, or smoking history. The authors reported that the association between 10-hour NO_2 exposure and mean QTc interval varied slightly by diabetic status, obesity status, and smoking history. Slightly larger positive associations between a 13 ppb increase in 10-hour NO_2 exposure and mean QTc interval were reported in diabetics compared to nondiabetics (diabetics: 7.10 msec increase [95% CI: -0.12, 14.32], non-diabetics: 1.22 msec increase [95% CI: -2.67, 5.12]), obese individuals compared to nonobese (obese: 4.02 msec increase [95% CI: -3.01, 11.04], non-obese: 2.28 msec increase [95% CI: -1.65, 6.21]), and in never smokers compared to ever smokers (never smoker: 4.18 msec increase [95% CI: -2.41, 10.77], ever smoker: 1.97 msec increase [95% CI: -2.02, 5.95]). The authors also stratified by genetic variation that can impact response to oxidative stress. Those with a high genetic susceptibility to oxidative stress had larger positive associations between 10-hour cumulative NO_2 and mean QTc interval than those with a low genetic susceptibility (high genetic susceptibility: 3.50 msec increase [95% CI: -1.86, 8.87], low genetic susceptibility: 0.29 msec increase [95% CI: -4.56, 5.14]). The reported estimates of modification are modest and require replication in additional populations.

5.10.2.1.2 Conclusions and Remaining Uncertainties and Data Gaps

The existing epidemiologic evidence of effects of NO_2 on QT interval is limited to a couple of studies and is not sufficient to draw broader conclusions on the potential effect of NO_2 exposure.

5.10.2.2 Controlled Human Exposure Studies of QT Interval Duration

While no recent studies looked at the effect of short-term NO_2 exposure on QT interval duration or variability, one study evaluated in the 2016 Oxides of Nitrogen ISA reported small decreases in QTc at one and 18 hours following a 2-hour exposure to 500 ppb NO_2 in healthy, exercising adults (Huang et al., 2012b). This trend is the opposite of the elongation of QT interval that is associated with ventricular arrhythmias. Exposure to NO_2 also caused a decrease in QT variability index. Co-exposure of subjects to both $PM_{2.5}$ and NO_2 resulted in increased QT variability.

5.10.2.2.1 Conclusions and Remaining Uncertainties

The existing controlled human exposure evidence of effects of NO_2 on QT interval is limited to a single study and is not sufficient to draw broader conclusions on the potential effect of NO_2 exposure.

5.10.2.3 Animal Toxicological Studies of QT Interval Duration

The 2016 Oxides of Nitrogen ISA did not evaluate any animal toxicological studies that measured the effects of NO_2 exposure on QT interval or QT variability. A recent study by Farraj et al. (2016) used implanted telemeters to measure the impact of NO_2 exposure alone or in sequence with O_3 on heart electrophysiology. The study exposed spontaneously hypertensive rats to NO_2 (500 ppb) or filtered air for three hours in the morning and then O_3 (300 ppb) or filtered air for three hours in the afternoon. The authors reported that exposure to NO_2 in the morning followed by filtered air in the afternoon did not impact the HR (RR interval), PR interval (time between atrial and ventricular depolarization) or QTc interval although there was a non-significant increase in QTc in the NO_2-Air group (see Appendix A – Appendix Table 5-12). A separate group of rats were challenged with aconitine 24 hours after the final exposure to induce arrythmia. The authors reported that NO_2 exposure alone reduced the concentration of aconitine needed to induce ventricular premature beats, ventricular tachycardia, and cardiac arrest. Exposure to NO_2 in the morning followed by O_3 in the afternoon resulted in statistically significant decrease in HR, increase in PR interval and QTc, and reduced threshold for aconitine-induced ventricular premature beats, ventricular tachycardia, ventricular fibrillation, and cardiac arrest suggesting that NO_2 exposure can prime the heart to subsequent arrhythmia.

5.10.2.3.1 Conclusions and Remaining Uncertainties

The existing animal toxicologic evidence of effects of NO_2 on QT interval is limited to a single study and is not sufficient to draw broader conclusions on the potential effect of NO_2 exposure.

5.10.3 Vascular Reactivity and Arterial Stiffness

Vascular reactivity refers to the ability of blood vessels to respond to various stimuli by adjusting vascular tone, which is crucial for maintaining blood flow and blood pressure regulation. The endothelium, a single layer of cells lining the blood vessels, plays a pivotal role in vascular reactivity. Endothelial dysfunction is characterized by an imbalance of vasodilators (e.g., nitric oxide) and vasoconstrictors (e.g., endothlin-1). High BP is often the result of an imbalance of these factors that leads to greater vasoconstriction. In a similar manner, increased stiffness in the vasculature can contribute to increased blood pressure. Arterial stiffness can be measured through markers of artery calcification, tonometry measures of arterial stiffness, and imaging of intima media thickness. Epidemiologic, controlled human exposure, and animal toxicologic studies have investigated the potential relationship between short-term oxides of nitrogen exposure and vascular reactivity and arterial stiffness.

5.10.3.1 Epidemiologic Studies of Vascular Reactivity and Arterial Stiffness

The 2016 Oxides of Nitrogen ISA evaluated a limited number of epidemiologic studies that examined associations between short-term NO_2 exposures and markers of vascular reactivity. These studies reported inconsistent findings. Increased personal NO_2 exposure on lag day 1 was associated with increased brachial artery diameter but the association became negative with lag 2 (Williams et al., 2012). Personal NO_2 exposure was also associated with increased flow mediated dilation. No associations were reported when ambient measures of NO_2 exposure were used. A study by (Ljungman et al., 2014) reported no consistent associations between 1-, 2-, 3-, 5-, and 7-day moving average NO_X exposure and peripheral tonometry ratio in the Framingham Heart Study. Evidence from these recent studies is summarized below, followed by additional discussion of lifestages and populations examined in these studies (see Section 5.10.3.1.1). Section 5.10.3.1.2 discusses conclusions from the epidemiologic evidence and summarizes remaining uncertainties. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 5-10.

Recent studies examining the association of short-term NO_2 exposure and vascular reactivity endpoints show mixed results.

- In a multi-country European panel study (median age = 65 years old), five-day average NO_2 exposure, estimated from monitoring stations and personal exposure samplers, was inversely

associated with distensibility (DC) and compliance (CC) coefficients, which are measures of elasticity related to arterial stiffness. A 10 μg/m^3 increase in NO_2 at fixed site monitors was reported to have a negative association with CC (-2.05 mm^2/kPa [95% CI: -3.54, -0.56]) and a negative association that included the null with DC (-1.58 10^{-3}/kPa [95% CI: -3.28, 0.12]). Similar observations were made with NO_2 measured with personal monitors. A 10 μg/m^3 (5.3 ppb) increase in personal NO_2 was reported to have a negative association with CC (-1.42 mm^2/kPa [95% CI: -2.64, -0.20]) and a negative association with DC that included the null (-1.31 10^{-3}/kPa [95% CI: -2.69, 0.07]) (Scheers et al., 2018).

- Recent Canadian studies in adults 55 years of age and older, a panel study of 70 participants and a randomized control trial of 72 participants, assessed the influence of exercise on air pollution-associated cardiorespiratory effects. These studies reported that NO_2 exposures, measured with fixed site monitors, occurring over the previous two-days were not consistently associated with RHI, a measure of endothelial function (Stieb et al., 2019; Stieb et al., 2018). In another Canadian study in younger adults (mean age = 24 years old), Biel et al. (2020) conducted a panel study of 46 non-smoking adults in Toronto with the aim of distinguishing the effects of noise and air pollution on subclinical cardiovascular endpoints including endothelial function. No discernable pattern of association between 24-hour regional NO_2 exposure (estimated from fixed site monitors) and RHI was reported, regardless of noise adjustment.
- A U.S. panel study of 62 participants (mean age = 38 years old) assessed the potential modulating effect of supplementation of omega-3 PUFA. For participants with high omega-3 intake, NO_2 exposures (lag 1-, lag 2-, lag 3-, lag 4-, 5-day moving average [5dMA]) were associated with higher flow-mediated dilation (FMD) and lower ET-1, indicators of improved vascular function (Chen et al., 2021). Among participants with low omega-3 intake, NO_2 exposure (5dMA) was associated with lower ET-1.
- In a study of Boston area residents (mean age = 63.9 years old) with Type 2 diabetes, baseline brachial artery diameter was negatively associated with 5-day mean NO_2 concentrations, although associations were imprecise (Zanobetti et al., 2014). Another study of older adults (mean age = 74.9 years old) in the Los Angeles, CA, U.S., metropolitan area reported an inverse association between 1-, 3-, 5, and 7-day average ambient NO_X concentration and RHI, a measure of endothelial reactivity (Zhang et al., 2016b). However, no association was reported between 7-day personal NO_X values and RHI.

Recent studies reporting associations with measures of arterial stiffness are mixed.

- A U.S. study of the Framingham Heart Study Offspring and Third Generation cohort (mean age = 51 years old) reported mixed associations between short-term oxides of nitrogen exposure and mean arterial pressure, carotid femoral pulse wave velocity, augmentation index, and forward pressure wave amplitude (Ljungman et al., 2018). The authors reported negative associations between short-term oxides of nitrogen exposure (one to 14 moving day averages) and carotid femoral pulse wave velocity and mean arterial pressure. However, no associations were reported between short-term oxides of nitrogen exposure and the augmentation index or forward pressure wave amplitude.
- A European study of healthy older adults (median age = 65 years old) by Scheers et al. (2018) estimated 24-hour NO_2 exposure from fixed site monitors and personal monitors and both exposure assessment methods yielded similar results. Authors reported an inverse association between short-term ambient NO_2 exposure and carotid artery compliance (-2.05 mm^2/kPa [95% CI: -3.54, -0.56]), suggestive of increased arterial stiffness. However, no associations

were observed with other measures of arterial stiffness (carotid distensibility, pulse wave velocity, or Young's Elastic Modulus).

- A study in the U.K. National Cohort Study of Idiopathic and Heritable Pulmonary Arterial Hypertension cohort (mean age = 51 years old) from 2014 to 2018 reported no association between 24-hour NO_2 exposure (lags 0 to 14 days) and pulmonary vascular resistance, mean pulmonary arterial pressure, cardiac index, or right arterial pressure (Sofianopoulou et al., 2019).

5.10.3.1.1 Lifestages and Populations

Recent studies have investigated the association of short-term NO_2 concentrations with measures of vascular reactivity and arterial stiffness in several populations at increased risk including older adults (59 years of age and older) (Stieb et al., 2019; Scheers et al., 2018; Stieb et al., 2018; Zhang et al., 2016b), Type-2 diabetes (Zanobetti et al., 2014), and idiopathic or heritable pulmonary arterial hypertension (Sofianopoulou et al., 2019). None of these studies compared how observations in these potentially at-risk populations compared to control populations.

5.10.3.1.2 Conclusions and Remaining Uncertainties

The overall evidence base for evaluating the association between oxides of nitrogen and indices of vascular reactivity and arterial stiffness remains limited, though recent studies provide some support for an inverse relationship with vascular reactivity. Heterogeneity in the indices of vascular reactivity and small study populations likely contributed to the inconsistent results. Additional studies are needed to assess the potential influence of traffic-related copollutants on the independent association between NO_2 exposure and vascular reactivity.

5.10.3.2 Controlled Human Exposure Studies of Vascular Reactivity and Arterial Stiffness

The 2016 Oxides of Nitrogen ISA evaluated one controlled human exposure study that looked at the impact of NO_2 exposure on vascular endothelial tone in response to multiple vasodilators. Langrish et al. (2010) exposed 10 healthy males to either filtered air or 4 ppm NO_2 for 1-hour with intermittent exercise in a crossover design. Four hours after the end of exposure, forearm blood flow was measured in response to infusion with the endothelial-dependent vasodilators bradykinin and acetylcholine and the endothelial-independent vasodilators sodium nitroprusside, and verapamil. The authors reported that the changes in blood flow in response to each of the vasodilators were not significantly different following NO_2 exposure compared to after air exposure. There are no recent controlled human exposure studies since the 2016 Oxides of Nitrogen ISA. Thus, the evidence remains very limited and suggests no impact of NO_2 exposure on vascular reactivity.

5.10.3.3 Animal Toxicological Studies of Vascular Reactivity and Arterial Stiffness

The 2016 Oxides of Nitrogen ISA did not evaluate any animal toxicologic studies related to NO_2 exposure and vascular reactivity. Since the completion of the 2016 Oxides of Nitrogen ISA, a recent study investigated the impact of a single or repeated NO_2 exposure on vascular reactivity. Karoui et al. (2020) exposed Wistar rats to either clean air of 5 ppm NO_2 for three hours. Vascular reactivity of the septal coronary artery was measured 1-hour post exposure with treatment with acetylcholine or nitroprusside. The study reported no difference in relaxation in response to acetylcholine in arteries of rats exposed to NO_2 compared to air controls. In another arm of the study, rats were exposed to 5 ppm NO_2 for three hours per day over three weeks. Repeat exposure to NO_2 impaired acetylcholine-induced relaxation with no effect of nitroprusside-induced relaxation. Acetylcholine vasorelaxation is mediated through actions on the endothelium whereas nitroprusside acts directly on the vascular smooth muscle, thus, these data suggest that exposure to NO_2 for three weeks alters the ability of the endothelium to trigger vasorelaxation but does not compromise the ability of the smooth muscle to respond to pro-relaxation stimuli. The onset of vasoreactivity changes with repeat exposure to NO_2 in this study corresponded to worsening cardiac functional measurements which were generally small and transient after one day of NO_2 exposure but were greater in magnitude and more prolonged following repeat exposures (Karoui et al., 2020) (see Appendix A – Appendix Table 5-13).

5.10.3.3.1 Conclusions and Remaining Uncertainties

The animal toxicologic database for NO_2-induced effects on vasoreactivity is very limited in number and requires further study to validate and expand on the findings presented. The one available study suggests that repeated NO_2 exposure could alter the ability of the endothelium to trigger vasorelaxation, though considerable uncertainty remains regarding the dose-response relationship and the replicability of this finding.

5.10.4 Blood and Other Markers of Cardiovascular Effects

Recent epidemiologic studies have explored the potential relationship between short-term oxides of nitrogen exposure and blood markers of cardiovascular risk. In particular, markers of inflammation, cell adhesion, coagulation, and thrombosis have been evaluated in a number of epidemiologic studies published since the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016). These blood markers also have been examined in controlled human exposure and animal toxicological studies.

5.10.4.1 Epidemiologic Studies of Blood and Other Markers of Cardiovascular Effects

The studies evaluated in the 2016 Oxides of Nitrogen ISA provided evidence of associations between short-term oxides of nitrogen exposure and several blood markers relevant to cardiovascular changes. These changes were reported predominantly in populations with a history of heart disease [see Table 5-49 in U.S. EPA (2016)]. The bullets below summarize information from studies included in the 2016 Oxides of Nitrogen ISA.

- In populations with a history of heart disease, outdoor NO_2 and NO_X concentrations were associated with some but not all inflammatory mediators (Wittkopp et al., 2013; Delfino et al., 2010; Delfino et al., 2009; Ljungman et al., 2009; Delfino et al., 2008).
- Some studies of patients with heart failure generally did not report associations between NO_2 concentrations and blood markers of inflammation, coagulation, hemoglobin, cellular changes indicative of systemic inflammation, or a general marker of cardiac functional status (Barclay et al., 2009; Wellenius et al., 2007).
- A German study reported mixed associations between short-term NO and NO_2 exposure and the vascular inflammation marker lipoprotein-associated phospholipase A2 (Lp-PLA2) (Brüske et al., 2011). Increases in 24-hour NO were reported to be associated with decreased Lp-PLA2 at lag 0. For short-term NO_2 exposure, negative association was reported at lag 2, while positive associations were reported at lags 4 and 5.
- Studies of healthy populations have generally reported associations between NO_2 or NO and decreases of some white blood cell types (Steenhof et al., 2014), increased inflammatory markers (Ren et al., 2011), and increased clotting-related factors (Strak et al., 2013; Zhang et al., 2013; Bind et al., 2012; Rich et al., 2012; Rudez et al., 2009). Despite this, no one individual marker seemed to be consistently measured or consistently associated with NO_2 in multiple studies and some studies reported no changes to clotting or inflammatory-related markers.
- Some studies reported positive associations of NO_2 with urinary 8-hydroxy-29-deoxyguanosine (8-OhdG) concentrations, a marker of oxidative stress resulting in deoxyribonucleic acid (DNA) damage (Ren et al., 2011), malondialdehyde, oxidized-LDL, conjugated diene (Kelishadi et al., 2009), and decreased antioxidant enzyme activity (Delfino et al., 2008).

Recent studies report additional mixed evidence for associations between short-term NO_2 or NO_X exposures and the increase of inflammatory or clotting markers in the blood. Recent studies are summarized below, followed by additional discussion of the lifestages and populations evaluated in these studies (see Section 5.10.4.1.1) and the potential impacts of copollutants on associations (see Section 5.10.4.1.2). Section 5.10.4.1.3 discusses conclusions from these studies and summarizes remaining uncertainties. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 5-10.

- In a study of 97 older (>65 year olds) non-smokers in the Los Angeles, CA, U.S., area from 2012 to 2014, NO_X exposure was estimated using personal and ambient NO_X exposure metrics from the South Coast Air Quality Management District monitoring stations (Zhang et al., 2016a). The authors reported that 24-hour ambient NO_X exposure was associated with

increased interleukin 6 (IL-6) (0.049% [95% CI: 0.005, 0.094]). Longer NO_X exposure averaging times ranging three to seven days were not reported to be associated with IL-6. Short-term NO_X exposure (one to seven day averages) were not reported to be associated with changes in changes in blood oxidized low density lipid (LDL) (Zhang et al., 2016a).

- In a study of school age children (six to eight year olds) in the Fresno, CA, U.S., authors reported an inverse relationship between short-term NO_2 and NO_X exposure and high-density lipoprotein (HDL) concentrations. HDL was negatively associated with both 1-day (-10.85 mg/dL [95% CI: -17.87, -3.83] per 20 ppb NO_2) and 1-week average (-9.11 mg/dL [95% CI: -17.13, -1.09] per 20 ppb NO_2) NO_2 exposure. HDL was also negatively associated with 1-week NO_X exposure (-9.40 mg/dL [95% CI: -17.80, -7.01] per 30 ppb NO_X) (Zhang et al., 2022).
- In a study of adolescents (14 to 18 year olds) in Fresno Asthmatic Children's Environment Study (FACES) cohort, Prunicki et al. (2020) used biomarker data and air quality information to report biomarkers that were associated with elevated pollutant exposures (over seven days before visit) using a partial least squares analysis. The study reported that increased 7-day average NO_2 exposures were associated with biomarkers of inflammation and oxidative stress, including myeloperoxidase, soluble vascular cell adhesion molecule-1 (VCAM-1), percentage of peripheral blood mononuclear cell (PBMC) Th1 t helper cells, and percentage of PBMC Th2 t helper cells. Increased 7-day average ambient NO was associated with increased myeloperoxidase, percentage of PBMC monocytes, RANTES, IL-7 and percentage of T reg cells and 7-day average NO concentrations were associated with reduced MCP-1 and VEGF-D.
- In a study of older adults (mean age = 63 years old) who were participants of the MESA cohort, 8-day average NO_2 exposure was positively associated with soluble LOX-1 (sLOX-1), but not associated with plasma nitrite concentrations. The association with sLOX-1 was positive but imprecise in the fully adjusted model (30.28 pg/mL [95% CI: -6.15, 66.71) (Ni et al., 2021).
- Recent studies included participants from the Framingham Offspring cohort and assessed the association between short-term NO_X exposure and changes in osteoprotegrin and myeloperoxidase, which contribute to tissue damage during inflammation and are considered to be related to the promotion of atherosclerosis (Li et al., 2019; Li et al., 2016). Li et al. (2019) reported a negative association between 5-day moving average NO_X and osteoprotegrin (-3.00 % [95% CI: -5.40, -0.60]) a protein whose increase is a marker for increased atherosclerotic plaque calcification. This association was reported to be robust to co-adjustment for ozone exposure. Li et al. (2016) reported no association between NO_X exposures (moving averages over one to seven days) and blood myeloperoxidase concentrations.
- In 24 healthy participants (mean age = 38 years old), Chen et al. (2022a) reported that daily average NO_2 exposure was not associated with plasma inflammation factors including C-Reactive Protein (CRP), interleukin 8 (IL-8), and TNFα.
- In a U.S. panel study of 62 participants assessing the potential modulating effect of supplementation of omega-3 PUFA, NO_2 exposure was associated with lowered HDL (lag 1-, 2-, 5-day moving average), LDL (5-day moving average), and total cholesterol (lag 2) but increased markers of the coagulation protein von Willebrand factor (lag 1, 5 dMA) in participants with high omega-3 intake (Chen et al., 2021). Participants with low omega-3 intake also exhibited positive associations between NO_2 concentrations and the clotting factors von Willebrand factor and D-dimer at lag 0 but associations with blood cholesterol,

HDL, and LDL were not statistically significant with 95% confidence intervals including the null.

- A study in school age children (six to eight years of age) reported a positive association between 1-day, 1-week, and 1-month average NO_2 concentrations and urinary, creatinine-adjusted, 8-isoprostane (Mann et al., 2021). Another study in school age children (eight to 10 years of age) reported no associations between creatinine-adjusted 8-isoprostane concentrations with short-term (1-week to 1-month) measures of NO_2 or NO_X in children (Zhang et al., 2022). In this study, some negative associations were seen with longer averaging times (three to six months).
- In Recent Canadian studies, a panel study of 70 older participants and an RCT of 72 participants that were both assessing the influence of exercise on air pollution-associated cardiorespiratory effects, reported NO_2 exposures occurring over the prior two days were not consistently associated with a urinary oxidative stress marker (Stieb et al., 2019; Stieb et al., 2018).
- A recent study in school age children (six to 11 years of age) examined the association between ambient NO_2 exposure and plasmatic protein levels. Hepatocyte growth factor, a marker of endothelial cell injury, was positively associated (log_2-fold change: 2.00 [95% CI: 0.78, 3.22] per 20 ppb NO_2) with 1-week home NO_2 concentrations for children in the HELIX project (6 countries around Europe) (de Prado-Bert et al., 2022).

5.10.4.1.1 Lifestages and Populations

The 2016 Oxides of Nitrogen ISA reported that levels of some circulating systemic inflammatory markers appeared to be related to short-term NO_2 concentrations in populations with a history of heart disease [see Section 5.3.10.4 of U.S. EPA (2016)]. Positive associations between NO_2 concentrations and systemic inflammatory markers were also seen in studies of patients with COPD and type 2 diabetes. Results were more heterogeneous for people without a history of heart disease. Recent studies have investigated the association of short-term NO_2 concentrations with measures of blood or other cardiovascular risk markers in several populations including older adults (Ni et al., 2021; Stieb et al., 2019; Stieb et al., 2018; Chi et al., 2016; Zhang et al., 2016a) or children and adolescents (de Prado-Bert et al., 2022; Zhang et al., 2022; Mann et al., 2021; Prunicki et al., 2020) with generally positive associations with NO_2.

5.10.4.1.2 Copollutants

Epidemiologic studies previously reviewed in the 2016 Oxides of Nitrogen ISA reported that some positive associations between NO_2 exposure and blood markers were robust to correction for other confounding pollutants. For example, positive associations between concentrations of NO_2 and P-selectin, a marker of endothelial dysfunction, or fibrinogen remained positive in models controlling for $PM_{2.5}$, CO, O_3, or SO_2 (Zhang et al., 2013; Rich et al., 2012). In a recently evaluated study, the associations between

short-term exposure to NO_2 and the biomarkers were robust in the two-air pollutant model after adjustment for simultaneous exposure $PM_{2.5}$ or O_3 (Chen et al., 2021).

5.10.4.1.3 Conclusions and Remaining Uncertainties

Epidemiologic evidence has investigated the associations between short term exposure to oxides of nitrogen (i.e. NO_2, NO, or NO_X) and blood markers of inflammation, oxidative stress, and clotting responses with outcomes related to inflammation and oxidative stress being the most common. Studies looking at associations between short-term NO_2 exposures and inflammatory markers was mixed with some studies showing statistically significant positive associations with individual inflammatory markers, however, coherent trends across multiple inflammatory measures within studies was not reported and no one inflammatory measure appeared consistently associated with short-term NO_2 exposure across studies. Epidemiologic associations between short-term NO_2 exposures and markers of oxidative stress were similarly mixed with some studies showing positive associations with markers like blood myeloperoxidase or 8-isporostane but other studies finding no association. Fewer studies have looked at clotting-related factors and the associations with short-term NO_2 exposures, but the existing studies do provide some evidence for positive associations with clotting factors however the results are not consistent across studies. Negative associations between NO_2 exposures and HDL have been reported in a limited number of epidemiology studies but changes in other cardiovascular-related lipid markers (LDL, total cholesterol) are not seen.

Associations between short-term NO and inflammation and oxidative stress are mixed with some evidence for positive associations with specific inflammatory or oxidative stress markers in individual studies but not with others. Like with NO_2, no single marker appeared consistently associated with short-term NO across multiple studies. The limited number of studies looking at circulating lipid changes and clotting factors prevents making judgements on the overall associations with short-term NO exposures.

Epidemiology studies of short-term NO_X exposures and cardiovascular related blood markers is limited in number. Studies related to inflammatory markers and oxidative stress show mostly no association with NO_X exposures. The limited number of studies looking at circulating lipid changes and clotting factors prevents making judgements on the overall associations with short-term NO_X exposures.

Overall, the evidence for associations between short-term oxides of nitrogen and blood or other markers of inflammation is relatively inconsistent with most studies focused on oxidative stress and inflammatory markers related to NO_2. No individual endpoint measure appeared to be consistency associated with NO_2 (or NO or NO_X) exposure across multiple studies. It is important to note that some papers showed null associations with NO_X as opposed to NO_2, suggesting that NO_2 may have different effects in a mixture with other oxides of nitrogen such as NO, and measures of NO_X, which include NO_2 and NO, may potentially mask the associations due to either pollutant alone. Small study populations and heterogeneity in the biomarkers of cardiovascular effects likely also contributed to the inconsistent

results. Additional studies in larger study populations are needed to assess the association between short-term exposures to NO_2 and these blood biomarkers and the potential influence of traffic-related copollutants. These studies relied upon central site monitors instead of individual monitoring to assess short-term exposures to oxides of nitrogen. Therefore, the extent to which concentrations at central site monitors captured the spatial and temporal variation in NO_2 concentrations in ambient air across the study area continues to be a source of uncertainty.

5.10.4.2 Controlled Human Exposure Studies of Blood and Other Markers of Cardiovascular Effects

Controlled human exposure studies have investigated several subclinical markers related to cardiovascular endpoints including inflammatory and oxidative stress markers in serum, changes in blood cell populations, and alterations in coagulation factors and fibrinolytic markers. The following sections summarize the evidence available for subclinical markers that may underlie development of cardiovascular disease or contribute to cardiovascular events investigated in epidemiologic studies.

Inflammatory and oxidative stress markers/endothelial activation

The 2016 Oxides of Nitrogen ISA described a few studies that investigated various markers of systemic inflammation, and although some inflammatory markers were shown to increase following NO_2 exposure in some studies, the results were not consistent across studies. Systemic inflammation is often indicated by increases in inflammatory cytokine and chemokine concentrations in blood plasma or serum, increases in endothelial adhesion markers, which promote the recruitment of circulating immune cells to the site of inflammation, and shifts in the number or proportion of circulating immune cells. The evidence for increases in inflammatory signaling are summarized below and results are in Appendix A – Appendix Table 5-14:

- Exposure to 500 ppb NO_2 for two hours with intermittent exercise did not alter IL-8 or IL-6 concentrations (Huang et al., 2012b).
- Primary human endothelial cells treated with plasma from volunteers exposed to 500 ppb NO_2 for two hours showed greater induction of mRNA for endothelial adhesion markers and IL-8 compared to treatment with plasma from air exposed volunteers (Channell et al., 2012). Plasma from NO_2-exposed volunteers had higher concentrations of soluble lectin-like oxLDL receptor (LOX-1) concentrations, a protein recently found to play a role in the pathogenesis of atherosclerosis.
- Exposure of healthy volunteers or individuals with asthma to 350 ppb NO_2 for two hours with exercise did not result in changes in intercellular adhesion molecule 1 (ICAM-1) or IL-6 in the blood the morning after exposure (Riedl et al., 2012).
- Changes in the number or percentage of circulating blood cells can be an indicator of systemic response to inflammation. Some studies showed changes in circulating blood cell subsets following NO_2 exposure for two or six hours (Frampton et al., 2002; Azadniv et al., 1998) while another study reported no changes in blood cell subsets following two hours of

NO_2 (Huang et al., 2012b). Repeated exposures to 2 ppm NO_2 for four hours per day for three or four days did not result in changes in blood cell subsets (Solomon et al., 2000; Blomberg et al., 1999).

- A recent study showed that exposure to 100, 500, and 1,500 ppb NO_2 for three hours in healthy, exercising adults did not result in increases in blood IL-6, IL-8, or endothelin-1. Similarly, there were not changes in the number or proportion of circulating blood immune cells (Brand et al., 2016).

Together, the controlled human exposure data provides little support for increased markers of systemic inflammation following NO_2 exposure.

Markers of coagulation and disruption of clot breakdown

Formation of blood clots (thrombi) can block blood vessels, contributing to a number of cardiovascular diseases including myocardial infarction, stroke, and deep vein thrombosis. Clot formation is regulated by a balance between pro-coagulation factors that promote clot formation and anticoagulation factors that breakdown clots. Exaggerated or ectopic clot formation (e.g., after a ruptured atherosclerotic plaque) can cause vessel occlusion. Experimental studies generally measure changes in clotting factors or activity of enzymes involved in activation of clotting factors or anticoagulation. While there are no recent studies related to short-term oxides of nitrogen exposure and coagulation, the controlled human exposure studies evaluated in the 2016 Oxides of Nitrogen ISA showed little effect of NO_2 exposure on clotting or fibrinolytic factors. A summary of the findings of these studies is provided below:

- Riedl et al. (2012) reported that exposure of people with mild asthma to 350 ppb NO_2 for two hours with exercise did not impact levels of clotting factors (factor VII, fibrinogen and von Willebrand factor) the morning after exposure.
- Similarly, concentrations of tissue-plasminogen activator and plasminogen activator inhibitor Type I were unchanged at baseline or four hours after exposure to 4,000 ppb NO_2 (Langrish et al., 2010).

Overall, the controlled human exposure data does not provide evidence that short term NO_2 leads to increased markers of coagulation.

Lipid profile changes

Atherosclerosis is related to changes in lipid metabolism and trafficking. Thus, some controlled human exposure studies have investigated the impact of NO_2 exposure on lipid profiles in the blood. One study evaluated in the 2016 Oxides of Nitrogen ISA showed changes in blood lipid profiles. Huang et al. (2012b) reported increases on blood cholesterol (4.1%) and HDL cholesterol (5.9%) 18 hours after exposure to 500 ppb NO_2 for two hours but observed no changes in LDL or vLDL cholesterol or triglycerides. No recent controlled human exposure studies were available looking at changes in blood lipids in response to NO_2 exposure.

Effects on hematocrit and hemoglobin

The 2016 Oxides of Nitrogen ISA described controlled human exposure studies that investigated the effect of NO_2 exposure on hematocrit and hemoglobin. No recent publications of controlled human exposure to NO_2 add to the evidence on hematologic effects resulting from exposure to short-term NO_2. A summary of the findings from previously reviewed studies are as follows:

- Exposure to 600 ppb and 1,500 ppb NO_2 for three hours in healthy volunteers with exercise resulted in decreased hematocrit, hemoglobin, and RBC count three and a half hours after exposure (Frampton et al., 2002).
- Hematocrit and hemoglobin levels were decreased in healthy young males exposed to 1,000 ppb of 2,000 ppb NO_2 for approximately two and a half hours with exercise (Posin et al., 1978).
- No change in hemoglobin levels were seen at four or six hours after a 1-hour exposure to 4,000 ppb NO_2 (Langrish et al., 2010).

5.10.4.2.1 Conclusions and Remaining Uncertainties

Taken together, controlled human exposure studies provide limited and mixed evidence that short-term oxides of nitrogen exposure increase systemic inflammation, no support for increased oxidative stress, and no indication that exposure alters lipid profiles to increase cardiovascular risk. There is limited evidence of acute decreases in hematocrit and hemoglobin levels following oxides of nitrogen exposure. While decreases in hematocrit and hemoglobin may have implications for populations with lower baseline blood oxygenation (e.g., people with COPD), the clinical significance of the reported effects is not well understood. There is also a lack of information regarding the cumulative effects of multiple exposures that could help to understand the clinical significance of the findings.

5.10.4.3 Animal Toxicological Studies of Blood and Other Markers of Cardiovascular Effects

Similar to controlled human exposure studies (see Section 5.10.4.2), animal studies can investigate underlying signaling events and subclinical biomarkers that correspond to the development of cardiovascular disease (see Appendix A – Appendix Table 5-15). The following sections outline the animal toxicologic data related to subclinical markers of cardiovascular disease.

Inflammatory and oxidative stress markers

The 2008 Oxides of Nitrogen ISA and 2016 Oxides of Nitrogen ISA evaluated several animal studies that looked at markers of inflammation, oxidative stress, and endothelial activation in response to NO_2 exposure. A summary of the previously reviewed studies is below:

- Exposure to a high concentration of NO_2 (5,320 ppb) for six hours/day over seven days led to increases in TNF mRNA in heart tissue and increased IL-1 gene expression and protein levels in rats (Li et al., 2011).
- Rats exposed to 2,660 or 5,320 ppb NO_2 for seven days had small but significant decreases in Cu/Zn-SOD activity which corresponded to increased malondialdehyde (an indicator of lipid peroxidation) at the higher exposure concentration. There was no change in MN-SOD or GPx activity and no change in protein carbonyl levels at either exposure concentration (Li et al., 2011).
- Apolipoprotein E knockout mice exposed six hours a day for seven days to 200 ppb or 2,000 ppb NO_2 showed a dose dependent decrease in heme oxygenase enzyme in the aorta (Campen et al., 2010).
- Dietary restriction of selenium (an integral component of antioxidant enzymes) led to decreases in plasma SOD activity following 5,000 ppb NO_2 for five days or 50,000 ppb for 30 minutes, but animals were protected against the effects of NO_2 exposure with a diet high in selenium (de Burbure et al., 2007).
- NO_2 exposure increased concentrations of the vasoconstrictor endothelin-1 at high exposure concentrations (2,660 or 5,320 ppb for seven days) (Li et al., 2011) but not at lower concentrations (200 ppb or 2,000 ppb for seven days) (Campen et al., 2010). Protein and RNA concentrations of the vasorelaxation-inducing enzyme eNOS and the endothelial adhesion marker ICAM-1 were increased following 7-day exposure to 2,660 ppb and 5,320 ppb but not at 10,640 ppb NO_2 exposure (Li et al., 2011).

Recent studies looked at markers of oxidative stress in animal models of NO_2 exposure.

- Farraj et al. (2016) reported that exposure to 500 ppb NO_2 for three hours resulted in increased total protein in the blood and increased GSH peroxidase activity but did not impact SOD or GSH transferase activity.
- Exposure of rats to 5,000 ppb NO_2 for three hours resulted in no changes in markers of mitochondrial activity and a transient increase in ROS in intrafibrillar, but not subsarcolemmal, mitochondria (Karoui et al., 2020). In the same study, exposure to 5,000 ppb NO_2 for three weeks resulted in statistically significant decreases in mitochondrial activity and more prolonged ROS production in cardiac intrafibrillar mitochondria. The mitochondrial ROS production was inversely correlated with cardiac output.

Together, there is some evidence for NO_2-induced systemic inflammation and oxidative stress, with the stronger evidence supporting oxidative stress.

Lipid profile changes

The 2016 Oxides of Nitrogen ISA did not evaluate any animal studies that looked at changes in blood lipid levels following NO_2 exposure. Recent studies evaluated changes in blood lipid concentrations following NO_2 exposure and the results are summarized below:

- Spontaneously hypertensive rats that were exposed to 500 ppb NO_2 for three hours had a statistically significant reduction in serum HDL cholesterol with no change to triglyceride or LDL cholesterol levels (Farraj et al., 2016).

- Rats exposed to 5,000 ppb NO_2 for two weeks had changes to the serum metabolome that suggested disruption of glycerophospholipid metabolism and choline metabolism that may underlie cardiovascular disease (Li et al., 2022b).

Overall, there remains limited evidence from animal studies to support NO_2 exposure-induced changes to blood lipid profiles and lipid metabolism that would suggest increased cardiovascular risk.

Effects on hematocrit and hemoglobin

The 2008 Oxides of Nitrogen ISA described a few studies that looked at the impact of short-term NO_2 exposure on hematocrit and hemoglobin-related endpoints and reported some evidence for NO_2 driven effects. Study specific details, exposure conditions, and endpoints examined are highlighted in the text below.

- Animal studies suggested an increase in younger RBC populations via increasing D-2,3-diphosphoglycerate and increased sialic acid on peripheral RBCs exposed to NO_2 at either 360 ppb for seven days (Mersch et al., 1973) or 4,000 ppb for 10 days (Kunimoto et al., 1984).
- Exposure of rats to NO_2 at 4,000 ppb for seven days resulted in a decrease in younger, less dense RBCs and a corresponding increase of older, denser, RBC (Mochitate and Miura, 1984).
- Nakajima and Kusumoto (1968) reported that, in mice exposed to 800 ppb NO_2 for five days, the amount of methemoglobin was not increased. Studies of higher exposure concentrations (>10,000 ppb) both in vivo and in vitro tend to show increases in methemoglobin (Gressent et al., 2014; U.S. EPA, 1993; Chiodi et al., 1983).

No recent studies have looked at the impact of short-term NO_2 exposure on hemoglobin or hematocrit. Older studies report contradictory results on RBC populations and provide some evidence for NO_2-induced decreased oxygen carrying capacity, however these effects have only been demonstrated at high NO_2 concentrations.

5.10.4.3.2 Conclusions and Remaining Uncertainties

Taken together, the animal toxicologic literature evaluating short-term NO_2 exposure provides some support for subclinical effects underlying cardiovascular disease, with the strongest support for systemic oxidative stress and inflammation. However, the literature base is limited, especially at ambient-relevant NO_2 concentrations. There remain inherent limitations related to translating findings in animals to those in humans, and changes in subclinical markers do not necessarily reflect certainty of developing CVD or experiencing a cardiovascular event.

5.10.5 Synthesis Across Lines of Evidence

Across lines of evidence, support for subclinical effects underlying cardiovascular disease following short-term exposures to NO_2 or other oxides of nitrogen remains limited and inconsistent. Epidemiologic studies provide some evidence of negative associations between short-term NO_2 exposure and markers of HRV, which is indicative of increased risk of cardiovascular disease and mortality. The epidemiologic evidence base for associations of short term NO_2 exposure and increased HR are mixed. There is limited toxicological evidence from controlled human and animal studies and the available findings do not support NO_2-mediated effects on either HRV or HR. Epidemiologic evidence for associations with NO_2 exposure and changes to QT interval, vascular reactivity, and arterial stiffness are very limited in number and with mixed results. There is a similar lack of controlled human or animal studies to provide experimental support for these subclinical effects. Evidence for changes in other blood markers related to inflammation and oxidative stress comes from a few studies, mostly conducted in animals, but there is inconsistency in the endpoints and markers measured across studies which makes interpreting the clinical importance of the observed results difficult. Without more consistent support from other biomarkers, it is not clear how these general indicators of systemic effects relate specifically to cardiovascular disease risk.

5.11 Cardiovascular Mortality

Studies that examine the association between short-term oxides of nitrogen exposure and cause-specific cardiovascular mortality provide additional evidence for oxides of nitrogen-related cardiovascular effects. This evidence is discussed below.

5.11.1 Epidemiologic Studies of Cardiovascular Mortality

The evidence evaluated in the 2016 Oxides of Nitrogen ISA supported a positive association between short-term increases in NO_2 exposure and cardiovascular mortality outcomes. Recent multicity studies consistently reported positive associations between short-term NO_2 exposure and cardiovascular mortality in Asia (Chen et al., 2013; Chen et al., 2012a; Wong et al., 2008) and Europe (Chiusolo et al., 2011; Bellini et al., 2007); these associations were slightly greater in magnitude than the associations with total mortality. There remains uncertainty about the independent nature of the NO_2 association given the high correlations between NO_2 and other traffic-related pollutants. Studies included in the 2016 Oxides of Nitrogen ISA that estimated potential confounding by copollutants reported positive but slightly attenuated effect estimates in copollutant models after adjusting for PM_{10}, O_3, and SO_2 (Chen et al., 2012a Chen, 2013, 1520807, Chiusolo, 2011, 732546), but copollutants more closely associated with traffic (e.g., $PM_{2.5}$, BC/EC, UFP, OC, VOCs, CO) were not evaluated. Evaluation of a seasonal influence on the NO_2-cardiovascular mortality relationship was limited, with a higher NO_2-related mortality risk reported

in warmer months in studies of European cities (Chiusolo et al., 2011; Bellini et al., 2007) and no observed seasonal difference in risk in a single-city U.S.-based study (Sacks et al., 2012).

Many recent studies further evaluate the relationship between short-term NO_2 exposure and cardiovascular mortality. In contrast to the findings of the 2016 Oxides of Nitrogen ISA, recent evidence indicates generally inconsistent associations between short-term NO_2 exposure and cardiovascular mortality (see Appendix A – Appendix Table 5-16 and Table 5-4). These studies include a wider geographic coverage than previously reported, including multi-country studies across over 300 cities, as well as additional multicity studies conducted across North America, Europe, and Asia. Recent studies also evaluated potential confounding by copollutant exposure, such as $PM_{2.5}$ and O_3.

Recent studies among large patient populations conducted globally (Schwarz et al., 2024; Meng et al., 2021) and in the United States (Liu et al., 2022), Canada (Shin et al., 2023; Shin et al., 2022; Vanos et al., 2014), Europe (Gariazzo et al., 2023; Corso et al., 2020; Stafoggia and Bellander, 2020; Lanzinger et al., 2016; Perez et al., 2015; Milojevic et al., 2014), and Asia (Xu et al., 2022; Kim and Lee, 2021; Li et al., 2021; Li et al., 2020; Yap et al., 2019; Chen et al., 2018) reported inconsistent evidence of an association between short-term NO_2 and cardiovascular mortality. Main findings included null effect estimates, negative and positive effect estimates with 95% CIs containing the null, negative and positive effect estimates with 95% CIs excluding the null. Recent studies included time series and case-crossover analyses, with little variation in model specifications. Models typically included adjustments for temporal trends, temperature, humidity, day-of-week, holidays, and vacation periods using flexible splines and categorical variables.

Evidence from recent studies is summarized in the bullets below, followed by additional discussion of the concentration-response relationships in these studies (see Section 5.11.1.1), the lifestages and populations evaluated (see Section 5.11.1.2), the potential impacts of copollutants on reported associations (see Section 5.11.1.3), exposure assignment approaches evaluated (see Section 5.11.1.4), lag structure (see Section 5.11.1.5), and seasonal and temperature impacts on associations (see Section 5.11.1.6). Section 5.11.1.7 discusses conclusions from these studies and summarizes remaining uncertainties. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 5-16.

- Two global analyses conducted using the Multi-City Multi-Country database reported positive associations between short-term NO_2 exposure and cardiovascular mortality. In a study of 362 cities in 16 countries, Meng et al. (2021) observed a 1.24% (95% CI: 0.50, 1.98) increase in pooled cardiovascular mortality risk per 20 ppb increase in 0- to1-day lag NO_2 concentrations. Positive associations between NO_2 and cardiovascular mortality were also detected at daily lags 1, 2, and 3 and were slightly larger in magnitude when restricted to U.S. cities (percent change per 20 ppb increase in daily lag 1 NO_2 of 1.70% [95% CI: 1.00, 1.98] in the United States compared to 1.40% [95% CI: 0.81, 1.99] globally). Similarly, and in 11 of the same countries, Schwarz et al. (2024) reported a comparable increase in risk of cardiovascular mortality in an analysis of 338 cities across 20 countries between 2009 and 2016 (1.32% [95% CI: 0.68, 1.97%] per 20 ppb increase in previous-day NO_2

concentrations). The authors additionally used a longitudinal meta-regression to pool effect estimates using a linear term for time (i.e., year) to assess potential long-term temporal variation and noted minimal variation in the percent increase in cardiovascular mortality risk associated with NO_2 from 2009 to 2016 compared to 1995 to 2001 (1.32% [95% CI: 0.60, 2.05%]) and 2002 to 2008 (1.40% [95% CI: 0.94, 1.86%]).

- In contrast, a multicity study conducted across seven U.S. states using state-wide mortality data reported a null association between short-term NO_2 exposure and cardiovascular mortality (percent change in risk per 20 ppb increase in lag 0 to 2 NO_2 concentration: -0.26 [95% CI: -0.96, 0.44]) (Liu et al., 2022). This study assigned high-resolution (1 km × 1 km) daily NO_2 concentrations using a hybrid prediction model with an out-of-sample predicted r^2 of 0.79 and leveraged a negative exposure and negative outcome control to account for the potential presence of bias by unmeasured confounding. No association between the negative exposure control (NO_2 concentration day following death) and cardiovascular mortality, suggesting omitted confounding was unlikely.
- Inconsistent associations were reported in studies conducted in Canada using central site monitors to estimate NO_2 exposures. Two time series studies conducted in 24 cities across Canada that examined sex-specific and season-specific associations by warm (April to September) and cold (October to March) seasons reported a mix of negative and positive effect estimates (Shin et al., 2023; Shin et al., 2022). Results varied across season, exposure lag period, and copollutant adjustment (see Sections 5.11.1.3, 5.11.1.5, and 5.11.1.6 for further details). Other recent Canadian studies reported positive associations between NO_2 and cardiovascular mortality. A season-stratified time series analysis conducted in 10 Canadian cities reported increased risks of cardiovascular mortality from lag 0 to 5 NO_2 exposure, with no differences observed by season (Vanos et al., 2014).
- Studies in European populations with similar exposure distributions used a mix of monitoring and modeled NO_2 data to estimate exposures and reported inconsistent findings. A study conducted in France in an area covering 15 million inhabitants used central site monitors and reported positive associations between short-term NO_2 exposure and cardiovascular mortality with an increased risk per 20 ppb NO_2 of 4.98% (95% CI: 2.03, 8.01) at lag 0 to 1 (Corso et al., 2020). At lag 0 to 7, the estimate was less precise and contained the null, 11.77% (95% CI: -1.59, 26.94). Other European studies using central and fixed-site monitors (Lanzinger et al., 2016; Perez et al., 2015; Milojevic et al., 2014), a random forest model (Stafoggia and Bellander, 2020), and a hybrid prediction model (Gariazzo et al., 2023) reported a mix of positive and negative associations across different exposure lag periods, copollutant adjustment criteria, and seasons, with 95% CIs containing the null. Given the range of results reported across these different study designs and NO_2 exposure assessments, the drivers of inconsistencies in the directionality and magnitude of associations between short-term NO_2 and cardiovascular mortality remain unclear.
- Studies conducted among populations in China using a hybrid prediction model (Xu et al., 2022) and central site monitors (Li et al., 2021; Li et al., 2020; Chen et al., 2018) reported consistently positive associations across varied exposure lag periods and copollutant adjustment, with some attenuation detected with copollutant adjustment (see Section 5.11.1.3). A study of all cardiovascular mortality and cause-specific endpoints (Chen et al., 2018) observed increased risk per 5.31 ppb NO_2 of 0.9% (95% CI: 0.7, 1.2) for lag 0 to 1 NO_2 exposure across total cardiovascular mortality. Stratified analyses in (Li et al., 2021) and (Chen et al., 2018) also provide evidence for effect measure modification by age, sex, and education level. However, other studies in Asia using central site monitors, including studies conducted across seven cities in South Korea (Kim and Lee, 2021) and across Singapore

(Yap et al., 2019) reported generally null associations across varied exposure lag periods and copollutant adjustment.

- Recent studies examined cause-specific cardiovascular mortality in analyses that may be limited in statistical power due to small case sizes. A case-crossover study conducted in the United Kingdom reported largely null associations between short-term NO_2 and cardiovascular mortality endpoints (total cardiovascular mortality, non-MI, stroke, IHD, MI, chronic IHD, arrhythmias, atrial fibrillation, pulmonary embolism, heart failure). In contrast, a nationwide study across China reported positive percent changes in total cardiovascular mortality risk per 20 ppb increase in short-term NO_2 (3.43 [95% CI: 2.47, 4.40]) as well as increased percent risks of various cause-specific outcomes: hypertension (5.37 [95% CI: 3.05, 7.74]), congenital heart defects (CHD) (3.43 [95% CI: 2.28, 4.59]), stroke (3.43 [95% CI: 2.09, 4.79]) (Chen et al., 2018). Studies of short-term NO_2 and stroke mortality conducted in China conducted in the Jiangsu Province (Xu et al., 2022) and nationwide (Li et al., 2020) reported positive associations between short-term NO_2 and total stroke mortality, with higher risks of ischemic stroke observed compared to hemorrhagic stroke.

Table 5-4 Association between short-term exposure to oxides of nitrogen and cardiovascular mortality

Study	Location	Ages	Lag	Averaging Time	Effect Estimate (95% CI)
†Meng et al. (2021)	362 cities in 16 countries	All ages	0–1 d	24-hr avg	% change in risk per 20 ppb NO_2: 1.24 (0.50, 1.98)
†Schwarz et al. (2024)	338 cities in 20 countries	All ages	1 d	24-hr avg	% change in risk per 20 ppb NO_2: 1.32 (0.68, 1.97)
†Liu et al. (2022)	7 U.S. states	All ages	0–2 d	24-hr avg	% change in risk per 20 ppb NO_2: -0.26 (-0.96, 0.44)
†Shin et al. (2022)	24 census divisions in Canada	All ages	0 d	24-hr avg	% change in risk per 20 ppb NO_2: Year-round Female: 4.65 (95% PI: 1.13, 8.29) Male: 4.86 (95% PI: 1.83, 7.98) Warm season Female: 1.81 (95% PI: -2.34, 6.14) Male: 1.20 (95% PI: -2.16, 4.68) Cold season Female: 1.00 (95% PI: -2.27, 4.39) Male: -1.79 (95% PI: -3.75, 0.21)
†Shin et al. (2023)	24 census divisions in Canada	All ages	0 d	24-hr avg	% change in risk per 20 ppb NO_2: Year-round: -0.40 (95% PI: -2.28, 1.52) Warm season: -3.17 (95% PI: -6.37, 0.13) Cold season: -1.20 (95% PI: -3.65, 1.32)
†Vanos et al. (2014)	10 Canadian cities	All ages	0–5 d	24-hr avg	% change in risk per 20 ppb NO_2: Winter: 3.03 (1.71, 4.36) Spring: 4.92 (3.91, 5.95)

Study	Location	Ages	Lag	Averaging Time	Effect Estimate (95% CI)
					Summer: 4.26 (2.42, 6.13) Fall: 4.07 (0.45, 7.82)
†Milojevic et al. (2014)	England and Wales, U.K.	All ages	0–4 d	24-hr avg	% change in risk per 20 ppb NO_2: -0.42 (-1.75, 0.93)
†Stafoggia and Bellander (2020)	Stockholm County, Sweden	All ages	0–1 d	24-hr avg	% change in risk per 20 ppb NO_2: -0.60 (-9.08, 8.67)
†Corso et al. (2020)	18 areas in France	All ages	0–1 d	24-hr avg	% change in risk per 20 ppb NO_2: 4.98 (2.03, 8.01)
†Perez et al. (2015)	13 regions in Switzerland	All ages	0–1 d	24-hr avg	% change in risk per 20 ppb NO_2: 1.51 (-0.19, 3.24)
†Lanzinger et al. (2016)	5 cities in Central Europe (Dresden, Augsburg, Prague, Ljubljana, Chernivtsi)	All ages	0–1 d	24-hr avg	% change in risk per 20 ppb NO_2: -2.67 (-10.18, 5.47)
†Gariazzo et al. (2023)	Italy	All ages	0–1 d	24-hr avg	% change in risk per 20 ppb NO_2: -5.20 (-9.38, -0.84)
†Xu et al. (2022)	Jiangsu province, China	All ages	0–1 d	24-hr avg	% change in risk per 20 ppb NO_2: Total Stroke: 5.61 (4.11, 7.13) Ischemic Stroke: 7.62 (5.49, 9.78) Hemorrhagic Stroke: 3.16 (0.78, 5.59)
†Li et al. (2021)	48 cities across 28 provinces in China	All ages	0–1 d	24-hr avg	% change in risk per 20 ppb NO_2: 2.28 (1.55, 3.01)
†Li et al. (2020)	48 cities across 28 provinces in China	All ages	0–1 d	24-hr avg	% change in risk per 20 ppb NO_2: Ischemic Stroke: 3.12 (0.40, 5.92) Hemorrhagic Stroke: 1.74 (0.32, 3.18)
†Chen et al. (2018)	272 cities in China	All ages	0–1 d	24-hr avg	% change in risk per 20 ppb NO_2: 3.43 (95% PI: 2.47, 4.40)
†Kim and Lee (2021)	7 cities in South Korea	All ages	0–7 d	24-hr avg	% change in risk per 20 ppb NO_2: 0.77 (-2.29, 3.93)
†Yap et al. (2019)	Singapore	All ages	0–5 d	24-hr avg	% change in risk per 20 ppb NO_2: -4.97 (-16.32, 7.93)

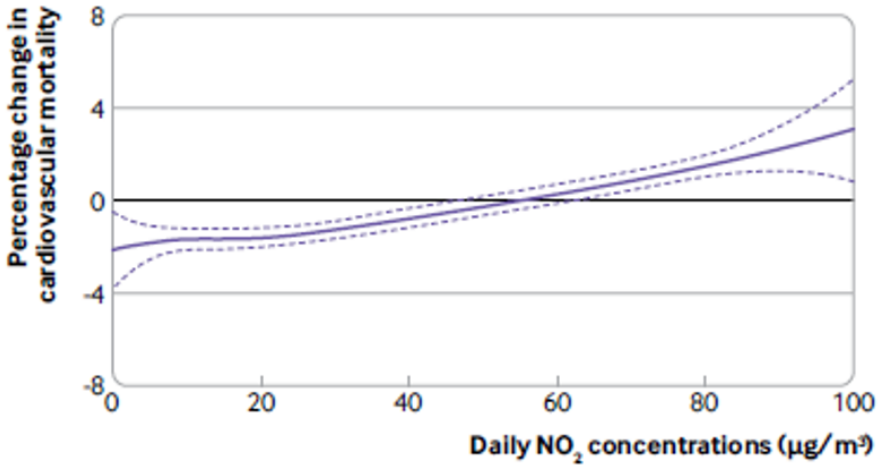
List of abbreviations in alphabetical order: avg = average; CI = confidence interval; d = day; HDI = highest density interval; hr = hour; max = maximum; NO_2 = nitrogen dioxide; NO_X = nitrogen oxides; OR = odds ratio; PI = posterior interval; ppb = parts per billion; U.K. = United Kingdom; U.S. = United States; yr = year.
†Studies published since the 2016 Oxides of Nitrogen ISA.

5.11.1.1 Concentration-Response

In the 2016 Oxides of Nitrogen ISA analyses detailing the shape of the concentration-response relationship between short-term NO_2 exposure and cardiovascular mortality were limited. A study

conducted across eight Chinese cities fit both a linear and natural cubic spline model to examine the overall NO_2-stroke mortality C-R relationship and evidence of non-linearity across all study cities (Chen et al., 2013). Visual inspection and examination of deviances between the linear and spline models did not indicate that the spline model improved the overall fit of the NO_2-stroke mortality C-R relationship over a linear model.

Recent studies conducted globally (Meng et al., 2021) and in China (Li et al., 2021; Li et al., 2020; Chen et al., 2018) have incorporated cubic and quadratic spline models to evaluate potential nonlinearities of the NO_2-cardiovascular mortality C-R relationship. In these studies, authors selected two spline knots, with the lower knot value of 10.64 ppb or 15.96 ppb and a higher knot value of 21.28 ppb or 26.6 ppb. A multicity study in China implemented a quadratic b-spline to examine the NO_2-stroke mortality C-R relationship with two spline knots at 15.96 ppb and 26.6 ppb (Li et al., 2020). Upon visual inspection, the C-R curves demonstrate approximately linear associations between short-term NO_2 exposure and both cardiovascular mortality and stroke mortality, without a discernible lower threshold present (see Figure 5-10 and Figure 5-11 for further details). Population mean (IQR) NO_2 concentrations ranged from 16.7 ppb in Meng et al. (2021) to 16.4 ppb in Li et al. (2020) and Li et al. (2021).



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

Figure 5-10 Concentration-response curve between nitrogen dioxide concentrations (lag 1) and cardiovascular mortality in 362 cities across 14 countries; estimated from a natural spline model with knots at 10.64 ppb and 21.28 ppb.

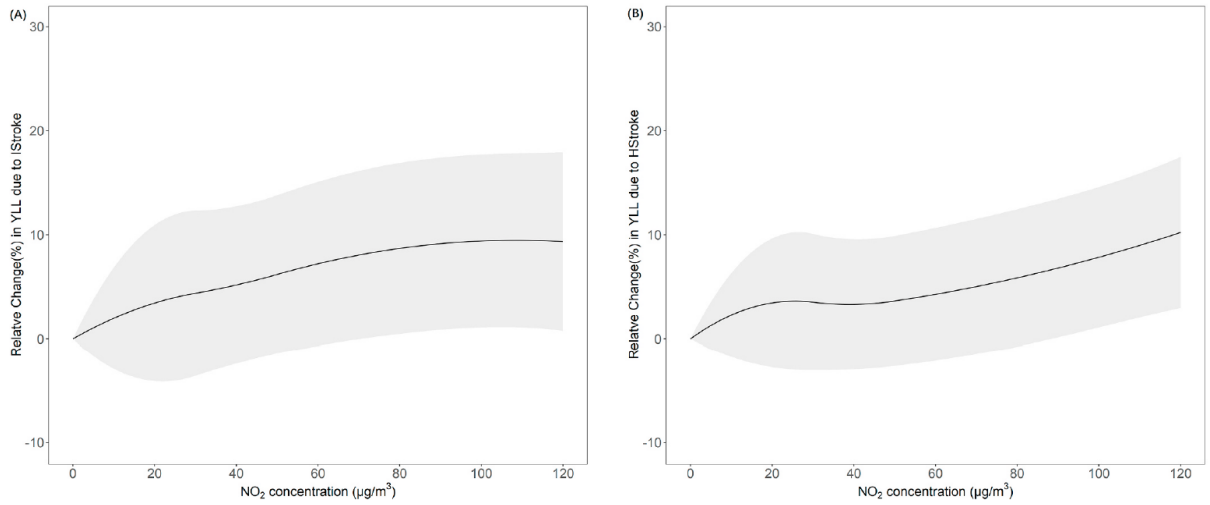


Fig. 3. The exposure-response curves for the associations between 2-day moving average of nitrogen dioxide concentrations and years of life lost from ischaemic and haemorrhagic stroke in 48 Chinese cities during 2013–2017. Note: IStroke and HStroke represent ischaemic and haemorrhagic stroke, respectively. The solid lines indicate the mean estimates, and the gray areas represent 95% confidence intervals.

List of abbreviations in alphabetical order: NO_2 = nitrogen dioxide; ppb = parts per billion; YLL = years of life lost; $\mu g/m^3$ = micrograms per cubic meter.
Source: Adapted from Li et al. (2020). Copyright permission pending.

Figure 5-11 Concentration-response curve between nitrogen dioxide concentrations (lag 0 to 1) and ischemic stroke and hemorrhagic stroke mortality in 48 Chinese cities; estimated from a natural spline model with knots at 15.96 ppb and 26.6 ppb.

5.11.1.2 Lifestages and Populations

Sex

In the 2016 Oxides of Nitrogen ISA, a limited number of studies examined sex-specific differences of cardiovascular mortality associated with short-term NO_2 exposure. A single study evaluated effect measure modification between male and female study populations and reported no modification by sex. Recent evidence examining effect modification by sex in studies of short-term exposures was inconsistent.

- Stronger (i.e., larger in magnitude) positive associations between short-term NO_2 and cardiovascular mortality reported among females compared to males in studies that reported positive associations overall (Li et al., 2021; Chen et al., 2018).
- No difference in risk was reported by sex in other studies that report inconsistent associations overall (Shin et al., 2022; Milojevic et al., 2014).
- A multicity time-series study of stroke mortality in China observed positive associations in the overall study population between short-term NO_2 and both ischemic and hemorrhagic stroke mortality, with somewhat higher risks in females compared to males for ischemic stroke and no difference by sex in hemorrhagic stroke risk (Li et al., 2020).

- Multi-city studies conducted in China reported positive associations between short-term NO_2 exposure and cardiovascular mortality, with slightly elevated risks of among females compared to males (Li et al., 2021; Chen et al., 2018).
- A case-crossover study conducted in England and Wales observed null associations and no sex-specific differences in the risk of mortality due to circulatory diseases, total cardiovascular mortality, or specific causes (non-MI, stroke, IHD, MI, chronic IHD, arrhythmias, atrial fibrillation, pulmonary embolism, heart failure) (Milojevic et al., 2014).
- In a time series study conducted across 24 census divisions in Canada, null associations were observed in season-specific analyses, and no sex-specific differences were reported for circulatory mortality associated with short-term NO_2 (Shin et al., 2022).

Lifestage

The 2016 Oxides of Nitrogen ISA included discussion of studies evaluating effect measure modification by lifestage for long-term NO_2 exposure but did not evaluate age-specific differences associated with short-term NO_2 exposure. A key limitation to evaluating effect measure modification by lifestages is the relatively small case size among younger individuals compared to the older adult population, which may contribute to unstable estimates among younger populations. Findings from recent studies examining effect modification by lifestage were inconsistent among studies that reported overall inconsistent findings across the study population.

- Studies conducted in China reported increased cardiovascular mortality risks associated with short-term NO_2 estimated in all age groups, with associations with larger estimated effects for adults over 75 years of age compared to those under 65 years of age (Li et al., 2021; Chen et al., 2018).
- A study in England and Wales that reported overall null associations between short-term NO_2 and cause-specific (non-MI, stroke, IHD, MI, chronic IHD, arrhythmias, atrial fibrillation, pulmonary embolism, heart failure) cardiovascular mortality risks. No differences in total or cause-specific cardiovascular mortality risks associated with short-term NO_2 were reported in participants over age 70 and below age 70 (Milojevic et al., 2014).
- A multicity study in China of stroke mortality reported positive associations between short-term NO_2 and both ischemic and hemorrhagic stroke mortality overall, with greater estimated risks for both ischemic and hemorrhagic stroke reported in adults over 65 years of age compared to adults under 65 years of age, though confidence intervals were overlapping (Li et al., 2020).

5.11.1.3 Copollutants

Given the high correlation between NO_2 and other traffic-related copollutants, it is difficult to discern independent associations between NO_2 exposure and cardiovascular mortality. Evidence from the 2016 Oxides of Nitrogen was limited and included a small number of studies adjusting for PM_{10}, SO_2, and O_3 that concluded associations between short-term NO_2 exposure and cardiovascular mortality remained relatively unchanged with copollutant adjustment. Some studies reported positive associations in single pollutant models with positive but slightly attenuated associations with PM_{10} and SO_2 adjustment (Chen

et al., 2013; Chen et al., 2012a), while others reported that the short-term NO_2-cardiovascular mortality association remained robust with adjustment for PM_{10} and O_3 (Chiusolo et al., 2011). No studies examined copollutants that are more closely tied to traffic-related emissions (e.g., $PM_{2.5}$, BC/EC, UFP, OC, VOCs, CO).

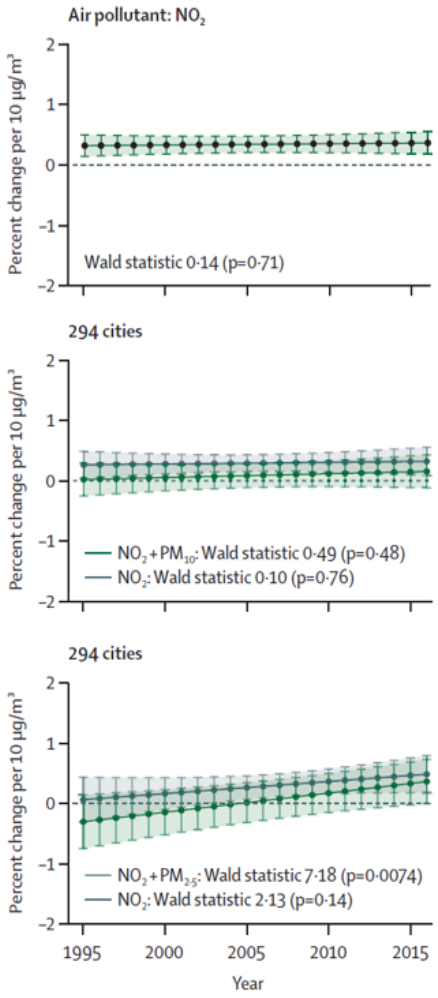
Recent studies, including a global study (Schwarz et al., 2024) and nationwide and multicity studies in North America (Shin et al., 2023), Europe (Gariazzo et al., 2023; Corso et al., 2020; Stafoggia and Bellander, 2020; Perez et al., 2015), and Asia (Xu et al., 2022; Kim and Lee, 2021; Li et al., 2021; Li et al., 2020; Chen et al., 2018), also examined copollutant models and adjusted for additional copollutants, such as CO and $PM_{2.5}$.

Many studies that reported overall null associations between short-term NO_2 exposure and cardiovascular mortality in single pollutant models observed effect estimates that were relatively unchanged with copollutant adjustment.

- A study in Canada reported null associations at lag 0 NO_2 exposure in both single and copollutant models adjusting for O_3, $PM_{2.5}$, and multipollutant models adjusting for both O_3 and $PM_{2.5}$ (Shin et al., 2023).
- Multicity studies in Sweden (Stafoggia and Bellander, 2020) and Switzerland (Perez et al., 2015) observed null associations in both single- and multipollutant models adjusting for $PM_{2.5}$, PM_{10} and O_3, although there was a positive increased association reported when adjusting for O_3 in Sweden (Stafoggia and Bellander, 2020).
- A nationwide study in Italy reported inverse associations in single pollutant model, which were somewhat attenuated when adjusting for PM_{10} and $PM_{2.5}$ (Gariazzo et al., 2023).

Other studies reported positive associations between short-term NO_2 exposure and cardiovascular mortality from single pollutant models but observed substantial attenuation of positive associations when applying copollutant models.

- Schwarz et al. (2024) reported positive associations between short-term NO_2 exposure and cardiovascular mortality that were stable over time in single pollutant analyses. Adjustment for PM_{10} yielded null associations with little temporal variation and adjustment for $PM_{2.5}$ yielded attenuated associations that became more positive over time (see Figure 5-12).
- Studies conducted in China reported positive associations between short-term NO_2 and stroke mortality (Xu et al., 2022; Li et al., 2021; Li et al., 2020) and short-term NO_2 and cardiovascular mortality (Chen et al., 2018) in single pollutant models, with positive but somewhat attenuated associations with adjustment for $PM_{2.5}$, PM_{10}, SO_2, and CO and no difference in associations with adjustment for O_3.



List of abbreviations in alphabetical order: NO_2 = nitrogen dioxide; ppb = parts per billion; $PM_{\#}$ = particulate matter with a nominal mean aerodynamic diameter less than or equal to # µm.
Source: Adapted from Schwarz et al. (2024). Copyright permission pending.

Figure 5-12 Relative risks of cardiovascular mortality associated with 5.32 ppb increase in nitrogen dioxide in single and copollutants models at lag day 1 pooled over they study period (1995 to 2016) across 362 cities in 16 countries.

5.11.1.4 Exposure Assignment

Exposure measurement error present in epidemiologic studies may result in biased health effect estimates. Chapter 2 (see Section 2.5.1) provides a thorough discussion of the bias resulting from particular exposure assessment methods common to epidemiologic literature and the impact on inferences that can be drawn about health effects (see Chapter 2, Section 2.3). Most studies of short-term NO_2 and cardiovascular mortality to date have assigned daily NO_2 concentrations using fixed and central site monitors. Some recent studies have leveraged predicted estimates from hybrid models (Gariazzo et al.,

2023; Liu et al., 2022) and spatiotemporal and geospatial models (Stafoggia and Bellander, 2020) as proxies for short-term NO_2 exposure. Hybrid prediction models used in recent studies have included daily NO_2 models at 1 km × 1 km resolution, that incorporate combinations of monitoring data, chemical transport modeling, meteorologic variables, and land use terms (Gariazzo et al., 2023; Liu et al., 2022). Other recent studies that utilized a hybrid model used atmospheric composition forecasts and incorporated data from satellites, ground monitoring, atmospheric reanalysis, and model simulations (Xu et al., 2022). The spatiotemporal and geospatial model utilized in Stafoggia and Bellander (2020) was trained from satellite, atmospheric, meteorological, population density, and road network data. Out-of-sample cross-validation values reported varied across prediction models, ranging $r^2 = 0.60$ to 0.84 for hybrid models (Gariazzo et al., 2023; Liu et al., 2022; Xu et al., 2022) and $r^2 = 0.74$ for the spatiotemporal and geospatial model (Stafoggia and Bellander, 2020).

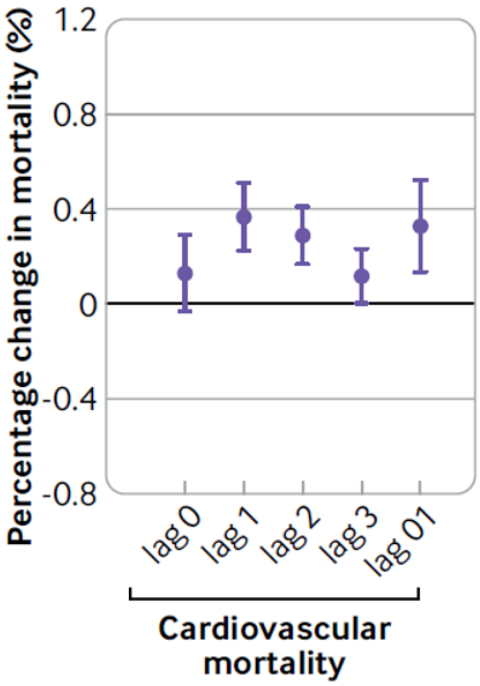
Generally, studies reported null associations between short-term NO_2 and cardiovascular mortality (Liu et al., 2022; Stafoggia and Bellander, 2020), with another study reporting negative associations (Gariazzo et al., 2023). In contrast in a cause-specific model using a hybrid prediction model to assign short-term NO_2, Xu et al. (2022) reported positive association between short-term NO_2 and stroke mortality. These overall inconsistencies in findings among studies that assigned exposure using model predictions are similar to the inconsistent results reported across studies using central site monitors, though direct comparisons are complicated by differences in study populations and other methodological approaches.

5.11.1.5 Lag Structure

Studies in the 2016 Oxides of Nitrogen ISA generally reported associations between short-term NO_2 exposure and cardiovascular mortality within the first few days of exposure. Shorter lag periods were most frequently examined, including immediate moving average exposure windows (e.g., lags 0 to 1, 0 to 2 days) and daily lags (e.g., lag 0, lag 1). Multicity studies in Italy (Chiusolo et al., 2011) and China (Chen et al., 2013; Chen et al., 2012a) examining multiple lags reported that the magnitude of associations was largest at lag 0–1, though associations were also reported at longer lags: lag 0 to 4 and 0 to 5, indicating a potential effect present after more prolonged exposure.

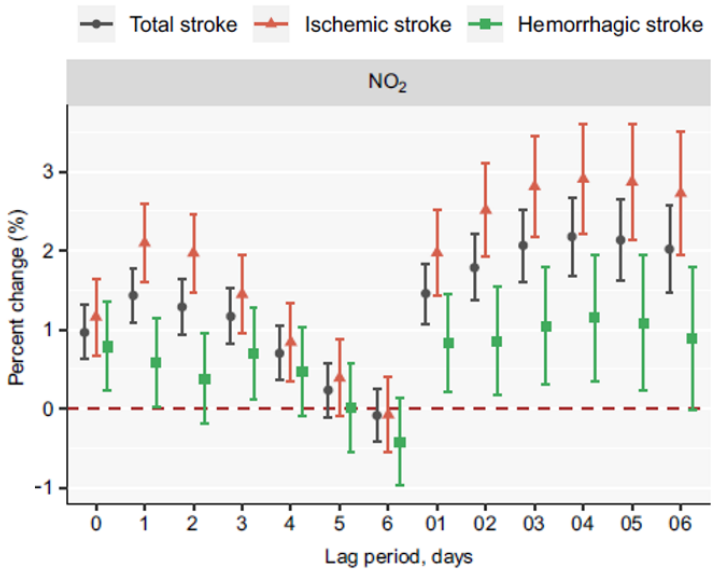
Recent studies examining cardiovascular mortality and stroke mortality commonly observed positive associations at a priori moving average daily lags of 0 to 1 and single day lags 0 and 1. Some studies compared associations using different lag structures ranging from zero to three days: a global analysis of 362 cities (Meng et al., 2021) and nationwide analysis of 272 cities in China (Chen et al., 2018) reported stronger positive associations between NO_2 exposure and cardiovascular mortality at more proximal lags (e.g., lag 0, lag 1, and lag 0 to 1) and attenuated estimates at more distal lags (e.g., lag 2 and lag 3) (see Figure 5-13). Similarly, in an analysis of stroke mortality across the Jiangsu province in China with lags ranging from 0 to 6, Xu et al. (2022) reported generally positive associations for total, ischemic,

and hemorrhagic stroke mortality at proximal single-day lags which decreased in magnitude at more distal single-day lags, and higher risks associated with moving average lags compared to single-day lags, with the strongest associations at lag 0–4 (see Figure 5-14).



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

Figure 5-13 Relative risks of cardiovascular mortality associated with 5.32 ppb increase in nitrogen dioxide on different lag days across 362 cities in 16 countries.



List of abbreviations in alphabetical order: NO_2 = nitrogen dioxide; ppb = parts per billion.
Note: Outcomes displayed include mortality related to total stroke (black circles), ischemic stroke (orange triangles), and hemorrhagic stroke (green squares).
Source: Adapted from Xu et al. (2022). Copyright permission pending.

Figure 5-14 Relative risks of cardiovascular mortality associated with 5.32 ppb increase in nitrogen dioxide on different lag days across Jiangsu Province, China.

Other studies reported inconsistent results when examining lag windows of up to 7 days before death. A couple of multicity studies in Canada reported overall null associations between short-term NO_2 exposure and cardiovascular mortality (Shin et al., 2023; Shin et al., 2022) across different single-day lags ranging from 0 to 6, with an inverse association reported at lag 3 in the warm season in Shin et al. (2023). A nationwide study in Italy observed negative associations at a moving average daily lag 0 to 1 exposure window and null associations at lag 2 to 5 and lag 0 to 5 exposure (Gariazzo et al., 2023). Similarly, studies that examined different lag windows conducted across Stockholm County, Sweden (Stafoggia and Bellander, 2020), South Korea (Kim and Lee, 2021), and Singapore (Yap et al., 2019) generally did not observe an association between short-term NO_2 exposure and cardiovascular mortality.

5.11.1.6 Seasonal and Temperature Differences

The 2016 Oxides of Nitrogen ISA included a limited number of multicity studies across Italy and a single-city study in the United States that examined potential seasonal differences in cardiovascular mortality risk associated with short-term NO_2 exposure (Sacks et al., 2012; Chiusolo et al., 2011; Bellini et al., 2007). Multicity studies conducted in Italy observed associations between NO_2 exposure and cardiovascular mortality that were larger in magnitude in summer compared to winter (Bellini et al., 2007) and in the warm season (i.e., April to September) compared to annual estimates (Chiusolo et al.,

2011). In contrast, the U.S. studies did not provide evidence of seasonal differences in Philadelphia, PA, U.S. (Sacks et al., 2012).

Recent studies examining seasonal and temperature differences in the NO_2-cardiovascular mortality relationship were inconsistent.

- Several multicity time series studies using fixed and central site monitors conducted globally (Meng et al., 2021) and in Canada (Vanos et al., 2014), Switzerland (Perez et al., 2015), and South Korea (Kim and Lee, 2021) observed no notable differences in cardiovascular mortality risk associated with short-term NO_2 exposure in season-stratified compared to year-round analyses.
- Two Canadian multicity time-series studies using central site monitors provided some evidence for confounding and effect measure modification of the relationship between short-term NO_2 exposure and cardiovascular mortality by warm (April to September) and cold (October to March) seasons (Shin et al., 2023; Shin et al., 2022). Sex-specific year-round analyses in Shin et al. (2022) reported increased risks among both males and females. Meanwhile, season-stratified analyses revealed largely attenuated, null associations among both males and females. These attenuated season-specific effect estimates in Shin et al. (2022) suggest the presence of residual confounding, despite a natural cubic spline fitted to account for the long-term effects of seasonality. Year-round analyses in Shin et al. (2023) indicated a null association between NO_2 and cardiovascular mortality risk across single-day lag periods ranging from zero to six days. However, season-stratified estimates yielded higher risk during the cold season compared to the warm season for lag 3, though these higher season-specific risks reported for lag 3 were attenuated once adjusted for O_3 and $PM_{2.5}$.

A few other studies outside the United States also reported evidence of varying degrees of effect measure modification by season.

- A multicity time series study using central site monitors conducted in France observed positive associations in year-round analyses and the warm period (from May to October) compared to null findings in the cold period (from November to April) (Corso et al., 2020).
- A case-crossover study using a random forest model conducted across Stockholm County, Sweden, reported associations with wide 95% confidence intervals overlapping with the null in year-round and season-stratified analyses, but more positive effect estimates in the warm season compared to the cold season (Stafoggia and Bellander, 2020).
- A case-crossover analysis of stroke mortality risk associated with short-term NO_2 exposure estimated with a hybrid model in Jiangsu Province, China reported positive associations year-round, with higher risks of ischemic stroke and lower risk of hemorrhagic stroke in the warm season (April to November) and no change in ischemic stroke risk and higher risks of hemorrhagic stroke in the cold season (December to March) (Xu et al., 2022).
- A time-series study conducted in China across 48 cities that stratified by temperature quartiles observed positive year-round associations and consistent positive associations in the upper three quartiles, with attenuated associations only in the lowest quartile temperature group (Li et al., 2021).

5.11.1.7 Conclusions and Remaining Uncertainties

Recent epidemiologic studies conducted among large patient populations across multiple countries continue to provide evidence of a positive association between short-term exposure to NO_2 and cardiovascular mortality. Supporting evidence from the 2016 Oxides of Nitrogen ISA came from multi-country and multicity studies from Asia and Europe. Recent studies provided expanded this evidence with multi-country and multicity studies representing populations in the United States, Asia, and Europe. Reporting on the concentration-response relationship between short-term exposure to NO_2 and cardiovascular mortality was limited in studies in the 2016 Oxides of Nitrogen ISA and in current studies; however, among prior and current studies that reported positive associations and examined potential non-linearity in the association, the concentration-response relationship is described as approximately linear.

Studies that reported positive associations in both the 2016 Oxides of Nitrogen ISA and recent evidence were congruent in highlighting the approximately linear concentration-response relationship and the relevance of lagged exposure in the immediate days prior to the outcome. Recent studies also expand on the limited evidence from the 2016 Oxides of Nitrogen on details of the short-term NO_2-cardiovascular mortality relationship, such as copollutant confounding, populations potentially at increased risk, and seasonal differences. However, uncertainties remain given the relatively inconsistent evidence reflected across recent study findings.

5.12 Biological Plausibility

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

One pathway potentially linking short-term exposure to NO_2 with cardiovascular morbidity and mortality is through the induction of systemic inflammation and oxidative stress. Animal toxicologic studies have shown increases in gene expression and protein levels of inflammatory mediators in heart tissue (Li et al., 2011). This corresponds to decreases in antioxidant enzyme activity and an increase in markers of oxidative damage (Li et al., 2011). A recently identified study supports the increase in

mitochondrial ROS production in cardiac tissue in animals following NO_2 exposure (Karoui et al., 2020). Controlled human exposure studies have not examined systemic markers of oxidative stress and provide little support for systemic inflammation following NO_2 exposure (see Section 5.10.4.2), adding uncertainty to the interpretation of the animal results. Thus, while animal studies indicate the occurrence of systemic oxidative stress and inflammation following NO_2 exposures, the relevance of these pathways in humans is uncertain.

Inflammation can lead to many physiological changes that can potentially result in cardiovascular effects, including metabolic changes. There is evidence from animal studies that NO_2 exposure can reduce the protective HDL cholesterol levels in the plasma of rats (Farraj et al., 2016) and can alter glycerophospholipid metabolism which is involved in the pathogenesis of cardiovascular disease (Li et al., 2022b). A controlled human exposure study showed altered lipid profiles including an increase in total blood cholesterol; however, this study also reported an increase in HDL cholesterol (Huang et al., 2012b). Alteration of lipid and cholesterol levels could promote the formation and growth of atherosclerotic plaques that could precipitate an increase in blood pressure, myocardial infarction, and ischemic stroke, all of which are linked to increases in cardiovascular mortality.

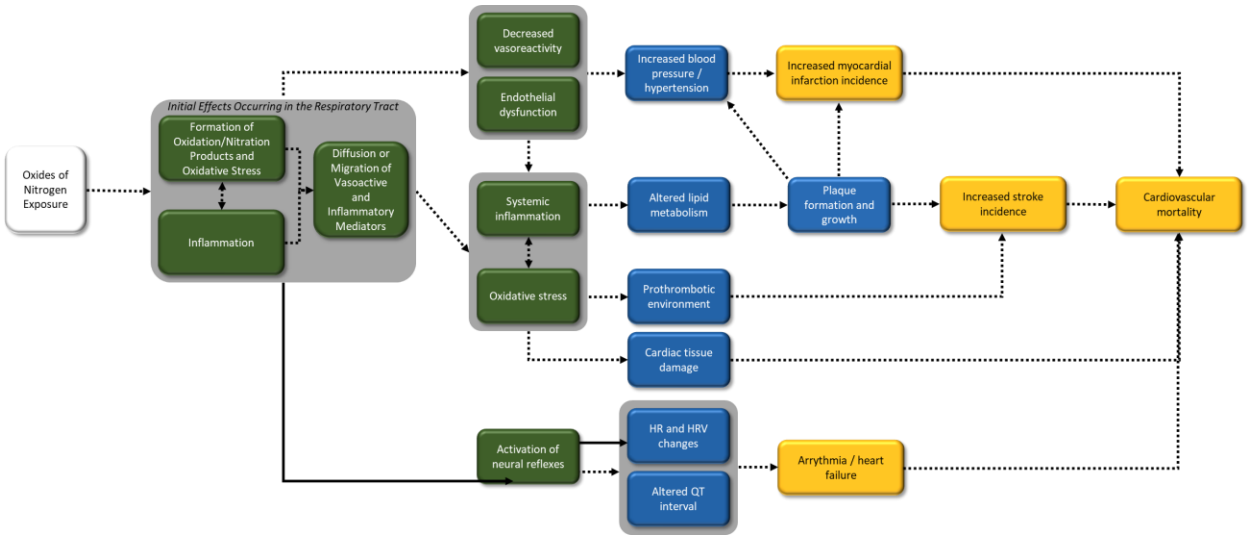
Excessive inflammation can also lead to death of myocardial cells and subsequent changes to heart tissue structure. These changes can impact cardiac function and contribute to the development and progression of cardiac disease. While the toxicologic evidence is limited, an animal study of short-term NO_2 exposure showed histologic evidence of cardiovascular cell death and changes to heart tissue architecture (Li et al., 2011).

Inflammation can also affect the balance between clotting and anticoagulation factors and promote the formation of blood clots that trigger infarction or stroke. Some, but not all, epidemiologic evidence has shown associations between short-term NO and NO_2 exposures and increases in clotting-related factors (Strak et al., 2013; Zhang et al., 2013; Bind et al., 2012; Rich et al., 2012; Rudez et al., 2009), suggesting that NO_2 exposure can impact blood clotting. However, results from controlled human exposure studies does not provide support for effects of NO_2 on clotting (Riedl et al., 2012; Langrish et al., 2010), adding uncertainty to the interpretation of epidemiologic associations.

Another potential pathway that could potentially induce cardiovascular effects includes activation of endothelial cells and changes to vascular reactivity. Endothelial cells produce mediators that can either relax or contract the smooth muscle surrounding blood vessels to help maintain proper blood flow. Increased vasoconstriction can lead to increased blood pressure which could result in hypertension which is a risk factor for other cardiovascular sequalae. An experimental animal study reported increased concentrations of the vasoconstrictor endothelin-1 following NO_2 exposure and increased expression of ICAM-1, a marker of endothelial activation (Li et al., 2011), though other evidence supporting this pathway is lacking.

Short-term NO_2 exposure could also potentially increase cardiovascular risk through activation of nervous system reflexes, which play an important role in coordinating heart contractions and regulating vascular tone. Changes in nervous system would thus have impacts on cardiovascular function. Animal studies of exposure to high concentrations (>1,000 ppb) of NO_2 have reported bradycardia (Tsubone et al., 1982; Suzuki et al., 1981). Treatment with atropine to block vagus nerve responses resulted in attenuation of the response suggesting a role for nervous system responses in NO_2-induced decrease in HR (Tsubone et al., 1982). This evidence supports the solid line connecting activation of neural reflexes and HR and HRV in Figure 5-15. Despite the animal evidence, controlled human studies have not shown changes in HR or cardiac output which calls into question the relevance of the animal results for humans. HR variability also can be impacted by changes in electrical signaling controlled by the nervous system. A controlled human exposure study showed decreased HF domain in humans following NO_2 exposure (Huang et al., 2012b); however, the limited animal toxicologic database showed little impact on HR or HRV following NO_2 exposure (Ji et al., 2022; Farraj et al., 2016). One controlled human exposure study showed changes in QT interval following NO_2 exposure, but the directionality of the effect is the opposite of what is typically associated with increased risk for arrhythmia (Huang et al., 2012b). In a recently reviewed study, NO_2 exposure in rats reduced the amount of aconitine needed to induce arrhythmia (Farraj et al., 2016) suggesting a reduced threshold required for the onset of arrhythmia.

Experimental and epidemiologic data suggest several plausible pathways by which short-term NO_2 exposure could result in cardiovascular morbidity and mortality. However, the experimental and epidemiologic evidence supporting several of the steps in these proposed pathways are limited and, in some cases, inconsistent with other studies making the proposed pathways, while plausible, more uncertain.



Note: The boxes represent the effects for which there is experimental or epidemiologic evidence related to oxides of nitrogen exposure, and the arrows indicate a proposed relationship between those effects. Solid arrows denote evidence of essentiality as provided, for example, by an inhibitor of the pathway used in an experimental study involving oxides of nitrogen exposure. Dotted arrows denote a possible relationship between effects. Shading around multiple boxes is used to denote a grouping of these effects. Arrows may connect individual boxes, groupings of boxes, and individual boxes within groupings of boxes. Progression of effects is generally depicted from left to right and color coded (white, exposure; green, initial effect; blue, intermediate effect; orange, effect at the population level or a key clinical effect). Here, population-level effects generally reflect results of epidemiologic studies. When there are gaps in the evidence, there are complementary gaps in the figure and the accompanying text below. The structure of the biological plausibility sections and the role of biological plausibility in contributing to the weight-of-evidence analysis used in the Oxides of Nitrogen ISA are discussed in Section 5.14.

Figure 5-15 Potential biological plausibility pathways for cardiovascular effects associated with exposure to oxides of nitrogen.

5.13 Summary of Lifestages and Population

Some populations and lifestages may be at greater risk of oxides of nitrogen-related health effects than the general population. Higher risks could be due to intrinsic,[5] extrinsic,[6] and/or exposure-related factors. In assessing the recent evidence, these factors are identified, evaluated, and characterized to inform conclusions on the populations and lifestages that may be at increased risk. The systematic approach used to evaluate factors that may increase the risk of a population or specific lifestage to an air pollutant-related health effect is described in more detail in the Preamble to the ISAs (U.S. EPA, 2015a) and the Integrated Review Plan for the Primary National Ambient Air Quality Standards for Oxides of Nitrogen Volume 2 (U.S. EPA, 2024). The evaluation of these factors focuses on health effect studies that conduct stratified analyses to compare populations or lifestages exposed to similar air pollutant

[5]Intrinsic factors such as genetics, lifestage, or disease status can contribute to larger or more serious responses to a particular pollutant exposure, and physiological differences between groups (e.g., differences in breathing patterns between children and adults) can contribute to higher internal pollutant doses in some populations.
[6]Extrinsic factors (e.g., nutritional status) and exposure-related factors (e.g., working outdoors, living near roadways or other pollution sources) can also contribute to increased risk, and many of the characteristics commonly used to classify populations potentially at increased risk (e.g., race, SES, educational attainment) are surrogates for the combined impact of several intrinsic, extrinsic, and exposure-related factors.

concentrations within the same study design. For the evaluation of these studies, important considerations include whether the stratified analyses were planned a priori or were post hoc analyses, whether the study conducted multiple comparisons, and whether there were small sample sizes in individual strata. These study design issues can increase the probability of finding associations by chance or reduce the power to detect associations in subgroup analyses. Other epidemiologic studies that do not stratify results but instead examine a specific population or lifestage can provide supporting evidence for the pattern of associations observed in studies that formally examine effect measure modification. Additionally, experimental studies that examine health effects exclusively in human subjects or animal models with a particular factor, such as pre-existing disease, are also important lines of evidence to evaluate because they inform judgments of the coherence and biological plausibility of effects observed in epidemiologic studies, as well as the independent effect of exposure to oxides of nitrogen.

The objective of this section is to integrate information from preceding sections and describe the basis for characterizing the evidence for particular factors to increase risk of NO_2-related cardiovascular effects as adequate, suggestive, inadequate, or no effect. The classification provides a consistent basis for evaluating the evidence that a population or lifestage may be at increased risk and for describing the overall confidence in the evidence. Consistent with the characterization of evidence for health effects in the general population, the greatest emphasis is placed on patterns in results across studies. These classifications are not direct assessments of causality but rather can inform consideration of whether a NAAQS provides an adequate margin of safety across groups in the population.[7] This section discusses available analyses examining whether pre-existing disease (see Section 5.13.1), age (see Section 5.13.2), sex (see Section 5.13.3), or other factors (see Section 5.13.4) may modify the risk of cardiovascular effects following short-term exposure to NO_2 or other oxides of nitrogen with a summary of conclusions in Table 5-1.

5.13.1 Pre-existing disease

Individuals with pre-existing diseases or medical conditions may be considered at greater risk for some air pollution-related health effects because they may be in a compromised biological state depending on the disease/condition and severity. The large proportions of the U.S. population affected by various chronic diseases or medical conditions underscore the importance of characterizing the potential risk of oxides of nitrogen-related, predominantly NO_2-related, health effects for affected populations.

[7]Although the at-risk classifications are not direct assessments of causality, evidence for at-risk groups or factors inform causality determinations in several ways. Consistent evidence that at least one population subgroup is at risk of an NO_2-related health effect provides strong support for causality determinations. Evidence for effect modification can also explain heterogeneity in results across studies, which could reduce uncertainties regarding inconsistent evidence. Finally, at-risk factors such as genetics or pre-existing conditions can provide supporting information on mechanisms contributing to NO_2-related health effects. Notably, the lack of evidence for differential effects in subgroups where there is otherwise evidence of an NO_2-related health effect in the general population does not weaken the overall evidence supporting a causality determination.

5.13.1.1 Pre-existing COPD

The 2016 Oxides of Nitrogen ISA identified two epidemiologic studies that stratified by presence or absence of pre-existing COPD and compared the associations between short-term NO_2 exposure and cardiovascular effects (Suh and Zanobetti, 2010; Peel et al., 2007). Suh and Zanobetti (2010) reported that positive associations between NO_2 exposure and pNN50 decrements were stronger in populations with COPD than in populations without the disease, but that there was no difference in rMSSD. Peel et al. (2007) additionally reported that those with COPD had larger associations with cardiovascular-related ED visits compared to those without COPD. A recently identified study reported similar NO_2-associated increases in the risk of acute coronary events in those with COPD compared to those without (Chen et al., 2022c). A controlled human exposure study reported that NO_2 exposure had little effect on HR both in those with and without COPD. Together, the limited and inconsistent evidence is inadequate to determine whether people with COPD are at increased risk of cardiovascular effects from short-term NO_2 exposure.

5.13.1.2 Pre-existing cardiovascular disease

Cardiovascular disease is the primary cause of death in the United States. Evidence described in the 2016 Oxides of Nitrogen ISA reported higher NO_2-related mortality among individuals with cardiovascular disease, however, the evidence for hospital admissions and ED visits was less consistent. Studies showed no difference in associations between NO_2 and MI when stratifying for prior hypertension, arrhythmia, or congestive heart failure. Studies of subclinical effects also showed no difference in associations between short-term NO_2 concentrations in groups with underlying cardiovascular disease, IHD, or hypertension. Animal toxicologic studies and controlled human exposure studies were limited in number and provided mixed results with a study of adults with CVD or in a mouse model of CVD.

A recently evaluated study reported that those with atrial fibrillation have a decreased NO_2-associated risk for hospital admissions for stroke (Ho et al., 2022a) while another reported similar NO_2-associated risk of stroke in those with either atrial fibrillation or hypertension compared to those without those conditions (Sun et al., 2019). The previous percutaneous coronary intervention did not obviously affect NO_2-related risk of acute coronary syndrome onset (Chen et al., 2022c). Together, the evidence is inadequate to determine whether people with underlying cardiovascular disease are at increased risk of cardiovascular effects from short-term NO_2 exposure.

5.13.1.3 Obesity

Obesity can be defined as a BMI of 30 kg/m^2 or greater. It is a public health issue of increasing importance as obesity rates have continually increased over several decades in the United States among

adults and children. Obesity or high BMI has been examined as a potential modifier of NO_2-related health effects only in adults. Among U.S. adults, the prevalence of both obesity and being overweight (BMI of 25 to 30 kg/m^2) is high (28% and 34.5%, respectively). Being obese or overweight could increase the risk of NO_2-related health effects through multiple mechanisms including persistent, low-grade inflammation. Poor diet and chronic diseases often occur with obesity and could be part of the pathway by which obesity increases the risk of NO_2-related health effects or could act in combination with obesity to increase risk.

The 2016 Oxides of Nitrogen reported that the evidence for effect modification by obesity status was inadequate. This was based on a single rat study showing increased markers of dyslipidemia in obese rats compared to non-obese rats, which was largely not supported by epidemiologic studies of cardiovascular disease that stratified by obesity status (Takano et al., 2004).

Recently evaluated studies that stratify for obesity status continue to provide mixed findings. One study reported that the risk of hemorrhagic stroke associated with increased 3-day moving average NO_2 was approximately null for non-obese individuals compared to an inverse relationship among obese participants (Sun et al., 2019). A study of blood pressure reported effect modification by BMI; however, the impact on associations between NO_2 exposure and SBP and DBP were in opposite directions (Wen et al., 2023). Together, the evidence is inadequate to determine whether obese individuals are at increased risk of cardiovascular effects from short-term NO_2 exposure.

5.13.1.4 Other preexisting diseases

The 2016 Oxides of Nitrogen ISA presented limited information on effect modification by other disease states. Diabetes showed no clear impact on associations between short-term NO_2 exposure and cardiovascular mortality, ED visits and HA for IHD, or subclinical markers of cardiovascular disease. Recent studies continued to show mixed results. Associations between NO_2 concentrations and coronary heart disease in stratified analyses showed similar associations in people with and without diabetes (Milojevic et al., 2014). Another study reported mixed results for diabetes status, including stronger positive associations with DBP and attenuated associations with SBP in those with diabetes compared to those without the disease (Wen et al., 2023). Associations with longer QTc were also stronger in diabetic individuals than in those without diabetes (Baja et al., 2010).

Evidence of effect modification of other disease states is very limited. One previously reviewed study reported a decrease in hospital admissions for MI among those with chronic renal failure (Milojevic et al., 2014). Chen et al. (2022c) reported similar increases in the risk for 0- to 24-hour average NO_2 concentration and acute coronary syndrome onset among individuals with thyroid disorders. Overall, the evidence is inadequate to determine whether people with other underlying diseases are at increased risk of cardiovascular effects from short-term NO_2 exposure.

5.13.2 Lifestage

The 2016 Oxides of Nitrogen ISA did not characterize lifestage-related effects, as only a single study assessed associations between short-term oxides of nitrogen exposure and cardiovascular outcomes across multiple age groups. The study from the 2016 Oxides of Nitrogen ISA reported increased NO_2-associated cardiovascular hospital admissions in adults 65 years of age and older. Recent studies expand the consideration of lifestage as an effect modifier for NO_2-induced cardiovascular effects. For the association between short-term NO_2 and cardiovascular-specific mortality, findings regarding effect modification by lifestage were generally mixed across studies. While recent studies suggested elevated NO_2-associated cardiovascular mortality in older adults, effect estimates were generally not statistically distinct from those in younger age groupings (see Section 5.11.1.2). However, similar to the 2016 Oxides of Nitrogen ISA, studies of other clinical or subclinical markers of cardiovascular morbidity were mixed with some studies showing greater associations (NSTEMI, OHCA, ischemic stroke, pulmonary embolism, aggregated, subclinical) while others reported no difference in associations when stratified by age (MI, ACS, STEMI, ischemic stroke, stroke, heart failure, aggregated). Overall, the current evidence weakly suggests that the risk of cardiovascular mortality and subclinical markers of cardiovascular morbidity from short-term NO_2 exposure is higher in older individuals, but the evidence remains limited and further studies are needed to strengthen the evidence base and clarify this trend.

5.13.3 Sex

A large number of health conditions and diseases have been shown to differ by sex, with some indication that there may be differences by sex in the relationship between air pollution and health effects. The 2016 Oxides of Nitrogen ISA evaluated a limited number of studies of short-term NO_2 exposures and cardiovascular effects that were stratified by sex. A couple of studies reported that HRV and out of hospital cardiac arrests had larger associations with NO_2 exposure in females compared to males (Wichmann et al., 2013; Huang et al., 2012a). Recently evaluated studies stratify by sex, but with inconsistent results on effect modification. Results for IHD and MI were mixed with some studies showing that males had a greater NO_2-associated risk of hospitalization for MI or IHD compared to females (Shin et al., 2022; Milojevic et al., 2014) while another showed similar risk among sexes (Chen et al., 2022c). Studies of short-term NO_2 concentrations and arrythmia or OHCA reported that females had larger associations with NO_2 exposure than men (Kranc et al., 2021; Milojevic et al., 2014). Associations between short term NO_2 and stroke were also mixed with one study showing increased risk in males compared to females (Tian et al., 2018) while other studies did not see sex-dependent differences (Liu et al., 2023; Shin et al., 2022). One study reported an increased NO_2-associated risk of heart failure in females compared to males (Milojevic et al., 2014). Sex-dependent differences in NO_2 associations were not observed in epidemiologic studies for other cardiovascular-related endpoints including blood pressure and hypertension, venous thromboembolism, and aggregated measures of cardiovascular disease. Overall, inconsistency of the findings both within and among cardiovascular endpoints and the lack of supporting

controlled human or animal toxicologic studies make the evidence inadequate to determine whether men or women are at higher risk of cardiovascular effects from short-term NO_2 exposure.

5.13.4 Other Factors

Some epidemiologic studies have looked at other factors, including potential genetic factors and smoking status, as potential effect modifiers. The 2016 Oxides of Nitrogen ISA reported that the evidence was inadequate to determine if any of these factors increased risk of cardiovascular effects from short-term oxides of nitrogen, predominantly NO_2, exposure. The recently identified literature remains limited in number for other effect-modifying factors. A recently identified study reported a greater positive association between NO_2 and longer QTc intervals in smokers compared to nonsmokers as well as in individuals with a high genetic susceptibility to oxidative stress compared to low susceptibility (Baja et al., 2010). The interpretation of such a limited evidence base was inadequate to determine the potential for increased risk of cardiovascular effects from short-term oxides of nitrogen, including NO_2, exposure.

Table 5-1 Summary of evidence for populations at increased risk of cardiovascular effects from oxides of nitrogen exposure

	Key Evidence	Key References
Suggestive Evidence		
Lifestage	A limited number of epidemiologic studies show generally, greater and more precise positive associations between short-term NO_2 exposure and cardiovascular mortality in older populations	†Requia et al. (2024) †Chen et al. (2018) †Li et al. (2021)
	Limited evidence of age as an effect modifier for cardiovascular morbidity and a lack of supporting animal or controlled human exposure evidence	
Inadequate Evidence		
Pre-existing COPD	Limited number of studies show inconsistent effects of COPD status on the association between short-term NO_2 exposure and cardiovascular endpoints	Suh and Zanobetti (2010) Peel et al. (2007) †Chen et al. (2022c)
Pre-existing CVD	Inconsistent effects of underlying CVD on the association between short-term NO_2 exposure and cardiovascular endpoints	Table 7-7 of U.S. EPA (2016) †Ho et al. (2022a) †Sun et al. (2019) †Chen et al. (2022c)

	Key Evidence	Key References
Obesity	Limited number of studies showing inconsistent effects of other underlying diseases on association between short-term NO_2 exposure and cardiovascular endpoints	Ljungman et al. (2008) Huang et al. (2012a) Baja et al. (2010) †Sun et al. (2019) †Wen et al. (2023)
	Lack of coherence with an animal toxicologic study showing greater dyslipidemia in obese mice	Takano et al. (2004)
Other preexisting diseases (diabetes, kidney disease, thyroid disorders)	Limited number of studies showing inconsistent effects of other underlying diseases on association between short-term NO_2 exposure and cardiovascular endpoints	†Milojevic et al. (2014) †Wen et al. (2023) †Chen et al. (2022c)
Sex	Inconsistent effects of sex on association between short-term NO_2 exposure and cardiovascular endpoints	Huang et al. (2012a) Wichmann et al. (2013) †Shin et al. (2022) †Milojevic et al. (2014) †Chen et al. (2022c) †Kranc et al. (2021) †Liu et al. (2023)
Other Factors (e.g. smoking, genetic factors)	Limited number of studies showing inconsistent effects of smoking status or genetic factors on association between short-term NO_2 exposure and cardiovascular endpoints	†Milojevic et al. (2014) †Chen et al. (2022c)

List of abbreviations in alphabetical order: COPD = chronic obstructive pulmonary disease; CVD = cardiovascular disease; NO_2 = nitrogen dioxide.
†Studies published since the 2016 Oxides of Nitrogen ISA.

5.14 Summary and Causality Determination

The 2016 Integrated Science Assessment for Oxides of Nitrogen concluded that the evidence was "suggestive of, but not sufficient to infer," a causal relationship between short-term NO_2 exposure and cardiovascular effects. This causality determination was primarily based on epidemiologic data that reported consistently positive associations between short-term increases in NO_2 concentrations and coronary events. This included positive associations between short-term increases in NO_2 concentrations and hospital admissions and ED visits for MI, IHD, and angina; ST segment alterations; and mortality from cardiovascular disease. Epidemiologic evidence of effects on the association of short-term NO_2 with other cardiovascular endpoints including cerebrovascular disease, stroke, arrythmia, hypertension were more inconsistent or lacked sufficient evidence to draw meaningful conclusions. Controlled human exposure studies and animal toxicologic studies provided limited biological plausibility of an underlying mechanism, although inflammation and oxidative stress were implicated. The remaining uncertainties

regarding epidemiologic studies were related to limited investigation of copollutant confounding, especially for other traffic-related pollutants. Animal and controlled human exposure evidence were limited in number and did not provide evidence of NO_2-driven effects on cardiovascular-relevant endpoints to support plausibility of epidemiologic findings.

Recent epidemiologic studies examining associations between short-term NO_2 exposures and MI or IHD consist primarily of case crossover studies that continue to show generally positive, although not entirely consistent, associations with short-term NO_2 exposures (see Table 5-2). These results come from studies in multiple countries including some large, multicity studies. Although still limited, recent evidence provides expanded analysis of copollutants effects on observed associations and suggest that associations are generally robust in copollutant models though some studies show attenuation of associations in models that account for other traffic-related pollutants.

In contrast, compared to studies in the 2016 Oxides of Nitrogen ISA, recent epidemiologic studies examining associations with cardiovascular mortality in large patient populations across multiple locations are less consistent in their support of a positive association with short-term NO_2 exposure. This evidence includes large studies that span a wider geographic range than was evaluated by studies in the 2016 Oxides of Nitrogen ISA, including several multicity studies across North America, Europe, and Asia.

Recent evidence expands the limited evidence base that was evaluated in the 2016 Oxides of Nitrogen ISA for associations between short-term NO_2 exposure and hospital admission or ED visits for arrythmia. While still limited in number, recent studies showed generally positive associations and were preformed over a wide geographic area. Positive associations with NO_2 concentrations were seen at lag times of 0–3-days. Results from studies that looked at associations with cardiac arrest were mixed. Recent studies looking at the relationship between heart failure and short term NO_2 concentrations expand the evidence of positive associations, but the evidence base as a whole remains limited in number and inconsistent.

Epidemiology studies of associations between short-term NO_2 concentrations and cerebrovascular disease, stroke, blood pressure, hypertension, and venous thromboembolism continue to show inconsistent results.

For epidemiology studies, utilization of exposure surrogates such as single monitors and unweighted or population-weighted averages of multiple monitors contributes to uncertainty in the measurement of exposure in epidemiologic studies that may result in biased health effect estimates. Exposure measurement error that does not adequately capture temporal variability in short-term NO_2 concentrations may underestimate the magnitude but not the directionality of the association

The evidence for subclinical effects that underlie the development of cardiovascular disease continues to be limited and inconsistent. Animal toxicologic and controlled human exposure studies continue to show little impact of acute NO_2 exposure on cardiovascular-relevant endpoints including HR,

HRV, QT interval measures, and vascular reactivity. Effects on more general markers of health such as inflammation, oxidative stress, and altered lipid metabolism were reported in animal studies but were not coherent with results of controlled human exposure studies.

Overall, the evidence is *suggestive of, but not sufficient to infer, a causal relationship* between short-term oxides of nitrogen exposure and cardiovascular effects. The strongest evidence for this determination comes from epidemiologic studies of associations between short-term NO_2 exposure and hospitalizations and ED visits for MI (see Table 5-2). The evidence supporting positive association between short-term NO_2 exposure and arrhythmias is also strengthened, but the continued lack of evidence of associations of NO_2 exposure with cardiac arrest and increased inconsistency in epidemiologic evidence of associations of NO_2 exposure with cardiovascular mortality add uncertainty to the evidence base. Epidemiologic evidence of associations with short-term NO_2 exposure and cerebrovascular disease, heart failure, blood pressure and hypertension, and venous thromboembolism remain inconsistent. Across the epidemiologic evidence, uncertainty remains over the potential for confounding by other traffic-related copollutants and the effect of exposure measurement error on the observed associations.

Experimental studies provide only limited support for the coherence of findings across disciplines and for biologically plausible pathways by which short-term NO_2 exposure could contribute to cardiovascular-related morbidity or mortality. In particular, controlled human exposure studies failed to find cardiovascular effects following short-term NO_2 exposures in both younger and older subjects, and in those with asthma and COPD. Thus, these studies do not support NO_2-induced cardiovascular effects at near-ambient concentrations. Animal toxicologic studies remain limited in number and provide little support for biological plausibility, although recent evidence of NO_2-induced formation of reactive oxygen species in cardiac tissue provides some support.

Table 5-2 Summary of evidence contributing to causality determinations for short-term oxides of nitrogen exposure and cardiovascular effects

Rationale for Causality Determination[a]	Key Evidence[b]	Key References	Oxides of Nitrogen Concentrations Associated with Effects
MI and IHD			
Generally consistent positive associations between short-term NO_2 exposure and MI and IHD in epidemiologic studies	Findings from a previously reviewed large multicohort prospective study along with more recent case crossover studies provides evidence that short-term NO_2 exposure is associated with MI and IHD events.	Section 5.3.1	Mean NO_2: 13.2–26.5 ppb for 1-hr max Mean NO_X: 24.5–56.0 ppb for 1-hr max

Rationale for Causality Determination[a]	Key Evidence[b]	Key References	Oxides of Nitrogen Concentrations Associated with Effects
Limited evidence of biological plausibility	Controlled human exposure studies and animal toxicology provide limited evidence for short-term NO_2 induced effects on vascular reactivity and arterial stiffness	†Karoui et al. (2020) †Li et al. (2011) Section 5.10.3.2 Section 5.10.3.3	Rats: 5 ppm NO_2 for 3 hrs or 2.66–5.32 ppm for 6 hr/d over 7 d Humans: 4 ppm NO_2 for 1 hr
	Animal studies provide limited evidence of increased ROS and inflammation in cardiac tissue in response to short-term NO_2 exposure	†Karoui et al. (2020) Section 5.10.4.3	Rats: 5 ppm NO_2 for 3 hrs
Cardiovascular Mortality			
Increased uncertainty in epidemiology studies of short-term NO_2 exposure and cardiovascular mortality	Recently identified studies show less consistent positive associations with cardiovascular mortality. These studies cover larger geographic regions.	Section 5.11.1	Mean NO_2: 6.27–34.6 ppb for 24-hr avg
Arrhythmia and Cardiac Arrest			
Epidemiology evidence for general positive associations	Recently reviewed case control studies show generally positive associations with short-term NO_2 concentrations	†Milojevic et al. (2014) †Santurtún et al. (2016) †Krall et al. (2018)	
Epidemiology evidence with inconsistent results	Inconsistent evidence of positive associations with cardiac arrest with some studies showing inverse relationships	Section 5.4.1	Mean NO_2: 6.96–23.1 ppb for 24-hr avg Mean NO_X: 24.5–56.0 ppb for 1-hr max
Limited support from controlled human exposure and animal tox studies	Predominantly null results for NO_2-induced effects on HRV or QT interval	Section 5.10.1.2 Section 5.10.2.2	Mean NO_2: 6.96–23.1 ppb for 24-hr avg Mean NO_X: 24.5–56.0 ppb for 1-hr max
	Evidence from an animal study that NO_2 reduced threshold to induce arrythmia and CA	†Farraj et al. (2016)	Rats: 500 ppb for 3 hrs
Cerebrovascular Disease and Stroke			
Inconsistent epidemiologic evidence	Mixed results from mostly time series and case crossover studies of stroke hospital admissions and ED visits	Section 5.5.1	Mean NO_2: 8.0–23.9 ppb for 24-hr avg Mean NO_2: 13.2–26.5 ppb for 1-hr max

Rationale for Causality Determination[a]	Key Evidence[b]	Key References	Oxides of Nitrogen Concentrations Associated with Effects
Heart Failure, Blood pressure, Hypertension, Venous Thromboembolism			
Inconsistent epidemiologic evidence	Limited number of epidemiology studies and with inconsistent results	Section 5.6.1 Section 5.7.1 Section 5.8.1	Mean NO_2: 1.8–24.5 ppb for 24-hr avg Mean NO_2: 13.2–26.5 ppb for 1-hr max Mean NO_X: 24.5–56.0 ppb for 1-hr max
Limited support for biological plausibility	Inconsistent findings from controlled human studies and animal studies on blood pressure	Section 5.10.1.2 Section 5.10.1.3	
Subclinical Effects			
Associations between short-term NO_2 exposure and HRV and HR mixed in epidemiology studies	Mixed results in recent epidemiology studies while positive associations between short-term NO_2 and HR and inverse relationships between short-term NO_2 and HRV are stronger in populations with underlying disease.	Section 5.10.1.1	Mean NO_2: 4.8–29.2 ppb for 24-hr avg Mean NO_2: 4.6–6.89 ppb for 1-hr max Mean NO_X: 35.4–39.1 ppb for 24-hr avg
Lack of epidemiology studies with mixed results for associations between short-term NO_2 and other subclinical markers of cardiovascular health	Few studies with inconsistent findings	Section 5.10.2.1 Section 5.10.3.1 Section 5.10.4.1	Mean NO_2: 4.8–29.2 ppb for 24-hr avg Mean NO_2: 4.6–6.89 ppb for 1-hr max Mean NO_X: 35.4–39.1 ppb for 24-hr avg
Lack of biological plausibility from animal and controlled human exposure studies	Few controlled human exposure studies of subclinical cardiovascular endpoints and results from existing studies generally are mixed or show no effect of NO_2 exposure	Section 5.10.1.2 Section 5.10.2.2 Section 5.10.3.2 Section 5.10.4.2	
	Animal studies of subclinical effects is strongest for effects of short-term NO_2 exposure on oxidative stress and inflammation although the number of studies is limited and the clinical importance of observed changes is uncertain	Section 5.10.1.3 Section 5.10.2.3 Section 5.10.3.3 Section 5.10.4.3	

List of abbreviations in alphabetical order: CA = cardiac arrest; d = day; ED = emergency department; hrs = hours; HR = heart rate; HRV = heart rate variability; IHD = ischemic heart disease; MI = myocardial infarction; NO_2 = nitrogen dioxide; ppm = parts per million; ROS = reactive oxygen species.

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

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

†Studies published since the 2016 Oxides of Nitrogen ISA.

5.15 References

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