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Integrated Science Assessment for Oxides of Nitrogen – Health Criteria

Chapter 7: Reproductive and Developmental Effects

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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

AC	abdominal circumference
AGD	anogenital distance
AQCD	Air Quality Criteria Document
ASD	atrial septal defects
ART	assisted reproductive technology
avg	average
BC	black carbon
BMI	body mass index
BPD	biparietal diameter
CI	confidence interval
CHD	congenital heart defects
CHT	congenital hypothyroidism
CMAQ	Community Multiscale Air Quality
CO	carbon monoxide
C-R	concentration-response
CRP	C-Reactive Protein
d	day(s)
e.g.	exempli gratia (for example)
EC	elemental carbon
ED	emergency department
ELF	epithelial lining fluid
EPA	U.S. Environmental Protection Agency
etc.	et cetera
F	female(s)
FGR	fetal growth restriction
FL	femur length
g	gram(s)
GD	Gestational day
GDM	Gestational diabetes mellitus
GTWR	gestational time window of risk
HC	head circumference
hr	hour(s)
HR	hazard ratio
i.e.	id est (that is)
IQR	interquartile range
ISA	Integrated Science Assessment
IUGR	intrauterine growth restriction
IVF	in vitro fertilization
L	liter(s)
LUR	land use regression
M	male
m	milligram
m	meter(s)
Max	maximum
Min	minimum
min	minute(s)
mL	milliliter
mo	month(s)
n	sample size
N	population size
NA	not applicable
NO	nitric oxide
NO_2	nitrogen dioxide
NO_X	NO_2 + NO
NR	not reported
O_3	ozone
OR	odds ratio
P10	10^{th} percentile
P25	25^{th} percentile
P5	5^{th} percentile
P75	75^{th} percentile
P95	95^{th} percentile
PECOS	Population, Exposure, Comparison, Outcome, and Study Design
PM	particulate matter
PM_{10}	particulate matter with a nominal mean aerodynamic diameter less than or equal to 10 μm
$PM_{2.5}$	particulate matter with a nominal mean aerodynamic diameter less than or equal to 2.5 μm
ppb	parts per billion
ppm	parts per million
PTB	Preterm birth
RR	relative risk or risk ratio
SD	standard deviation
SE	standard error
SES	socioeconomic status
SGA	small for gestational age
SO_2	sulfur dioxide
TGA	the great arteries
ToF	tetralogy of Fallot
U.K.	United Kingdom
U.S.	United States
UFP	ultra fine particles
vs.	versus
VPTB	very preterm birth
VSD	ventricular septal defects
WQS	weighted quantile sum
wk	week(s)
yr	year(s)
μg	microgram
β	beta
$\mu g/m^3$	micrograms per cubic meter
μm	micrometer
ρ	rho

CHAPTER 7 Reproductive and Developmental Effects

Summary of Causality Determinations for Oxides of Nitrogen Exposure and Reproductive and Developmental Effects

This chapter characterizes the scientific evidence that supports causality determinations for oxides of nitrogen exposure and Reproductive and Developmental 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 detail 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
Male Reproductive Function	Inadequate to infer a causal relationship
Female Reproductive Function	Inadequate to infer a causal relationship
Pregnancy Outcomes	Suggestive of, but not sufficient to infer, a causal relationship
Birth Outcomes	Suggestive of, but not sufficient to infer, a causal relationship
Postnatal Development	Inadequate 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.

7.1 Introduction

7.1.1 Summary of the Previous ISA

The U.S. Environmental Protection Agency's (EPA) 2016 Integrated Science Assessment for Oxides of Nitrogen – Health Criteria (hereafter referred to as the 2016 Oxides of Nitrogen ISA) issued causality determinations for the effects of oxides of nitrogen exposure and reproductive and developmental effects (U.S. EPA, 2016). The evidence underpinning these causality determinations is briefly summarized below.

The 2016 Oxides of Nitrogen ISA included three causality determinations for reproductive and developmental effects: (1) fertility, reproduction, and pregnancy; (2) birth outcomes; and (3) postnatal development. Overall, the evidence was determined to be suggestive of, but not sufficient to infer, a causal relationship between oxides of nitrogen exposure and birth outcomes, and the evidence was inadequate to infer the presence or absence of causal relationships with fertility, reproduction, and pregnancy as well as with postnatal development. The strongest evidence for reproductive and developmental effects came from epidemiologic studies that assessed the relationship between nitrogen dioxide (NO_2) exposure and birth outcomes, specifically fetal growth restrictions. These epidemiologic studies were generally supportive but inconsistent, with the strongest evidence from well-conducted Spanish cohort studies supported by consistent evidence for associations with small for gestational age (SGA) and intrauterine growth restriction (IUGR). The remaining uncertainty for epidemiologic studies of NO_2 exposure and reproductive and developmental effects was regarding the independent effect of NO_2 exposure, as potential copollutant confounding was not evaluated. Toxicological evidence for the effects of oxides of nitrogen on reproductive and developmental endpoints was severely lacking. Very few animal toxicological studies were available in the 2016 Oxides of Nitrogen ISA. The limited available toxicological evidence made it challenging to judge consistency and coherence among studies and added to the uncertainty regarding the independent reproductive and developmental effects of exposure to NO_2 or other oxides of nitrogen.

7.1.2 Chapter Organization and Conventions

This chapter evaluates recent evidence examining the relationship between oxides of nitrogen exposure and reproductive and developmental effects. Its conclusions are drawn from the integration of the full body of evidence available in the 2016 Oxides of Nitrogen ISA and the evidence that has become available since the 2016 Oxides of Nitrogen ISA. The following sections provide an overview of the study inclusion criteria for this chapter (see Section 7.2) followed by summaries of recent health effects evidence for male reproductive function (see Section 7.3), female reproductive function (see Section 7.4), pregnancy outcomes (see Section 7.5), birth outcomes (see Section 7.6), and postnatal development (see

Section 7.7). 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 pregnancy outcomes and birth outcomes, there is a summary of lifestages and population. Each health outcome section includes a discussion of the causality determination for that outcome category (see Section 7.3.4 and Table 7-1; Section 7.4.6 and Table 7-2; Section 7.5.10, Table 7-3 and Table 7-4; Section 7.6.9 and Table 7-5 and Table 7-6; and Section 7.7.5 and Table 7-7, respectively). For outcomes with larger bodies of supporting evidence (e.g., pregnancy outcomes, birth outcomes), studies related to biological plausibility are discussed separately (see Sections 7.5.8 and 7.6.7), and for outcomes supported by evidence from multiple disciplines, subsections titled "Synthesis Across Lines of Evidence" are included.

7.1.2.1 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 two-pollutant models or co-adjusted models, can reduce concern for confounding by copollutants when correlations between the copollutants are relatively low (e.g., $\rho<0.4$). However, when correlations are high (e.g., $\rho>0.7$), 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.

Unless otherwise specified, throughout this chapter, exposure to oxides of nitrogen is considered a long-term exposure (e.g., more than 30 days). There were a limited number of recent epidemiologic studies that evaluated the days immediately preceding birth or on specific weeks during gestation (short-term exposure). While some recent epidemiologic studies evaluate short-term exposures (e.g., gestational weeks), these shorter exposure periods are thought to contribute to the long exposure period (e.g., entire pregnancy). Additionally, in the summaries of the recent epidemiologic evidence, the abbreviation "NO_X" is widely used in the epidemiologic literature and is assumed to be the sum of nitric oxide (NO) and NO_2, which are the dominant forms of oxides of nitrogen emitted from major sources (see Chapter 1, Section 1.3.1 and Chapter 2, Section 2.2).

To increase comparability of results across epidemiologic studies, this Oxides of Nitrogen ISA presents effect estimates for associations with health outcomes scaled to the same increment of oxide of nitrogen concentration.[1] The increments for standardization for short-term exposure vary by averaging time (e.g., 24-hour avg, 8-hour max, 1-hour max) and oxide of nitrogen. For 24-hour average, effect estimates were scaled to a 20 parts per billion (ppb) increase for NO_2 or NO and a 30 ppb increase for NO_X. For a 1-hour maximum, effect estimates were scaled to a 25 ppb increase for NO_2, a 60 ppb increase for NO, and an 80 ppb increase for NO_X. For an 8-hour maximum, the increments for standardization are 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, 2015b). There were common exceptions to this standardization method. Averaging times other than 24-hour average or 1-hour maximum were examined, for example, 2- to 15-hour averages. Effect estimates based on these averaging times were not standardized but are presented in the ISA as reported in their respective studies.

For long-term exposure metrics, effect estimates are scaled to a 10 ppb increase in NO_2, a 15 ppb increase in NO, and a 20 ppb increase in NO_X. These increments were derived by calculating the U.S. nationwide percentile distributions for annual average concentrations and then calculating the approximate difference between the median (a typical pollution year) and the 95th percentile (a more polluted year) concentrations among monitors in the State and Local Air Monitoring Stations network (SLAMS). Long-term average concentrations of oxides of nitrogen in ambient air are less variable across time, and do not differ widely among multi-month averages, annual averages, or multi-year averages. Thus, all long-term exposure metrics were scaled to the same increment.

Some epidemiologic 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

[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.

concentrations in μg/m^3 at a national scale for the United States or other countries. Therefore, the ISA presents effect estimates based on μg/m^3 of NO_X as they are reported in their respective studies.

7.1.2.2 Animal Toxicological Studies

In the assessment of the recent toxicological evidence, this chapter includes targeted discussions of analyses examining dose-response relationships, relevance of toxicological responses to human responses, and multipollutant exposures. For apical endpoints with no or limited evidence on these topics, subsections addressing them are not included.

7.2 Scope

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

[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 new 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 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).

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 reproductive and developmental health effects.

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

Animal Toxicological Studies:

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

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

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

Outcome: Reproductive and developmental outcomes.

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

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

[4]Five ppm is approximately two orders of magnitude higher than peak NO_2 concentrations in the ambient air in the United States. As discussed in the 2015 Preamble to the ISAs (U.S. EPA, 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.

7.3 Effects on Male Reproductive Function

The 2016 oxides of nitrogen (U.S. EPA, 2016) concluded that the evidence was inadequate to infer the presence or absence of a causal relationship between oxides of nitrogen exposure and male reproductive function. There was little and inconsistent epidemiologic evidence and no toxicological evidence of effects of NO_2 exposure on sperm or semen quality. The recent evidence continues to be limited. The recent epidemiologic and animal toxicological studies that examined the relationship between exposure to oxides of nitrogen and male reproductive function are summarized in the text below. Details of the recent epidemiologic studies are included in Appendix A – Appendix Table 7-1 and the recent animal toxicological studies are in Appendix A – Appendix Table 7-2.

7.3.1 Sperm and Semen Production, Quality, and Function

In the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016), there was a limited number of epidemiologic studies that evaluated the relationships between exposure to NO_2[5] and sperm and semen production, quality, and function. There were no animal toxicological studies. Overall, the epidemiologic evidence was limited and inconsistent for effects on sperm and no examination of copollutant confounding or concentration-response relationship. There continues to be limited evidence examining the associations between oxides of nitrogen and sperm and semen production, quality, and function across the recent epidemiologic and animal toxicological studies.

7.3.1.1 Epidemiologic Studies of Sperm and Semen Production, Quality, and Function

Evidence from the recent epidemiologic studies is summarized below. Study specific details including study population characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), potential confounders, and select results are in Appendix A – Appendix Table 7-1. Overall, the recent epidemiologic evidence of sperm and semen production, quality, and function remains limited. The few recent studies evaluated different apical endpoints, making it difficult to judge consistency and coherence across the studies.

- A recent epidemiologic study evaluated the association of residential exposure to ambient air pollutants, estimated using the Community Multiscale Air Quality (CMAQ) model, with 35 semen quality parameters among men enrolled in a longitudinal time-to-pregnancy study in the U.S. study participants were part of the Longitudinal Investigation of Fertility and the Environment (LIFE) Study (Nobles et al., 2018). There was decreased total sperm count (β: -20.64 million [95% CI: -38.76, -2.53] per 4.6 ppb increase in NO_2), decreased number of immature sperm (β: -0.61 [95% CI: -1.09, -0.14]), and increased sperm head length (β: 0.031 µm [95% CI: 0.005, 0.056]) associated with exposure to NO_2 72 days before ejaculation, but

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

no associations between exposure to NO_X and any of the semen quality parameters. Nobles et al. (2018) also evaluated associations with NO_X in a multipollutant model including SO_2, O_3, CO, PM_{10}, and $PM_{2.5}$ in a single model and the 35 semen quality parameters. In the multipollutant model, there was decreased micro head size (β: -0.241% [95% CI: -0.411%, -0.071%] per 8.3 ppb) and decreased number of immature sperm (β: -0.66 [95% CI: -1.22, -0.09]). The association in the multipollutant model may be a result of the moderate to high correlations between the different pollutants, resulting in bias amplification in the effect estimates.

- Among sperm donors in a multi-center study in six provinces in China, Cai et al. (2023) used ensemble machine learning to estimate daily average concentrations of NO_2 in association with four sperm parameters. There was decreased sperm concentration (β: -0.0369 $x10^6$/mL [95% CI: -0.0729, -0.0001]) overall. There were also negative, but imprecise, associations with sperm count (β: -0.0043 $x10^6$ [95% CI: -0.0398, 0.0311]), progressive motility (β: -0.0073% [95% CI: -0.0441, 0.0295]), and total motility (β: -0.0153% [95% CI: -0.0568, 0.0262]). Additionally, Cai et al. (2023) also evaluated the associations in co-adjusted models for CO, SO_2, and O_3. In two-pollutant models with CO, SO_2, and O_3, the associations between NO_2 exposure and decreased sperm concentration were similar to those in single pollutant models. The estimates were additionally unchanged for sperm count in the two-pollutant models with CO, SO_2, and O_3. In a two-pollutant model adjusted for CO or SO_2, there was an imprecise positive association for progressive motility, but a null association when adjusted for O_3. For total motility, when adjusted for CO or SO_2, there was an imprecise positive association, but adjusting for O_3, the association was similar to the single exposure model for NO_2.

7.3.1.1.1 Conclusions and Remaining Uncertainties

There is limited evidence examining the associations between exposure to oxides of nitrogen and sperm and semen production, quality, and function. While the few recent studies provide some evidence of effects on sperm and semen production, quality, and function, there are still remaining uncertainties regarding the concentration-response relationship, lifestages and population differences, and potential of confounding by copollutants. The different apical endpoints assessed by a few of the recent epidemiologic studies make it difficult to judge consistency and coherence across studies.

7.3.1.2 Animal Toxicological Studies of Sperm and Semen Production, Quality, and Function

The 1993 Oxides of Nitrogen Air Quality Criteria Document (AQCD) summarized two studies that investigated the effects of NO_2 on sperm. Gooch et al. (1977) exposed adult C3H male mice to 0.1, 1, or 5 ppm of NO_2 for six hours and observed no abnormalities in the chromosomal health of their spermatocytes eight weeks after the exposure, when compared to that of unexposed controls. Similarly, Kripke and Sherwin (1984) exposed adult LEW/f rats to 1 ppm NO_2 for seven hours/day, five days/week, for three weeks and reported no effects on spermatogenesis or testicular histopathology. The 2016 Oxides of Nitrogen ISA and the 2008 Oxides of Nitrogen ISA did not include additional toxicological studies that

examined the effects of oxides of nitrogen exposure on sperm or semen quality, and no recent animal toxicological studies have investigated these effects following NO_2 exposure.

7.3.1.2.1 Conclusions and Remaining Uncertainties

There is limited animal toxicological evidence examining the effects of exposure to oxides of nitrogen on sperm and semen production, quality, and function. Although the few available studies both report a lack of effects on the investigated outcomes (chromosomal health of spermatocytes and testicular histopathology), there remains a large amount of uncertainty due to the lack of available data. Specifically, studies assessing the effects of exposure to oxides of nitrogen on parameters such as sperm count, sperm motility, sperm morphology, and sperm viability are completely absent. Additional studies are needed to fully elucidate the potential impact of exposure to oxides of nitrogen on testicular function and sperm and semen parameters, especially those that assess a potential dose-response relationship.

7.3.2 Other Male Reproductive Function Outcomes

There were a few recent studies of male reproductive function that evaluated endpoints other than sperm and semen production, quality, and function. Those studies are summarized below and more specific study details, including study population characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), and selected results are in Appendix A – Appendix Table 7-1.

7.3.2.1 Epidemiologic Studies of Other Male Reproductive Outcomes

Evidence for other male reproductive outcomes from recent epidemiologic studies are summarized below. Study specific details including study population characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), potential confounders, and select results are in Appendix A – Appendix Table 7-1. There was a single recent study of erectile dysfunction and a single recent study of male programming windows and mini-puberty with anogenital distance and penile width at birth. The few recent studies evaluated different apical endpoints, making it difficult to judge consistency and coherence across the studies.

- Tallon et al. (2017) conducted a cross-sectional analysis of the National Social Life, Health, and Aging Project (NSHAP) cohort to investigate the association of 1- to 7-year moving average NO_2 exposure, estimated from EPA's Air Quality System (AQS), and self-reported erectile dysfunction. Associations were generally consistent across averaging times, with positive, but imprecise, associations between exposure to NO_2 and erectile dysfunction. The strongest association reported was with the 6-year moving average for erectile dysfunction (OR: 1.16 [95% CI: 0.65, 2.07]).

- Barrett et al. (2023) examined exposure to NO_2 in relation to human anogenital distance (AGD) at birth and at one year of age, focusing on exposures during critical windows of reproductive development including male programming window (gestational weeks eight to 14) and mini-puberty (postnatal months one to three), among participants of The Infant Development and Environment Study (TIDES) in four U.S. sites. AGD is an indicator of the prenatal androgen environment and can serve as a signal (predictor) of reproductive health and prenatal exposures like endocrine disrupting chemicals that interfere with in utero hormone signaling result in shorter, less androgenized AGD in males at birth. When AGD metrics were measured at birth for the male programming window, Barrett et al. (2023) reported a decreased AGD-anus to penis (β: -0.003 mm [95% CI: -0.14, 0.13] per 1 ppb increase in NO_2), increased AGD-anus to scrotum (β: 0.04 mm [95% CI: -0.07, 0.15] per 1 ppb increase in NO_2), and increased penile width (β: 0.02 mm [95% CI: -0.02, 0.05] per 1 ppb increase in NO_2), but all CIs include the null. When AGD metrics were measured at age one for the male programming window, there was decreased penial width (β: -0.07 mm [95% CI: -0.12, -0.02] per 1 ppb increase in NO_2), and imprecise associations with AGD-anus to penis (β: -0.12 mm [95% CI: -0.31, 0.07] per 1 ppb increase in NO_2) and AGD-anus to scrotum (β: 0.03 mm [95% CI: -0.13, 0.20] per 1 ppb increase in NO_2). When AGD metrics were measured at age one for mini-puberty window, there were consistent negative, but imprecise, associations for AGD-anus to penis (β: -0.06 mm [95% CI: -0.23, 0.12] per 1 ppb increase in NO_2), AGD-anus to scrotum (β: -0.05 mm [95% CI: -0.20, 0.11] per 1 ppb increase in NO_2), and increased penile width (β: -0.04 mm [95% CI: -0.08, 0.01] per 1 ppb increase in NO_2).

7.3.2.1.1 Conclusions and Remaining Uncertainties

There was a small body of recent epidemiologic studies of other male reproductive functions; however, the small number of studies of different apical outcomes limits the ability to judge coherence and consistency across these studies. There remains uncertainty regarding concentration-response relationship, lifestages and population differences, and potential confounding by copollutants.

7.3.3 Synthesis Across Lines of Evidence

There remains limited evidence regarding the effects related to exposure to oxides of nitrogen and male reproductive function. No recent toxicological studies have investigated the effects of exposure to oxides of nitrogen, including NO_2 exposure, on male reproductive function. There were only a few recent epidemiologic studies that evaluated associations between oxides of nitrogen and sperm and semen production, quality, and function. While the recent epidemiologic studies provide some evidence of effects on sperm and semen production, quality, and function, the evidence is limited and there are remaining uncertainties regarding the concentration-response relationship, lifestages and population differences, and potential of confounding by copollutants. While there were no recent toxicological studies of male reproductive function, older evidence did not report effects relating to sperm and semen production, quality, and function. The limited evidence from both epidemiologic and toxicological studies makes it difficult to judge consistency and coherence across the multiple lines of evidence.

7.3.4 Summary and Causality Determination

The 2016 Oxides of Nitrogen ISA concluded that there was insufficient evidence to infer a causal relationship between exposure to oxides of nitrogen, primarily NO_2 exposure, and effects on male reproductive function. Epidemiologic studies summarized in the 2016 Oxides of Nitrogen ISA investigated sperm and semen quality parameters. Toxicological studies assessed the effects of NO_2 exposure on spermatogenic processes and health of germinal and interstitial cells in the testis of rats.

Epidemiologic evidence regarding NO_2 exposure and male reproductive function in the 2016 Oxides of Nitrogen ISA tended to be inconsistent. One study in occupationally exposed males reported that NO_2 exposure was associated with lower sperm motility (Boggia et al., 2009), but reported a null association with sperm count. Another study reported that NO_2 exposure was associated with negative impacts on sperm morphology and motility (Zhou et al., 2014). In contrast, two other epidemiologic studies reported null associations between NO_2 exposure and semen quality parameters (Rubes et al., 2010; Sokol et al., 2006). Toxicological evidence was limited to a single study, which reported no effects on spermatogenesis or on the germinal and interstitial cells of the testes in exposed rats (Kripke and Sherwin, 1984).

Among the recent evidence, there were no toxicological studies investigating the effects of exposure to oxides of nitrogen, including NO_2 exposure, on male reproductive function (see Table 7-1). While the few recent epidemiologic studies provide some evidence of effects on sperm and semen production, quality, and function, there are remaining uncertainties regarding concentration-response relationship, lifestages and population differences, and potential of confounding by copollutants. However, the limited evidence from both the epidemiologic and animal toxicological studies makes it difficult to judge consistency and coherence across studies. **Overall, the evidence is *inadequate to infer the presence or absence of a causal relationship* between oxides of nitrogen exposure and male reproductive function.**

Table 7-1 Summary of evidence contributing to causality determinations for oxides of nitrogen exposure and effects on male reproductive function

Rationale for Causality Determination[a]	Key Evidence[b]	Key References	Oxides of Nitrogen Concentrations Associated with Effects
Sperm and Semen Production, Quality, and Function			
Lack of supporting epidemiologic and toxicological evidence for effects on sperm	Limited number of animal toxicological and epidemiologic studies provide no evidence for effects on sperm count or motility, spermatogenesis.	Rats: Kripke and Sherwin (1984) Gooch et al. (1977)	0.1–5 ppm NO_2

Rationale for Causality Determination[a]	Key Evidence[b]	Key References	Oxides of Nitrogen Concentrations Associated with Effects
		Humans: †Nobles et al. (2018)	Median (IQR) NO_2: 7.3 (4.6) ppb Median (IQR) NO_X: 11.2 (8.3) ppb
		Humans: †Cai et al. (2023)	Median NO_2:23.60 ppb for NO_2

List of abbreviations in alphabetical order: IQR = interquartile range; NO_2 = nitrogen dioxide; NO_X = oxides of nitrogen; ppb = parts per billion.
[a]Based on application of the causality framework described in section 3.3.4 of Volume 2 of the Integrated Review Plan for the Primary National Ambient Air Quality Standards for Oxides of Nitrogen (U.S. EPA, 2024).
[b]Describes the key evidence and provides references contributing to the causality determination. References to earlier sections indicate where the full body of evidence is described.
†Studies published since the 2016 Oxides of Nitrogen ISA.

7.4 Effects on Female Reproductive Function

In the 2016 Oxides of Nitrogen ISA, there were a limited number of epidemiologic and animal toxicological studies examining female reproductive function. Of the epidemiologic studies, most focused on in vitro fertilization (IVF) outcomes. In the animal toxicological studies, NO_2-exposed rats showed impaired estrus cyclicity and a decrease in the number of primordial follicles, which could indicate an effect on reproduction. Overall, the 2016 Oxides of Nitrogen ISA concluded that there was limited and inconsistent evidence across the epidemiologic and toxicological studies. There remains limited evidence across the epidemiologic and toxicological studies evaluating associations between oxides of nitrogen and female reproductive function in the recent literature.

7.4.1 Fertility and Reproduction

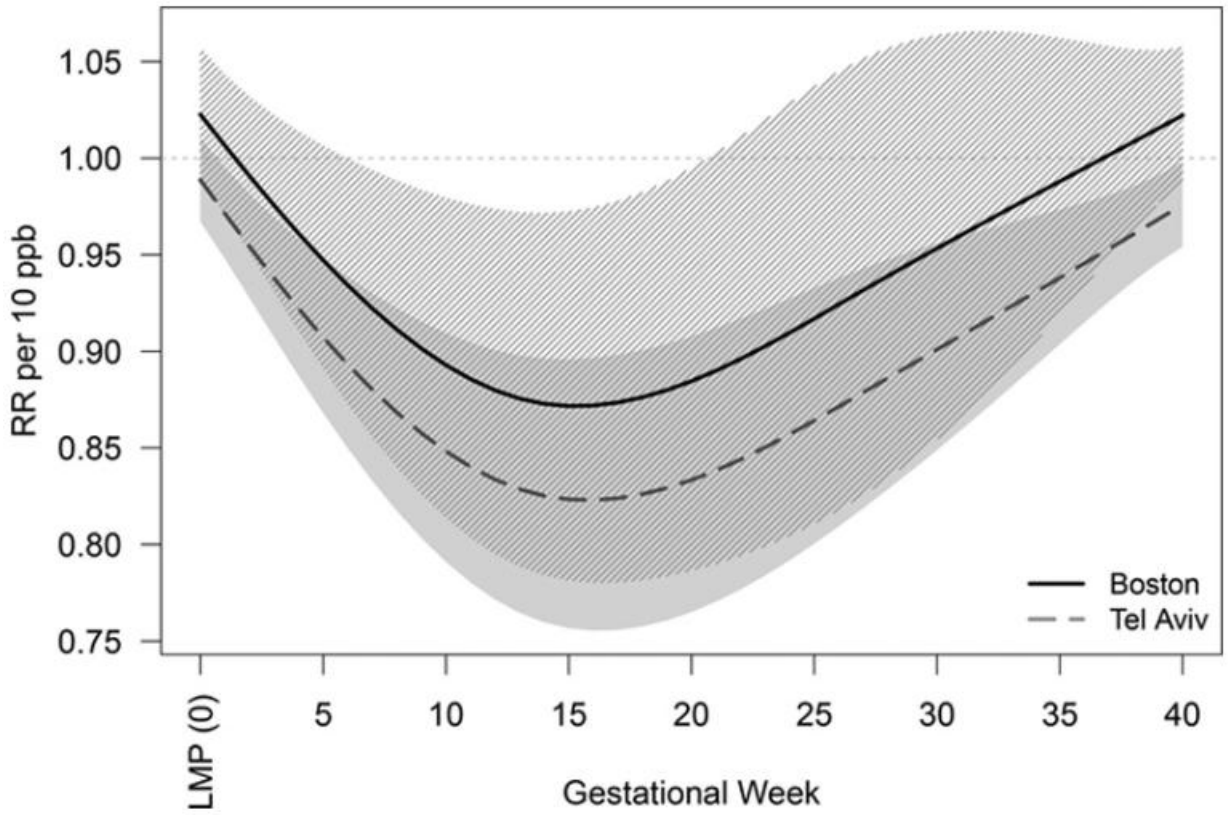
The recent epidemiologic and toxicological studies examining the relationship between exposure to oxides of nitrogen and fertility and reproduction are summarized in the text below. Study details of the recent epidemiologic studies are included in Appendix A – Appendix Table 7-3 and the recent animal toxicological studies are in Appendix A – Appendix Table 7-2.

7.4.1.1 Epidemiologic Studies of Fertility and Reproduction

There were a limited number of epidemiologic studies reviewed in the 2016 Oxides of Nitrogen ISA and most focused on in vitro fertilization (IVF) outcomes (Legro et al., 2010; Slama et al., 2013). Overall, there was inconsistent evidence for IVF failure, with only a few studies reporting lower odds of live birth associated with higher NO_2 concentrations during ovulation induction and the period after embryo transfer.

Evidence from recent studies is summarized below. Study-specific details including study population characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), potential confounders, and select results are in Appendix A – Appendix Table 7-3. In the recent epidemiologic studies, there were some studies of IVF-related outcomes in association with exposure to NO_2 and/or NO_X. The most commonly evaluated IVF outcomes were pregnancy loss or live births, and there were inconsistent associations among the small number of recent studies examining these endpoints. Most studies evaluated U.S. populations, with a variety of exposure assessment metrics, and exposure windows considered. While most studies were conducted in cohorts related to IVF or assisted reproductive technology (ART) (Quraishi et al., 2019; Ha et al., 2017), a single study explored associations of pregnancy loss in the general population (Kioumourtzoglou et al., 2019). Additionally, Kioumourtzoglou et al. (2019) utilized a time series approach to evaluate week-to-week traffic-related air pollution and conceptions resulting in live births, as well as potential critical windows of exposure. Among the studies conducted in cohorts for IVF or ART, the associations were inconsistent overall.

- In the Longitudinal Investigation of Fertility and the Environment study, Ha et al. (2017) investigated the association between exposure to NO_2 and NO_X and incidence of pregnancy loss in a cohort of couples attempting pregnancy. Exposure to NO_2 and NO_X was estimated from CMAQ. Average entire pregnancy exposure to NO_2 had a positive, but imprecise, association with the risk of pregnancy loss (HR for NO_2: 1.03 [95% CI: 0.98, 1.08] per 4.8 ppb increase in NO_2), but there was a null association with risk of pregnancy loss with average entire pregnancy exposure to NO_X (HR for NO_X: 0.98 [95% CI: 0.95, 1.02] per 7.5 ppb increase in NO_X). Considering short-term exposures for the gestational week of the loss and for the prior week appeared to have no impact on the associations.
- Quraishi et al. (2019) used medical records from women initiating an IVF cycle in one of six participating clinics and NO_2 exposure estimates for the year before the start of the IVF cycle from a hybrid model. There was a 0.04% (95% CI: -0.08%, -0.004%) lower oocyte fertilization per 5-ppb increase in NO_2. However, for other markers of IVF, there were null associations with embryo grade, positive hCG test, positive ultrasound, and live birth.
- Kioumourtzoglou et al. (2019) explored pregnancy loss in the general population based on live births from hospitals in Boston, Massachusetts, U.S., and Tel Aviv District, Israel. This study utilized a novel variation on a time-series design in which the outcome metric was live birth-identified conceptions. For each calendar week, the number of conceptions that resulted in live births (live birth-identified conceptions) were determined using data on gestational age at birth. Exposure to NO_2 in Boston was estimated from monitoring data on NO_2 concentrations in ambient air from EPA's AQS database, while an optimized dispersion model (leave-one-out cross-validation $r^2 = 0.62$) was used to estimate exposure to NO_2 in Tel Aviv District. In the two study locations, the median concentrations of NO_2 were similar, 17.4 ppb for Boston and 16.3 ppb in Tel Aviv District. In Boston, the strongest association was during the 15th week of gestation. For every 10 ppb of NO_2 increase during that week, there was decreased rate in live birth-identified conception (RR: 0.87 [95% CI: 0.78, 0.97]). Overall, among the Boston births, there were decreases in live birth-identified conception in association with NO_2 exposures between gestational weeks six and 19. In Tel Aviv District, the strongest association was during gestational week 16, with decreased rate in live birth-identified conception (RR: 0.82 [95% CI: 0.76, 0.90] per 10 ppb increase in NO_2) (see Figure 7-1).



List of abbreviations in alphabetical order: LMP = last menstrual period; ppb = part per billion; RR = rate ratio.
Notes: The shaded areas represent the 95% confidence intervals. Solid black line is for Boston and striped, black line is for Tel Aviv.
Source: Kioumourtzoglou et al. (2019). Copyright permission pending.

Figure 7-1 Associations between weekly nitrogen dioxide over gestation and the rate ratio for live birth identified conceptions.

7.4.1.1.1 Conclusions and Remaining Uncertainties

There was a small body of recent epidemiologic studies evaluating various IVF outcomes; however, the small number of studies limits the ability to judge coherence and consistency across these studies. However, the associations reported demonstrate that exposure to oxides of nitrogen could result in physiological responses that contribute to adverse outcomes related to pregnancy loss or live births. There are remaining uncertainties regarding the concentration-response relationship, lifestages and population differences, and potential confounding by copollutants.

7.4.1.2 Animal Toxicological Studies of Fertility and Reproduction

The 1993 Oxides of Nitrogen AQCD summarized a single study that assessed the effects of NO_2 on female fertility and aspects of female reproduction using rats (Shalamberidze and Tsereteli, 1971). Shalamberidze and Tsereteli (1971) exposed female albino rats to 0.126 or 2.36 mg/m^3 NO_2 (0.067 or 1.25 ppm NO_2) for 12 hours/day over three months and assessed reproductive outcomes in mating trials immediately after dosing was complete. When compared to unexposed controls, the ability of females to conceive and maintain a pregnancy was unaffected in both treatment groups. However, females in the higher treatment group (1.25 ppm NO_2) produced significantly smaller litters than the unexposed control group. Although it is not a direct measure, litter size in rodents can correlate with female fertility, indicating that overall female fertility may be impacted by exposure to 1.25 ppm NO_2 (Shalamberidze and Tsereteli, 1971). The 2008 Oxides of Nitrogen ISA also summarized a single study that evaluated effects of NO_2 on relevant endpoints (Di Giovanni et al., 1994). Di Giovanni et al. (1994) exposed female Wistar rats to 1.5 or 3 ppm NO_2 throughout gestation (gestational day [GD] 0 to GD 20) and observed no effects on the percentage of dams giving birth or litter size at birth when compared to unexposed controls, indicating that NO_2 exposure during gestation did not impact female fertility or reproduction. No additional toxicological studies that investigated the effects of oxides of nitrogen exposure on female fertility and reproduction were reported in the 2016 Oxides of Nitrogen ISA.

Since the 2016 Oxides of Nitrogen ISA, several recent toxicological studies were identified that did not corroborate the findings of Shalamberidze and Tsereteli (1971) but, similar to Di Giovanni et al. (1994), largely reported null effects of NO_2 exposure on female fertility parameters (Li et al., 2022; Yan et al., 2020; Yue et al., 2018; Yue et al., 2017). Evidence from recent studies, including study specific details, exposure conditions, and endpoints examined, are highlighted in the text below and described in more detail in Appendix A – Appendix Table 7-2. Notably, all recent studies were conducted by the same research group and utilized the same doses and dosing regimen (dams were dosed with 2.5 ppm NO_2 for 5 hours/day throughout gestation (GD 0 to postnatal day [PND] 0).

- Two studies utilized BALB/c mice and reported no effects on delivery rate (interpreted as percent of dams who successfully gave birth) or litter size (Yue et al., 2018; Yue et al., 2017).
- The other studies utilized C57BL/6 (Yan et al., 2020) and C57BL/6J mice (Li et al., 2022) and both reported no effects of NO_2 exposure on litter size.

The contrast between the findings of Shalamberidze and Tsereteli (1971) and those available for the current Oxides of Nitrogen ISA and 2008 Oxides of Nitrogen ISA may be attributed to differences in dosing regimen and exposure window. Although Shalamberidze and Tsereteli (1971) utilized lower doses (1.25 ppm) than more recent studies (1.5 to 3 ppm), it is possible that the shorter duration of exposure used in recent studies (20 to 21 days) is insufficient to elicit the effects observed in the previous study, which used a much longer dosing duration (three months prior to mating). Furthermore, exposure during the premating window may be necessary to elicit effects such as the reduced litter size observed by Shalamberidze and Tsereteli (1971). Indeed, Shalamberidze and Tsereteli (1971) reported that treated rats

also exhibited altered estrous cyclicity and altered ovarian follicle populations, indicating that NO_2 may disrupt hormonal cycles and negatively impact the ovary, both of which could manifest as a reduction in litter size if ovulation is disrupted or if oocyte/ovum health is compromised.

7.4.1.2.1 Conclusions and Remaining Uncertainties

There is limited toxicological evidence examining the effects of exposure to oxides of nitrogen on female fertility and reproduction, and contrasting findings were reported between studies from the 1993 Oxides of Nitrogen AQCD and more recent ISAs (reduced litter size and no effects on litter size, respectively). The observed contrast in results could be attributed to differences in dose, dosing duration, and exposure timing. Thus, more studies are needed to investigate the impacts of exposure to oxides of nitrogen on female fertility and to determine what exposure windows may be the most sensitive. Specifically, studies investigating a dose-response relationship and studies utilizing a variety of exposure windows (e.g., premating/preconception, gestational, lactational) would be particularly informative. Further, additional studies to interrogate what biological processes are affected to impart reduced fertility (e.g., impaired ovulation, impaired implantation, altered reproductive hormones) would also greatly contribute to the understanding of the impacts of exposure to oxides of nitrogen.

7.4.2 Menstrual and Estrous Cycle

Recent epidemiologic studies evaluating associations between exposure to oxides of nitrogen and menstrual cycle are discussed below. Study details for the recent epidemiologic studies are included in Appendix A – Appendix Table 7-3. There were no recent toxicological studies examining exposure to oxides of nitrogen and effects on the estrous cycle.

7.4.2.1 Epidemiologic Studies of Menstrual and Estrous Cycle

There were no epidemiologic studies of menstrual and estrous cycle in the 2016 Oxides of Nitrogen ISA. The recent literature is limited. Study-specific details including study population characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), potential confounders, and select results are in Appendix A – Appendix Table 7-3.

- Giorgis-Allemand et al. (2020) evaluated the short-term effects of NO_2 on the length of the follicular and luteal phases and the duration of the menstrual cycle on a small number of participants in the Observatory of fecundity in France (Obseff) study. Short-term exposure to NO_2 was estimated from a spatial model, with three windows of exposures: 1 to 30 days before cycle start, days 1 to 10 before cycle start, and days 11 to 30 before cycle starts. Among women not using hormonal contraception, the strongest association was in days 1 to 10 before cycle start, with an increase in mean follicular length of 0.81 days (95% CI: 0.37,

1.25) and evidence of linear associations among the tertiles (p = 0.03). There were null associations between NO_2 and luteal phase length or total cycle length across any exposure windows and no evidence of a linear association across tertiles. In addition, when adjusted for $PM_{2.5}$ in days 1 to 30 before cycle start, there was attenuation in the associations for follicular phase length and total cycle, and a slight increase in luteal phase length, but all the CIs included the null.

- In a nationwide study in Taiwan, Lin et al. (2021) evaluated associations between exposure to air pollutants and risk of dysmenorrhea, which is defined as painful cramps of the uterus during menstruation (ICD-9 code 625.3). Exposure to NO_X, NO_2, and NO was estimated from the Taiwan Air Quality Monitoring Database and was categorized into quartiles of exposure. There was an increased risk of dysmenorrhea in the highest quartile of exposure to NO_X (HR: 27.9 [95% CI: 21.6, 31.3]), NO_2 (HR: 33.1 [95% CI: 30.9, 37.4]), and NO (HR: 16.7 [95% CI: 15.4, 18.4]), respectively, compared to the lowest quartile of exposure, or referent. When stratified by age (<30, 30 to 44, and ≥45), in the highest quartile of exposure there were increased HRs for NO_X, NO_2, and NO across all age groups. High urbanization and occupation class not white collar (occupations involving working in an office and doing work that requires mental rather than physical effort) or blue collar (occupations such as farmers or fishermen, who do work that requires strength or physical skill) had increased risk of dysmenorrhea in the highest quartile of exposure to NO_X, NO_2, and NO.

7.4.2.1.1 Conclusions and Remaining Uncertainties

There was a small body of recent epidemiologic studies of the menstrual cycle; however, the small number of studies limits the ability to judge coherence and consistency across these studies, although the associations reported demonstrate that exposure to oxides of nitrogen could result in physiological responses that contribute to effects on menstrual cycles. There remains uncertainty regarding concentration-response relationships, lifestages and population differences, and the potential of confounding by copollutants.

7.4.2.2 Animal Toxicological Studies of Menstrual and Estrous Cycle

The 1993 Oxides of Nitrogen AQCD summarized a single study that assessed the effects of NO_2 on the estrous cycle (Shalamberidze and Tsereteli, 1971). No additional toxicological studies that investigated the effects of oxides of nitrogen exposure on the menstrual or estrous cycle were reported in the 2016 Oxides of Nitrogen ISA or the 2008 Oxides of Nitrogen ISA. The lone study dosed female albino rats to 0.126 or 2.36 mg/m^3 NO_2 (0.067 or 1.25 ppm NO_2) for 12 hours a day for three months (Shalamberidze and Tsereteli, 1971). Estrous cyclicity was continuously monitored from 24 days prior to the start of dosing through a 3-month recovery period following completion of dosing. Following completion of dosing, rats in the highest dose group (1.25 ppm NO_2) exhibited increased length of estrus, increased duration of estrous cycles overall, a decreased number of normal estrous cycles in a month, and overall fewer estrous cycles per month when compared to control (Shalamberidze and Tsereteli, 1971). Of note is that authors also reported that these effects were recoverable. Estrous cycle duration returned to

levels similar to controls after seven months of no additional NO_2 exposure. The number of normal and total estrous cycles per month returned to levels similar to controls after three months of no additional NO_2 exposure (Shalamberidze and Tsereteli, 1971). However, no recent toxicological studies on the effects of oxides of nitrogen exposure on the menstrual or estrous cycle were available for assessment.

7.4.2.2.1 Conclusions and Remaining Uncertainties

There is limited toxicological evidence examining the effects of NO_2 exposure on the menstrual and estrous cyclicity. The only available study reported disrupted estrous cyclicity (increased length of estrus, increased duration of estrous cycle, decreased number of normal estrous cycles in a month) in rats exposed to 1.25 ppm (but not 0.067 ppm) NO_2. Of note is that the reported cyclicity outcomes were coherent with outcomes in other biologically-related endpoints such as altered ovarian histopathology and reduced overall fertility. Still, with no other available studies to corroborate or expand upon these findings, considerable uncertainty remains. Additional studies that investigate the impacts of NO_2 exposure on estrous cyclicity could reduce this uncertainty. Further, it is possible that hormonal disruption may play a mediating role in some or all of these outcomes. Additional studies to investigate the effects of NO_2 on gonadotropin levels (luteinizing hormone [LH] and follicle stimulating hormone [FSH]) and sex steroid hormones (e.g., estradiol, testosterone, progesterone) could inform a potential mechanism of action.

7.4.3 Morphology and Histology of Female Sex Organs (Ovaries, Uterus, Fallopian Tubes/Oviducts, Vagina, and Mammary Glands)

The recent epidemiologic studies evaluating associations between exposure to oxides of nitrogen and morphology or histology of female sex organs (ovaries, uterus, fallopian tubes/oviducts, cervix, vagina, and/or mammary glands) are detailed below. Study details for the recent epidemiologic studies are included in Appendix A – Appendix Table 7-3. There were no recent toxicological studies of exposure to oxides of nitrogen with morphology or histology of female sex organs.

7.4.3.1 Epidemiologic Studies of Morphology and Histology of Female Sex Organs (Ovaries, Uterus, Fallopian Tubes/Oviducts, Vagina, and Mammary Glands)

In the 2016 Oxides of Nitrogen ISA, there were no epidemiologic studies available that evaluated exposure to oxides of nitrogen and associations with morphology or histology of female sex organs. For the single recent study available, study-specific details including study population characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), potential confounders, and select results are in Appendix A – Appendix Table 7-3.

- A single recent study evaluated the association between NO_2 and ovarian reserve, measured by antral follicle count (AFC). In a small observational study, exposure to air pollutants, estimated from central site monitors, was assessed with AFC in women of reproductive age in Shanxi Province, China (Feng et al., 2021). Three exposure periods were considered: (1) development stage from primary follicle to antral follicle; (2) early transition stage from primary follicle to prenatal follicle stage; and (3) later transition stage from prenatal follicle to antral follicle stage. Regardless of exposure period, there were decreases in AFC with exposure to NO_2, but the associations were imprecise (e.g., included the null). In copollutant models for exposure period 1, there was attenuation of the association with adjustment for SO_2 and PM_{10}, but the risk was unchanged when adjusted for $PM_{2.5}$, CO, and O_3.

7.4.3.1.1 Conclusions and Remaining Uncertainties

There was a single recent epidemiologic study of the morphology or histology of female sex organs. The availability of only a single study limits the ability to judge coherence and consistency. In addition to limitations in the number of studies, there remains uncertainty regarding the concentration-response relationship, lifestages and population differences, and potential confounding by copollutants.

7.4.3.2 Animal Toxicological Studies of Morphology and Histology of Female Sex Organs (Ovaries, Uterus, Fallopian Tubes/Oviducts, Vagina, and Mammary Glands)

The 1993 Oxides of Nitrogen AQCD summarized a single study that assessed the effects of NO_2 on the histology of female sex organs (Shalamberidze and Tsereteli, 1971). No additional toxicological studies that investigated the effects of oxides of nitrogen exposure on the morphology and histology of female sex organs were reported in the 2016 Oxides of Nitrogen ISA or the 2008 Oxides of Nitrogen ISA. The single available study dosed female albino rats to 0.126 or 2.36 mg/m^3 NO_2 (0.067 or 1.25 ppm NO_2) for 12 hours a day for three months and assessed the effects of NO_2 exposure on the histopathology of the ovary and the uterus (Shalamberidze and Tsereteli, 1971). Of note is that reproductive organ histopathology was only altered in the highest treatment group (1.25 ppm NO_2). Specifically, authors noted that uterine sections from animals in the high dose group exhibited reduced glandular epithelium. Further, the ovary and the uterus both displayed sporadic mild cellular degeneration. Authors also assessed the effects of NO_2 on ovarian follicles. Ovarian follicles are the functional unit of the ovary and consist of the oocyte and the surrounding, supportive somatic cells (granulosa and theca cells). They can be categorized into distinct growth stages: primordial (quiescent follicles that serve as the ovarian reserve), primary (the first stage of growth after activation from the primordial stage), secondary/preantral, antral/tertiary/Graafian. Although no quantitative or qualitative supporting data were presented, the authors report that primordial follicle populations were reduced in the highest dose group (1.25 ppm NO_2) which, due to the non-renewable nature of the ovarian reserve, could impact the timing of reproductive senescence onset. Additional studies are required to better understand the impacts of NO_2

exposure on outcomes later in life. However, no recent toxicological studies on the effects of oxides of nitrogen exposure on morphology or histology of female sex organs were available for assessment.

7.4.3.2.1 Conclusions and Remaining Uncertainties

There is limited toxicological evidence examining the effects of NO_2 exposure on morphology and histology of female sex organs. A single study in rats reported altered ovarian and uterine histopathology following 3 months of dosing at 1.25 ppm (but not 0.067 ppm) NO_2. These findings were coherent with changes observed in other biologically-related outcomes, including disrupted estrous cyclicity and reduced fertility, but with no other available studies to corroborate or expand upon these findings, considerable uncertainty remains. Additional animal studies that investigate the impacts of NO_2 exposure on female sex organ morphology, specifically the ovary and uterus, could reduce this uncertainty. Further, it is possible that hormonal disruption may play a mediating role in some or all these outcomes, and additional studies to investigate the effects of NO_2 on gonadotropin levels (LH and FSH) and sex steroid hormones (e.g., estradiol, testosterone, progesterone) could inform a potential mechanism of action.

7.4.4 Other Female Reproductive Functions Outcomes

There was a single epidemiologic study that evaluated female reproductive endpoints other than fertility and reproduction, menstrual and estrous cycle, and morphology and histology of female reproductive organs. This study is summarized below and more specific study details, including study population characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), and selected results are in Appendix A – Appendix Table 7-3.

Barrett et al. (2023) examined exposure to NO_2 in relation to human anogenital distance (AGD) at birth among participants of the TIDES cohort. Little is known about the critical windows for the development or programming of the female reproductive system. However, some research indicates that shorter AGD in females is associated with endometriosis and longer AGD is associated with polycystic ovarian syndrome (Barrett et al., 2023). When AGD was measured in females at birth, there was increased AGD-anus to clitoris (β: 0.05 [95% CI: -0.05, 0.15] per 1 ppb increase in NO_2) and AGD-anus to fourchette (β: 0.03 [95% CI: -0.05, 0.11] per 1 ppb increase in NO_2), but the CIs included the null.

7.4.4.1 Conclusions and Remaining Uncertainties

The recent epidemiologic study of AGD provides limited insight into potential associations between NO_2 and the development or programming of the female reproductive system, and a single study limits the ability to judge the coherence and consistency of associations. In addition, there remains

uncertainty regarding concentration-response relationship, lifestages and population differences, and potential confounding by copollutants.

7.4.5 Synthesis Across Lines of Evidence

There remains limited evidence for the effects of exposure to oxides of nitrogen on female reproductive function. In the recent epidemiologic studies, there was a small body of evidence supporting associations between exposure to oxides of nitrogen and female fertility. There was limited evidence supporting physiological responses that contribute to adverse outcomes related to pregnancy loss or live births. There is limited toxicological evidence examining the effects of exposure to oxides of nitrogen on female fertility and reproduction, and previously and recently published studies reported contrasting findings (reduced litter size and no effects on litter size, respectively). Among the recent animal toxicological studies, there were largely null effects reported for NO_2 exposure on female fertility parameters. However, the small number of studies across the epidemiologic and animal toxicology studies limit the ability to judge coherence and consistency across these studies. There were no recent toxicological studies that investigated the effects of oxides of nitrogen exposure on menstrual and estrous cyclicity or morphology and histology of female sex organs. Furthermore, there are a very limited number of epidemiologic studies evaluating the associations of oxides of nitrogen and menstrual and estrous cyclicity or morphology and histology of female sex organs. Overall, the recent epidemiologic and animal toxicological evidence remains limited. Among the epidemiologic studies, there remain uncertainties regarding concentration-response, lifestages and population differences, and potential of confounding by copollutants.

7.4.6 Summary and Causality Determination

The 2016 Oxides of Nitrogen ISA concluded that there was insufficient evidence to infer a causal relationship between NO_2 exposure and effects on female reproductive function. Epidemiologic evidence for NO_2 exposure and female reproductive outcomes in the 2016 Oxides of Nitrogen ISA was limited. Overall, there was inconsistent evidence for IVF failure, with only a few studies reporting lower odds of live birth associated with higher NO_2 concentrations during ovulation induction and the period after embryo transfer. None of the available epidemiologic studies examined confounding by copollutants, introducing additional uncertainty. Toxicological studies were limited, but a single study in rats reported disrupted estrous cyclicity (cycle prolongation, increased duration of diestrus, decreased number of normal and total estrous cycles) and a reduction in the number of primordial follicles in the ovary.

The recent evidence remains limited across the epidemiologic studies and animal toxicological studies (see Table 7-2). In the recent epidemiologic studies, there was a small body of evidence supporting associations between exposure to oxides of nitrogen and physiological responses that

contribute to adverse outcomes related to pregnancy loss or live births. However, the small number of studies limits the ability to judge coherence and consistency across these studies. Among the recent animal toxicological studies, there were largely null effects reported for NO_2 exposure on female fertility parameters. Overall, the recent epidemiologic and animal toxicological evidence remains limited. Among the epidemiologic studies, uncertainties remain regarding concentration- response relationship, lifestages and population differences, and potential for confounding by copollutants. **Overall, the evidence is *inadequate to infer the presence or absence of a causal relationship* between oxides of nitrogen exposure and female reproductive function.**

Table 7-2 Summary of evidence contributing to causality determinations for oxides of nitrogen exposure and effects on female reproductive function

Rationale for Causality Determination[a]	Key Evidence[b]	Key References	Oxides of Nitrogen Concentrations Associated with Effects
Fertility and Reproductive Function			
Inconsistent epidemiologic evidence for in vitro fertilization failure	Decreased odds of live birth associated with higher NO_2 concentrations during ovulation induction and the period after embryo transfer. Uncertainties regarding concentration response and exposure response, lifestages and population differences, and copollutant confounding.	Legro et al. (2010) Slama et al. (2013) Section 7.4.1.1	Mean NO_2: 19 ppb for 3.6- to 200-day avg Median NO_2: 19 ppb for 1 mo-avg
Limited and inconsistent toxicological evidence for effects on fertility and reproduction	Mixed evidence for effects on litter size in rats and mice. Uncertainties regarding dose-response, dosing duration, and exposure timing.	Di Giovanni et al. (1994) Shalamberidze and Tsereteli (1971) †Yue et al. (2017) †Yan et al. (2020) †Yue et al. (2018) †Li et al. (2022) Section 7.4.1.2	0.067–3 ppm NO_2
Menstrual and Estrous Cycle			
Limited epidemiologic evidence of effects on menstrual cycle	Increased mean follicular length, but null associations for luteal phase length and total cycle length. Uncertainties regarding concentration response and exposure response, lifestages and population differences, and copollutant confounding.	†Giorgis-Allemand et al. (2020)	Mean NO_2: 13.34 ppb for 30-day window

Rationale for Causality Determination[a]	Key Evidence[b]	Key References	Oxides of Nitrogen Concentrations Associated with Effects
	Increased risk for dysmenorrhea. Uncertainties regarding concentration response and exposure response, lifestages and population differences, and copollutant confounding.	†Lin et al. (2021)	Median NO_X: 24.8 ppb for 24-hr avg Median NO_2: 18.9 ppb for 24-hr avg Median NO: 5.53 ppb for 24-hr avg
Limited toxicological evidence of effects on menstrual cycle	Increased length of estrus, increased duration of overall estrous cycles, decreased number of overall estrous cycles in a month, and overall fewer estrous cycles per month in rats.	Shalamberidze and Tsereteli (1971) Section 7.4.2.2	1.25 ppm NO_2
Morphology and Histology of Female Sex Organs			
Limited epidemiologic evidence of effects on morphology and histology of female sex organs	A single study reported decreased ovarian reserve, as antral follicle count. Uncertainties regarding concentration response and exposure response, lifestages and population differences, and copollutant confounding.	Feng et al. (2021)	Median NO_2: 19.13 ppb for annual average
Limited toxicological evidence of effects on morphology and histology of female sex organs	Reduced glandular epithelium in uterine tissue, and sporadic mild cellular degeneration in ovary and uterus of mice. Authors report primordial follicle populations reduced in mice.	Shalamberidze and Tsereteli (1971) Section 7.4.3.2	1.25 ppm NO_2

List of abbreviations in alphabetical order: avg = average; hr = hour; mo = month; NO = nitrogen monoxide; NO_2 = nitrogen dioxide; NO_X = oxides of nitrogen; ppb = parts per billion; ppm = parts per million.
[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.

7.5 Effects on Pregnancy Outcomes

Pregnancy outcomes encompass a wide range of health effects (e.g., gestational diabetes, gestational hypertension and preeclampsia, placental growth and function, pregnancy-related mental health, and pregnancy loss and stillbirths). The details of the recent epidemiologic and animal toxicological studies evaluating the association between oxides of nitrogen exposure and pregnancy outcomes are discussed in Sections 7.5.1 to 7.5.6 and are provided in Appendix A – Appendix Table 7-4 through Appendix A – Appendix Table 7-9 and Appendix A – Appendix Table 7-2, respectively.

7.5.1 Gestational Diabetes Mellitus

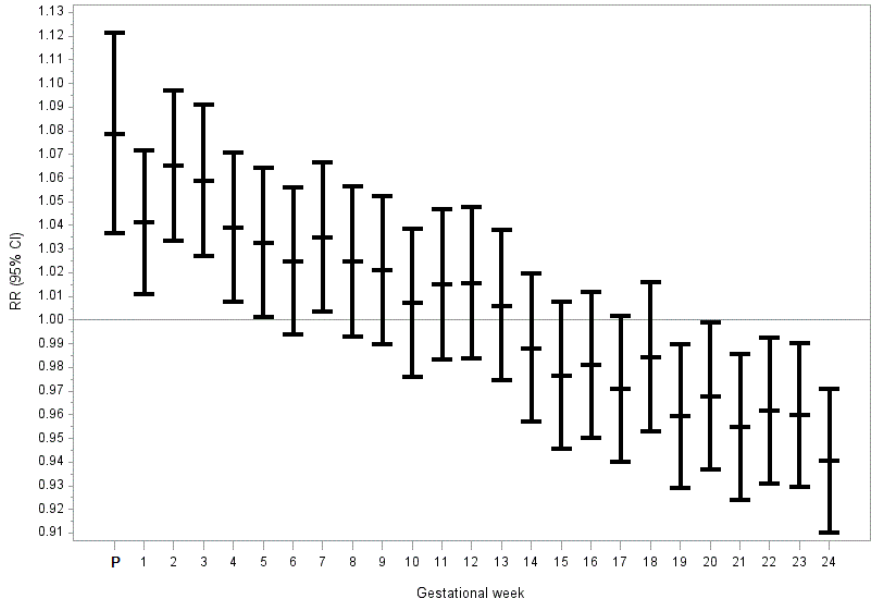
Gestational diabetes mellitus (GDM) is generally diagnosed during the second or third trimester of pregnancy. No toxicological studies and a single epidemiologic study investigated the effects of oxides of nitrogen exposure on GDM in previous ISAs and AQCDs (U.S. EPA, 2016, 2008, 1993). While there have been no recent toxicological studies, several recent epidemiologic studies evaluating the association between oxides of nitrogen and GDM were available. The evidence from these recent studies is generally consistent for U.S. populations indicating a positive association between oxides of nitrogen and GDM; however, results are more inconsistent for other countries with a mix of null-to-positive associations.

7.5.1.1 Epidemiologic Studies of Gestational Diabetes Mellitus

A single epidemiologic study reviewed in the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016) examined the association between NO_X and GDM. Malmqvist et al. (2013) observed a positive association with adjusted ORs for GDM increasing monotonically with increasing NO_X exposure concentration during the second trimester for the second, third, and fourth quartiles (OR: 1.19 [95% CI: 0.99, 1.44]; OR: 1.52 [95% CI 1.28, 1.82]; and OR: 1.69 [95% CI: 1.41, 2.03], respectively) compared to the first quartile in a Swedish cohort of women who had singleton deliveries between 1999 and 2005.

Since the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016), several studies evaluating U.S. study populations have generally reported positive associations between oxides of nitrogen and GDM. Recent studies are summarized below, followed by additional discussion of concentration-response relationships in these studies (see Section 7.5.1.1.1), the lifestages and populations evaluated (see Section 7.5.1.1.2), and impacts of copollutants (see Section 7.5.1.1.3). Section 7.5.1.1.4 discusses conclusions from available studies and summarizes remaining uncertainties. Study specific details including study population characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), potential confounders, and select results are in Appendix A – Appendix Table 7-4.

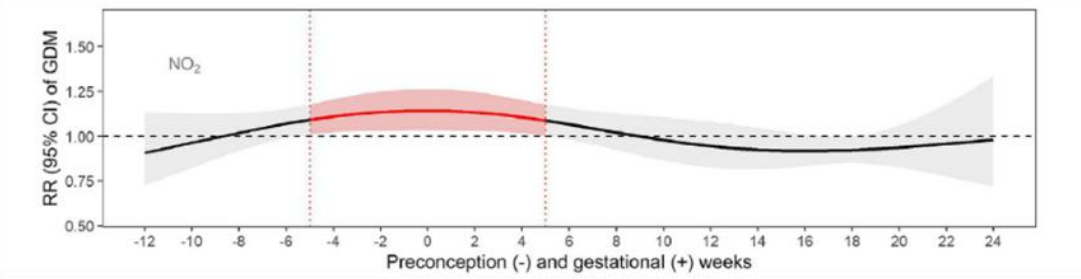
- Robledo et al. (2015) investigated associations between short- and long-term exposure to NO_X and GDM in the Consortium of Safe Labor (CSL: 2002 to 2008), a retrospective cohort study of labor and delivery across the U.S. authors observed positive associations between preconception (i.e., 91 days prior to last menstrual period) or first trimester exposure periods and GDM (RR: 1.09 [95% CI: 1.04, 1.13) per 25.55 ppb and RR: 1.06 [95% CI: 1.01, 1.10] per 30.21 ppb, respectively). For weekly averages, the preconception and first approximately nine weeks of gestation exposure windows were positively associated with GDM, after which risk estimates were attenuated and became negatively associated with GDM starting around 15 weeks gestation (see Figure 7-2).



List of abbreviations in alphabetical order: CI = confidence interval; GDM = gestational diabetes mellitus; IQR = interquartile range; P = conception; RR = relative risk.
Note: P represents the relative risk estimate for the average of three months prior to conception. All estimates adjusted for maternal age, race, and study site. Point estimates show relative risk of GDM for each IQR increase in air pollutant.
Source: Robledo et al. (2015). Copyright permission pending.

Figure 7-2 Adjusted relative risks and their 95% confidence intervals for the association between gestational diabetes mellitus and each IQR increase in NO_X from three months prior to conception through gestational week 24.

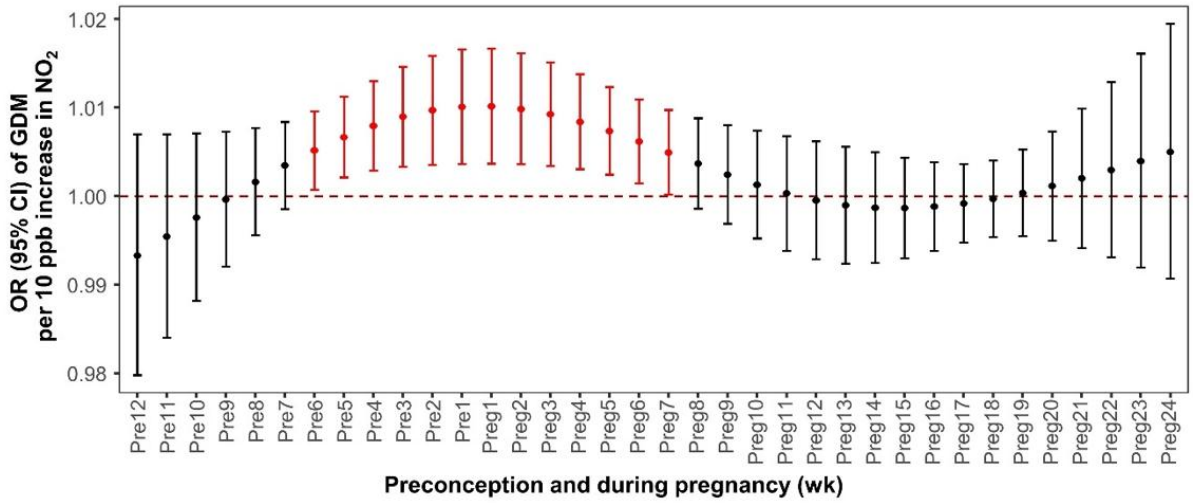
- Sun et al. (2022) conducted a retrospective cohort study that included women who gave birth between January 1, 2008, and December 31, 2018, in Southern California, U.S. Modeled NO_2 (kriging) was used to estimate exposure during preconception, first trimester, second trimester, both first and second trimester, and entire pregnancy periods. Sun et al. (2022) observed increased odds of GDM across all exposure periods evaluated with the strongest effects observed for the entire pregnancy (OR: 1.176 [95% CI: 1.147, 1.205] per 3.05 ppb).
- A prospective pregnancy cohort that predominantly enrolled low-income Hispanic/Latina women in Los Angeles, California, U.S., between 2015 and 2020 evaluated weekly gestational NO_2 exposures (from preconception -12 to zero weeks as well as post-conception from one to 24 weeks) and associations with GDM (Niu et al., 2023) (see Figure 7-3). Niu et al. (2023) observed increased risk of GDM associated with exposures to NO_2 from five weeks before conception to five weeks after conception (RR: 1.15 [95% CI: 1.14, 1.16] per 11 ppb).



List of abbreviations in alphabetical order: CI = confidence interval; DLM = distributed lag model; GDM = gestational diabetes mellitus; IQR = interquartile range; MADRES = Maternal And Developmental Risks from Environmental and Social Stressors Cohort; NO_2 = nitrogen dioxide; ppb = parts per billion; RR = risk ratio.
Note: All results were from DLMs adjusted for year, season of conception, weekly temperature, household income, maternal age, pre-pregnancy body mass index, maternal ethnicity and nativity, parity, and enrollment timepoint. Effect estimation was based on per IQR increases in weekly NO_2 concentrations (i.e., 11 ppb). Figure caption in sentence case. Gray bands indicate 95% CI. Week 0 indicates conception.
Source: Niu et al. (2023). Copyright permission pending.

Figure 7-3 Associations of pre-conception and prenatal weekly exposure to nitrogen dioxide with risk of gestational diabetes mellitus among participants in the MADRES cohort overall.

- An administrative cohort of singleton, live births in Kansas, U.S., from 2000 to 2016 was created to examine associations between NO_2 concentrations and GDM for each of the following exposure windows: first four weeks of pregnancy, first six weeks of pregnancy, first trimester, and second trimester (Hao et al., 2023). There was a consistent pattern of a positive association between NO_2 and GDM although all exposure window results included the null, except for the first trimester (OR first trimester: 1.059 [95% CI: 1.002, 1.120] per 5.8 ppb).
- Another administrative cohort of singleton, live births in Western New York, U.S., from 2004 to 2016 evaluated the associations between NO_2 concentrations and prevalence of GDM for many windows of exposure including, but not limited to, three months preconception, one to three months of pregnancy, four to six months of pregnancy, one to six months of pregnancy, and preconception to six months of pregnancy. All of which indicated positive associations (e.g., OR for preconception to six months of pregnancy: 1.12 [95% CI: 1.08, 1.16]) (Zhu et al., 2024). Additionally, in the distributed lag models (DLM) with weekly lags, Zhu et al. (2024) observed sensitive exposure windows from six weeks before to seven weeks after conception for NO_2 exposures (see Figure 7-4).



List of abbreviations in alphabetical order: CI = confidence interval; DLM = distributed lag model; GDM = gestational diabetes mellitus; NO_2 = nitrogen dioxide; OR = odds ratio; wk = week.
Note: Models were adjusted for year of birth, maternal age, race and ethnicity, education, pre-pregnancy body mass index, trimester when prenatal care started, parity, hypertensive disorders of pregnancy, smoking status, Area Deprivation Index 2013, and season of conception. Pre(n) represents the nth week before pregnancy; Preg(n) represents the nth week of pregnancy.
Source: Zhu et al. (2024). Copyright permission pending.

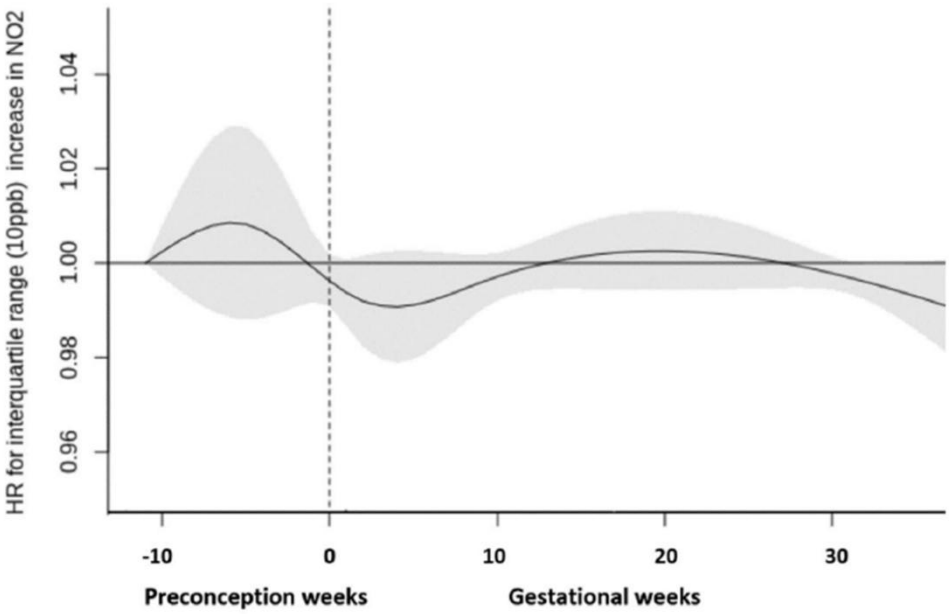
Figure 7-4 Associations of exposure to nitrogen dioxide by week with gestational diabetes using distributed lag models in Western New York, United States, 2004 to 2016.

In contrast to the other recent studies based in the United States that reported positive associations (Zhu et al., 2024; Hao et al., 2023; Niu et al., 2023; Sun et al., 2022; Robledo et al., 2015), a single recent study observed negative-to-positive associations between gestational NO_2 or NO_X exposures and GDM depending on the exposure assessment applied (Wu et al., 2016).

- In a nested case-control study in California, U.S., from 2006 to 2008 birth records, GDM was negatively associated, although the CI included the null, with NO_X estimated using CALINE4 dispersion modelling during the first or second trimester (OR: 0.983 [95% CI: 0.966, 1.000] and OR: 0.985 [0.967, 1.003], respectively) (Wu et al., 2016). Similarly, GDM was negatively associated with exposure to NO_2 concentrations interpolated by empirical Bayesian kriging during the first and second trimester (OR: 0.923 [95% CI: 0.881, 0.967] and OR: 0.943 [95% CI: 0.890, 0.999], respectively). Wu et al. (2016) cautions that their observed results regardless of exposure method utilized may be because of the outcome misclassification related to substantial underreporting of GDM in birth certificate data, which would attenuate effect estimates to the null due to misclassification of cases as non-cases. However, in a sub-analysis that used spatiotemporal NO_2 regression models in Los Angeles County only to estimate exposure, Wu et al. (2016) observed positive associations for the first and second trimester (OR: 1.053 [95% CI: 1.019, 1.089] and OR: 1.046 [95% CI: 1.003, 1.09], respectively). These results were more consistent with the reported results from Sun et al. (2022) and Niu et al. (2023), which were both geographically located in Southern California.

There have also been recent studies conducted outside of the United States in Taiwan, China, Denmark, and Canada with reported null-to-positive associations.

- In a Danish cohort enrolled from 1996 to 2002, Pedersen et al. (2017c) reported no evidence for an association between exposure to NO_2 during the first trimester, second trimester, third trimester, or entire pregnancy and GDM using the common Danish criterion for GDM (OR: 0.89 [95% CI: 0.76, 1.03]); however, NO_2 exposure during the first trimester, second trimester, third trimester, and entire pregnancy were associated with a higher odds of developing GDM when the WHO criterion was used for outcome ascertainment (e.g., first trimester OR: 1.24 [95% CI: 1.03, 1.49]). According to Pedersen et al. (2017c), "the WHO criterion is based on a 75g oral glucose tolerance test with either a fasting venous plasma glucose equal to or >7.0 mmol/L and/or a 120 minute value equal or >7.8 mmol/L. The WHO criterion is not directly comparable with the Danish criterion since for the WHO criterion venous plasma is needed, the diagnostic fasting value is higher, the 120 min value is lower than in the Danish criterion and more than one abnormal value is needed to make the GDM diagnosis by the Danish criterion."
- Yan et al. (2022) observed a positive association between first trimester NO_2 exposure and GDM among a Chinese cohort of pregnant women (OR: 1.114 [95% CI: 1.006, 1.233]); the association was also positive although more imprecise and crossed the null for the second trimester and entire pregnancy exposure periods (OR: 1.018 [95% CI: 0.887, 1.169] and OR: 1.044 [95% CI: 0.911, 1.197], respectively).
- (Shen et al., 2017) reported null associations between exposures to NO_2 within the 12-week period prior to pregnancy, first trimester, and second trimester and GDM from a case-control study in Taiwan (OR: 0.96 [95% CI: 0.90, 1.02], OR: 0.93 [95% CI: 0.88, 1.00], and OR: 0.97 [95% CI: 0.93, 1.02], respectively). NO_2 exposures were assigned by fixed site monitors in contrast to the other recent studies which incorporated some type of modeling methodology to estimate NO_2 concentrations for exposure assignment (e.g., kriging, inverse distance weighting (IDW), chemical transport model).
- Miron-Celis et al. (2023) conducted a retrospective cohort study using administrative data on singleton live births that occurred between April 1, 2006, and March 31, 2018, in Ontario, Canada, to evaluate the association between weekly NO_2 exposures during pregnancy and the preconception period (i.e., 12 weeks before estimated conception) and incident cases of GDM. Miron-Celis et al. (2023) reported largely null and imprecise associations (HR preconception period: 1.07 [95% CI: 0.88,1.23], HR pregnancy period: 1.00 [95% CI: 0.85, 1.16] per 10 ppb increase in NO_2), and the authors did not identify any distributed lag model sensitive windows for NO_2 (see Figure 7-5).



List of abbreviations in alphabetical order: HR = Hazard ratio; NO_2 = nitrogen dioxide; ppb = parts per billion.
Note: Gray shade indicates 95% confidence intervals; dashed vertical line demarcates preconception and post conception weeks. All the models were adjusted for maternal age, parity, maternal smoking status, prepregnancy body mass index, weekly ambient temperatures, month of birth, year of birth, residing in the Greater Toronto Area, community size, deprivation quintiles, instability quintiles, dependency quintiles and ethnic quintiles.
Source: Miron-Celis et al. (2023). Copyright permission pending.

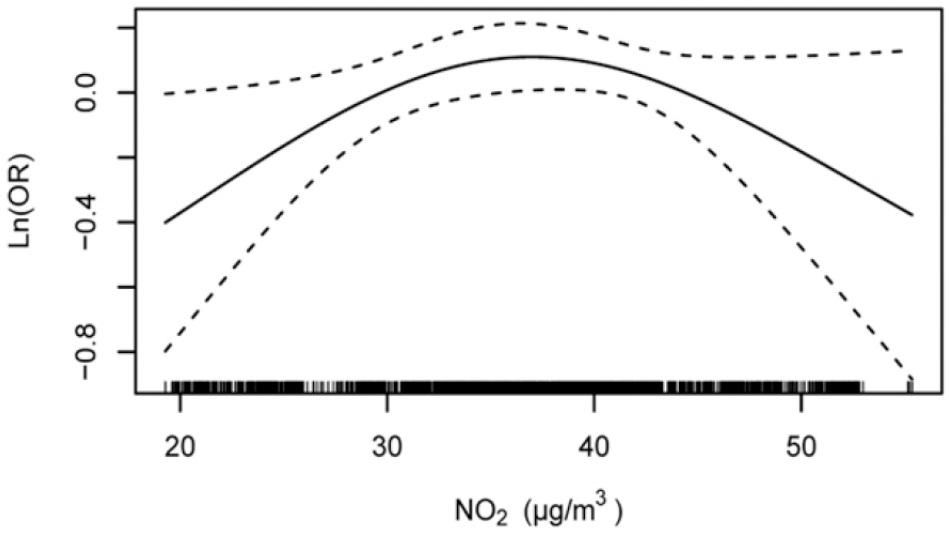
Figure 7-5 Weekly associated hazard ratios associated with weekly nitrogen dioxide exposures over the preconception period and the gestational period with risk of gestational diabetes in the overall cohort (n = 1,310,807).

7.5.1.1.1 Concentration-Response

There was limited evaluation of the concentration-response relationship in current studies; however, the studies that evaluated categorical exposure to NO_2 reported evidence of the strongest effect among the fourth versus the first quartile of exposure compared to the second quartile versus the first quartile of exposure (i.e., the first quartile was the referent).

- Pedersen et al. (2017c) observed that the OR for GDM when using the WHO criterion was strongest for the highest quartile of first trimester NO_2 exposure compared to the lowest quartile (OR: 2.07 [95% CI: 1.34, 3.19]), while ORs for the second and third quartiles compared to the first quartile were approximately equivalent.
- Zhu et al. (2024) reported that the concentration response relationship between NO_2 and increased odds of GDM was found in quartiles two, three, and four compared to quartile one for all exposure windows evaluated including three months preconception, one to three months of pregnancy, four to six months of pregnancy, one to six months of pregnancy, and preconception to six months of pregnancy (e.g., OR for preconception to 6 months of pregnancy: 1.14 [95% CI: 1.07, 1.22], 1.22 [95% CI: 1.14, 1.30], and 1.22 [95% CI: 1.13, 1.32], respectively).

- Yan et al. (2022) reported an inverted U-shaped concentration response curve for the association between entire pregnancy NO_2 exposure and GDM (see Figure 7-6).



List of abbreviations in alphabetical order: CI = confidence interval; Ln(OR) = natural logarithm of odds ratio; NO_2 = nitrogen dioxide; $\mu g/m^3$ = micrograms per cubic meter.
Note: The X-axis shows the exposure levels of NO_2. The black ticks on the x-axis show the distribution of air pollution concentrations. The Y-axis represents natural logarithm (Ln) of the odds ratio (OR). Solid lines show the point estimates of Ln(OR). Dashed lines show the 95% CI for Ln(OR).
Source: Yan et al. (2022). Copyright permission pending.

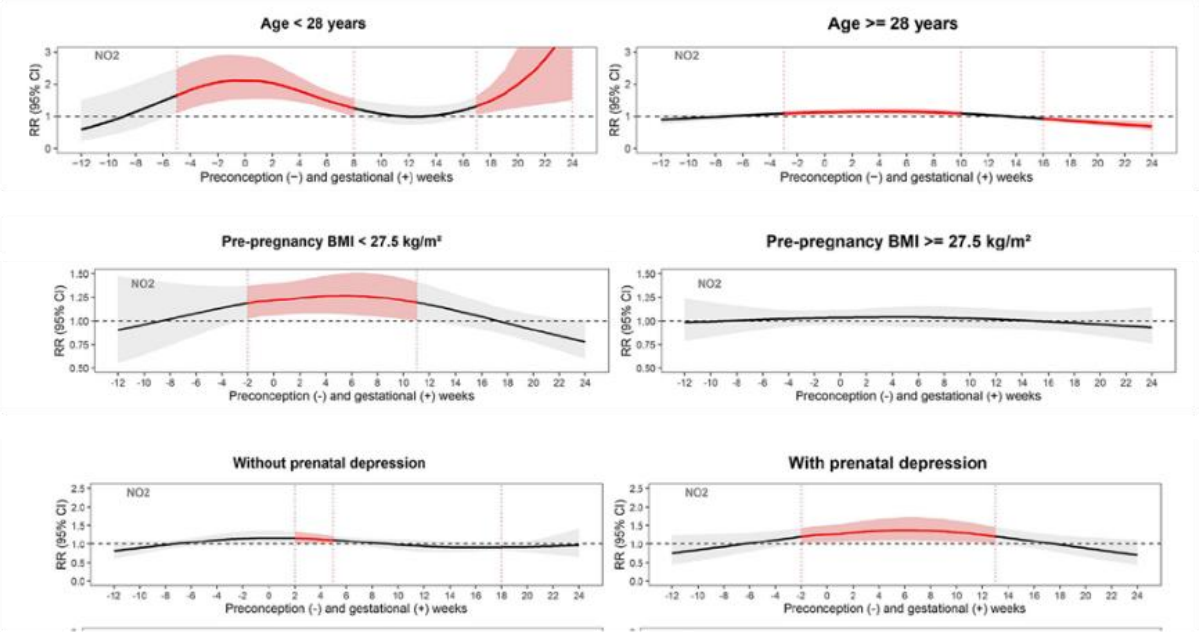
Figure 7-6 Nonlinear effects on gestational diabetes mellitus from exposure to ambient nitrogen dioxide during the entire pregnancy.

7.5.1.1.2 Lifestages and Populations

While several studies evaluated factors that may put certain populations at increased risk for GDM (i.e., road traffic noise, maternal race/ethnicity, pre-pregnancy BMI, household income, education, co-morbidity of chronic hypertension, parity, physical activity, depression, age), few factors were evaluated across multiple studies (i.e., maternal education (Pedersen et al., 2017c; Wu et al., 2016), race/ethnicity (Zhu et al., 2024; Sun et al., 2022; Wu et al., 2016), and pre-pregnancy BMI (Zhu et al., 2024; Niu et al., 2023; Sun et al., 2022; Pedersen et al., 2017c; Wu et al., 2016), and there was no consistency observed across studies.

- Robledo et al. (2015) did not observe effect measure modification by pre-pregnancy BMI; the RRs and 95% CIs for the normal weight stratum and overweight/obese stratum were of the same magnitude and direction for both preconception and first trimester exposure windows.
- Niu et al. (2023) observed that increased GDM risk from NO_2 was modified by maternal depression status, age, and pre-pregnancy BMI with stronger associations observed in participants with prenatal depression, younger participants as well as the participants with lower pre-pregnancy BMI (see Figure 7-7). Among the participants with prenatal depression, increased risk of GDM was associated with exposure to NO_2 during two weeks before to 13 weeks after conception (RR: 1.31 [95% CI: 1.29, 1.33] per 11 ppb); whereas a relatively

smaller increased risk of GDM was associated with exposure to NO_2 (RR: 1.14 [95% CI: 1.12, 1.17] per 11 ppb) during two to five weeks after conception for those without prenatal depression (Niu et al., 2023). Among the younger participants (<28 years), Niu et al. (2023) observed large positive associations during two windows of exposure to NO_2 (during five weeks before to eight weeks after conception (RR: 1.82 [95% CI: 1.77, 1.86] and during 17 to 24 weeks after conception (RR: 2.21 [95% CI: 2.06, 2.36] per 11 ppb). However, among the older participants (≥28 years), GDM risk was increased with NO_2 exposure during the periconceptional period from three weeks before to 10 weeks after conception (RR: 1.12 [95% CI: 1.11, 1.13] per 11 ppb), but GDM risk was decreased with NO_2 exposure in mid-pregnancy (RR: 0.80 [95% CI: 0.79, 0.82] per 11 ppb). Similarly, stronger positive associations of NO_2 exposure were observed in the lower pre-pregnancy BMI (<27.5 kg/m^2) participants during two weeks before to 11 weeks after conception, and null associations were observed in the higher pre-pregnancy BMI participants (≥27.5 kg/m^2) (Niu et al., 2023).



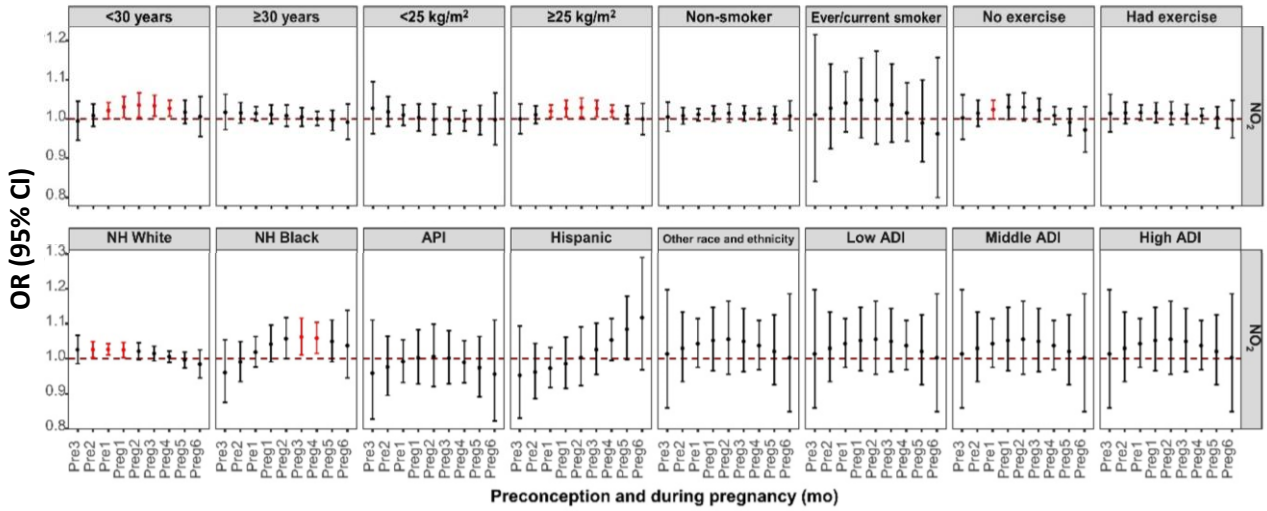
List of abbreviations in alphabetical order: CI = 95% confidence interval; BMI = body mass index; DLM = distributed lag model; GDM = gestational diabetes mellitus; IQR = interquartile range; kg/m^2 = kilograms per meter squared; MADRES = Maternal And Developmental Risks from Environmental and Social Stressors Cohort; NO_2 = nitrogen dioxide; ppb = parts per billion; RR = risk ratio.
Note: All results were from DLMs adjusted for year, season of conception, weekly temperature, household income, maternal age, pre-pregnancy body mass index, maternal ethnicity and nativity, parity, and enrollment timepoint. Effect estimation was based on per IQR increases in weekly NO_2 concentrations (i.e., 11 ppb). Figure caption in sentence case. Gray bands indicate 95% CI. Week 0 indicates conception.
Source: Niu et al. (2023). Copyright permission pending.

Figure 7-7 Associations of pre-conception and prenatal weekly exposure to nitrogen dioxide with risk of gestational diabetes mellitus among participants in the MADRES cohort stratified by maternal age, pre-pregnancy BMI, and prenatal depression.

- Pedersen et al. (2017c) investigated effect modification by road traffic noise, pre-pregnancy BMI, parity, education, and physical activity. Similar to Niu et al. (2023), Pedersen et al. (2017c) observed evidence of effect modification for pre-pregnancy BMI. However, while Niu et al. (2023) reported stronger positive associations in lower pre-pregnancy BMI (<27.5

kg/m^2), Pedersen et al. (2017c) reported stronger positive associations for participants with higher pre-pregnancy BMI (≥25 kg/m^2 OR: 1.42 [95%CI: 1.15, 1.76]; ≥18.5 to 25 kg/m^2 OR: 0.96 [95% CI: 0.69, 1.34]). Pedersen et al. (2017c) did not observe modification by road traffic noise, parity, education, or physical activity.

- Sun et al. (2022) evaluated effect measure modification by maternal race/ethnicity, pre-pregnancy BMI, and co-morbidity of chronic hypertension. Compared to the overall study population (OR: 1.176 [95%CI: 1.147, 1.205] per 3.05 ppb), Sun et al. (2022) reported that entire pregnancy NO_2 was associated with an increase in odds of GDM among Hispanic mothers (OR: 1.239 [95% CI: 1.198, 1.281] per 3.05 ppb) and African American mothers (OR: 1.213 [95% CI: 1.088, 1.352] per 3.05 ppb) as well as obese mothers (OR: 1.243 [95% CI: 1.196, 1.292] per 3.05 ppb).
- Wu et al. (2016) observed a negative association between entire pregnancy NO_2 and GDM with no statistically significant evidence of modification for BMI, race/ethnicity, household income, and maternal education. However, ORs tended to be more strongly negative in the subgroups with the lowest education or the lowest income. In contrast to the other U.S. based studies, Wu et al. (2016) did not report clear evidence of modification by race/ethnicity.
- Hao et al. (2023) observed elevated positive associations between first trimester NO_2 exposure and GDM for Black, Asian, and Hispanic participants (OR: 1.271 [95% CI: 1.027, 1.573], OR: 1.155 [95% CI: 0.901, 1.481], OR: 1.157 [95% CI: 1.051, 1.273], respectively, per 5.8 ppb) compared to the overall study population (OR: 1.059 [95% CI: 1.002, 1.120] per 5.8 ppb) as well as elevated positive association between second trimester NO_2 exposure and GDM for those greater than 27 years old (OR: 1.033 [95% CI: 0.969, 1.103] per 5.8 ppb), while attenuated to a negative association for those 27 years old or less (OR: 0.967 [95% CI: 0.867, 1.078] per 10.88 ppb), compared to the overall study population (OR: 1.014 [95% CI: 0.959, 1.073] per 5.8 ppb).
- Zhu et al. (2024) reported that the associations between NO_2 and GDM were magnified for the overweight/obese BMI (≥25 kg/m^2) participants compared to under/normal weight participants and magnified for younger women (<30 years old) compared to participants who were older (>30 years old). Authors also observed some evidence of modification by race/ethnicity, although no clear overall pattern (see Figure 7-8).



List of abbreviations in alphabetical order: CI = confidence interval; ADI = Area Deprivation Index; API = Asian/Pacific Islander; NH = non-Hispanic; NO_2 = nitrogen dioxide; mo = month; OR = odds ratio; ppb = parts per billion.
Note: Other race and ethnicity include Native American/Alaskan and mothers with multiple races and ethnicities. Models were adjusted for year of birth, maternal age, race and ethnicity, education, pre-pregnancy body mass index, trimester when prenatal care started, parity, hypertensive disorders of pregnancy, smoking status, ADI 2013, and season of conception. Pre(n) represents the nth month before pregnancy; Preg(n) represents the nth month of pregnancy. OR and 95% CI were estimated for each 10 ppb increase in NO_2 levels.
Source: Zhu et al. (2024). Copyright permission pending.

Figure 7-8 Associations of exposure to nitrogen dioxide by month by potential modifiers with gestational diabetes using distributed lag models by subgroups in Western New York, United States, 2004 to 2016.

7.5.1.1.3 Copollutants

There was limited evaluation of copollutants as well as no consistency for which copollutant was adjusted for across studies; however, within the studies, the associations remained robust after selected copollutant adjustment.

- Pedersen et al. (2017c) reported that in copollutant models mutually adjusted for road traffic noise, effect estimates were unchanged although slightly more imprecise (OR for single-pollutant Danish criterion: 0.89 [95% CI: 0.76, 1.03] vs. OR for copollutant Danish criterion: 0.89 [95% CI: 0.75, 1.06]; OR for single-pollutant WHO criterion: 1.24 [95% CI: 1.03, 1.49] vs. OR for copollutant WHO criterion: 1.22 [95% CI: 0.98, 1.53]).
- Hao et al. (2023) observed robust effect estimates after multipollutant adjustment for $PM_{2.5}$ and O_3 (OR first trimester: 1.065 [95% CI: 1.007, 1.125] per IQR and OR second trimester: 1.023 [95% CI: 0.967, 1.082] per 5.8 ppb).
- Zhu et al. (2024) observed robust effect estimates after copollutant adjustment for $PM_{2.5}$ (e.g., OR for preconception to 6 months of pregnancy: 1.12 [95% CI: 1.07, 1.17] per 10 ppb increase in NO_2).

A single study included mixture analyses. The overall mixture effect is not directly comparable but provides a comprehensive evaluation of joint exposures.

- Sun et al. (2022) evaluated an overall mixture effect for NO_2, $PM_{2.5}$, and PM_{10}. They reported that in multipollutant models using g-computation, entire pregnancy exposure to $PM_{2.5}$, PM_{10}, and NO_2 mixture was positively associated with GDM (OR: 1.095 [95% CI: 1.082, 1.108]). This mixture effect cannot be directly compared to the single-pollutant NO_2 effect. The mixture effect is a joint effect of all included exposures and the independent effect of any single included exposure cannot be differentiated; however, the mixture effect better approximates real-world exposures.

7.5.1.1.4 Conclusions and Remaining Uncertainties

Recent epidemiologic evidence was generally consistent for U.S. populations, suggesting a positive association between oxides of nitrogen and GDM. However, results were more inconsistent in studies conducted outside the United States, with null-to-positive associations. There were differences in outcome ascertainment definitions and exposure modeling, which may explain the heterogeneity across studies. Some of the recent studies included exposure windows that occur after outcome ascertainment, e.g., the exposure window should not include the third trimester given that GDM is usually screened for between 24 and 28 weeks of gestation, or earlier if known risk-factors. However, this may be why the critical windows of exposure most often identified included the preconception or pre-pregnancy period as well as the first approximately eight weeks of pregnancy (Zhu et al., 2024; Niu et al., 2023; Robledo et al., 2015). There was limited evaluation of the concentration-response relationship among recent studies. While several recent studies evaluated numerous factors that could potentially result in lifestages and population differences, there was limited overlap of the same factor being evaluated across multiple studies, and there was no consistency observed across those studies. Finally, there was limited evaluation of copollutants with no consistent copollutants adjusted for across studies; however, within the studies, the associations remained robust after selected copollutant adjustment.

7.5.2 Hypertensive Disorders of Pregnancy

Hypertensive disorders of pregnancy include chronic hypertension, gestational hypertension, preeclampsia, and chronic hypertension with superimposed preeclampsia. Pregnancy-associated hypertension is a leading cause of perinatal and maternal mortality and morbidity. A large body of research has linked changes in blood pressure to ambient air pollution; however, evidence is inconsistent for oxides of nitrogen (see Chapter 5, Section 5.7.1 and Chapter 6, Section 6.5.1). Gestational hypertension and preeclampsia are indicated by newly elevated blood pressure in the second half of pregnancy among women without clinically diagnosed hypertension (Nobles et al., 2019b); preeclampsia additionally involves the development of systemic multiorgan endothelial dysfunction, commonly diagnosed as the presence of proteinuria (Nobles et al., 2019b). No toxicological studies and a limited number of epidemiologic studies investigated the effects of oxides of nitrogen exposure on hypertensive

disorders of pregnancy in previous ISA and AQCDs. There are no recent toxicological studies examining the effects of oxides of nitrogen exposure on hypertensive disorders of pregnancy.

7.5.2.1 Epidemiologic Studies of Hypertensive Disorders of Pregnancy

This section discusses available epidemiologic studies that examine associations between oxides of nitrogen exposure and hypertensive disorders during pregnancy. Available studies are summarized in the sections below, followed by additional discussion of concentration-response relationships in these studies (see Section 7.5.2.1.2), the lifestages and populations evaluated (see Section 7.5.2.1.3), and impacts of copollutants (see Section 7.5.2.1.4). Section 7.5.2.1.5 discusses conclusions from available studies and summarizes remaining uncertainties.

Hypertensive disorders (combined)

In the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016), there was limited and inconsistent evidence for associations between oxides of nitrogen and hypertensive disorders of pregnancy (e.g., chronic hypertension, gestational hypertension, preeclampsia, and chronic hypertension with superimposed preeclampsia). However, there was a key study identified in the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016) that reported positive associations between NO_2 concentrations measured at central site monitors during the entire pregnancy and first trimester and hypertensive disorders of pregnancy (OR: 1.21 [95% CI: 1.09, 1.35] and OR: 1.14 [95% CI: 1.01, 1.29], respectively) (Xu et al., 2014).

Recent studies report inconsistent results across geographic regions for the association between gestational exposure to NO_2 and hypertensive disorders of pregnancy. Study specific details including study population characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), potential confounders, and select results are in Appendix A – Appendix Table 7-5. The few studies in the United States primarily reported null associations.

- In an administrative birth cohort examining data from 2000 to 2006 in the San Joaquin Valley, California, U.S. Weber et al. (2019) observed a null association between gestational NO_2 exposures (entire pregnancy, first trimester, second trimester) and pregnancy-induced hypertension, which they defined as gestational hypertension and preeclampsia, when the highest quartile of exposure (>19.47 ppb; central site monitors) was compared to the lower three quartiles.
- Similarly, in a 2014 to 2016 administrative birth cohort of mothers who lived within 30 meters of a street segment measured by the Google Street View cars in West and Downtown Oakland, California, U.S. Goin et al. (2021) used G-computation with log-binomial regression to estimate the effect of a potential intervention in which NO_2 concentrations were reduced to the 25th percentile of concentrations observed across the study participants. Goin et al. (2021) reported null associations with wide 95% CIs between NO_2 and risk of hypertensive disorders (RD: -0.3 cases per 100 women [95% CI: -1.9, 1.2]).
- Savitz et al. (2015) also observed null associations between first trimester and second trimester NO_2 exposures and hypertensive disorders of pregnancy (OR: 1.0 [95% CI: 1.0, 1.1]

and OR: 1.1 [95% CI: 1.0, 1.1] per 10 ppb, respectively) in a New York City, NY, U.S., administrative birth cohort from 2007 to 2010. In the same study population using a similar study design, although slightly different analytic inclusion criteria, Savitz et al. (2019) observed comparable null associations to Savitz et al. (2015) when first trimester NO_2 exposures and gestational hypertension were evaluated.

Studies outside of the United States (i.e., China and Denmark) observed positive associations between NO_2 exposure and hypertensive disorders in pregnancy.

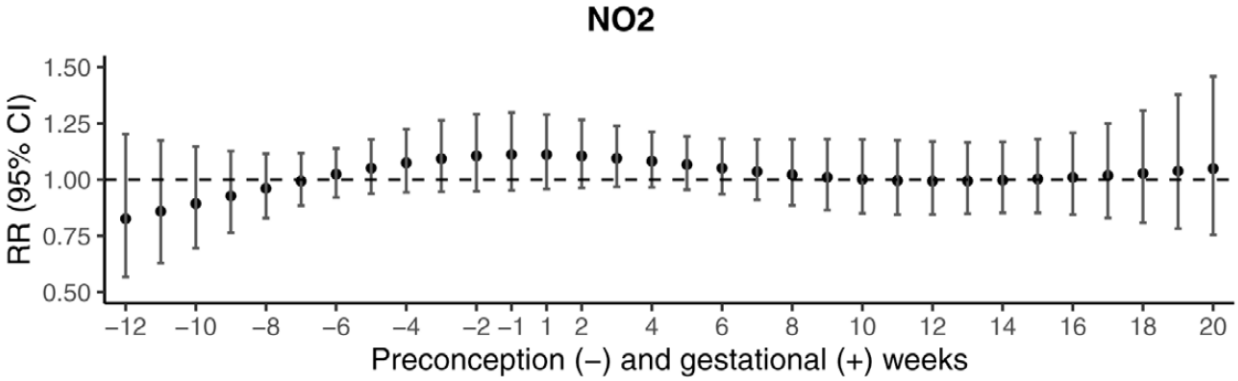
- In a Danish birth cohort enrolled from 1996 to 2002, Pedersen et al. (2017b) used dispersion modeling to estimate gestational exposure to NO_2 to evaluate the association between NO_2 exposure during first trimester and hypertensive disorders. They observed a positive association (OR: 1.07 [95 % CI: 1.01, 1.13], per 5.3 ppb); similar results were observed for second trimester, third trimester, and entire pregnancy exposure windows (Pedersen et al., 2017b).
- Jiang et al. (2023) reported a positive association between second trimester NO_2 exposure and hypertensive disorders (OR: 1.13 [95% CI: 1.01, 1.27]) in a multicenter hospital study in China with participants recruited between 2015 and 2016. However, associations between first trimester NO_2 exposure and hypertensive disorders were null, while the first and second trimester exposure period was positive, but the 95% CI crossed the null.

Gestational Hypertension

Recent studies have also evaluated specific hypertensive disorders of pregnancies, such as gestational hypertension (i.e., high blood pressure that develops during pregnancy usually after 20 weeks of gestation without the presence of protein in the urine or other signs of urine damage) or preeclampsia (i.e., high blood pressure after 20 weeks of pregnancy as well as signs of damage to organs, such as the kidneys or liver). In the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016), there was no evidence for associations between oxides of nitrogen and gestational hypertension. Recent studies for gestational hypertension among U.S. study participants report generally consistent positive results, especially during the second trimester exposure period. Study specific details including study population characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), potential confounders, and select results are in Appendix A – Appendix Table 7-5.

- Within a retrospective U.S. cohort enrolled from 2002 to 2008, Zhu et al. (2017) reported that exposures to NO_X during three months preconception, the first trimester, and gestational weeks zero to 20 were associated with higher risk of gestational hypertension (RR: 1.07 [95% CI: 0.98, 1.17], RR: 1.11 [95% CI: 1.01, 1.21], and RR: 1.17 [95% CI: 1.06, 1.29] per IQR, respectively).
- In a retrospective cohort study between 2002 to 2010 from northern Utah, U.S., and southern Idaho, U.S., Nobles et al. (2019b) investigated exposure to NO_2 and NO_X during five exposure windows (3-months preconception, first 20 weeks gestation, first trimester, second trimester, and entire pregnancy) and associations with gestational hypertension. For both NO_2 and NO_X, the strongest positive associations were observed in the second trimester (OR: 1.14 [95% CI: 1.06, 1.22] and OR: 1.17 [95% CI: 1.08, 1.27], respectively).

- In a New York City, NY, U.S. administrative birth cohort, Savitz et al. (2015) observed that first and second trimester NO_2 exposures were associated with an increased odds of gestational hypertension (OR: 1.2 [95% CI: 1.2, 1.3] and OR: 1.3 [95% CI: 1.2, 1.4] per 10 ppb, respectively). In the same study population using a similar study design, although slightly different analytic inclusion criteria, Savitz et al. (2019) observed comparable associations to Savitz et al. (2015) when associations between first trimester NO_2 exposures and gestational hypertension were evaluated.
- An administrative cohort of singleton, live births in Kansas, U.S., from 2000 to 2016 was created to examine associations between NO_2 concentrations and gestational hypertension for each of the following exposure windows: first four weeks of pregnancy, first six weeks of pregnancy, first trimester, second trimester, third trimester, and entire pregnancy (Hao et al., 2023). Effect estimates across those exposure windows ranged from negative to null with all estimates having 95% CIs crossing the null (e.g., OR first trimester: 0.967 [95% CI: 0.924, 1.013] per IQR, OR third trimester: 1.000 [95% CI: 0.954, 1.047] per IQR).
- A prospective pregnancy cohort that predominantly enrolled low-income Hispanic women in California (the MADRES study) evaluated weekly preconception and gestational exposure to NO_2 and associations with gestational hypertension (Niu et al., 2024). Niu et al. (2024) reported positive associations, although 95% CIs crossed the null, for weekly exposures from approximately four weeks prior to conception until six weeks of gestation (see Figure 7-9).



List of abbreviations in alphabetical order: CI = confidence interval; NO_2 = nitrogen dioxide; RR = risk ratio.
Note: All results were from distributed lag models adjusted for maternal age, ethnicity and nativity, marital status, pre-pregnancy body mass index, annual household income, parity, fetal sex, temperature, year and season of conception, and recruitment time point. Susceptible windows were marked with a star on the top. Effect estimation was based on per interquartile range increases in weekly air pollutant concentrations (i.e., 11 ppb for 24-hour average NO_2).
Source (adapted from): Niu et al. (2024). Copyright permission pending.

Figure 7-9 Associations of preconception and prenatal weekly exposure to nitrogen dioxide with risk of gestational hypertension in the MADRES cohort.

In studies conducted outside of the United States, associations between NO_2 exposure and gestational hypertension were more inconsistent than studies conducted within the United States.

- Among a cohort of Chinese pregnant women, Yan et al. (2022) observed positive associations, although imprecise and included the null, between first trimester (OR: 1.032 [95% CI: 0.840, 1.267]), second trimester (OR: 1.107 [95% CI: 0.844, 1.452]), third trimester (OR: 1.016 [95% CI: 0.768, 1.344]), and entire pregnancy (OR: 1.094 [95% CI: 0.820, 1.458]) NO_2 exposure concentrations and gestational hypertension, with the strongest effects observed for the second trimester exposure window.

- In a multicenter hospital study in China, Jiang et al. (2023) reported that second trimester NO_2 exposure was positively associated with gestational hypertension, although the confidence interval included the null (second trimester OR: 1.12 [0.95, 1.32] per 10.7 ppb).
- In contrast, in a Danish birth cohort enrolled from 1996 to 2002, Pedersen et al. (2017b) used dispersion modeling to estimate gestational exposure to NO_2 to evaluate the association between NO_2 exposure during first trimester and gestational hypertension. They observed near null associations (OR: 1.03 [95% CI: 0.93, 1.14], per 5.3 ppb); similar results were observed for second trimester, third trimester, and entire pregnancy exposure windows (Pedersen et al., 2017b).

Preeclampsia

In the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016), there were several studies reporting positive associations between gestational exposures to oxides of nitrogen and preeclampsia, especially for third trimester or entire pregnancy exposure windows (Dadvand et al., 2013; Malmqvist et al., 2013; Wu et al., 2011; Wu et al., 2009).

Evidence from recent studies is summarized below. Study specific details including study population characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), potential confounders, and select results are in Appendix A – Appendix Table 7-5. Several recent studies evaluated the association between oxides of nitrogen and preeclampsia. Evidence from these studies is inconsistent, with observed associations negative, null, and positive across geographies of the United States, China, and Nordic countries. While Savitz et al. (2015), (Nobles et al., 2019b), and Yan et al. (2022) observed positive associations between oxides of nitrogen and gestational hypertension, they observed negative associations between oxides of nitrogen and preeclampsia.

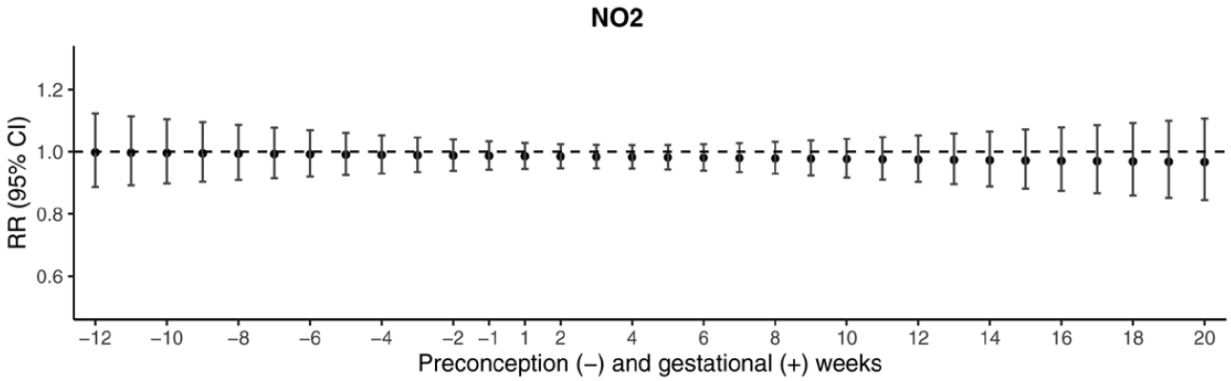
- Using a land use regression (LUR) exposure model, Savitz et al. (2015) observed that first and second trimester NO_2 exposures were associated with a decreased odds of mild preeclampsia (OR: 0.89 [95% CI: 0.85, 0.93] and OR: 0.93 [95% CI: 0.89, 0.98] per 10 ppb, respectively) and null associations with severe preeclampsia (OR: 1.0 [95% CI: 0.98, 1.1] and OR: 1.0 [95% CI: 0.97, 1.1] per 10 ppb, respectively).

Savitz et al. (2015) assigned participants exclusively to the diagnosis reflecting the most severe form when participants had codes for more than one category of hypertensive disorder (i.e., ordered as severe preeclampsia/eclampsia, mild preeclampsia, and gestational hypertension). While the biological mechanisms of gestational hypertension and preeclampsia may be different, often a pregnant person is diagnosed with gestational hypertension first then monitored for indications of preeclampsia (e.g., protein in urine). If preeclampsia is identified early enough, it may be initially coded as mild but could progress to severe by the time of delivery. The methodological decision to exclusively assign the categories of hypertensive disorders may result in outcome misclassification for gestational hypertension and mild preeclampsia with some cases coded as non-cases.

- Using a fused-CMAQ exposure model, Nobles et al. (2019b) did not exclusively assign categories of hypertensive disorders and they also reported negative-to-null associations with gestational exposures to NO_2 and NO_X (for NO_2, three months preconception RR: 0.91 [95% CI: 0.84, 0.99], first 20 weeks of gestation RR: 0.92 [95% CI: 0.84, 1.00], and second trimester RR: 0.96 [95% CI: 0.89, 1.04]; for NO_X, three months preconception RR: 0.85 [95% CI: 0.77, 0.93], first 20 weeks of gestation RR: 0.91 [95% CI: 0.82, 1.01], and second trimester RR: 0.99 [95% CI: 0.90, 1.09]).
- Yan et al. (2022) also observed negative associations, although they were imprecise and crossed the null, between first trimester (OR: 0.873 [95% CI: 0.597, 1.278]), third trimester (OR: 0.831 [95% CI: 0.486, 1.420]), and entire pregnancy (OR: 0.926 [95% CI: 0.547,1.567]) NO_2 exposure concentrations and preeclampsia among a cohort of Chinese pregnant women. For the second trimester, Yan et al. (2022) reported imprecise positive associations with preeclampsia (OR: 1.064 [95% CI: 0.646, 1.753]).
- In a 2002 to 2008 U.S. administrative cohort study that evaluated gestational exposures to NO_X (modeled by CMAQ) and associations with preeclampsia among women with Type 1 diabetes and women with no known autoimmune disease, Williams et al. (2021) observed null associations for all exposure windows assessed (i.e., preconception, first trimester, second trimester, entire pregnancy).
- In a nested case-control study in California that examined 2006 to 2008 birth records with NO_2 interpolated by empirical Bayesian kriging, Wu et al. (2016) observed near-null associations with preeclampsia, with no consistent pattern for the direction of the ORs for all NO_2 exposure windows evaluated (each trimester and the entire pregnancy, e.g., second trimester OR: 1.031 [95% CI: 0.963, 1.103], entire pregnancy OR: 0.940 [95% CI: 0.820, 1.078]). However, in a sub-analysis that used spatiotemporal NO_2 regression models in Los Angeles County only, Wu et al. (2016) observed negative associations for all exposure windows examined (e.g., second trimester OR: 0.924 [95% CI: 0.874, 0.977]; entire pregnancy OR: 0.904 [95% CI: 0.850, 0.961]). When evaluating NO_X using CALINE4 dispersion modelling for exposure assignment, Wu et al. (2016) observed near null associations with a consistent pattern for the direction of the ORs, but with 95% CIs that crossed the null (e.g., second trimester OR: 0.990 [95% CI: 0.965, 1.015]; entire pregnancy OR: 0.983 [95% CI: 0.957, 1.010]).
- In a multicenter hospital study in China with participants recruited between 2015 and 2016, Jiang et al. (2023) reported null-to-positive associations with gestational hypertension and preeclampsia. For second trimester and first and second trimester exposure windows, NO_2 exposures were positively associated with preeclampsia, although the confidence interval crossed the null (second trimester OR: 1.15 [95% CI: 0.98, 1.34] and first and second trimester OR: 1.10 [95% CI: 0.94, 1.28] per 10.7 ppb).
- In a 2014 to 2016 administrative birth cohort of mothers living within 30 meters of a street segment sampled by the Google Street View cars in West and Downtown Oakland, California, U.S, Goin et al. (2021) used G-computation with log-binomial regression to estimate the effect of a potential intervention in which NO_2 or NO_X concentrations were reduced to the 25th percentile of concentrations observed across the study participants. Goin et al. (2021) reported estimated reductions in risk of preeclampsia for NO_2 (RD: -1.5 cases per 100 women [95% CI: -2.5, -0.5]) and NO_X (RD: -1.8 cases per 100 women [95% CI: -2.9, -0.7]).
- In a case-control study from 2000 to 2006 in the San Joaquin Valley, California, U.S, Weber et al. (2021) evaluated the association between entire pregnancy NO_2 exposure (measured by

IDW of AQS monitors) and sub-categories of preeclampsia exclusively assigned (e.g., women with more than one preeclampsia code were assigned to the most severe condition). For the highest quartile of NO_2 concentrations compared to the other three quartiles, positive, although crosses the null, associations were observed for mild preeclampsia (OR: 1.13 [95% CI: 0.97, 1.31]) and severe preeclampsia (OR: 1.11 [95% CI: 0.96, 1.29]), while a null association was observed for superimposed preeclampsia (i.e., preeclampsia or eclampsia superimposed onto pre-existing hypertension (ICD-9 code: 642.7); OR: 0.99 [95% CI: 0.74, 1.34]).

- In a Danish birth cohort enrolled from 1996 to 2002, Pedersen et al. (2017b) used dispersion modeling to estimate gestational exposure to NO_2 to evaluate the association between NO_2 exposure during the first trimester and preeclampsia. Authors observed positive associations (OR: 1.07 [95% CI: 1.01, 1.14] per 5.3 ppb). Similar results were observed for the second trimester, the third trimester, and the entire pregnancy exposure windows (Pedersen et al., 2017b). When stratified by subtypes, a positive association was observed for mild preeclampsia (OR: 1.12 [95% CI: 1.05, 1.20]) while a negative association was observed for severe preeclampsia, although the 95% CI crossed the null (OR: 0.92 [95% CI: 0.81, 1.05]). When stratifying by onset, early onset preeclampsia was positive (OR: 1.16 [95% CI: 1.05, 1.27]), while late onset was attenuated to near null (OR: 1.02 [95% CI: 0.94, 1.10]).
- A prospective pregnancy cohort that predominantly enrolled low-income Hispanic women in California (the MADRES study) evaluated weekly preconception and gestational exposure to NO_2 and associations with preeclampsia (Niu et al., 2024). Niu et al. (2024) reported null associations for all weekly exposures evaluated from twelve weeks prior to conception until gestational week twenty (see Figure 7-10).



List of abbreviations in alphabetical order: CI = confidence interval; NO_2 = nitrogen dioxide; RR = risk ratio.
Note: All results were from distributed lag models adjusted for maternal age, ethnicity and nativity, marital status, prepregnancy body mass index, annual household income, parity, fetal sex, temperature, year and season of conception, and recruitment time point. Effect estimation was based on per interquartile range increases in weekly air pollutant concentrations (i.e., 11 ppb for 24-hour average NO_2. RR indicates risk ratio.
Source (adapted from): Niu et al. (2024). Copyright permission pending.

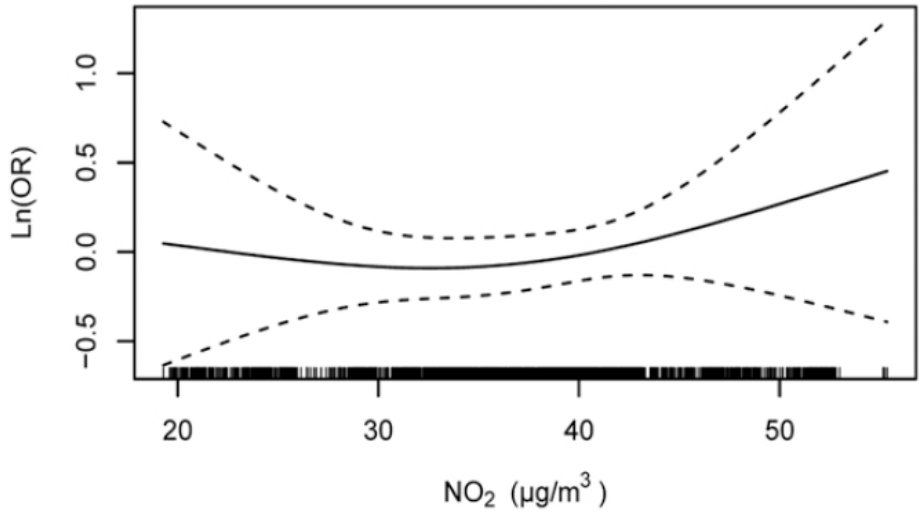
Figure 7-10 Associations of preconception and prenatal weekly exposure to nitrogen dioxide with risk of preeclampsia in the MADRES cohort.

7.5.2.1.2 Concentration-Response

Gestational Hypertension

There was limited evaluation of concentration-response relationship in recent studies for the association between oxides of nitrogen and gestational hypertension.

- In a categorical analysis of quartiles, a positive linear concentration response relationship was observed for the association between NO_2 and gestational hypertension (Savitz et al., 2015).
- Yan et al. (2022) reported a nonlinear concentration response curve with elevated associations at higher concentrations (>40 µg/m^3) between entire pregnancy NO_2 exposure and gestational hypertension (see Figure 7-11).



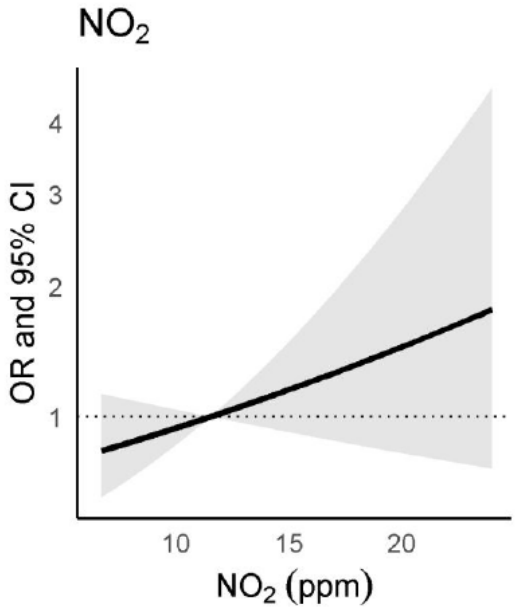
List of abbreviations in alphabetical order: Ln(OR) = natural logarithm of odds ratio; NO_2 = nitrogen dioxide; µg/m^3 = micrograms per cubic meter.
Note: X-axis shows the exposure levels of NO_2. The black ticks on the x-axis show the distribution of air pollution concentrations. Y-axis represents natural logarithm (Ln) of odds ratio (OR). Solid lines show the point estimates of Ln(OR). Dashed lines show the 95% CI for Ln(OR).
Source: Yan et al. (2022). Copyright permission pending.

Figure 7-11 Nonlinear effects on gestational hypertension from exposure to ambient nitrogen dioxide during the entire pregnancy.

Preeclampsia

There was limited evaluation with inconsistent results of concentration response relationship in current studies for the association between oxides of nitrogen and preeclampsia.

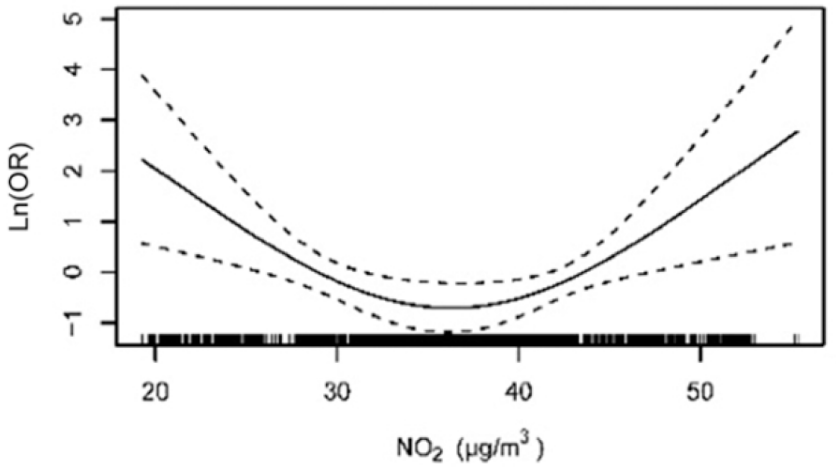
- Savitz et al. (2015) In categorical analyses of NO_2 quartiles, a negative linear concentration response relationship was observed for mild preeclampsia regardless of hospital adjustment while a positive linear concentration response was observed for severe preeclampsia in the models without hospital adjustment. With hospital adjustment, the concentration response observed for severe preeclampsia was attenuated to flat-to negative linear concentration responses with negative-to-null associations for the three quartiles.
- Goin et al. (2021) observed a linear relationship between NO_2 and the odds of preeclampsia (see Figure 7-12).



List of abbreviations in alphabetical order: 95% CI = 95% confidence interval; NO_2 = nitrogen dioxide; OR = odds ratio; ppm = parts per million.
Source: Goin et al. (2021). Copyright permission pending.

Figure 7-12 Estimated odds ratio and 95% confidence interval for preeclampsia across distribution of nitrogen dioxide concentrations.

- Yan et al. (2022) reported a U-shaped concentration response curve with elevated associations at lower (<30 µg/m^3) and higher (>45 µg/m^3) concentrations between entire pregnancy NO_2 exposure and preeclampsia (see Figure 7-13).



List of abbreviations in alphabetical order: Ln(OR) = natural logarithm of odds ratio; NO_2 = nitrogen dioxide; $\mu g/m^3$ = micrograms per cubic meter.
Note: X-axis shows the exposure levels of NO_2. The black ticks on the x-axis show the distribution of air pollution concentrations. Y-axis represents natural logarithm (Ln) of odds ratio (OR). Solid lines show the point estimates of Ln(OR). Dashed lines show the 95% CI for Ln(OR).
Source (adapted from): Yan et al. (2022). Copyright permission pending.

Figure 7-13 Nonlinear effects on preeclampsia from exposure to ambient nitrogen dioxide during the entire pregnancy.

7.5.2.1.3 Lifestages and Populations

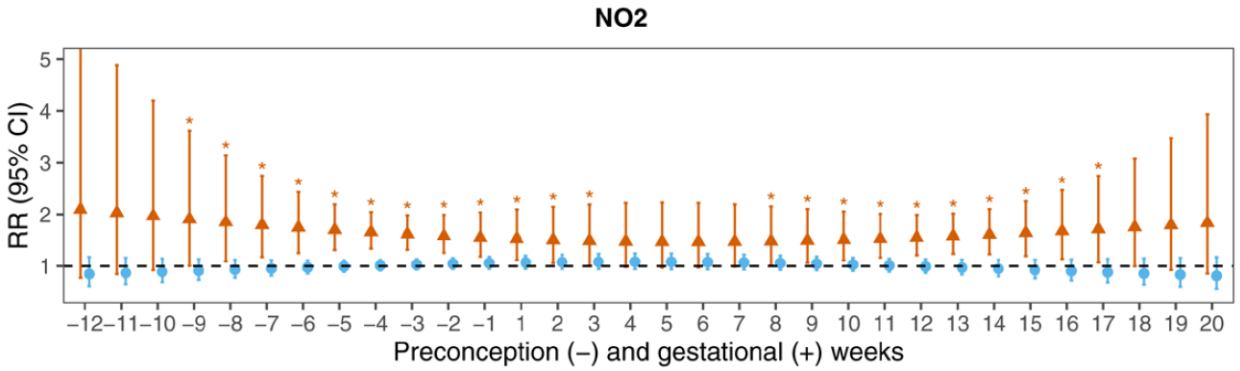
Hypertensive disorders of pregnancy

- Jiang et al. (2023) stratified analyses by maternal age, fetal sex, mode of conception, maternal BMI, and season of conception. Maternal age showed the strongest evidence of modification for the second and first and second trimesters; participants ≤35 yr had higher odds of overall NO_2-associated hypertensive disorders than those >35 yr (second trimester, ≤35 years OR: 1.18 [95% CI: 1.04, 1.33]; second trimester, >35 years OR: 0.88 [95% CI: 0.70, 1.09]). There was no evidence of modification by fetal sex, mode of conception, maternal BMI, or season of conception.
- Goin et al. (2021) reported that when stratifying by race/ethnicity, NO_2-associated results for the hypothetical intervention reducing the pollutant concentrations to the 25th percentile versus the observed concentrations were stronger for both non-Latina Black and non-Latina Asian mothers than non-Latina white, Latina, or other mothers when using the composite outcome that included all mothers who were classified as experiencing either preeclampsia and/or gestational hypertension.

Gestational Hypertension

- Hao et al. (2023) observed elevated positive associations between first trimester NO_2 exposure and gestational hypertension for Hispanic participants (OR: 1.026 [95% CI: 0.937, 1.123] per IQR) as well as elevated positive associated between first or second trimester NO_2 exposure and gestational hypertension for participants residing in zip codes with the level of poverty greater than 12% (OR: 1.028 [95% CI: 0.964, 1.098] per IQR and OR: 1.031 [95% CI: 0.965, 1.101] per IQR, respectively).

- Among participants with prenatal depression, Niu et al. (2024) reported there was a stronger association between NO_2 exposure and gestational hypertension than participants without prenatal depression (see Figure 7-14). NO_2 exposure was associated with an increased risk of gestational hypertension for two exposure windows identified as sensitive by authors (i.e., nine weeks before to three weeks after conception OR: 1.65 [95% CI: 1.17, 2.34] and eight to 17 gestational weeks OR: 1.58 [95% CI: 1.14, 2.18] per 11 ppb NO_2).



List of abbreviations in alphabetical order: CI = confidence interval; NO_2 = nitrogen dioxide; RR = risk ratio.
Note: All results were from distributed lag models adjusted for maternal age, ethnicity and nativity, marital status, prepregnancy body mass index, annual household income, parity, fetal sex, temperature, year and season of conception, and recruitment time point. Susceptible windows were marked with a star on the top. Triangles and bars in orange indicated the risk ratio (RR) and 95% CI among people with prenatal depression (n=163). Dots and bars in blue indicated the RR and 95% CI among people without prenatal depression (n = 349). Effect estimation was scaled by interquartile range increases in weekly air pollutant concentrations (i.e., 11 ppb for 24-hour average NO_2).
Source (adapted from): Niu et al. (2024). Copyright permission pending.

Figure 7-14 Stratified analyses by prenatal depression for the associations of preconception and prenatal weekly exposure to nitrogen dioxide with risk of gestational hypertension in the MADRES cohort.

Preeclampsia

- Williams et al. (2021) reported no effect measure modification by Type 1 diabetes status in a U.S. administrative cohort study from 2002 to 2008 that evaluated gestational exposures to NO_X (modeled by CMAQ) and associations with preeclampsia.
- Wu et al. (2016) observed no clear evidence of modification by maternal education, race/ethnicity, pre-pregnancy BMI, and household income.
- When evaluating modification by season of conception, there was no clear patterning for NO_2 and preeclampsia (Goin et al., 2021). However, when stratifying by race/ethnicity, the main effects only persisted in Black women; Goin et al. (2021) reported that the hypothetical intervention reducing the pollutant concentrations to the 25th percentile versus the observed concentrations for NO_2 would result in a preeclampsia risk difference among Black women of -2.8 (95% CI: -5.2, -0.3).
- In a case-control study from 2000 to 2006 in the San Joaquin Valley, California, U.S., Weber et al. (2021) evaluated the association between entire pregnancy NO_2 exposure and sub-categories of preeclampsia. No modification was observed for mild or severe preeclampsia when stratified by poverty level or income level neighborhoods. Superimposed preeclampsia had evidence of modification with participants in low-income neighborhoods having a null association (1.01 [95% CI: 0.57, 1.81]) while an inverse association was reported for participants in high-income neighborhoods (0.42 [95% CI: 0.27, 0.65]).

- For mild preeclampsia, (Pedersen et al., 2017b) observed no evidence of effect modification by urbanicity, physical activity, smoking status, education, pre-pregnancy BMI, or parity in a Danish birth cohort enrolled from 1996 to 2002 to evaluate the association between NO_2 exposure during first trimester and preeclampsia. Some evidence of effect modification by road traffic noise with association being positive for high (≥57.5 dB OR: 1.11 [95% CI: 1.03, 1.20]), but negative, although crossed the null, for low road traffic noise (<57.5 dB OR: 0.93 [95% CI: 0.73, 1.16]).

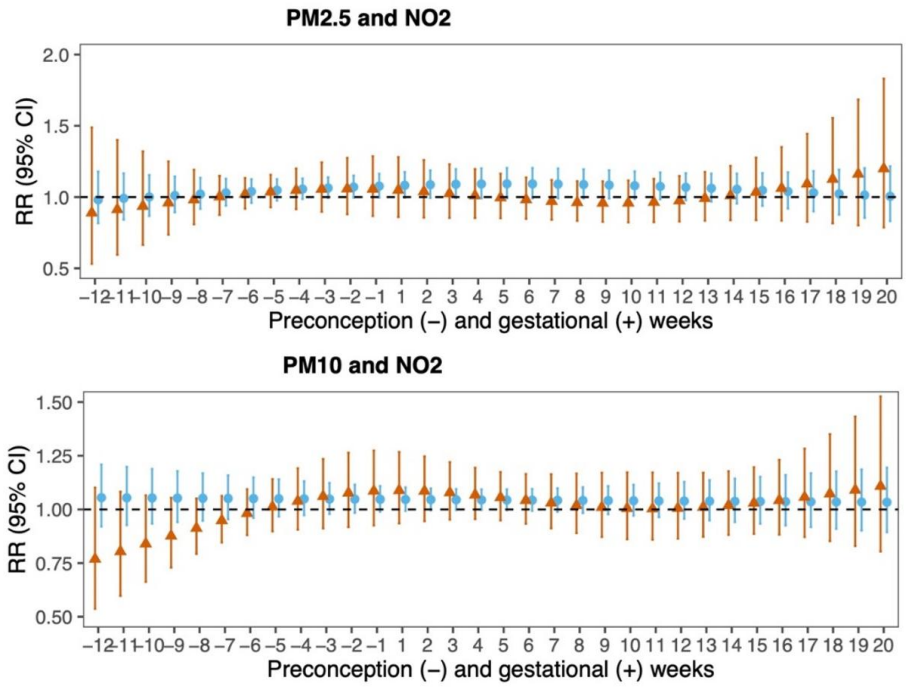
7.5.2.1.4 Copollutants

Hypertensive disorders of pregnancy

- Xu et al. (2014) reported that associations remained positive after copollutant adjustment for CO or $PM_{2.5}$.
- The positive associations reported by Pedersen et al. (2017b) were attenuated when copollutant adjusted for road traffic noise (OR: 1.04 [95% CI: 0.97, 1.11], per 10 $\mu g/m^3$ increment).
- Jiang et al. (2023) considered a mixture analysis (including CO, NO_2, O_3, $PM_{2.5}$, SO_2) using weighted quantile sum (WQS), which assesses pollutants as a mixture rather than an independent effect adjusted for by other copollutants. The magnitude and precision of the associations for first, second, and first and second trimester exposure windows were comparable to each other for the WQS in the positive direction (meaning, all included pollutants are assumed to have effects in the same direction). When the WQS was modeled in the positive direction, odds of gestational hypertension risk was elevated by 7 to 11% for an IQR increase in air pollutant mixture during early pregnancy (e.g., 2nd trimester OR: 1.08 [95% CI: 0.98, 1.19]; first and second trimester OR: 1.11 [95% CI: 1.01, 1.22]).

Gestational hypertension

- Pedersen et al. (2017b) observed near-null associations that were unchanged when copollutant adjusted for road traffic noise (OR: 1:01 [95% CI: 0.90, 1.14]).
- Similar to the single pollutant NO_2 models, Hao et al. (2023) observed near-null effect estimates after multipollutant adjustment for $PM_{2.5}$ and O_3 (e.g., OR first trimester: 0.978 [95% CI: 0.933, 1.025] per IQR, OR third trimester: 1.008 [95% CI: 0.961, 1.057] per IQR) when evaluated the association between NO_2 and gestational hypertension.
- For the association between exposure to NO_2 and gestational hypertension, Niu et al. (2024) observed similar patterns across the weekly preconception through 20 gestational weeks of exposure between the single pollutant and co-pollutant models. However, results were attenuated following copollutant adjustment of $PM_{2.5}$, while results for co-pollutant adjustment for PM_{10} remained robust (see Figure 7-15).



List of abbreviations in alphabetical order: CI = confidence interval; NO_2 = nitrogen dioxide; RR = risk ratio.
Note: Orange triangles are the results for NO_2. Blue circles are the results for $PM_{2.5}$ or PM_{10}. All results were from DLM additionally adjusted for maternal age, ethnicity and nativity, marital cohabitation status, pre-pregnancy BMI, annual household income, parity, fetal sex, weekly temperature in a cross-basis function, year and season of conception, and recruitment timepoint. Effect estimation was based on per IQR increases in each air pollutant (i.e., 5 µg/m^3, 12 µg/m^3, and 11 ppb for $PM_{2.5}$, PM_{10}, and NO_2, respectively). Week 0 indicates conception. $PM_{2.5}$ = particulate matter with aerodynamic diameter <2.5 µm; PM_{10} = particulate matter with aerodynamic diameter <10 µm.
Source (adapted from): Niu et al. (2024). Copyright permission pending.

Figure 7-15 Two pollutants model where each pair of air pollutants were included in the same model simultaneously.

- Jiang et al. (2023) considered a mixture analysis (including CO, NO_2, O_3, $PM_{2.5}$, SO_2) using WQS, which assesses pollutants as a mixture rather than an independent effect adjusted for by other copollutants. When the WQS was modeled in the positive direction (meaning, all included pollutants are assumed to have effects in the same direction), Jiang et al. (2023) reported odds of gestational hypertension were elevated by 7 to 11% for an IQR increase in air pollutant mixture during early pregnancy (first trimester OR: 1.07 [95% CI: 0.98, 1.17]; first and second trimester OR: 1.11 [95% CI: 1.01, 1.22]).

Preeclampsia

- In joint evaluation of the NO_2, BC, and UFP as a mixture using quantile G-computation, which estimates the expected change in risk of preeclampsia if all pollutant concentrations increased by one quartile, Goin et al. (2021) observed similar associations (RD: 1.6 cases per 100 women [95% CI: -0.2, 3.4]) to NO_2 alone; these results were driven equally by BC and NO_2, while contributions of UFP to the overall association were minimal.
- Pedersen et al. (2017b) observed positive associations (OR: 1.07 [95% CI: 1.01, 1.14] per 5.3 ppb), although this association was attenuated when additionally adjusted for road traffic noise (OR: 1.03 [95% CI: 0.96, 1.11] per 5.3 ppb) as a copollutant.

- Jiang et al. (2023) considered a mixture analysis (including CO, NO_2, O_3, $PM_{2.5}$, SO_2) using WQS, which assesses pollutants as a mixture rather than an independent effect adjusted for by other copollutants. When the WQS was modeled in the positive direction (meaning, all included pollutants are assumed to have effects in the same direction), Jiang et al. (2023) reported that the odds of preeclampsia was elevated by 14 to 25% for an IQR increase in air pollutant mixture during early pregnancy (second trimester OR: 1.25 [95% CI: 1.11, 1.41]; first and second trimester OR: 1.14 [95% CI: 1.05, 1.24]).

7.5.2.1.5 Conclusions and Remaining Uncertainties

The recent studies evaluating associations between NO_2 and NO_X exposures and preeclampsia were inconsistent, with reported associations ranging from negative to positive. There does not appear to be a consistent pattern across study geography, analytic exclusion criteria, or adjustment for pregnant persons with pre-pregnancy hypertension or chronic hypertension, or analytic creation of mutually exclusive preeclampsia outcomes based on severity.

7.5.3 Placental Growth and Function

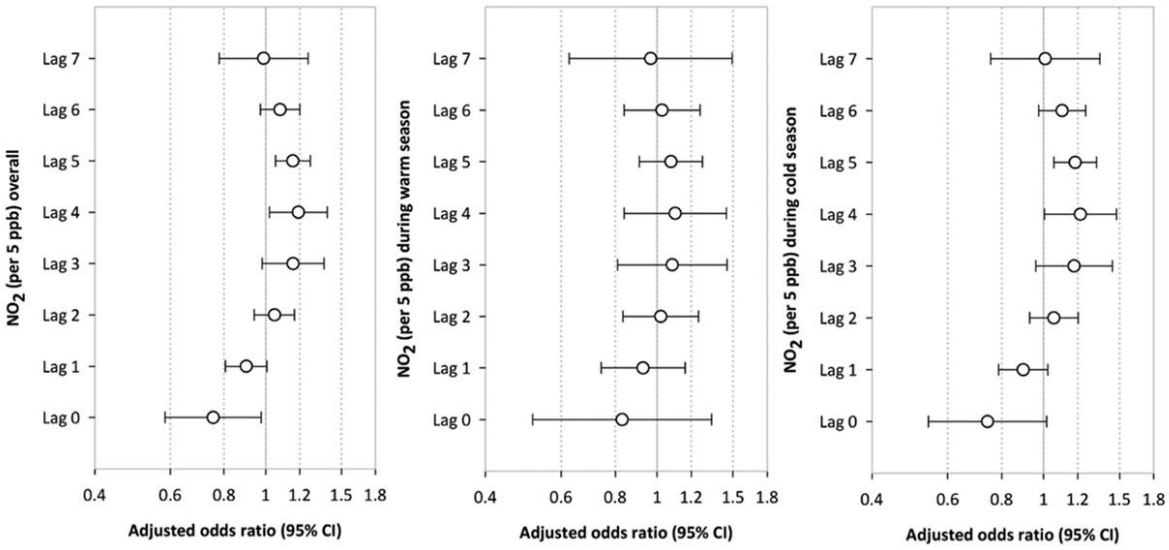
In the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016), there were inconsistent results in the limited epidemiologic studies evaluating oxides of nitrogen exposure and placental growth and function. No toxicological studies that investigate the effects of oxides of nitrogen exposure on placental function were reported in the past ISAs and AQCDs. A small number of recent epidemiologic and toxicological studies have evaluated the association between oxides of nitrogen exposure and placental growth and function. Details for the recent epidemiologic studies are included in Appendix A – Appendix Table 7-6 and the animal toxicological studies are included in Appendix A – Appendix Table 7-2.

7.5.3.1 Epidemiologic Studies of Placental Growth and Function

There were a limited number of studies with inconsistent evidence evaluating oxides of nitrogen exposure and placental growth and function in the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016). A key study identified in the 2016 Oxides of Nitrogen ISA reported NO_2 was associated with markers of placental growth and function (van den Hooven et al., 2012b). Other studies reported null associations. Recent studies are summarized below, followed by additional discussion of the lifestages and populations evaluated in these studies (see Section 7.5.3.1.1) and the impacts of copollutants (see Section 7.5.3.1.2). Section 7.5.3.1.3 discusses conclusions from available studies and summarizes remaining uncertainties. Study specific details including study population characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), potential confounders, and select results are in Appendix A – Appendix Table 7-6.

Recent studies evaluated short-term exposure to oxides of nitrogen and associations with placental vascular resistance or placental abruption and observed positive associations, although on different lag days.

- Michikawa et al. (2017) conducted a case-crossover study in a 2005 to 2010 Japanese administrative cohort. Using central site monitors to link to the maternal hospital of delivery, Michikawa et al. (2017) observed that short-term NO_2 exposure at lag 2 was associated with placental abruption with an increased odds of 1.4 (95% CI: 1.1., 1.8) per 10 ppb increase in NO_2. When evaluating the cumulative effects of NO_2, the odds ratio estimates consistently showed a positive direction, although attenuated (OR at lag1 to 2: 1.2 [95% CI: 1.0, 1.5]; OR at lag1 to 3: 1.2 [95% CI: 0.9, 1.6]; OR at lag1 to 4: 1.3 [95% CI: 0.9, 1.7]; and OR at lag 1 to 5: 1.2 [95% CI: 0.9, 1.8]). However, the associations were near null for individual lag days 1, 3, 4, and 5 (OR: 1.0 [95% CI: 0.8, 1.2], OR: 0.9 [95% CI: 0.7, 1.1], OR: 1.1 [95% CI: 0.9, 1.5], and OR: 0.9 [95% CI: 0.7, 1.1], respectively.
- In a New York City, NY, U.S., based case-crossover study, Ananth et al. (2018) observed decreased odds of placental abruption for women exposed to NO_2 on lag day 0 (OR: 0.75 [95% CI: 0.58, 0.98] per 5 ppb). However, Ananth et al. (2018) observed increased odds of placental abruption for women exposed to NO_2 on lag day 3 (OR 1.16 [95% CI: 0.98, 1.37]), lag day 4 (OR 1.19 [95% CI: 1.02, 1.39]), and lag day 5 (OR 1.16 [95% CI: 1.05, 1.27]) (see Figure 7-16).



List of abbreviations in alphabetical order: CI = 95% confidence interval; NO_2 = nitrogen dioxide; ppb = parts per billion.
Note: Case-crossover analysis of singleton live births in New York City, 2008–2014. Left panel indicated all abruption births; middle panel refers to abruption births in the warm season; and right panel refers to abruption births in the cold season CI indicates confidence interval.
Source: Ananth et al. (2018). Copyright permission pending.

Figure 7-16 Association between nitrogen dioxide at lags 0–7 days with case-day defined as the day prior to delivery and placental abruption of acute onset from distributed lag nonlinear models.

7.5.3.1.1 Lifestages and Populations

There was limited evaluation of lifestages and populations potentially at increased risk with no consistent patterns.

- Michikawa et al. (2017) did not observe evidence of effect modification for season of conception, age, parity, smoking during pregnancy, or hypertensive disorders in pregnancy.
- Ananth et al. (2018) reported that associations persisted only in the cold season when stratified by season of birth (see Figure 7-16 above).

7.5.3.1.2 Copollutants

There was limited evaluation of copollutants as well as no consistency for which copollutant was adjusted for across studies; however, within the studies, the associations remained robust after selected copollutant adjustment.

- Michikawa et al. (2017) reported that the association observed at lag 2 between short-term NO_2 exposure and placental abruption was robust after copollutant adjustment for suspended particulate matter (SPM), O_3, or SO_2.
- Similarly, Ananth et al. (2018) reported that after controlling for $PM_{2.5}$ in copollutant adjusted models, NO_2 exposure at lag day 4 (OR 1.30 [95% CI: 1.01, 1.67]), lag day 6 (OR 1.32 [95% CI: 1.03, 1.70]), and lag day 7 (OR 1.28 [95% CI: 1.01, 1.65] per 5 ppb) were associated with placental abruption.

7.5.3.1.3 Conclusions and Remaining Uncertainties

Recent studies evaluating short-term exposure to NO_2 and associations with placental abruption observed positive associations, although the evidence base remains small and there is inconsistency regarding the lags for which positive associations were reported. Limited studies evaluated potential effect modification. A single study did not observe evidence of modification by age, parity, smoking during pregnancy, or hypertensive disorders in pregnancy. Few studies evaluated the potential effect modification by season of birth/conception. While a single study reported that associations persisted only in the cold season when stratified by season of birth, another study that evaluated potential effect modification by season of conception did not observe evidence of modification. Overall, the epidemiologic evidence for associations between oxides of nitrogen exposure and effects on placental growth and function remains limited.

7.5.3.2 Animal Toxicological Studies of Placental Growth and Function

No toxicological studies that investigated the effects of oxides of nitrogen exposure on placental function were reported in the 2016 Oxides of Nitrogen ISA, the 2008 Oxides of Nitrogen ISA, or the 1993 Oxides of Nitrogen AQCD. A single, recent toxicological study was available that assessed outcomes regarding placental function in which C57BL/6 mice were exposed to 2.5 ppm NO_2 for either 13.5, 15.5, or 18.5 days starting on GD 0 (Zhu et al., 2022). Increased placental fibrosis, increased placental calcification, and decreased placental telomere length were observed in placentas from dams exposed for 18.5 days (Zhu et al., 2022). Study specific details, exposure conditions, and endpoints examined are described further in Appendix A – Appendix Table 7-2.

7.5.3.2.1 Conclusions and Remaining Uncertainties

There is limited toxicological evidence examining the effects of NO_2 exposure on placental growth and function. A single study reported markers of reduced placental health and function in mice exposed to 2.5 ppm NO_2 starting on GD 0 through sample collection. However, with no other available studies to corroborate or expand upon these findings, considerable uncertainty remains. Future studies that investigate the impacts of NO_2 exposure on placental outcomes would reduce this uncertainty. Additionally, using multiple doses and assessing placentae separately for male and female fetuses would help determine if a dose-response relationship exists and if effects are sex-specific, respectively.

7.5.4 Pregnancy-related Mental Health

There were no epidemiologic or toxicological studies evaluating oxides of nitrogen exposure and pregnancy-related mental health in the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016). The recent epidemiologic evidence is summarized below. There were no recent toxicological studies evaluating oxides of nitrogen exposure and pregnancy-related mental health.

7.5.4.1 Epidemiologic Studies of Pregnancy-related Mental Health

In the recent epidemiologic literature, there were a few studies that evaluated associations between exposure to NO_2 and postpartum depression and the evidence is inconsistent. Evidence from recent studies is summarized below, followed by additional discussion of the lifestages and populations evaluated in these studies (see Section 7.5.4.1.1) and the impacts of copollutants (see Section 7.5.4.1.2). Section 7.5.4.1.3 discusses conclusions from available studies and summarizes remaining uncertainties. Study specific details including study population characteristics, exposure details (assessment metrics,

temporal scale, and spatial scale), potential confounders, and select results are in Appendix A – Appendix Table 7-7.

- In a Taiwanese cohort from 2005, Shih et al. (2021) evaluated the associations between gestational and postpartum long-term exposures to NO_2 and self-reported postpartum depression at or before 6 months postpartum. Shih et al. (2021) reported a pattern of positive associations for trimesters as well as 3-month postpartum exposure to NO_2, with the strongest associations observed for first trimester exposure (OR: 1.008 [95% CI: 1.001, 1.014]) and 3-month postpartum exposure (OR: 1.006 [95% CI: 1.000, 1.013] per 1 ppb NO_2).
- A prospective pregnancy cohort that predominantly enrolled low-income Hispanic/Latina women in California between 2015 and 2020 evaluated long-term gestational exposures to NO_2 and associations with depression 12 months postpartum (Bastain et al., 2021). Bastain et al. (2021) observed an increased odds of depression at 12 months postpartum associated with second trimester NO_2 exposure (OR: 2.63 [95% CI: 1.41, 4.89] per 5.2 ppb NO_2) and entire pregnancy NO_2 exposure (OR: 2.04 [95% CI: 1.13, 3.69] per 2.7 ppb NO_2).
- Sun et al. (2023) constructed a retrospective cohort using Kaiser Permanente Southern California (KPSC) electronic health records for women who had births between January 1, 2008, and December 31, 2016, to evaluate the association between long-term NO_2 exposure and depression six months postpartum. Authors reported negative-to-null associations for exposure windows evaluated, which included first trimester, second trimester, third trimester, entire pregnancy, third trimester plus after delivery, and during pregnancy plus after delivery (e.g., during pregnancy plus the postpartum period OR: 0.97 [95% CI: 0.94, 1.00] per 5.38 ppb NO_2).

7.5.4.1.1 Lifestages and Population Differences

There was limited evaluation of factors that could modify associations among different lifestages and populations for the association between oxides of nitrogen and pregnancy-related mental health endpoints. Sun et al. (2023) evaluated modification by maternal age, race and ethnicity, educational level, neighborhood household income, and pre-pregnancy BMI. Authors reported no evidence of modification. Within the MADRES cohort, which was comprised of a majority (79.4%) of Hispanic participants, Bastain et al. (2021) reported positive associations between second trimester and entire pregnancy exposure to NO_2 and depression 12 months postpartum.

7.5.4.1.2 Copollutants

A single recent study evaluated copollutant adjustment. For the association between NO_2 and self-reported postpartum depression at or before six months postpartum, Shih et al. (2021) reported essentially unchanged ORs with additional model adjustment for copollutant $PM_{2.5}$ (e.g., 3-month postpartum OR for copollutant model: 1.007 [95% CI: 1.000, 1.014 versus 3-month postpartum for single-pollutant model (OR: 1.006 [95% CI: 1.000, 1.013] per 1 ppb NO_2).

7.5.4.1.3 Conclusions and Remaining Uncertainties

There was a small body of recent epidemiologic studies across pregnancy-related mental health outcomes. The small number of studies limits the ability to judge coherence and consistency across studies, although the associations reported demonstrate that exposure to oxides of nitrogen could result in physiological responses that contribute to effects on pregnancy-related mental health. There remain uncertainties regarding the concentration response relationship, populations at increased risk, and copollutant confounding.

7.5.5 Pregnancy Loss and Stillbirth

Fetal mortality is the intrauterine death of a fetus. Often these pregnancy losses are divided into those occurring before 20 weeks of gestation (spontaneous abortion/miscarriage) and those occurring after (stillbirth). Most stillbirths happen before a pregnant person goes into labor, but a small number happen during labor and birth. Studies that examine spontaneous abortion (i.e., pregnancy loss) are difficult to conduct, as many pregnancy losses occur during the first trimester. Pregnancy loss or miscarriages may occur before study enrollment and/or before the pregnancy is known, further limiting the ability to detect subtle effects, especially if higher oxides of nitrogen exposures do lead to increased risk of early spontaneous abortions. The limited number of epidemiologic studies reviewed in the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016) that evaluated the associations between oxides of nitrogen and pregnancy loss or stillbirth reported inconsistent results. In rodent toxicological studies, pregnancy loss is measured by counting pup loss or resorption sites, which can impact litter size. Therefore, reduced litter size may indicate the occurrence of in utero death, although this is not a direct measure. A limited number of toxicological studies in the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016) investigated the effects of oxides of nitrogen exposure on litter size, an indicator of potential fetal death, and reported inconsistent results. A small number of recent epidemiologic studies continue to indicate inconsistent associations with pregnancy loss or stillbirth, while recent toxicological studies have primarily demonstrated null effects on pregnancy loss.

7.5.5.1 Epidemiologic Studies of Pregnancy Loss and Stillbirth

The few epidemiologic studies reviewed in the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016) that examined the associations between NO_2 and pregnancy loss or stillbirth were inconsistent, with associations generally ranging from positive to null. These studies differed in geography (i.e., Taiwan or United States), exposure duration (e.g., entire pregnancy or within days preceding stillbirth), and outcome (e.g., stillbirth or spontaneous abortion). Faiz et al. (2012) observed positive associations between long-term exposure to NO_2 and stillbirth, while Faiz et al. (2013) observed positive, although imprecise, associations between short-term exposure to NO_2 and stillbirth.

Evidence from recent studies is summarized below, followed by additional discussion of the lifestages and populations evaluated in these studies (see Section 7.5.5.1.1). Section 7.5.5.1.2 discusses conclusions from available studies and summarizes remaining uncertainties. Study specific details including study population characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), potential confounders, and select results are in Appendix A – Appendix Table 7-8.

- A case-crossover study in Utah, U.S., from 2007 to 2015 evaluated short-term exposure to NO_2 (from EPA's Air Quality System Data Mart) and spontaneous pregnancy loss. Authors reported a positive association for the 7-day average NO_2 exposure window (OR: 1.35 [95% CI 1.02, 1.77]) as well as positive associations for the 3-day average, 3-day maximum, and 7-day maximum NO_2 exposure windows, although 95% CIs crossed the null (quantitative results not reported) (Leiser et al., 2019).
- Another case-crossover study conducted in California from 1999 to 2009 evaluated short-term exposure to NO_2 (via fixed site monitors) and stillbirth (Sarovar et al., 2020). Sarovar et al. (2020) reported a null association of stillbirth associated with IQR increase in NO_2 (4-day lag OR: 1.00 [95 % CI: 0.96, 1.05]).
- A U.S. administrative cohort study from 2002 to 2008 evaluated gestational exposures to NO_X (modeled by CMAQ) and associations with perinatal mortality (defined as pregnancy loss ≥23 weeks of gestation through neonatal mortality ≤7 days) among women with Type 1 diabetes and women with no known autoimmune disease Williams et al. (2021). This study observed null associations for preconception, first trimester, and second trimester exposure windows. In contrast, entire pregnancy exposure to NO_X was associated with a decrease in risk of perinatal mortality for Type 1 diabetes (RR: 0.92 [95% CI: 0.86, 0.98]) and no known autoimmune disease (RR: 0.99 [95% CI: 0.98, 1.00] per IQR NO_X).

7.5.5.1.1 Lifestages and Population Differences

The recent studies evaluated effect measure modification by maternal characteristics (i.e., race/ethnicity, maternal age, education, Type 1 diabetes status) as well as fetal characteristics (i.e., fetal sex, cause of death, gestational weeks). There was no observed evidence of modification for the factors evaluated, except for a single study that reported modification by Type 1 diabetes status.

- Leiser et al. (2019) observed no effect measure modification by Hispanic ethnicity.
- Sarovar et al. (2020) did not observe evidence of effect measure modification by maternal age, education, race/ethnicity, fetal sex, cause of death, or gestational weeks.
- Williams et al. (2021) observed no effect measure modification by Type 1 diabetes status for preconception, first trimester, and second trimester exposure windows. In contrast, there was some evidence of effect measure modification by Type 1 diabetes status for the entire pregnancy exposure to NO_X, with a decrease in risk of perinatal mortality for women with Type 1 diabetes.

7.5.5.1.2 Conclusions and Remaining Uncertainties

The recent literature continues to provide null to positive associations between oxides of nitrogen and pregnancy loss and/or stillbirth. It remains difficult to compare across studies given differences in exposure window assignments and outcome ascertainment. Studies that select from general population emergency department visits to identify cases of spontaneous abortion are likely missing many cases. Beyond study limitations and the inconsistent results across available studies, remaining uncertainties result from the lack of information on populations that may be at highest risk, on the shape of the concentration-response relationship, and on the potential impacts of copollutant confounding on reported associations.

7.5.5.2 Animal Toxicological Studies of Pregnancy Loss and Stillbirth

In rodent toxicological studies, fetal death is measured by counting pup loss or resorption sites, which can impact litter size. Therefore, reduced litter size may indicate the occurrence of in utero death, although this is not a direct measure. The 1993 Oxides of Nitrogen AQCD summarized a study evaluating the effects of oxides of nitrogen exposure on litter size in rats (Shalamberidze and Tsereteli, 1971). Shalamberidze and Tsereteli (1971) exposed female albino rats to 0.126 or 2.36 mg/m^3 NO_2 (0.067 or 1.25 ppm NO_2) for 12 hours/day over three months and assessed reproductive outcomes in mating trials immediately after dosing was complete. When compared to unexposed controls, females in the higher treatment group (1.25 ppm NO_2) produced significantly smaller litter sizes, indicating that fetal loss may have occurred (Shalamberidze and Tsereteli, 1971). Conversely, in the 2008 Oxides of Nitrogen ISA, a single study was summarized that reported null effects of NO_2 exposure on litter size (Di Giovanni et al., 1994). Di Giovanni et al. (1994) exposed female Wistar rats to 1.5 or 3 ppm NO_2 throughout gestation (GD 0 to GD 20) and observed no difference in litter size at birth when compared to unexposed controls. No additional toxicological studies that investigated the effects of oxides of nitrogen exposure on pregnancy loss were reported in the 2016 Oxides of Nitrogen ISA.

Since the 2016 Oxides of Nitrogen ISA, several recent toxicological studies were identified that, similar to Di Giovanni et al. (1994), largely reported null effects of NO_2 exposure on pregnancy loss (Li et al., 2022; Yan et al., 2020; Yue et al., 2018; Yue et al., 2017). Study specific details, exposure conditions, and endpoints examined are highlighted in the text below. Studies supporting causality determinations are described in more detail in Appendix A – Appendix Table 7-2. Notably, all recent studies were from the same research group and utilized the same dosing regimen: 2.5 ppm of NO_2 for five hours/day throughout gestation (GD 0 to PND 0). NO_2 exposure did not affect litter size in C57BL/6 mice (Yan et al., 2020), C57BL/6J mice (Li et al., 2022), or in BALB/c mice (Yue et al., 2018; Yue et al., 2017). Given that Shalamberidze and Tsereteli (1971) utilized lower doses (1.25 ppm) than more recent studies (1.5 to 3 ppm), the contrast in results may be attributable to differences in the exposure window or duration. It is possible that the shorter duration of exposure used in more recent studies (20 to 21 days) is

insufficient to elicit the effects observed in Shalamberidze and Tsereteli (1971), which used a much longer dosing duration (three months prior to mating). Furthermore, exposure during the premating window may be necessary to elicit effects leading to the reduced litter size observed by Shalamberidze and Tsereteli (1971). Indeed, Shalamberidze and Tsereteli (1971) reported that treated rats also exhibited altered estrous cyclicity and altered ovarian follicle populations, indicating that NO_2 may disrupt hormonal cycles and negatively impact the ovary, both of which could increase the risk of pregnancy loss.

7.5.5.2.1 Conclusions and Remaining Uncertainties

There is limited toxicological evidence examining the effects of exposure to oxides of nitrogen on pregnancy loss and still birth, and contrasting findings were reported between studies from the 1993 Oxides of Nitrogen AQCD and more recent ISAs (reduced litter size and no effects on litter size, respectively). The observed contrast in results could be attributed to differences in dose, dosing duration, and exposure timing. Thus, more studies are needed to investigate the impacts of exposure to oxides of nitrogen on pregnancy loss and to determine what exposure windows may be the most sensitive. Specifically, studies investigating a dose-response relationship and studies utilizing a variety of exposure windows (e.g., premating/preconception, gestational) would be particularly informative. Further, additional studies to interrogate what biological processes are affected to impart reduced litter size (e.g., hormonal imbalances) would also greatly contribute to the understanding of the impacts of exposure to oxides of nitrogen.

7.5.6 Other Pregnancy Outcomes

In the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016), the other pregnancy outcomes section included gestational diabetes mellitus and placental growth and function. In this ISA, those endpoints are discussed in Sections 7.5.1 and 7.5.3, respectively. Recent epidemiologic studies included in this section evaluate gestational thyroid function and cardiovascular events during delivery (see Section 7.5.6.1). Recent animal toxicological studies included in this section evaluate the effects of NO_2 exposure on sex ratio (see Section 7.5.6.2). Study details for epidemiological studies of other pregnancy outcomes are in Appendix A – Appendix Table 7-9. Study details for animal toxicological studies of other pregnancy outcomes are in Appendix A – Appendix Table 7-2.

7.5.6.1 Epidemiologic Studies of Other Pregnancy Outcomes

There were no epidemiologic studies evaluating oxides of nitrogen exposure and gestational thyroid function or cardiovascular events during delivery in the 2016 Oxides of Nitrogen ISA. Among the recent epidemiologic evidence, there were pregnancy outcomes other than GDM, hypertensive disorders

of pregnancy, pregnancy-related mental health, and pregnancy loss and stillbirth. The other pregnancy-related outcomes include gestational thyroid function and maternal cardiovascular events during delivery. There is limited evidence regarding these apical endpoints, making it difficult to judge consistency and coherence. Evidence from recent studies is summarized in the bullets below. Study details including study population characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), potential confounders, and select results are in Appendix A – Appendix Table 7-9.

- A single recent study investigated the association between oxides of nitrogen and gestational thyroid function. Ghassabian et al. (2019) combined four birth cohorts (three European cohorts and one U.S. cohort with enrollment that ranged from 1999 to 2008 depending on the cohort) for their random-effects meta-analysis evaluating first trimester NO_X and NO_2 exposures and associations with gestational thyroid function. Ghassabian et al. (2019) observed inverse associations between NO_X and NO_2 concentrations and hypothyroxinemia during pregnancy although 95% CIs crossed the null. However, for high thyroid stimulating hormone (high TSH defined as concentrations higher than the 95th percentile of the cohort) they observed a positive association for NO_2 although the 95% CI crossed the null, and a null association for NO_X.
- Another single recent study evaluated the association between oxides of nitrogen and maternal cardiovascular events during delivery. In a U.S. cohort from 2002 to 2008, Männistö et al. (2015) reported increased odds for maternal cardiovascular events during hospital delivery with recent (5- or 6-day lags) exposure to NO_X. These associations were magnified, although imprecise, in multipollutant models that included adjustment for PM_{10}, CO, SO_2, and O_3 (Männistö et al., 2015).

7.5.6.1.1 Conclusions and Remaining Uncertainties

There is limited evidence examining the associations between exposure to oxides of nitrogen and other pregnancy outcomes not covered in Sections 7.5.1 to 7.5.5. While recent single studies provide some evidence of effects on cardiovascular events during delivery, there remain uncertainties regarding concentration/exposure response, lifestages and populations potentially at increased risk, and the potential of confounding by copollutants. It is not plausible to judge consistency and coherency across studies given the evidence is limited to single studies for those other health endpoints.

7.5.6.2 Animal Toxicological Studies of Other Pregnancy Outcomes

One study measured effects of oxides of nitrogen exposure on other pregnancy outcomes in the 2008 Oxides of Nitrogen ISA (Di Giovanni et al., 1994). Di Giovanni et al. (1994) exposed female Wistar rats to 1.5 or 3 ppm NO_2 throughout gestation (GD 0 to GD 20) and observed no effect on maternal weight gain during pregnancy (measured as the percentage of body weight at conception) when compared to unexposed controls. No additional toxicological studies investigating the effects of oxides of nitrogen exposure on other pregnancy outcomes were reported in the 2016 Oxides of Nitrogen ISA or the 1993 Oxides of Nitrogen AQCD. Some recent toxicological studies assessed the effects of NO_2 exposure on

sex ratio. All available studies reported no effects on sex ratio after dosing pregnant mice with 2.5 ppm NO_2 starting at the beginning of pregnancy until birth (approximately 20 days) (Li et al., 2022; Yan et al., 2020).

7.5.6.2.1 Conclusions and Remaining Uncertainties

There is limited toxicological evidence examining the effects of NO_2 exposure on other pregnancy outcomes. The only two outcomes assessed in this category by animal toxicological studies were maternal weight gain and sex ratio, for which only a few recent studies were available, and all available studies reported null effects. Although the available evidence did not report treatment-related effects on sex ratio of pups born to pregnant mice dosed during pregnancy, considerable uncertainty remains regarding the potential effects that exposure in sires may have on sex ratio. The available studies suggest that it is unlikely that NO_2 exposure imparts sex-specific pressures on implantation and survival of embryos/fetuses when only the dam is exposed, and exposure of the dam during pregnancy may not be a sensitive period for eliciting effects on sex ratio. However, because the sex of the offspring is determined by the sire, additional research into any potential effects of NO_2 exposure on the health and/or selection of X-chromosome-carrying or Y-chromosome-carrying sperm in the male would be particularly informative.

7.5.7 Synthesis Across Lines of Evidence

Pregnancy outcomes evaluated for epidemiologic evidence included GDM, hypertensive disorders of pregnancy, placental growth and function, pregnancy-related mental health, pregnancy loss and stillbirth, and other pregnancy outcomes. Toxicological evidence was more narrowly available for placental growth and function, pregnancy loss and stillbirth, and other pregnancy outcomes. As expected, there were no controlled human exposure studies for any of the pregnancy outcome endpoints evaluated.

Overall, the epidemiologic evidence assessing the impacts of exposure to oxides of nitrogen on pregnancy outcomes is inconsistent, and for some specific outcomes the evidence is limited. However, the strongest, most consistent evidence comes from epidemiologic studies reporting a generally consistent pattern of positive associations between exposure to NO_2 or NO_X and GDM within the United States. Additionally, several studies reported the strongest positive associations during the preconception or first trimester exposure windows with sensitive exposure windows identified between five to twelve weeks before conception as well as zero to nine weeks after conception. Additional uncertainties result from the limited information on the shapes of concentration-response relationships and confidence in those relationships over the distribution of exposures, the potential modifying impact of maternal comorbidities, and the limited information available on potential confounding by copollutants, particularly those most closely associated with traffic emissions. Animal toxicological evidence assessing the impacts of oxides

of nitrogen exposure on pregnancy outcomes is limited to studies of NO_2. A single recent study reported reduced markers of placental health in NO_2-exposed rats and several recent studies reported inconsistent effects of NO_2 exposure on litter size in rats and mice. Additionally, few recent studies reported no effects on maternal weight gain or the sex ratio of offspring born to NO_2-exposed dams.

Lines of evidence other than epidemiologic evidence assessing the impacts of exposure to oxides of nitrogen on pregnancy outcomes are limited. Additionally, the bulk of the epidemiologic evidence is for apical outcomes that lack toxicological evidence, and the apical outcomes with toxicological evidence have relatively limited corresponding epidemiologic evidence. Thus, the collective evidence is overall limited to judge coherence across lines of evidence.

7.5.8 Biological Plausibility

This section describes biological pathways that potentially underlie effects on pregnancy outcomes resulting from exposure to oxides of nitrogen. 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 placenta. Evidence supporting these proposed pathways was derived from Sections 7.5.1 through 7.5.6 of this ISA, evidence reviewed in the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016), and recent evidence collected from studies that may not meet the current PECOS criteria but contain mechanistic information supporting these pathways. Discussion of how exposure to oxides of nitrogen may lead to reproductive and developmental effects contributes to an understanding of the biological plausibility of epidemiologic results.

There is limited experimental evidence available that examines potential pathways by which NO_2 exposure could result in adverse effects on pregnancy outcomes. In the 2016 Oxides of Nitrogen ISA, hypertensive disorders during pregnancy were the most well-studied, but associations were not consistently observed and there were no available toxicological studies evaluating hypertensive disorders during pregnancy to support any epidemiologic findings. Recent epidemiologic evidence also primarily evaluated the effects of NO_2 exposure on gestational hypertension or preeclampsia (Jiang et al., 2023; Nobles et al., 2019b; Savitz et al., 2019; Zhu et al., 2017; Savitz et al., 2015), but there were no recent toxicological studies evaluating related endpoints. The toxicological evidence available from recent studies provided little or no evidence of effects on placental growth and function (Zhu et al., 2022) and sex ratio (Li et al., 2022; Yan et al., 2020).

7.5.9 Summary of Lifestages and Populations

Some populations and lifestages may be at greater risk of oxides of nitrogen related health effects than the general population. Higher risks could be due to intrinsic,[6] extrinsic,[7] 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 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. 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 oxides of nitrogen exposure. Additionally, studies examining whether factors may result in differential exposure to oxides of nitrogen are included. Table 7-3 at the end of this section summarizes the evidence for each factor to increase the risk of oxides of nitrogen-related health effects.

7.5.9.1 Pre-existing Comorbidities

In the 2016 Oxides of Nitrogen ISA, there were no studies available that evaluated the potential for maternal pre-existing comorbidities to modify associations between exposure to oxides of nitrogen and pregnancy outcomes. Among the recent epidemiologic evidence, maternal pre-existing comorbidities (i.e., maternal Type 1 diabetes, hypertensive disorders, depression, BMI) were evaluated as potential modifiers for the associations between oxides of nitrogen and pregnancy outcomes; however, the evidence is limited overall. A single study evaluated maternal Type 1 diabetes status as a modifier to the association between oxides of nitrogen and pregnancy outcomes, specifically preeclampsia as well as pregnancy loss and stillbirth (Williams et al., 2021). Williams et al. (2021) reported no effect measure

[6]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.
[7]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.

modification by Type 1 diabetes status in a U.S. administrative cohort study from 2002 to 2008 that evaluated gestational exposures to NO_X and associations with preeclampsia; however, Williams et al. (2021) reported some evidence of effect measure modification by Type 1 diabetes status for the entire pregnancy exposure to NO_X and perinatal mortality, with a decrease in risk of perinatal mortality for women with Type 1 diabetes. Few studies evaluated hypertensive disorders in pregnancy as a modifier to the association between oxides of nitrogen and pregnancy outcomes, specifically GDM as well as placental growth and function, and those studies did not observe evidence of effect measure modification Sun et al. (2022); Michikawa et al. (2017). Few studies evaluated maternal depression as a modifier to the association between oxides of nitrogen and pregnancy outcomes. A single study evaluated the association between oxides of nitrogen and GDM modified by maternal depression (Niu et al., 2023). Niu et al. (2023) reported that among the participants with depression, an increased risk of GDM was associated with exposure to NO_2 during two weeks before to 13 weeks after conception (RR: 1.31 [95% CI: 1.29, 1.33]). A single study evaluated the association between oxides of nitrogen and hypertensive disorders of pregnancy by prenatal maternal depression (Niu et al., 2024). Among participants with prenatal maternal depression, Niu et al. (2024) reported there was a stronger association between NO_2 exposure and gestational hypertension than participants without prenatal depression.

Several recent studies evaluated the association between oxides of nitrogen and pregnancy outcomes modified by BMI, especially for GDM. While several studies (Zhu et al., 2024; Niu et al., 2023; Sun et al., 2022; Pedersen et al., 2017c; Wu et al., 2016; Robledo et al., 2015) evaluated effect measure modification by BMI for the association between oxides of nitrogen and GDM, the evidence was inconsistent with some studies reporting no evidence of effect measure modification (Wu et al., 2016; Robledo et al., 2015), evidence of effect measure modification with stronger associations among those with lower pre-pregnancy BMI Niu et al. (2023), or evidence of effect measure modification with the stronger associations among those with higher pre-pregnancy BMI Zhu et al. (2024); Sun et al. (2022); Pedersen et al. (2017c) (refer Section 7.5.1.1.2 for additional study details). The few studies that evaluated effect measure modification by BMI for the association between oxides of nitrogen and pregnancy-related mental health (Sun et al., 2023), hypertensive disorders of pregnancy (Jiang et al., 2023), and preeclampsia (Pedersen et al., 2017b; Wu et al., 2016) did not report effect measure modification.

Overall, the collective evidence is inadequate to determine whether maternal pre-existing comorbidities result in modification of the associations between exposure to oxides of nitrogen and pregnancy outcomes.

7.5.9.2 Sociodemographic Factors

Sociodemographic factors encompass the broad categories of lifestages (e.g., children, adults), socioeconomic status (SES), race/ethnicity, sex, residence in an urban area, and/or proximity to roadways.

In the 2016 Oxides of Nitrogen ISA, there were no studies available that evaluated the potential for sociodemographic factors to modify associations between exposure to oxides of nitrogen and pregnancy outcomes. Overall, the collective evidence is inadequate to determine whether sociodemographic factors result in modification of the associations between exposure to oxides of nitrogen and pregnancy outcomes.

Lifestages

The few studies that evaluated effect measure modification by maternal age for the association between oxides of nitrogen and GDM were inconsistent. Niu et al. (2023) observed that increased GDM risk from NO_2 was modified by age, with stronger associations observed in younger participants (<28 years). Similarly, Zhu et al. (2024) reported that the associations between NO_2 and GDM were magnified for younger women (<30 years old) compared to participants who were older (>30 years old). However, Hao et al. (2023) observed elevated positive association between NO_2 exposure and GDM for those >27 years old. A single study that evaluated effect measure modification by maternal age for the association between oxides of nitrogen and hypertensive disorders of pregnancy observed evidence of modification, with participants ≤35 years having higher odds of overall NO_2-associated hypertensive disorders than participants >35 years (Jiang et al., 2023). No evidence of effect measure modification by maternal age was reported for the associations between oxides of nitrogen and placental growth and function (Michikawa et al., 2017), pregnancy-related mental health (Sun et al., 2023), or pregnancy loss and stillbirth Sarovar et al. (2020).

Fetal Sex

No evidence of effect measure modification by fetal sex was reported for the association between oxides of nitrogen and hypertensive disorders of pregnancy (Jiang et al., 2023) as well as pregnancy loss and stillbirth (Sarovar et al., 2020).

Traffic noise/ urbanicity

A single study that evaluated the association between oxides of nitrogen and GDM did not observe evidence of effect measure modification by road traffic noise (Pedersen et al., 2017c). A single study that evaluated the association between oxides of nitrogen and mild preeclampsia observed some evidence of effect measure modification by road traffic noise with a positive association for high road traffic noise (≥57.5 dB), but a negative association, although included the null, for low road traffic noise (<57.5 dB) (Pedersen et al., 2017b). That same study observed no evidence of effect measure modification by urbanicity for the association between NO_2 and mild preeclampsia (Pedersen et al., 2017b).

Income

A single study evaluated effect measure modification by household income for the association between NO_2 and GDM (Wu et al., 2016) with limited evidence of effect measure modification. The

studies that evaluated effect measure modification by income for the association between oxides of nitrogen and hypertensive disorders of pregnancy (i.e., gestational hypertension and preeclampsia) were inconsistent. A single study evaluated effect measure modification by income for the association between oxides of nitrogen and gestational hypertension. Hao et al. (2023) observed an elevated positive association between first or second trimester NO_2 exposure and gestational hypertension for participants residing in zip codes with the level of poverty greater than 12%. Wu et al. (2016) observed no clear evidence of effect measure modification by household income for the association between oxides of nitrogen and preeclampsia. Weber et al. (2021) similarly observed no effect measure modification for mild or severe preeclampsia when stratified by poverty level or income level neighborhoods. However, Weber et al. (2021) did observe evidence of effect measure modification for superimposed preeclampsia with participants in low-income neighborhoods having a null association while an inverse association was reported for participants in high-income neighborhoods. A single study that evaluated effect measure modification by neighborhood household income for the association between oxides of nitrogen and pregnancy-related mental health (Sun et al., 2023) with no observed evidence of effect measure modification.

Education

The few studies that evaluated effect measure modification by maternal education for the association between oxides of nitrogen and pregnancy-related mental health (Sun et al., 2023), pregnancy loss and stillbirth (Sarovar et al., 2020), preeclampsia (Wu et al., 2016) (Pedersen et al., 2017b), and GDM (Pedersen et al., 2017c) (Wu et al., 2016) did not observe effect measure modification.

Race/ethnicity

Race and ethnicity are complex factors that are often closely related to other factors including particular genetics, diet, and SES. Therefore, race and ethnicity may influence NO_2-related health effects through both intrinsic and extrinsic mechanisms. Several studies evaluated effect measure modification by race/ethnicity for the association between oxides of nitrogen and GDM with inconsistent evidence. Hao et al. (2023) observed elevated positive associations between first trimester NO_2 exposure and GDM for Black, Asian, and Hispanic participants compared to the overall study population. Similarly, Sun et al. (2022) observed entire pregnancy NO_2 was associated with an increase in odds of GDM among Hispanic mothers and African American mothers compared to the overall study population. In contrast, Zhu et al. (2024) observed some evidence of modification by race/ethnicity, although no clear overall pattern, while Wu et al. (2016) reported no clear evidence of modification for race/ethnicity.

Few studies evaluated effect measure modification by race/ethnicity for the association between oxides of nitrogen and hypertensive disorders of pregnancy (e.g., hypertension and preeclampsia). For overall hypertensive disorders of pregnancy, Goin et al. (2021) reported that when stratifying by race/ethnicity, NO_2-associated results for the hypothetical intervention of reducing the pollutant concentrations to the 25th percentile versus the observed concentrations were stronger for both non-Latina Black and non-Latina Asian mothers than non-Latina white, Latina, or other mothers when using the

composite outcome that included all mothers who were classified as experiencing either preeclampsia and/or gestational hypertension. For preeclampsia only, when stratifying by race/ethnicity, the main effects only persisted in Black women (Goin et al., 2021). In contrast, Wu et al. (2016) observed no clear evidence of modification by maternal race/ethnicity for preeclampsia. Meanwhile, Hao et al. (2023) observed elevated positive associations between first trimester NO_2 exposure and gestational hypertension for Hispanic participants.

Sun et al. (2023) reported no effect measure modification by race/ethnicity for the association between oxides of nitrogen and pregnancy-related mental health. Within a study of a majority (79.4%) of Hispanic participants, Bastain et al. (2021) reported positive associations between second trimester and entire pregnancy exposure to NO_2 and depression 12 months postpartum. The few studies that evaluated effect measure modification by race/ethnicity for the association between oxides of nitrogen and pregnancy loss and stillbirth (Sarovar et al., 2020; Leiser et al., 2019) did not observe effect measure modification.

7.5.9.3 Behavioral Factors

Behavioral and other factors, such as physical activity and smoking, may modify the association between exposure to oxides of nitrogen and pregnancy outcomes. In the 2016 Oxides of Nitrogen ISA, there were no studies available that evaluated the potential for behavioral and other factors to modify associations between exposure to oxides of nitrogen and pregnancy outcomes. Among the recent epidemiologic evidence, physical activity was evaluated as a potential modifier for the associations between oxides of nitrogen and GDM (Pedersen et al., 2017b) or preeclampsia (Pedersen et al., 2017c). However, no evidence of modification was observed.

Smoking is a well-documented risk factor for many diseases, but it is unclear whether smoking exacerbates health effects associated with air pollutant exposures, including oxides of nitrogen. Information on oxides of nitrogen exposure or internal dose differences between smokers and nonsmokers is also lacking. Among the recent epidemiologic evidence, smoking was evaluated as a potential modifier for the associations between oxides of nitrogen and preeclampsia (Pedersen et al., 2017b) or placental growth and function (Michikawa et al., 2017). However, no evidence of modification was observed.

Overall, the collective evidence is inadequate to determine whether behaviors factors result in modification of the associations between exposure to oxides of nitrogen and pregnancy outcomes.

Table 7-3 Summary of evidence for lifestages and population differences effects on pregnancy outcomes from oxides of nitrogen exposure

	Key Evidence		Key References
Inadequate evidence			
Maternal Preexisting comorbidities	GDM: Limited and inconsistent evidence	BMI	†Niu et al. (2023); †Pedersen et al. (2017c); †Zhu et al. (2024); †Sun et al. (2022); †Robledo et al. (2015); †Wu et al. (2016)
		Maternal depression	†Niu et al. (2023)
	Hypertensive disorders of pregnancy: Limited evidence of EMM	Maternal depression	†Niu et al. (2024)
	Pregnancy loss and still birth: Limited evidence of EMM	T1D	†Williams et al. (2021)
Sociodemographic factors	GDM: Limited evidence with no observed EMM	Maternal age	†Niu et al. (2023); †Zhu et al. (2024); †Hao et al. (2023)
		Maternal education	†Pedersen et al. (2017c); †Wu et al. (2016)
		Income	†Wu et al. (2016)
		Road traffic noise	†Pedersen et al. (2017c)
	Limited and inconsistent evidence	Maternal race/ethnicity	†Hao et al. (2023); †Sun et al. (2022); †Zhu et al. (2024); †Wu et al. (2016)
	Hypertensive disorders of pregnancy: Limited evidence with no observed EMM	Maternal age	†Jiang et al. (2023)
		Fetal sex	†Jiang et al. (2023)
	Limited evidence of EMM	Maternal race/ethnicity	†Goin et al. (2021)

	Key Evidence		Key References
	Gestational hypertension: Limited evidence of EMM	Income, Maternal race/ethnicity	†Hao et al. (2023)
	Preeclampsia:		
	Limited evidence with no observed EMM	Maternal education	†Wu et al. (2016); †Pedersen et al. (2017b)
		Urbanicity	†Pedersen et al. (2017b)
	Limited and inconsistent evidence	Income	†Wu et al. (2016); †Weber et al. (2021)
		Maternal race/ethnicity	†Hao et al. (2023) †Wu et al. (2016)
	Limited evidence of EMM	Road traffic noise	†Pedersen et al. (2017b)
	Placental growth and function: Limited evidence with no observed EMM	Maternal age	†Michikawa et al. (2017)
	Pregnancy-related mental health: Limited evidence with no observed EMM	Maternal age, Maternal education, Income	†Sun et al. (2023)
	Limited and inconsistent evidence	Maternal race/ethnicity	†Sun et al. (2023); †Bastain et al. (2021)
	Pregnancy loss and stillbirth: Limited evidence with no observed EMM	Maternal age, Maternal education, Fetal sex, Maternal race/ethnicity	†Sarovar et al. (2020); †Leiser et al. (2019)
Behavioral factors	GDM: Limited evidence with no observed EMM	Physical activity	†Pedersen et al. (2017b)
	Preeclampsia: Limited evidence with no observed EMM	Physical activity	†Pedersen et al. (2017c)
		Smoking	†Pedersen et al. (2017b)

	Key Evidence		Key References
	Placental growth and function: Limited evidence with no observed EMM	Smoking	†Michikawa et al. (2017)

List of abbreviations in alphabetical order: EMM = effect measure modification; GDM = gestational diabetes mellitus; T1D = Type 1 Diabetes.
†Studies published since the 2016 Oxides of Nitrogen ISA.

7.5.10 Summary and Causality Determination

The 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016) concluded that there was insufficient evidence to infer a causal relationship between oxides of nitrogen and effects on pregnancy outcomes. Epidemiologic studies investigated whether exposure to oxides of nitrogen was associated with systemic inflammation during pregnancy, gestational hypertension, pre-eclampsia, gestational diabetes, and placental growth and function. Few toxicological studies were available, and the outcomes investigated in these studies were limited to gestation duration, maternal weight, and fetal death.

Epidemiologic studies evaluating NO_2 exposure and pregnancy outcomes in the 2016 Oxides of Nitrogen ISA tended to be inconsistent. Associations between NO_2 exposure and C-Reactive Protein (CRP, a marker of systemic inflammation) were generally null, although some relationships between NO_2 and fetal CRP levels were observed in one study. Relationships between NO_2 exposure and changes in blood pressure during pregnancy were also inconsistent, with some studies reporting positive associations with blood pressure and hypertensive disorders during pregnancy, while others reported negative or null associations. Positive associations between exposure to oxides of nitrogen and preeclampsia were reported in some epidemiological studies, but nearly as many studies (of similar quality and design) also reported null associations. Although some studies reported associations between NO_2 exposure and gestational diabetes and markers of placental function and growth, the evidence base was extremely limited. Toxicological studies that investigated the effects of exposure to NO_2 were inconsistent. No effects were observed on gestation duration or maternal weight, and some, but not all, studies reported decreased litter size (an indicator of fetal death).

Overall, among the recent evidence, the epidemiologic evidence assessing the impacts of exposure to oxides of nitrogen on pregnancy outcomes is inconsistent, and for some specific outcomes, the evidence is limited. However, the strongest, most consistent evidence comes from epidemiologic studies reporting a generally consistent pattern of positive associations between exposure to NO_2 or NO_X and GDM within the United States. This pattern holds across different U.S. geographies and populations as well as study design and analysis decisions. Additionally, several studies evaluating GDM reported the strongest positive associations during the preconception or first trimester exposure windows with sensitive exposure windows identified between five to twelve weeks before conception as well as zero to nine weeks after conception. The epidemiologic evidence assessing the impacts of exposure to oxides of nitrogen and GMD was more inconsistent in studies conducted outside of the United States, with positive-

to-null associations. However, there were differences in outcome ascertainment definitions and exposure modeling, which may help explain the heterogeneity across studies. Additionally, there is no animal toxicological evidence to provide coherence.

The recent evidence for the other pregnancy outcomes evaluated was either more inconsistent than what was reported for GDM or the evidence was limited in quantity making it challenging to draw too much inference from those few studies. Recent studies evaluating short-term exposure to NO_2 and associations with placental abruption observed positive associations, although the evidence base remains small and there is inconsistency regarding the lags for which associations were reported. While coherence is limited, a single recent toxicological study reported reduced markers of placental health in NO_2-exposed dams. There was no corresponding recent toxicological evidence for pregnancy-related mental health or hypertensive disorders of pregnancy. There was a small body of recent epidemiologic studies across pregnancy-related mental health outcomes. The small number of studies limits the ability to judge consistency across studies, although the associations reported demonstrate that exposure to oxides of nitrogen could result in physiological responses that contribute to effects on pregnancy-related mental health. The recent literature continues to provide positive-to-null associations between oxides of nitrogen and pregnancy loss and/or stillbirth. It remains difficult to compare across studies given differences in exposure window assignments and outcome ascertainment. Studies that select from general population emergency department visits to identify cases of spontaneous abortion are likely missing many cases. Several recent animal toxicological studies reported inconsistent effects on litter size, an indicator of fetal death. The recent studies evaluating associations between NO_2 and NO_X exposures and preeclampsia were inconsistent, with reported associations ranging from negative to positive. There does not appear to be a consistent pattern across study geography, analytic exclusion criteria, or adjustment for pregnant persons with pre-pregnancy hypertension or chronic hypertension, or analytic creation of mutually exclusive preeclampsia outcomes based on severity.

Overall, the evidence is *suggestive of, but not sufficient to infer, a causal relationship* between oxides of nitrogen exposure and pregnancy outcomes. The strongest evidence comes from epidemiologic studies within the United States reporting a generally consistent pattern of positive associations between exposure to NO_2 or NO_X and GDM, especially within the preconception and first trimester exposure windows (see Table 7-4). Among the epidemiologic studies, there remains uncertainty regarding the concentration-response relationship, potential differences across lifestages and populations, and the potential for copollutant confounding. Furthermore, there are limited animal toxicological studies available, and when available, for outcomes that have limited epidemiologic evidence. Animal toxicological evidence assessing the impacts of oxides of nitrogen exposure on pregnancy outcomes is limited to studies of NO_2. A single recent study reported reduced markers of placental health in NO_2-exposed rats and several recent studies reported inconsistent effects of NO_2 exposure on litter size in rats and mice. Additionally, few recent studies reported no effects on maternal weight gain or the sex ratio of offspring born to NO_2-exposed dams. Thus, the evidence is limited overall to judge consistency and coherence across the lines of evidence.

Table 7-4 Summary of evidence contributing to causality determinations for oxides of nitrogen exposure and effects on pregnancy outcomes

Rationale for Causality Determination[a]	Key Evidence[b]	Key References	Oxides of Nitrogen Concentrations Associated with Effects
Gestational Diabetes Mellitus			
Evidence from epidemiologic studies is generally supportive but not consistent for GDM	Generally consistent positive associations within the U.S. studies.	†Robledo et al. (2015) †Sun et al. (2022) †Niu et al. (2023) †Hao et al. (2023) †Zhu et al. (2024) Section 7.5.1.1	Mean NO_2: 10.16 – 16.69 ppb for entire pregnancy Mean NO_X: 25.18 ppb for first trimester
	Potential critical window of exposure: preconception or first trimester	†Robledo et al. (2015) †Niu et al. (2023) †Hao et al. (2023) †Zhu et al. (2024)	Mean NO_2: 10.9 ppb for first trimester Median NO_2: 25.18 ppb for first trimester Mean NO_X: 25.18 ppb for first trimester
Hypertensive Disorders of Pregnancy			
Inconsistent epidemiologic evidence for hypertensive disorders of pregnancy	Inconsistent associations for hypertensive disorders of pregnancy combined (null-to-positive), only gestational hypertension (null-to-positive), or only preeclampsia (negative-to null-to-positive).	Section 7.5.2.1	Mean NO_2: 10.16 – 37.8 ppb for entire pregnancy Median NO_2: 10.8 – 17.4 ppb for entire pregnancy Mean NO_X: 29.8 ppb for entire pregnancy
Placental Growth and Function			
Limited epidemiologic evidence for placental growth and function	Positive associations for placental vascular resistance and placental abruption, although for different lag days	†Michikawa et al. (2017) †Ananth et al. (2018) Section 7.5.3.1	Mean NO_2: 9.9 – 21.5 ppb for entire pregnancy
Limited toxicological evidence of effects on placental growth and function	Increased placental fibrosis, increased placental calcification, and decreased placental telomere length.	†Zhu et al. (2022) Section 7.5.3.2	2.5 ppm NO_2
Pregnancy-related Mental Health			
Limited and inconsistent epidemiologic evidence for pregnancy-related mental health	Some studies reported positive associations with pregnancy-related mental health, while other studies reported no associations or negative associations.	†Bastain et al. (2021) †Sun et al. (2023) Section 7.5.4.1	Mean NO_2: 15.86 – 17 ppb for entire pregnancy

Rationale for Causality Determination[a]	Key Evidence[b]	Key References	Oxides of Nitrogen Concentrations Associated with Effects
Pregnancy Loss and Stillbirth			
Limited and inconsistent epidemiologic evidence for pregnancy loss and stillbirth	Studies reported positive associations or no associations between NO_2 and pregnancy loss and stillbirth	†Sarovar et al. (2020) †Leiser et al. (2019)	Mean NO_2: 18 ppb for 7-d avg Mean NO_2: 36.8 ppb for entire pregnancy
	Single study reported negative-to-no association between NO_X and pregnancy loss and stillbirth	†Williams et al. (2021)	Mean NO_X: 29.8 ppb for entire pregnancy
Inconsistent toxicological evidence for pregnancy loss and stillbirth	Mixed evidence for effects on litter size in rats and mice. Uncertainties regarding dose-response, dosing duration, and exposure timing.	Di Giovanni et al. (1994) Shalamberidze and Tsereteli (1971) †Yue et al. (2017) †Yan et al. (2020) †Yue et al. (2018) †Li et al. (2022) Section 7.5.5.2	0.067–3 ppm NO_2

List of abbreviations in alphabetical order: d = day, GD = gestational day, hr = hour, NO_2 = nitrogen dioxide, NO_X = oxides of nitrogen , ppb = parts per billion, ppm = parts per million.
[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.

7.6 Effects on Birth Outcomes

In the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016), there was generally consistent evidence for an association between exposure to NO_2 and fetal growth restriction, including evidence from studies that used fetal anthropometric measurements made via ultrasound and anthropometric measurements made immediately after birth (Ritz et al., 2014; Pedersen et al., 2013; Iñiguez et al., 2012; Estarlich et al., 2011; Aguilera et al., 2010; Ballester et al., 2010). These included studies using validated exposure models to estimate residential NO_2 exposures for individual subjects. Results of these studies were consistent with the results of studies of the clinical measurement of IUGR and the statistical definition of SGA. The evidence was less certain regarding the time period of pregnancy when exposure to NO_2 is associated with the highest risks. Some studies reported the highest risks associated with NO_2 exposures in early pregnancy, while in other studies the exposure period associated with the greatest risk was toward the end of pregnancy. Others reported the greatest risk when exposure was assigned for the entire pregnancy period. A major uncertainty was whether NO_2 exposure has an independent effect on fetal growth restriction because epidemiologic studies did not examine the potential for confounding by traffic-

related copollutants and toxicological investigation of these outcomes following NO_2 exposure was lacking. Among the other birth outcomes, preterm birth, birth weight, birth defects, and early life mortality, there was limited evidence and inconsistent associations overall.

7.6.1 Prenatal Growth

Prenatal growth incorporates multiple apical endpoints related to size at birth. Apical endpoints include, but are not limited to, birth weight, head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC), femur length (FL), SGA, and fetal growth restriction (FGR). The critical windows of exposure for these apical outcomes are not well established and it is often seen in the literature to have varying prenatal exposure periods analyzed. This section discusses epidemiologic studies (see Section 7.6.1.1) and animal toxicological studies (see Section 7.6.1.2) examining the relationship between oxides of nitrogen exposure and prenatal growth.

7.6.1.1 Epidemiologic Studies of Prenatal Growth

In the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016), there were a number of studies that evaluated the association between exposure to NO_2 and birth weight, and the associations were generally inconsistent. When birth weight was examined as a continuous variable, several studies reported decreases in birth weight associated with increases in NO_2 exposure. Generally, these studies reported the largest decreases in birth weight when exposure to NO_2 was averaged over the entire pregnancy. However, there were also a number of studies that examined birth weight as a continuous variable that reported no consistent decreases in birth weight associated with increases in NO_2 exposure averaged over the entire pregnancy or specific trimesters of pregnancy. Several recent studies evaluated associations between NO_2 exposures and birthweight. Evidence from recent studies is summarized below, followed by additional discussion of the lifestages and populations evaluated in these studies (see Section 7.6.1.1.2) and the potential impacts of copollutants (see Section 7.6.1.1.3). Section 7.6.1.1.4 discusses conclusions from these studies and summarizes remaining uncertainties. Study specific details including study population characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), potential confounders, and select results are in Appendix A – Appendix Table 7-10.

Birth Weight

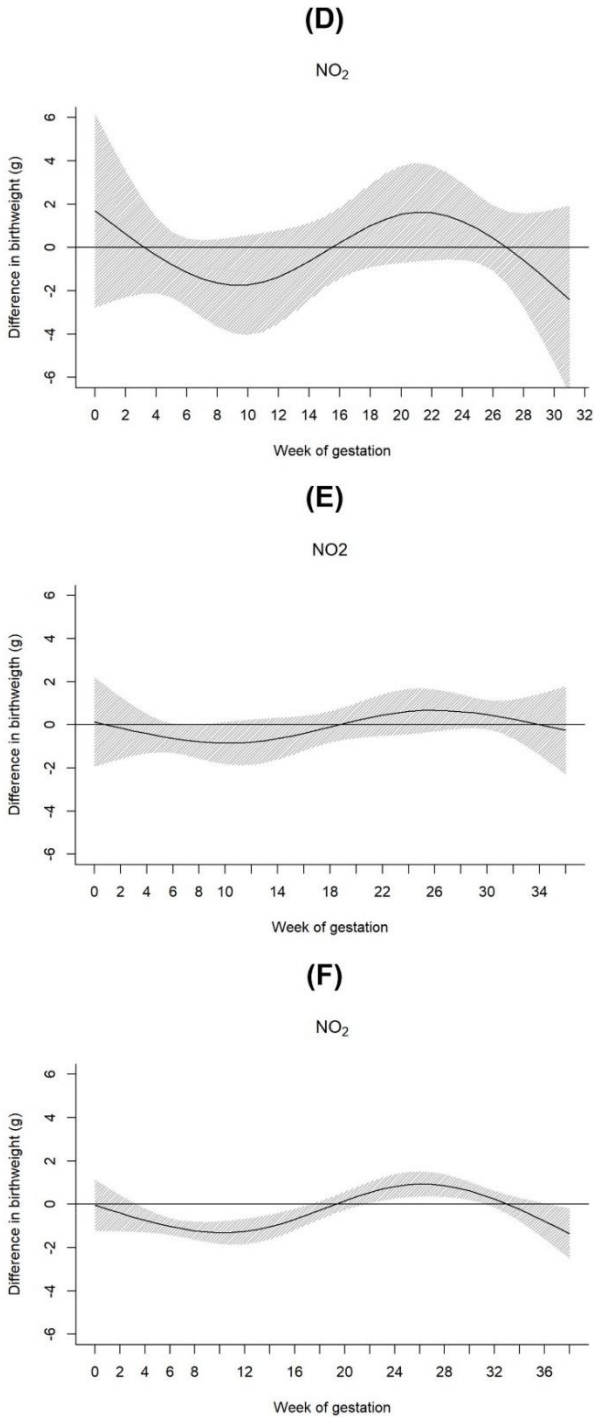
Among the recent studies that evaluated the associations between oxides of nitrogen and birth weight, most studies reported decreased birth weight with exposure to NO_2 (Bravo et al., 2024; Dong et al., 2022; Johnson et al., 2022; Peterson et al., 2022; Villeneuve et al., 2022; Guo et al., 2020; Savitz et al., 2019; Hjortebjerg et al., 2016; Li et al., 2016a; Stieb et al., 2016; Clemente et al., 2015), while a few studies reported positive, but imprecise, associations (Hao et al., 2023; Ouidir et al., 2021a; Mendoza-Ramirez et al., 2018). In addition, there were a few recent studies that evaluated associations between

NO_X and birth weight (Mendoza-Ramirez et al., 2018; Hjortebjerg et al., 2016; Li et al., 2016a), with inconsistent associations overall. There was also a single study that reported a reduction in birth weight associated with gestational exposure to NO (Mendoza-Ramirez et al., 2018).

In addition, a single study evaluated the associations between prenatal exposure to NO_2 and attained infant weight gain (Ji et al., 2023). In a study amongst participants in the MADRES cohort, Ji et al. (2023) estimated exposure to NO_2 by using inverse distance squared weighted spatial interpolation based on AQS data and examined associations with infant growth. When comparing the 10^{th} percentile of pregnancy-averaged NO_2 exposure to the 90th percentile, there was a 10.94% (95% CI: -16.44, -5.07) decrease in infant attained weight at the third trimester and a 6.45% (95% CI: -10.47, -2.24) decrease at birth in infant attained weight.

There were a variety of different exposure assessment metrics used to estimate NO_2, NO_X, and NO, and associations were evaluated across different geographies. Of note, and detailed in bullets below, several studies used distributed lag models or alternative methods for confounder control (causal inference framework) to identify critical windows of exposure and/or reduce bias, (Bravo et al., 2024; Hao et al., 2023; Dong et al., 2022; Johnson et al., 2022; Peterson et al., 2022; Ouidir et al., 2021a; Li et al., 2016a). However, even with these statistical approaches, there was a general pattern of decreased birth weight associated with exposure to oxides of nitrogen, but overall, the associations were inconsistent.

- Peterson et al. (2022) used distributed lag models to explore associations between NO_2 exposures and estimated fetal weight among participants in the Maternal and Development Risks from Environmental and Social Stressors (MADRES) cohort. The study examined NO_2 exposures from 12 weeks preconception to 32 weeks of gestation. In the linear regression model, there was a negative, but imprecise, association between NO_2 and estimated fetal weight (β: -35.60 g [95% CI: -98.70, 27.50] per 5.6 ppb NO_2), and there were no associations for any gestational week exposure to NO_2 and estimated fetal weight in the distributed lag models.
- Bravo et al. (2024) used distributed lag nonlinear models to evaluate associations between weekly gestational exposure to NO_2 and birth weight in all singleton, live births in the Lower Peninsula of Michigan. There was a negative cumulative difference in birth weight (β: -11.69 g [95% CI: -14.46, -8.92]) among full term births (births with gestational age between 39 to 42 weeks). While there were also negative cumulative differences in birth weight for preterm births (gestation age between 32 to 36 weeks) and early term births (gestational age between 37 to 38 weeks), the CIs included the null. Among full term births, the largest decrements in birth weight were associated with NO_2 exposure between weeks six to 18 of pregnancy (see Figure 7-17).



List of abbreviations in alphabetical order: g = grams; NO_2 = nitrogen dioxide.
Note: D displays preterm births; E displays early term births; and F displays full term births.
Source (adapted from): Bravo et al. (2024). Copyright permission pending.

Figure 7-17 Difference in birth weight (in grams) associated with a 10 ppb increase in NO_2 exposure for preterm births, early term births, and full-term births.

- Li et al. (2016a) used two-stage Bayesian hierarchical non-linear models to examine the associations between NO_2 and NO_X, estimated from spatiotemporal models, and birth weight

in term births (≥37 weeks gestation) in Los Angeles County, California, U.S. This statistical approach takes into account non-linear associations and spatial effects and can combine a priori knowledge with new data to minimize potential overfitting. Overall, lower term birth weight was associated with NO_2 (average posterior effect: -14.7 g [95% CI: -19.8, -9.7] per 10 ppb increase in NO_2) and NO_X (average posterior effect: -6.9 g [95% CI: -12.9, -0.9] per 10 ppb in NO_X). Furthermore, when considering the influence of spatial factors, there were attenuating influences for distance from freeways, mean normalized difference vegetation index (NDVI) of the census tract, and median household income. There were aggravating influences (factors that strengthened the associations) for populations driving to work, heavy industry land-use, and electricity facility land-use (see Figure 7-18).

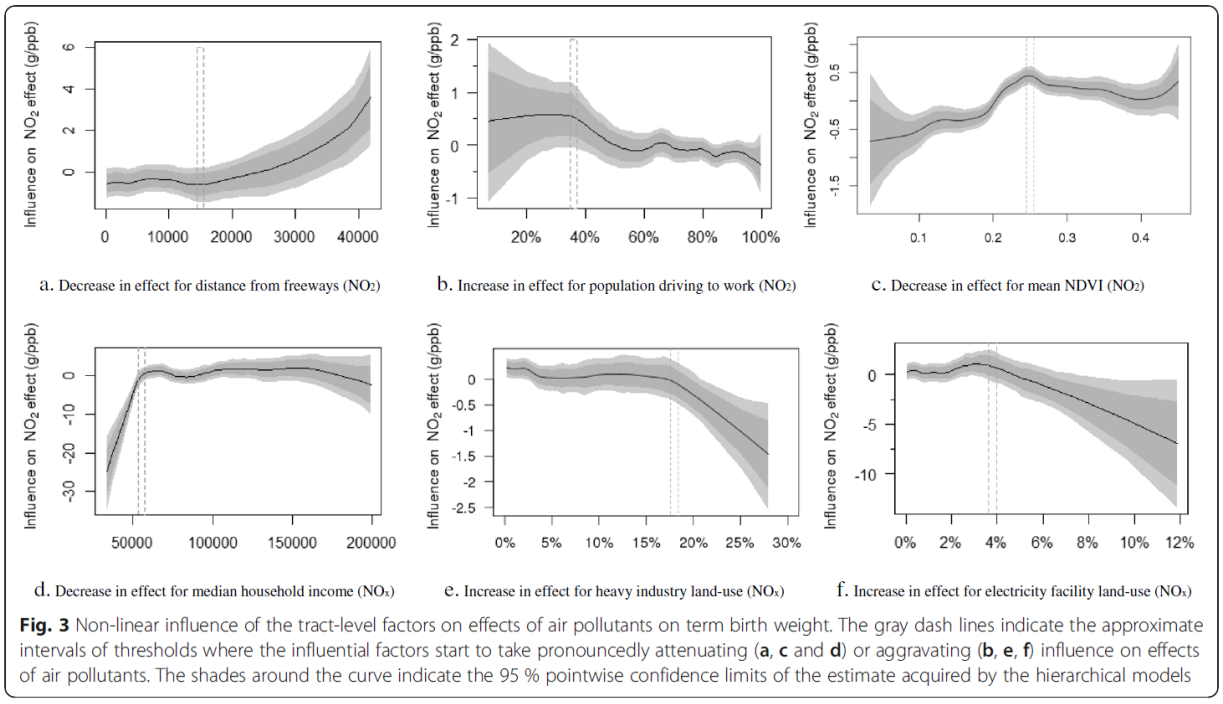


Fig. 3 Non-linear influence of the tract-level factors on effects of air pollutants on term birth weight. The gray dash lines indicate the approximate intervals of thresholds where the influential factors start to take pronouncedly attenuating (**a**, **c** and **d**) or aggravating (**b**, **e**, **f**) influence on effects of air pollutants. The shades around the curve indicate the 95 % pointwise confidence limits of the estimate acquired by the hierarchical models

List of abbreviations in alphabetical order: NO_2 = nitrogen dioxide; ppb = parts per billion.
Source: Li et al. (2016a). Copyright permission pending.

Figure 7-18 Non-linear influence of the tract-level factors on effects of air pollutants on term birth weight.

- Ouidir et al. (2021a) used inverse probability scores and doubly robust methods in generalized additive models accounting for spatial autocorrelation to evaluate the associations of NO_2, estimated from a spatiotemporal exposure model, and term birth weight. There were null associations between term birth weight and exposure to NO_2 across any of the exposure windows (entire pregnancy, first trimester, second trimester, third trimester, 60 days before delivery, or 90 days before delivery) from the weighted generalized additive model. With using inverse probability scores with the doubly robust method, the associations remained null.
- Johnson et al. (2022) investigated time-varying vulnerability of birth weight to NO_2 exposure, estimated from an LUR model, using flexible time intervals among participants of the Canadian Maternal-Infant Research on Environmental Chemicals (MIREC) cohort. A two-stage Bayesian Metropolis-Hastings (M-H) algorithm coupled with an empirical density

threshold was used to identify critical time windows without relying on predetermined time intervals (e.g., trimesters) or distributional assumptions (e.g., distributed lag nonlinear model time lags). Exposure to NO_2 during mid-pregnancy (days 55 to 145) was associated with a 3 g (95% credible interval: -5, -1) reduction in birth weight per 1 ppb increase in NO_2.

- Dong et al. (2022) used stabilized inverse probability weights based on a propensity score approach to estimate the marginal effects of maternal exposure to NO_2 on birth weight. NO_2 exposures were predicted by high-resolution spatial-temporal models. The marginal structural models regressed NO_2 exposure with corresponding stabilized inverse-probability weights calculated from GPS models using gradient boosting with the parameter set determined by the grid search algorithm, which incorporates high-level interactions and nonlinearities. The new algorithm was developed by the authors to replace the usual goal of machine learning algorithms (minimizing mean squared error) with minimizing the correlation between covariates and exposure. For NO_2 exposure during days 0 to 30, there was an 8.2 g (95% CI: -10.4, -6.1) decrease in birth weight per 12.8 ppb increase in NO_2 and a 14.0 g (95% CI: -16.1, -11.8) decrease in birth weight per 10.1 ppb increase in NO_2 for days 31 to 280.
- Hao et al. (2023) examined associations between exposure to NO_2 and birth outcomes for each of the following exposure windows: first four weeks of pregnancy, first six weeks of pregnancy, first trimester, second trimester, third trimester, and total pregnancy. Except for total pregnancy, for which there was a decreased mean difference in birth, there was a consistent pattern of increased mean difference in birth weight (range of 0.994 g to 3.371 g).

In addition to birth weight, several recent studies examined the associations between NO_2 exposure and term low birth weight as a binary indicator, rather than a continuous variable. Overall, the associations were inconsistent across studies, with most studies reporting null associations (Guo et al., 2020; Stieb et al., 2016; Wu et al., 2016), but a few studies reported associations with increased risk (Dong et al., 2022; Fu et al., 2022; Villeneuve et al., 2022), while a single study reported decreased risk (Lavigne et al., 2016b). Additionally, a single study evaluated the associations between exposure to NO_X and term low birth weight, reporting a positive, but imprecise, association (OR: 1.003 [95% CI: 0.991, 1.015]) (Wu et al., 2016). These recent studies were mostly conducted in North America, but there was a single study from China and a single study from the United Kingdom. Despite the geographical differences, the median/mean NO_2 concentrations were similar (see Appendix Table 7-10). Among these studies, exposure to NO_2 was estimated from IDW (Guo et al., 2020), LUR (Villeneuve et al., 2022; Lavigne et al., 2016b; Stieb et al., 2016), ensemble machine learning model (Dong et al., 2022), and interpolated by empirical Bayesian kriging (Wu et al., 2016).

- A recent study utilized an alternative method for confounder control (causal inference framework) to investigate the effects of maternal exposure to NO_2 on birth weight using stabilized inverse probability weights based on a propensity score approach that incorporates high-level interactions and nonlinearities. Dong et al. (2022) developed a new algorithm to replace the usual goal of machine learning algorithms (minimizing mean squared error) with minimizing the correlation between covariates and exposure. For NO_2 exposure during days 0 to 30 and 31 to 280 prior to the delivery date, there was increased odds of low birth weight, with OR of 1.047 (95% CI: 1.016, 1.079) per 12.8 ppb increase in NO_2 and 1.046 (95% CI: 1.015, 1.078) per 10.1 ppb increase in NO_2, respectively.
- A single study among participants in the UK Biobank cohort, assessed the association between gestational exposure to NO_2 and NO_X and low birth weight, defined as birth weight

below 2500 g regardless of gestational age (Fu et al., 2022). Exposure to NO_2 and NO_X was estimated from an LUR model developed from the European Study of Cohorts for Air Pollution Effects (ESCAPE) project. There were increased odds of low birth weight for both exposure to NO2 (OR: 1.01 [95% CI: 0.99, 1.04] per 10 μg/m^3 increase in NO_2) and NO_X (OR: 1.01 [95% CI: 0.99, 1.02] per10 μg/m^3 increase in NO_X), but the CIs included the null.

Other Prenatal Growth Indicators

In the 2016 Oxides of Nitrogen ISA, the strongest evidence of associations with anthropometric fetal measurements, such as HC, BPD, AC, and FL, was among a well-conducted Spanish cohort (Iñiguez et al., 2012; Estarlich et al., 2011; Aguilera et al., 2010; Ballester et al., 2010). In the recent epidemiologic evidence, anthropometric fetal measurements were evaluated in association with exposure to NO_2 (Leung et al., 2023; Peterson et al., 2022; Hjortebjerg et al., 2016). Overall, although the CIs included the null, exposure to NO_2 was associated with decreased HC and decreased AC, and associations with BPD and FL were inconsistent.

- Leung et al. (2023) fitted distributed lag models to estimate the time-varying association between ultrasound parameters of fetal growth and weekly NO_2 exposure, separately for each fetal growth parameter and time interval in which the parameter was measured, in an Eastern Massachusetts pregnancy cohort. Exposure to NO_2 was estimated from ensemble machine learning algorithms. NO_2 exposures were associated with decreased head parameters (BPD and HC) throughout pregnancy, decreased FL during mid- to late-pregnancy (gestational weeks 5 to 20), and decreased AC (see Appendix A – Appendix Table 7-10).
- In the Danish National Birth Cohort, Hjortebjerg et al. (2016) estimated exposure to NO_2 from a dispersion model and evaluated associations with several prenatal growth metrics. NO_2 exposure was associated with an increase in placental weight of 3.92 g (95% CI: 2.17, 5.68) per 10 μg/m^3 increase in NO_2, a decrease of 0.42 mm (95% CI: -0.61, -0.23) for HC, and a decrease of 0.43 mm (95% CI: -0.68, -0.17) in AC.
- In the Maternal And Developmental Risks from Environmental and Social Stressors (MADRES) cohort, Peterson et al. (2022) reported no associations between prenatal exposure to NO_2 and HC (β: -1.2 mm [95% CI: -4.0, 1.6] per 5.6 ppb NO_2), BPD (β: 0.001 [95% CI: -0.9, 0.9]), AC (β: -2.6 mm [95% CI: -6.3, 1.2]), or FL (β: 0.1 [95% CI: -0.6, 09]) in the linear models. In the distributed lag models, there were no critical windows of exposure identified for NO_2 exposure.

Small-for-gestational age

Several recent epidemiologic studies evaluated the associations between NO_2 and SGA. SGA is typically defined as falling below the 10th percentile for birth weight dependent on gestational age. The associations with SGA were inconsistent overall, with some studies reporting positive associations (Villeneuve et al., 2022; Savitz et al., 2019; Stieb et al., 2016), positive, but imprecise associations (Nobles et al., 2019a), and others reporting null associations (Guo et al., 2020), and a single study reporting inverse associations (Lavigne et al., 2016b). These studies were conducted among cohorts in the United States and Canada. Among these studies, exposure to NO_2 was estimated from IDW (Guo et al., 2020), LUR (Villeneuve et al., 2022; Lavigne et al., 2016b; Stieb et al., 2016), and a spatiotemporal model (Savitz et al., 2019) with similar distributions (see Appendix A – Appendix Table 7-10).

In addition, a few studies evaluated associations between NO_2 and NO_X and SGA, with null associations reported (Williams et al., 2021; Nobles et al., 2019a). FGR is the failure of a fetus to reach its full growth potential. Although FGR and SGA are used interchangeably, SGA includes constitutionally small but healthy infants and not all infants with FGR will meet criteria for SGA (Nobles et al., 2019a). When employing surrogate metrics for fetal growth like SGA or even low birth weight, infants may be incorrectly classified with FGR.

- Because of the potential for misclassification, Nobles et al. (2019a) assessed exposure to NO_2 and NO_X in relation to physician-diagnosed fetal growth restriction and population-based SGA in the Consecutive Pregnancy Study in Utah, U.S. NO_2 and NO_X were estimated from CMAQ, and multiple exposure windows were constructed: 3-month preconception period, entire pregnancy, and each trimester (first, second, third). FGR was positively associated with an IQR increase of NO_X during the 3-month preconception, second trimester, third trimester, and whole pregnancy, with the strongest association during the 3-month preconception period (RR: 1.23 [95% CI: 1.10, 1.38]). For an IQR increase in NO_2, fetal growth restriction was positively associated with exposure during each exposure window, with the strongest association during the 3-month preconception period (RR: 1.19 [95% CI: 1.08, 1.31]). However, positive associations between SGA and NO_X (RR: 1.08 [95% CI: 1.03, 1.14]) and NO_2 (RR: 1.05 [95% CI: 1.01, 1.10]) were reported only during the third trimester. When evaluating whether the association of NO_2 with FGR might differ by concurrent diagnosis with SGA, the strongest associations were FGR without SGA, across all exposure windows. Additionally, there were positive associations with FGR with SGA, with the strongest associations for entire pregnancy and third trimester. The magnitude of the associations of FGR without SGA were stronger than those for FGR with SGA.

7.6.1.1.2 Lifestages and Populations

Overall, there were limited studies that evaluated potential difference among lifestages and populations in the associations between exposure to NO_2 and/or NO_X and prenatal growth. Only maternal comorbidities were evaluated as potential modifiers in the recent epidemiologic literature. In general, there was no evidence of effect measure modification of maternal comorbidities on the associations between NO_2 and prenatal growth, specifically term low birth weight and SGA.

- Lavigne et al. (2016b) reported no evidence of effect measure modification by maternal comorbidities of asthma, hypertension, heart disease, diabetes, gestational diabetes, or pre-eclampsia for exposure to NO_2 and term low birth weight over the entire pregnancy or by trimester. All associations were null (see Appendix A – Appendix Table 7-10).
- Lavigne et al. (2016b) reported no evidence of effect measure modification by maternal comorbidities of asthma, hypertension, heart disease, diabetes, gestational diabetes, or pre-eclampsia for exposure to NO_2 and SGA over the entire pregnancy or by trimester. All associations were null (see Appendix A – Appendix Table 7-10).
- Williams et al. (2021) hypothesized that women with Type 1 diabetes and their infants would have greater risk of poor outcomes when exposed to air pollution compared to women without autoimmune disorders and their infants. Overall, there were null associations with exposure to NO_X among women with Type 1 diabetes compared to women without

autoimmune disorders for SGA across preconception exposure period, first trimester, second trimester, or entire pregnancy (see Appendix A – Appendix Table 7-10).

7.6.1.1.3 Copollutants

A few recent studies evaluated potential confounding by copollutants on the associations between exposure to NO_2 and prenatal growth, specifically birth weight. Overall, there is limited evidence and inconsistent associations.

- Guo et al. (2020) investigated the associations of exposure to air pollutants with term birth weight (continuous), risk of term low birth weight (binary indicator), and risk of SGA. In a single pollutant model, exposure to NO_2 was associated with a decrease in term birth weight (β: -2.71 g [95% CI: -5.53, 0.11]). When adjusted for SO_2 (ρ = 0.16), the association was unchanged; however, adjustment for CO (ρ = 0.25) attenuated the association towards the null (β: -0.84 g [95% CI: -3.73, 2.05]), while adjustment for O_3 (ρ = -0.64) further strengthened the reduction in birth weight (β: -4.39 g [95% CI: -7.52, -1.26]). Adjustment for $PM_{2.5}$ (ρ = 0.53) resulted in a null association (β: 0.45 [95% CI: -2.68, 3.58]) and adjustment for PM_{10} (ρ = 0.59), there was a positive, but imprecise, association (β: 2.01 g [95% CI: -1.24, 5.26]). For term low birth weight (binary indicator) and SGA, adjusting for SO_2, CO, O3, $PM_{2.5}$, and PM_{10} did not change the null association in the single pollutant model (see Appendix A – Appendix Table 7-10).
- Bravo et al. (2024) estimated the difference in birth weight among preterm, early term, and full term births associated with exposure to NO_2 throughout gestation. Among preterm births and early term births, there was an attenuation of association towards null when co-adjusted for $PM_{2.5}$ (ρ^2 = 0.368), but the reduction in birth weight remained overall (β: -2.46 g [95% CI: -9.78, 9.28] and β: -2.10 g [95% CI: -7.10, 2.90], respectively). However, among full term births, the reduction in birth weight was slightly strengthened when co-adjusted for $PM_{2.5}$ (β: -11.80 [95% CI: -14.66, -8.94]).
- Hjortebjerg et al. (2016) investigated the associations between residential exposure to NO_2 and traffic noise (ρ = 0.47) during pregnancy on markers of prenatal growth, birth weight, head circumference, and abdominal circumference. Adjustment for traffic noise resulted in strengthened associations for birth weight and abdominal circumference, but attenuation towards the null for the association for head circumference (see Appendix A – Appendix Table 7-10).

7.6.1.1.4 Conclusions and Remaining Uncertainties

Overall, the associations between exposure to NO_2 and prenatal growth were inconsistent across the different apical endpoints in the epidemiologic studies. Among the recent evidence, birth weight was the most common metric of prenatal growth evaluated. While there was a general pattern of decreased birth weight, there were inconsistent associations across other prenatal growth indicators including SGA. While the evidence is limited, there was also a consistent pattern of decreased head circumference and abdominal circumference, but more studies are needed to judge consistency and coherence of this pattern of association. Additional uncertainties result from the lack of information on the shapes of concentration-

response relationships and confidence in those relationships over the distribution of exposures, the potential modifying impact of maternal comorbidities, and the limited information available on potential confounding by copollutants, particularly those most closely associated with traffic emissions. These uncertainties limit the ability to judge coherence and consistency across studies of prenatal growth.

7.6.1.2 Animal Toxicological Studies of Prenatal Growth

No animal toxicological studies that investigated the effects of oxides of nitrogen exposure on prenatal growth were reported in the 2016 Oxides of Nitrogen ISA or 2008 Oxides of Nitrogen ISA. A single study in the 1993 Oxides of Nitrogen AQCD reported on the effects of oxides of nitrogen exposure on prenatal growth in animals. Shalamberidze and Tsereteli (1971) exposed female albino rats to 0.126 and 2.36 mg/m^3 (0.067 and 1.25 ppm) NO_2 for 12 hours/day for three months and mated them with untreated male controls when exposure was complete. Birth weight was significantly reduced in the 1.25 ppm group, but no effects on birth weight were observed in the 0.067 ppm group.

Only one recent animal toxicological study that investigated the effects of NO_2 exposure on prenatal growth was available. Immediately following mating with untreated males, Yan et al. (2020) exposed C57BL/6 female mice to 2.5 ppm NO_2 for five hours/day throughout gestation (GD 0 to PND 0) and reported that, similar to Shalamberidze and Tsereteli (1971), offspring body weight on PND 1 was significantly reduced in both male and female offspring. Study-specific details, exposure conditions, and endpoints examined are highlighted in Appendix A – Appendix Table 7-2.

7.6.1.2.1 Conclusions and Remaining Uncertainties

There is limited animal toxicological evidence examining the effects of oxides of nitrogen exposure on prenatal growth. One previous study in rats and a recent study in mice both reported reductions in birth weight, suggesting this may be a potentially sensitive outcome for exposure to oxides of nitrogen, but future studies are still needed to confirm and corroborate these findings. Additional studies to investigate a potential dose-response relationship and a potential mechanism of action could also address existing gaps in knowledge.

7.6.2 Preterm Birth

The recent epidemiologic and toxicological studies that examined the relationship between exposure to oxides of nitrogen and preterm birth are summarized in Sections 7.6.2.1 and 7.6.2.2, respectively. Study details of the recent epidemiologic studies are included in Appendix A – Appendix Table 7-11 and the recent toxicological studies are in Appendix A – Appendix Table 7-2.

7.6.2.1 Epidemiologic Studies of Preterm Birth

Preterm birth (PTB) is defined as delivery at less than 37 weeks of completed gestation. In the 2016 Oxides of Nitrogen ISA, the associations between exposure to NO_2 and PTB were inconsistent overall. The inconsistent associations may be due to the different exposure assessment metrics (LUR, central site monitor, IDW) considered, study designs, and windows of exposure (entire pregnancy, third trimester last eight weeks of pregnancy, last six weeks of pregnancy, month of birth, and last week of pregnancy). In addition to PTB, very preterm birth (VPTB, <30 weeks gestation) was evaluated. While a single study reported a positive association with NO_2 and VPTB when none were observed for PTB, a few studies reported stronger associations for NO_2 and NO_X with VPTB compared to those for PTB.

Among the recent epidemiologic studies, there was an inconsistent pattern of associations between NO_2 and PTB overall. Several studies reported positive associations between NO_2 and PTB (Xiao et al., 2023; Genin et al., 2022; Weber et al., 2019; Estarlich et al., 2016; Hao et al., 2016; Laurent et al., 2016; Lavigne et al., 2016b; Li et al., 2016b; Wu et al., 2016), but there were several other studies that reported null associations (Hao et al., 2023; Escoto et al., 2022; Riddell et al., 2022; Cassidy-Bushrow et al., 2020; Garshol et al., 2020; Giorgis-Allemand et al., 2017), as well as inverse associations (Cowan et al., 2024; Krajewski et al., 2023; Villeneuve et al., 2022; Savitz et al., 2019; Stieb et al., 2019; Johnson et al., 2016; Stieb et al., 2016; Wu et al., 2016). There were a variety of different exposure assessment metrics used to estimate NO_2 (CMAQ, LUR, central site monitors, empirical Bayesian kriging, spatiotemporal/geospatial model, hybrid model), across different geographies (United States, Canada, Europe), and different exposure windows (hourly, trimester, entire pregnancy).

In addition to NO_2, there were a few recent studies that evaluated associations between exposure to NO_X and PTB (Williams et al., 2021; Giorgis-Allemand et al., 2017; Laurent et al., 2016; Mendola et al., 2016; Wu et al., 2016). Overall, the associations are inconsistent, with a few studies reporting a positive association between exposure to NO_X and PTB (Laurent et al., 2016; Mendola et al., 2016) and a few studies reporting null associations (Williams et al., 2021; Giorgis-Allemand et al., 2017). These studies were conducted among large cohorts, with a variety of exposure assessment metrics (CMAQ, dispersion model, and LUR), and exposure windows (entire pregnancy, trimester, weekly).

The critical window of exposure(s) for PTB in humans remains unknown. Because of this, there is a variation in the exposure windows considered in analyses for PTB. While trimester-specific or entire pregnancy are common exposure windows evaluated for PTB, shorter-term exposures, such as hourly and weekly, have additionally been evaluated in recent studies. Of the recent studies that reported positive associations with NO_2 and PTB, there were several studies that utilized study designs to examine short-term exposure of NO_2 in association with PTB to identify critical windows of exposure. Recent studies are described in the summary bullets below, followed by additional discussion of concentration-response relationships in these studies (see Section 7.6.2.1.1), the lifestages and populations evaluated (see Section 7.6.2.1.2), and copollutant impacts (see Section 7.6.2.1.3). Section 7.6.2.1.4 discusses conclusions from these studies and summarizes remaining uncertainties. Study specific details including study population

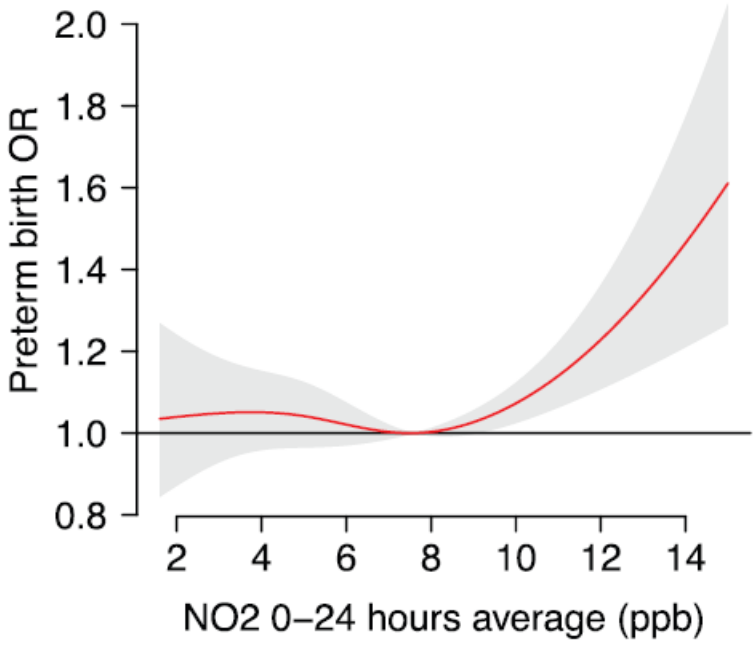
characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), potential confounders, and select results are in Appendix A – Appendix Table 7-11.

- Hao et al. (2016) used discrete-time survival models to examine associations between trimester and total pregnancy NO_2 exposure, estimated from CMAQ, and PTB among births in Georgia. There were increased odds for PTB for exposure in the first trimester (OR: 1.009 [95% CI: 1.005, 1.013] per 1.81 ppb NO_2), second trimester (OR: 1.008 [95% CI: 1.004, 1.0121]), third trimester (OR: 1.010 [95% CI: 1.007, 1.014]), and entire pregnancy (OR: 1.012 [95% CI: 1.007, 1.017]).
- Li et al. (2016b) used a time-stratified case-crossover design to examine the relationship between hourly ambient air pollution a few hours before the onset of labor, estimated from monitoring sites, and the risk of PTB in Brisbane, Australia. There was increased risk of PTB of 1.17 (95% CI: 1.08, 1.27) for the 95th percentile (11.30 ppb) of NO_2 exposure, compared to the threshold concentration (7.6 ppb), for 0-24 hours preceding the time of labor onset.
- To study the acute effects of exposure to NO_X on PTB, Mendola et al. (2016) used conditional logistic regression for each gestational week from 23 to 36, conditioning on women being at risk for delivery at that week, comparing exposure among deliveries at that week versus the same gestational week of exposure for all ongoing pregnancies for women with and without asthma in the Consortium of Safe Labor cohort. Among women with asthma, there was increased risk of PTB with NO_X exposure at gestational week 23 (OR: 1.21 [95% CI: 0.76, 1.92] per IQR), gestational week 29 (OR: 1.23 [95% CI: 0.91, 1.67]), gestational week 34 (OR: 1.16 [95% CI: 0.98, 1.38]), and gestational week 35 (OR: 1.13 [95% CI: 0.98, 1.30]), but the association was imprecise. When evaluating longer-term exposures, there was increased risk for early PTB (<34 weeks) during the 3-month preconception period (with exposure to NO_X among women with asthma (OR: 1.29 [95% CI: 1.06, 1.57]) and without asthma (OR: 1.09 [95% CI: 0.99, 1.20]). For gestational weeks 1-7, there was increased risk of early PTB for women with asthma (OR: 1.22 [95% CI: 1.08, 1.37]) with exposure to NO_X, but null for women without asthma (OR: 1.01 [95% CI: 0.96, 1.07]). Among women without asthma, there was a consistent pattern of inverse associations between exposure to NO_X and early PTB for the exposure periods of gestational weeks 8 to 14, 15 to 21, 22 to 28, last six weeks of pregnancy, and whole pregnancy. For women with asthma, there was no consistent pattern of associations between exposure to NO_X and early PTB for the exposure periods of gestational weeks 8 to 14, 15 to 21, 22 to 28, last six weeks of pregnancy, and whole pregnancy, with CIs crossing the null. Similarly, there was increased risk for total preterm births (<37 weeks) with exposure to NO_X during the 3-month preconception period and gestational weeks one to seven for women with asthma and without asthma, respectively.
- Stieb et al. (2019) examined the influence of short-term exposure to NO_2, using a time-to-event analysis, the week prior to delivery and PTB in 24 Canadian cities. Overall, there were inconsistent associations across the lag days (0 to 6) with PTB. There were inverse associations with NO_2 exposures on lag 0, 1, and 6 and null associations for lag days 2 to 5.

In addition to PTB, a few recent studies evaluated the associations between exposure to NO_2 and spontaneous premature rupture of membranes (SPROM), which can be related to PTB, but also can increase maternal and fetal mortality and morbidity (Jiao et al., 2023).

7.6.2.1.1 Concentration-Response

There was only a single study that evaluated the concentration-response relationship between NO_2 and PTB. Li et al. (2016b) used a time-stratified case-crossover design to investigate the association between acute increases in exposure to NO_2 a few hours before onset of labor and the risk of PTB. Using natural cubic splines with 3 degrees of freedom for mean values of 0 to 24 hour preceding the time of labor onset, Li et al. (2016b) reported a U-shaped curve, with a threshold of 7.6 ppb for NO_2 (see Figure 7-19).



List of abbreviations: NO_2 = nitrogen dioxide; OR = odds ratio; ppb = parts per billion.
Source: Li et al. (2016b). Copyright permission pending.

Figure 7-19 The relationship between NO_2 and preterm birth in single pollutant model with 3 degrees of freedom natural cubic spline for air pollutants.

7.6.2.1.2 Lifestages and Populations

Race/ethnicity

There were a limited number of studies that evaluated the potential for race/ethnicity to modify the associations between exposure to NO_2 and NO_X and PTB. Overall, the associations were inconsistent, with evidence from the recent epidemiologic studies summarized below.

- While the association between exposure to NO_2 and PTB overall was null (OR: 0.975 [95% CI: 0.92, 1.03]) among a subset of participants in the GeoBirth Cohort in the Philadelphia area, when stratified by maternal race/ethnicity non-Hispanic Asians and other races had increased odds (OR: 1.12 [95% CI: 0.857, 1.46] and OR: 1.11 [95% CI: 0.850, 1.45],

respectively), although the CIs included the null (Escoto et al., 2022). Associations remained null for non-Hispanic Black, Hispanic, and non-Hispanic white mothers.

- Hao et al. (2016) reported higher odds of PTB among Black mothers (OR: 1.018 [95% CI: 1.011, 1.025] per 1.81 ppb NO_2) compared to other races (OR: 1.001 [95% CI: 0.994, 1.008] per 1.81 ppb NO_2).
- Among pregnant women in Oakland and San Jose, California, U.S., (2013 to 2015), Riddell et al. (2022) reported inconsistent and imprecise associations among non-Latina whites, non-Latina Blacks, non-Latina Asians, and Latinas. There were positive, but imprecise associations for non-Latina whites (RD: 0.8 [95% CI: -4.0, 5.7]), non-Latina Blacks (RD: 0.6 [95% CI: -3.8, 5.0]), and Latinas (RD: 3.3 [95% CI: -0.1, 6.6]), but an inverse, imprecise, association for non-Latina Asians (RD: -1.0 [95% CI: -5.4, 3.4]).

Socioeconomic Status

SES is a composite measure that usually consists of economic status measured by income, social status measured by education, and work status measured by occupation. There were a few different SES metrics that were evaluated as factors that may modify associations between exposure to NO_2 and PTB. Overall, the impact of modification by SES metrics was inconsistent, with evidence from the recent epidemiologic studies summarized below.

- Among participants in France, Genin et al. (2022) included an ecological adjustment for social deprivation and reported higher magnitude of risk of overall PTB (NO_2 RR: 1.019 [95% CI: 1.014, 1.023] and NO_2 plus social deprivation RR: 1.054 [95% CI: 1.048, 1.060]). However, in stratified analyses for SES index (low level and high level), Li et al. (2016b) reported similar positive associations with the adjustment for SES index (see Appendix A – Appendix Table 7-11).

- Maternal education has been used as a proxy measure for SES. Among births in Georgia, U.S., in 2002 to 2006, Hao et al. (2016) reported among mothers with less than high school education there were higher odds (OR: 1.024 [95% CI: 1.017, 1.030] per 1.81 ppb NO_2) for PTB, compared to mothers with higher than high school education (OR: 0.994 [95%: 0.987, 1.001] per 1.81 ppb NO_2).

Pre-existing comorbidities

Several recent studies evaluated the potential modification of the association between NO_2 or NO_X exposure and PTB by maternal pre-existing comorbidities (Williams et al., 2021; Weber et al., 2019; Lavigne et al., 2016b; Li et al., 2016b; Mendola et al., 2016). While Li et al. (2016b) included adjustment for an aggregate for pre-pregnancy medical conditions (yes/no), other studies evaluated the impact of specific conditions, including asthma (Lavigne et al., 2016b; Mendola et al., 2016), hypertension (Weber et al., 2019; Lavigne et al., 2016b); diabetes (Lavigne et al., 2016b), and Type 1 diabetes (Williams et al., 2021). Some pre-existing maternal comorbidities modified the association between NO_2 or NO_X exposure and PTB, but not consistently across studies.

- Among women who reported having any pre-pregnancy medical conditions, Li et al. (2016b) reported increased risk of NO_2-associated PTB (OR: 1.90 [95% CI: 1.18, 3.04]) compared to women who reported having no pre-pregnancy medical conditions (OR: 1.18 [95% CI: 1.07, 1.30]) in Brisbane, Australia.
- Mendola et al. (2016) evaluated associations between women with and without asthma in the Consortium of Safe Labor. Overall, the pattern of association between exposure to NO_X and PTB suggested that PTB risk was higher among women with asthma compared to women without asthma (see Appendix A – Appendix Table 7-11).
- Among births in Ontario, Canada Lavigne et al. (2016b) reported that women with chronic diabetes had a larger risk of NO_2-associated PTB (OR: 1.238 [95% CI: 1.055, 1.448]) compared to women without chronic diabetes (OR: 1.065 [95% CI: 1.037, 1.084]). However, there was no evidence of effect modification for maternal comorbidities of asthma, hypertension, heart disease, gestational diabetes, or preeclampsia.
- Among women living in San Joaquin Valley, California, U.S., Weber et al. (2019) reported an inconsistent pattern of associations across preterm births occuring at gestational weeks 20 to 27, 28 to 31, 32 to 33, and 34 to 36 and six NO_2 exposure periods (entire pregnancy, first trimester, second trimester, third trimester, and last 6 weeks of pregnancy). Maternal hypertension increased the odds of first trimester NO_2 exposure and PTB during gestational weeks 32 to 33 (OR: 1.22 [95% CI: 1.02, 1.46]).
- Williams et al. (2021) hypothesized that women with Type 1 diabetes and their infants would have a greater risk of poor outcomes when exposed to air pollution compared to women without autoimmune disorders and their infants. Overall, there were null associations with exposure to NO_X among women with Type 1 diabetes compared to women without autoimmune disorders for preterm birth across preconception exposure period, first trimester, second trimester, or entire pregnancy (see Appendix A – Appendix Table 7-11).

7.6.2.1.3 Copollutants

There were a few recent studies that evaluated the potential confounding by copollutants of the associations between NO_2 and PTB. In the recent epidemiologic evidence, the copollutants evaluated were $PM_{2.5}$, O_3, BC, UFPs, SO_2, CO, PM_{10}, and benzene. There were different statistical methods utilized to consider confounding by copollutants, but most studies co-adjusted for copollutants. Overall, the associations between NO_2 and PTB were inconsistent in co-adjusted models or mixture analysis.

- Laurent et al. (2016) used co-adjusted models to explore the associations between exposure to NO_2 and $PM_{2.5}$ and NO_2 and O_3, estimated from interpolation by empirical Bayesian kriging, and PTB in CA. In the co-adjusted model for NO_2 and $PM_{2.5}$, the magnitude of the association was decreased (NO_2 OR: 1.079 [95% CI: 1.065, 1.093] and NO_2 and $PM_{2.5}$ OR: 0.986 [95% CI: 0.971, 1.001]) ($\rho = 0.81$). However, in the two-pollutant model for NO_2 and O_3, the magnitude of the association was increased (NO_2 OR: 1.079 [95% CI: 1.065, 1.093] and NO_2 and O_3 OR: 1.083 [95% CI: 1.069, 1.098]) ($\rho = -0.07$).
- In a co-adjusted model with SO_2 ($\rho = -0.03$), the association between exposure to NO_2 and PTB was unchanged (Li et al., 2016b). In a two-pollutant model with CO ($\rho = 0.43$), there was a slight attenuation in the association with PTB, but the association remained positive (NO_2 OR: 1.17 [95% CI: 1.08, 1.27] and NO_2 and CO OR: 1.13 [95% CI: 1.01, 1.26]). In the

multipollutant model, NO_2, CO, and SO_2, the association with PTB was slightly attenuated, but the association remained positive (multipollutant OR: 1.13 [95% CI: 1.01, 1.26]).

- In a two-pollutant model with benzene, Estarlich et al. (2016) reported positive associations larger in magnitude with co-adjustment for benzene compared to the single pollutant NO_2 model across all exposure periods (first trimester, second trimester, third trimester, and entire pregnancy) in a multicenter Spanish birth cohort (see Appendix A – Appendix Table 7-11). However, NO_2 and benzene were moderately to highly correlated (ρ = 0.54 to 0.78) across exposure periods.
- Riddell et al. (2022) co-adjusted for BC and UFPs in multipollutant models with NO_2 in association with PTB. There were no correlation values reported. In the multipollutant model, for non-Latina whites, the association was strengthened, but the association was imprecise (main effect RD: 0.8 [95% CI: -4.0, 5.7] and co-adjusted RD: 4.0 [95% CI: -2.6, 10.6]). For non-Latina Blacks, the association was strengthened, but the association was imprecise (main effect RD: 0.6 [95% CI: -3.8, 5.0] and co-adjusted RD: 1.2 [95% CI: -4.7, 7.1]). For Latinas, the association was attenuated towards the null with co-adjusted for BC and UFPs, but the association was imprecise (main effect RD: 3.3 [95% CI: -0.1, 6.6] and co-adjusted RD: 1.3 [95% CI: -2.5, 5.0]). In the multipollutant model, for non-Latina Asians, the magnitude increased, and the association was imprecise (main effect RD: -1.0 [95% CI: -5.4, 3.4] and co-adjusted RD: 1.1 [95% CI: -4.7, 6.8]).
- Jiao et al. (2023) evaluated the joint effects of air pollution mixture on spontaneous premature rupture of membranes (SPROM) using quantile-based g computation. In a mixture of NO_2, O_3, $PM_{2.5}$, and PM_{10}, the overall mixture effect was associated with 1.15 (95% CI: 1.12,1.18) higher odds of SPROM for exposure throughout the entire pregnancy. While the mixture effect is not directly comparable to the single pollutant effect of NO_2, the mixture effect represents more real-life exposures and the potential for how NO_2 interacts with other co-exposures.

7.6.2.1.4 Conclusions and Remaining Uncertainties

Overall, the associations between exposure to NO_2 and PTB were inconsistent in the epidemiologic studies. Among the recent evidence, there were different exposure periods evaluated (trimester, entire pregnancy, hourly), different exposure assessment metrics (CMAQ, LUR, central site monitors, empirical Bayesian kriging, spatiotemporal/geospatial model, hybrid model), and across different geographies (United States, Canada, Australia, Europe). While some recent studies utilized study designs to examine short-term exposure of NO_2 in association with PTB to identify critical windows of exposure, there was no clear critical window of exposure. A single study in Australia evaluated the concentration response relationship of NO_2 on associations with PTB, reporting a threshold at 7.6 ppb. However, a single study makes it difficult to judge consistency or coherence. Additionally, several recent studies also considered exposure to NO_X in association with PTB, but the associations were inconsistent overall. Furthermore, there was limited evidence evaluating factors (SES, race/ethnicity, pre-existing comorbidities) that could modify associations between exposure to NO_2 and PTB, but the evidence was inconsistent and limited. While there were a few recent studies that evaluated the potential of copollutant confounding, there were inconsistent associations overall. Among the studies evaluating copollutants,

most of the studies only considered co-adjusted models, with moderate to high correlations among copollutants, which may bias the estimates. Overall, there were inconsistent associations with PTB and exposure to NO_2 and NO_X in the recent epidemiologic evidence.

7.6.2.2 Animal Toxicological Studies of Preterm Birth

Although some outcomes in animal toxicological studies such as gestation duration can be used as a proxy for preterm birth in humans, there exists no direct corollary in animal toxicological studies for preterm birth. The 2008 Oxides of Nitrogen ISA summarized a single study that evaluated effects of NO_2 gestation duration (Di Giovanni et al., 1994). Di Giovanni et al. (1994) exposed female Wistar rats to 1.5 or 3 ppm NO_2 from GD 0 to GD 20 and reported null effects on gestation duration. No additional animal toxicological studies that investigated the effects of oxides of nitrogen exposure on preterm birth or gestation duration were reported in the 2016 Oxides of Nitrogen ISA or the 1993 Oxides of Nitrogen AQCD. The few recent toxicological studies that investigated the effects of oxides of nitrogen on preterm birth were also limited to assessments of gestation duration and reported no effects. Study specific details, exposure conditions, and endpoints examined are highlighted in the text below. Studies supporting causality determinations are described in more detail in Appendix A – Appendix Table 7-2. Li et al. (2022) reported that exposure to 2.5 ppm of NO_2 for five hours/day from GD 0 to birth (PND 0) did not affect gestation duration in C57BL/6J mice. In agreement, Yan et al. (2020) reported that NO_2 exposure (2.5 ppm for five hours/day from GD 0 to PND 0) in C57BL/6J mice did not alter gestation duration.

7.6.2.2.1 Conclusions and Remaining Uncertainties

There is limited animal toxicological evidence examining the effects of oxides of nitrogen on preterm birth. The only available studies are recently published studies in mice which both reported no effects on the duration of gestation. Although no effects were observed, there is a lack of sufficient evidence to determine that there are no negative effects. Thus, additional studies are still needed to fully assess the potential impacts of NO_2 exposure on preterm birth and gestation duration in animal models.

7.6.3 Birth Defects

Overall, the evidence from the 2016 Oxides of Nitrogen ISA was limited. There were no animal toxicological studies and a limited number of epidemiologic studies. The epidemiologic studies reviewed in the 2016 Oxides of Nitrogen ISA reported that there were overall inconsistent associations between oxides of nitrogen exposure and birth defects. There were a limited number of studies, with most focused on cardiac defects (Lin et al., 2014; Stingone et al., 2014). The inconsistencies in the associations with cardiac defects could have been due to the absence of true associations between NO_2 and risks of

cardiovascular malformations. It could also have been due to issues relating to statistical power or measurement error or due to differences across studies in populations, pollution concentrations, outcome definitions, or analytical approaches. Also, the lack of consistency of associations between NO_2 and cardiovascular malformations might be due to issues relating to statistical power or measurement error. None of the birth defect studies of NO_2, including meta-analyses, examined confounding by $PM_{2.5}$ or traffic-related pollution. There were several recent epidemiologic studies examining associations between oxides of nitrogen and a number of different birth defects. However, there were no recent toxicological studies of birth defects.

7.6.3.1 Epidemiologic Studies of Birth Defects

Overall, there were inconsistent associations between exposure to oxides of nitrogen and birth defects in the recent epidemiologic studies. Across the studies, there were differences in the exposure estimation approaches (LUR, AQS, dispersion models, ensemble machine learning, and central site monitors), different species of oxides of nitrogen examined (NO_2, NO_X, and NO), different geographies (Europe, United States, Canada, Taiwan, and Israel), different windows of exposure, and different birth defects. Additionally, there were very small numbers of cases of birth defects in some of the studies, which may have led to imprecise associations.

Evidence from recent studies is summarized below. Study specific details including study population characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), potential confounders, and select results are in Appendix A – Appendix Table 7-12. There were no recent studies of birth defects that evaluated the concentration response relationship with exposure to NO_2, factors (genetics, race/ethnicity, diet) that may modify associations, or the potential for confounding by copollutants.

Aggregate Birth Defects

Only a few recent studies examined associations with birth defects overall (Cowan et al., 2024; Al Noaimi et al., 2021). In a Lebanese national study, NO_2 estimates from monitors were used to build a two-dimensional approach for exposure assessment. In this approach, the following variables were used to build the base: the number of gestational weeks, date of birth of the infant, type of birth defect in each case, mother's area of residence, and weekly mean air pollution concentration in the mother's area of residence. Then, the assigned exposure of each mother-infant pair for each pollutant during the gestational time window of risk (GTWR) specific for each birth defect type was used in the analysis. The assigned exposure during the GTWR specific for each birth defect was used for each case and its matched controls. There was a null association with NO_2 exposure and birth defects overall (OR: 1.008 [95% CI: 0.98, 1.04]). In a North Carolina, U.S., birth cohort, in general, there was no evidence of associations between exposure to NO_2 and birth defects, and the associations did not differ across NDI levels or trimesters (Cowan et al., 2024).

Congenital Heart Defects

Several studies evaluated the associations between NO_2 and congenital heart defects (CHD), both overall and for subtypes (Cowan et al., 2024; Buteau et al., 2023; Al Noaimi et al., 2021; Yan et al., 2021; Huang et al., 2019; Lavigne et al., 2019; Pedersen et al., 2017a). Overall, the associations were inconsistent for CHD overall and by CHD subtype. The recent epidemiologic studies were conducted in Canada, Lebanon, Denmark, Taiwan, United States, and China. These recent studies evaluated similar critical windows of exposure (gestational weeks two to eight and first trimester). There were a variety of different exposure assessment metrics used (dispersion model, fixed site monitors, LUR, ensemble machine learning, AQS) with reported similar means/medians (see Appendix A – Appendix Table 7-12). A summary of the recent epidemiologic evidence follows below.

- In a Lebanese national study, exposure to NO_2 was estimated from monitors and used to build a two-dimensional approach for exposure assessment (Al Noaimi et al., 2021). In this approach, the following variables were used to build the base: the number of gestational weeks, date of birth of the infant, type of birth defect in each case, mother's area of residence, and weekly mean air pollution concentration in the mother's area of residence. Then, the assigned exposure of each mother-infant pair for each pollutant during the GTWR specific for each birth defect type was used in the analysis. The assigned exposure during the GTWR specific for CHD was used for each case (n = 47) and its matched controls (n = 1,431). There was an imprecise positive association between exposure to NO_2 and CHD (OR: 1.02 [95% CI: 0.95, 1.09] per 1 $\mu g/m^3$ NO_2).
- In the Danish National Birth Cohort, Pedersen et al. (2017a) estimated exposure to NO_2 from a dispersion model to evaluate the association with total CHD and six CHD subtypes (ventricular septal defects (VSD), atrial septal defects (ASD), coarctation of aorta, pulmonary valve stenosis, tetralogy of Fallot (ToF), or severe CHD). There was an imprecise inverse association with first trimester exposure to NO_2 and CHD overall (OR: 0.93 [95% CI: 0.83, 1.04]). There was a positive, but imprecise, association with ToF (OR: 1.08 [95% CI: 0.61, 1.93] per 10 $\mu g/m^3$ NO_2). The imprecise association may be due to the small number of cases (n = 22). There were imprecise inverse associations with VSD, ASD, coarctation of the aorta, pulmonary valve stenosis, and severe CHD.
- In population-based case-control study in Taiwan, Huang et al. (2019) reported positive, but imprecise, associations with endocardial cushion defect (OR: 1.07 [95% CI: 0.43, 2.66] per 5.72 ppb NO_2) and pulmonary artery and valve stenosis (1.20 [95% CI: 0.71, 2.04] per 5.72 ppb NO_2), but no associations with VSD, ToF, or transposition of the great arteries (TGA) and an imprecise inverse association with ASD.
- Among singleton live births in Toronto, Lavigne et al. (2019) reported positive, but imprecise, associations between exposure to NO_2 during gestational weeks 2 to 8 and VSD (OR: 1.01 [95% CI: 0.85, 1.20] per 7.4 ppb increase in NO_2), and ASD (OR: 1.05 [95% CI: 0.93, 1.19] 7.4 ppb increase in NO_2).
- In another study in Canada, Buteau et al. (2023) reported increased odds with monthly average first trimester NO_2 and any heart defect (OR: 1.10 [95% CI: 1.07, 1.13] per 11.4 ppb increase in NO_2), with a slight increase when adjusting for $PM_{2.5}$ (OR: 1.11 [95% CI: 1.08, 1.15]). There were increased odds for ASD (OR: 1.18 [95% CI: 1.12, 1.25]) and NO_2, but null associations for VSD and ToF, and a positive, but imprecise, association for coarctation

of the aorta. Additionally, there was evidence of effect measure modification for any maternal comorbidity, maternal epilepsy and mood disorders, and maternal anemia.

- Yan et al. (2021) evaluated ASD birth defects from the China National Population-based Birth Defects Surveillance System to gestational weekly exposure to NO_2, estimated from ensemble machine learning methods. There were increased odds (OR: 1.33 [95% CI: 1.22, 1.45]) of isolated ASD per 10 μg/m^3 increase of NO_2 over the entire pregnancy. In addition, maternal exposure to NO_2 in any week of pregnancy was positively associated with ASD, with the strongest associations observed during the gestational weeks 1-8.
- Among a North Carolina birth cohort, Cowan et al. (2024) reported decreased risk differences for pulmonary valve atresia/stenosis (RD: -15.96 [95% CI: -28.83, -3.09] per 7 ppb increase in NO_2), tetralogy of Fallot (RD: -11.56 [95% CI: -33.85, 10.74] per 7 ppb increase in NO_2), and atrioventricular septal defects (RD: -12.45 [95% CI: -24.24,-0.65) ppb increase in NO_2) per every IQR (7 ppb) increase in NO_2 exposure during the first trimester, which was estimated from an ensemble machine learning model.

Neural Tube Defects (NTDs)

There were only a few studies that evaluated the associations between exposure to NO_2 and NTDs. Overall, there were inconsistent associations for NTDs overall and by NTD subtype. The recent epidemiologic studies were conducted in Lebanon and Denmark, and the inconsistent associations may be due to differences in critical windows examined (gestational weeks two to seven vs. first trimester), different exposure metrics (monitor network vs. dispersion model), and a small number of cases. A summary of the recent epidemiologic evidence follows below.

- In a Lebanese national study, exposure to NO_2 was estimated from monitors that were used to build a two-dimensional approach for exposure assessment (Al Noaimi et al., 2021). In this approach, the following variables were used to build the base: the number of gestational weeks, date of birth of the infant, type of birth defect in each case, mother's area of residence, and weekly mean air pollution concentration in the mother's area of residence. Then, the assigned exposure of each mother-infant pair for each pollutant during the gestational time window of risk (GTWR) specific for each birth defect type was used in the analysis. The assigned exposure during the GTWR specific for NTDs was used for each case (n = 73) and its matched controls (n = 2,102). There was an inverse association between NO_2 and NTDs (OR: 0.94 [95% CI: 0.90, 0.99] per 1 μg/m^3 of NO_2).
- In the Danish National Birth Cohort, Pedersen et al. (2017a) estimated exposure to NO_2 from a dispersion model and evaluated the association of exposure to NO_2 with nervous system anomalies, including NTDs. There was an inverse association between first trimester exposure to NO_2 and nervous system anomalies (OR: 0.77 [95% CI: 0.55, 1.06] per 10 μg/m^3 NO_2), but the CI was wide, which may be due to the small number of cases (n = 127).

Orofacial Defects

Overall, there were inconsistent associations between exposure to NO_2 and NO_X and orofacial birth defects, including cleft lip with or without cleft palate and cleft palate among the few recent epidemiologic studies. These studies were conducted in the United States and Denmark. Detailed summaries are in the following bullets.

- In the Danish National Birth Cohort, Pedersen et al. (2017a) reported no associations between first trimester exposure to NO_2, estimated from a dispersion model, and orofacial clefts defects overall (OR: 0.95 [95% CI: 0.77, 1.18]), cleft lip with or without cleft palate (OR: 0.98 [95% CI: 0.77, 1.26]), or cleft palate (OR: 0.89 [95% CI: 0.56, 1.35)].
- In the Consortium on Safe Labor cohort, Zhu et al. (2015) estimated exposure to NO_X from CMAQ, and reported increased odds of isolated cleft palate (OR: 3.64 [95% CI: 1.73, 7.66] per 22.97 ppb increase in NO_X) with NO_X during gestational weeks three to eight, and increased odds for isolated cleft lip with or without cleft palate (OR: 1.58 [95% CI: 0.70, 3.55]) during the 3-month preconception period, but CI includes the null. These imprecise associations for isolated cleft palate defects may be due to the small number of cases (n = 63). With isolated cleft lip with or without cleft palate (n = 159), there was a positive, but imprecise, association during gestational weeks three to eight (OR: 1.37 [95% CI: 0.88, 2.13]) and no association during the 3-month preconception period (OR: 0.89 [95% CI: 0.53, 1.050).

Other Birth Defects

In addition to associations with the CHDs, NTDs, and orofacial birth defects, several studies examined other subtypes of birth defects, but the evidence base is limited.

- Harari-Kremer et al. (2021) examined associations of trimester-specific exposure to NO_2 and NO_X with congenital hypothyroidism (CHT), using data from the national program for neonatal screening in Israel. To evaluate residual confounding, postnatal exposures were used to the same pollutants in the same address as negative control exposures. These exposures cannot affect CHT status at birth because they occur after birth, but they are presumably affected by the same potential unobserved confounders as the prenatal exposures. Higher third-trimester exposure to NO_2 and NO_X was positively associated with CHT (NO_2 OR: 1.25 [95% CI: 1.06, 1.47] and NO_X OR: 1.22 [95% CI: 1.07, 1.39]), but there were imprecise associations for the first trimester, second trimester, and postnatal periods. When mutually adjusting for all three trimesters, the third trimester association with NO_2 was slightly increased (OR: 1.34 [95% CI: 1.11, 1.62]) for CHT and although positive, the association with NO_X was slightly attenuated (OR: 1.12 [95% CI: 1.03, 1.21]) for CHT. The associations with the first trimester, second trimester, and postnatal periods were unchanged. The matching negative control exposure periods for the first and second trimester NO_2 and NO_X exposures resulted in imprecise associations with CHT. However, the matching negative control exposure periods for the third trimester exposures resulted in positive associations of NO_2 and NO_X with CHT (NO_2 OR: 1.28 [95% CI: 1.09, 1.51] and NO_X OR: 1.23 [95% CI: 1.08, 1.41]) and inverse, but imprecise, associations with the postnatal period (NO_2 OR: 0.92 [95% CI: 0.84, 1.01] and NO_X OR: 0.88 [95% CI: 0.73, 1.02]).The authors note that the associations detected were not a result of residual confounding, given that the matching postnatal exposures show null or negative associations in mutually adjusted models. If residual confounding was present, it works in the opposite direction of the "causal effect", producing a negative association for the negative control exposure, and therefore, that the "causal effect" might be more substantial.
- Al Noaimi et al. (2021) reported imprecise associations between NO_2 and genital and urinary tract defects (OR: 1.008 [95% CI: 0.96, 1.06]) and musculoskeletal defects (OR: 1.04 [95% CI: 0.98, 1.09]) in a Lebanese national case-control study. The imprecise estimates are likely due to the small number of cases (n = 65).

- Among births in the Taiwanese Birth Registry database, there were no associations with hypospadias and exposure to NO_2 or NO_X in any exposure window: pre-conception (zero to one months, one to two months, two to three months, and zero to three months) and post-conception (zero to one months, one to two months, two to three months, and zero to three months, three to four months, four to five months, five to six months, and three to six months) exposure periods for NO_2 and NO_X (Huang et al., 2020). The small number of cases may have contributed to the wide CIs.
- In the Danish National Birth Cohort, Pedersen et al. (2017a) reported positive, but imprecise, associations with ear, face, and neck anomalies (OR: 1.22 [95% CI: 0.98, 1.52] per 10 $\mu g/m^3$ NO_2), urinary anomalies (OR: 1.14 [95% CI: 0.98, 1.33] per 10 $\mu g/m^3$ NO_2), and genital anomalies (OR: 1.06 [95% CI: 0.90, 1.25] per 10 $\mu g/m^3$ NO_2), but no associations with eye anomalies, respiratory system anomalies, digestive system anomalies, limb anomalies, multiple congenital anomalies, or chromosomal anomalies.
- Among participants of the National Birth Defects Prevention Study, there was a positive, but imprecise, association between congenital limb defects and NO_2 (OR: 1.10 [95% CI: 0.96, 1.26] per 8.5 ppb increase in NO_2) and NO_2 daily max (OR: 1.04 [0.90, 1.21] per 12.6 ppb increase in NO_2 daily max) averaged across gestational weeks two to eight (Choi et al., 2019).
- Among all live births in England and Wales, the highest quintile of annual average exposure to NO_2 was associated with infant deaths from congenital malformations (OR: 1.159 [95% CI: 1.063, 1.264]), compared to the lowest quintile (referent) (Kotecha et al., 2020).
- In a North Carolina birth cohort, Cowan et al. (2024) found no evidence of associations between exposure to NO_2 and prevalence of limb reduction defects or gastroschisis and these associations did not differ across NDI levels or trimesters (see Appendix A – Appendix Table 7-12).

7.6.3.1.2 Conclusions and Remaining Uncertainties

Overall, the epidemiologic evidence is limited for birth defects and the associations across all birth defect subtypes were inconsistent. The small body of evidence limits the ability to judge coherence and consistency across these studies. These recent studies of other birth defects did not evaluate the concentration-response relationship between exposure to oxides of nitrogen and these birth defects, factors (maternal age, race/ethnicity, SES) that may modify associations, or potential confounding by copollutants.

7.6.4 Infant Mortality

Infant mortality is a death occurring in the first year of life and is divided into two periods: neonatal (i.e., death during the first 28 days) and post-neonatal (i.e., death after the first month of life and before the first birthday). The recent epidemiologic and toxicological studies examining the relationship between oxides of nitrogen exposure and infant mortality are summarized in the text below. Details of the

recent epidemiologic studies are included in Appendix A – Appendix Table 7-13 and the recent animal toxicological studies are in Appendix A – Appendix Table 7-2, respectively.

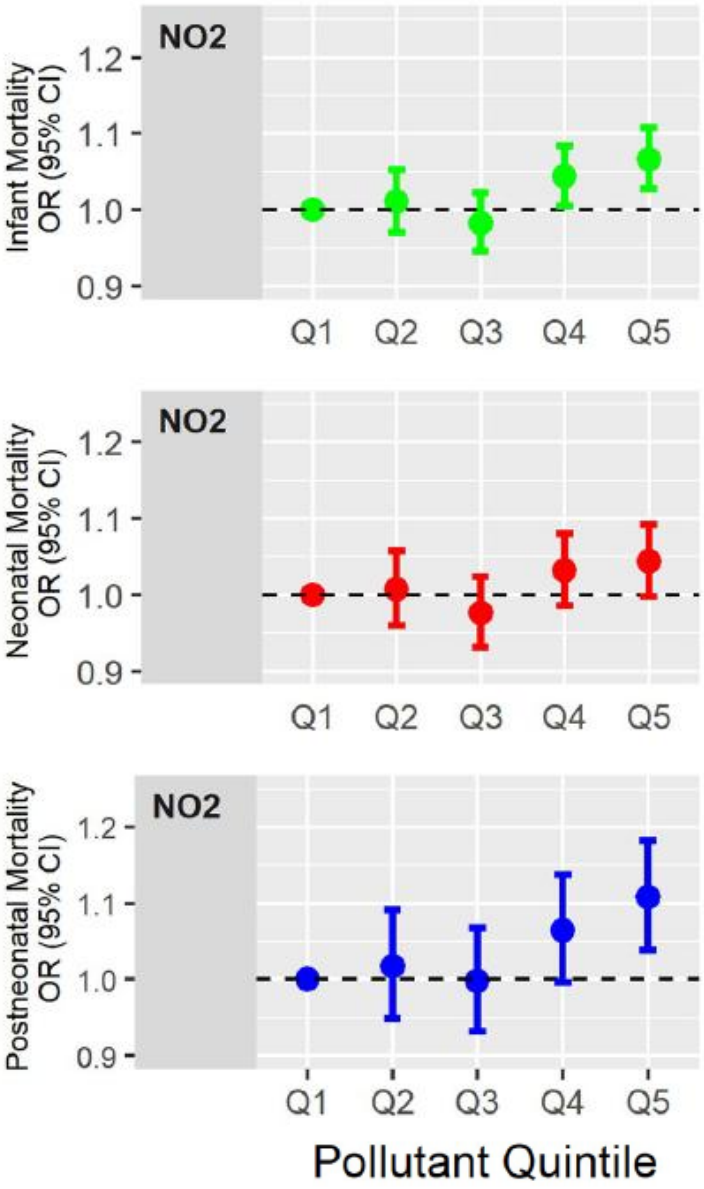
7.6.4.1 Epidemiologic Studies of Infant Mortality

There were a few epidemiologic studies reviewed in the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016) that examined the associations between oxides of nitrogen and infant mortality. Overall, the evidence for an association between oxides of nitrogen and infant mortality was inconsistent with a mix of positive and null associations. However, this was not unexpected given the inconsistency across studies with regards to exposure windows (e.g., short-term vs. long-term) and outcome definitions (e.g., infant mortality versus neonatal mortality vs. postneonatal mortality). Recent studies were limited in number.

- An administrative cohort study in England and Wales from 2001 to 2012 evaluated modeled annual average NO_2 exposure for year of birth (Kotecha et al., 2020). Kotecha et al. (2020) observed positive associations between long-term exposure to NO_2 and infant mortality, neonatal mortality, and postneonatal mortality for quintile 4 (OR: 1.044 [95% CI: 1.005, 1.084], OR: 1.032 [95% CI: 0.986, 1.080], OR: 1.064 [95% CI: 0.996, 1.137], respectively) and quintile 5 (OR: 1.066 [95% CI: 1.027, 1.107], OR: 1.044 [95% CI: 0.998, 1.092], OR: 1.108 [95% CI: 1.038, 1.182], respectively) compared to quintile 1 in the fully adjusted models. The strongest effects were observed for postneonatal mortality. Additionally, authors observed positive associations for cause-specific infant mortality in quintiles 4 and 5 for congenital malformations, endocrine disorders, and blood disorders, although the ORs were imprecise.
- A case-control study in Taiwan from 1997 to 2002 observed positive associations between short-term exposures to NO_2 (1- to 14-day lags) and sudden infant death syndrome (SIDS) incidence (e.g., 1-day lag OR: 1.30 [95% CI: 1.00, 1.69]) (Chen et al., 2021). Authors observed null associations between short-term exposures to NO_2 and respiratory-related infant mortality (e.g., 1-day lag OR: 0.94 [95% CI: 0.59, 1.50]). The mean concentrations of NO_2 ranged from 22.5 ppb to 26.0 ppb depending on the lag day and outcome evaluated.

7.6.4.1.1 Concentration-Response

A single study evaluated concentration-response by assigning exposure categorically (quintiles) and comparing quintiles 2 to 5 to quintile 1. Kotecha et al. (2020) did not observe associations between long-term exposure to NO_2 and infant mortality, neonatal mortality, and postneonatal mortality for quintiles 2 and 3, but did observe positive associations for quartiles 4 and 5 (see Figure 7-20).



List of abbreviations in alphabetical order: LMP = last menstrual period; ppb = part per billion; RR = rate ratio.
Note: Adjusted odds ratios (OR) and 95% CIs are shown. Models adjusted for Index of Multiple Deprivation, birthweight, maternal age, sex, and multiple birth. Source: Kotecha et al. (2020). Copyright permission pending.

Figure 7-20 Associations between NO_2 by quintile and infant mortality, including neonatal and postneonatal mortality.

7.6.4.1.2 Copollutants

A single study evaluated copollutant confounding for the association between oxides of nitrogen and infant mortality. Chen et al. (2021) observed that the magnitude of associations between short-term exposures to NO_2 (1- to 14-day lags) and SIDS infant mortality as well as respiratory-related infant

mortality were strengthened after multipollutant adjustment for O_3 and PM_{10} (e.g., SIDS 1-day lag OR: 1.49 [95% CI: 1.08, 2.05]). For 1-day to 14-day lag results, Pearson correlation coefficients ranged from -0.18 to -0.13 for O_3 and 0.51 to 0.55 for $PM_{2.5}$. Magnitude of associations strengthened after adjustment for copollutants.

7.6.4.1.3 Conclusions and Remaining Uncertainties

There was a small body of recent epidemiologic studies for infant mortality although the studies lacked consistency across exposure windows (short-term vs. long-term) as well as outcome definitions (e.g., infant mortality versus neonatal mortality versus postneonatal mortality). The small number of studies limits the ability to judge coherence and consistency across these studies. There are remaining uncertainties regarding concentration response, lifestages and populations potentially at increased risk, and potential confounding by copollutants.

7.6.4.2 Animal Toxicological Studies of Infant Mortality

No animal toxicological studies that investigated the effects of oxides of nitrogen exposure on offspring mortality parameters were reported in the 2016 Oxides of Nitrogen ISA. A single study reported effects on infant mortality in the 2008 Oxides of Nitrogen ISA (Di Giovanni et al., 1994). Di Giovanni et al. (1994) exposed female Wistar rats to 1.5 or 3 ppm NO_2 from GD 0 to GD 20, and observed null effects on male rat pup mortality at PND 21. The 1993 Oxides of Nitrogen AQCD summarized animal toxicological studies that investigated the effects of oxides of nitrogen exposure on offspring mortality. One study reported that viability of offspring born to Wistar rats exposed to 10 mg/m^3 (5.32 ppm) of NO_2 (but not 1 mg/m^3 (0.532 ppm) of NO_2) for six hours/day throughout gestation (GD 0 to birth was reduced on PND 21 (but not PND 1, 4, or 7) (Tabacova et al., 1985). In support, another study reported that pregnant rats continuously exposed to 10 ppm NO_2 starting on GD 14 had significantly reduced litter sizes, indicating fetal loss may have occurred (Freeman et al., 1974). Additionally, authors report in a qualitative statement that offspring mortality remained elevated until PND 15. However, of note is that the high exposure concentration and long exposure duration resulted in overt toxicity in the dams, and authors report that some pregnant rats died prior to delivery.

Similar to the results of Di Giovanni et al. (1994), the available recent animal toxicological studies did not report that exposure to oxides of nitrogen affected offspring mortality. Study specific details, exposure conditions, and endpoints examined are highlighted in the text below. Studies supporting causality determinations are described in more detail in Appendix A – Appendix Table 7-2**Error! Reference source not found.**. Notably, all recent studies were from the same research group and u tilized the same dosing regimen: 2.5 ppm of NO_2 for five hours/day throughout gestation (GD 0 to PND 0). NO_2 exposure did not affect litter size or offspring mortality in C57BL/6 mice (Yan et al., 2020) or in

BALB/c mice (Yue et al., 2018; Yue et al., 2017). The contrast in results from Freeman et al. (1974) may be attributable to the use of higher doses in the previous studies, especially in the case of Freeman et al. (1974) which resulted in overt toxicity and death in some treated animals. Thus, it is possible that negative impacts on offspring mortality may only occur at doses greater than 2.5 ppm of NO_2. Further, the previous studies were both conducted in rats while recent studies were conducted in mice, indicating that there may be species-dependent sensitivity for this particular outcome.

7.6.4.2.1 Conclusions and Remaining Uncertainties

There is limited animal toxicological evidence examining the effects of exposure to oxides of nitrogen on offspring mortality. The exposure windows used in all of the available studies are the same (GD 0 to PND 0) with the exception of one study that began dosing at GD 14. Thus, the primary driver for the observed contrast in results is likely more attributable to other differences in study design such as dose, species, and, in the case of (Di Giovanni et al., 1994), the sex of pups evaluated. Indeed, no effects were observed at doses of 3 ppm or lower, suggesting that higher doses may be required to elicit effects on offspring mortality. Further, effects were only observed in rats, and none were observed in mice, suggesting there may be species-dependent sensitivity to NO_2. Additional studies to confirm a dose-response relationship and any inter-species differences in sensitivity would meaningfully contribute to this gap in knowledge.

7.6.5 Other Birth Outcomes

There were some recent studies of birth outcomes other than prenatal growth, preterm birth, birth defect, or infant mortality. These studies are summarized below and more specific study details, including study population characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), and selected results are in Appendix A – Appendix Table 7-14.

7.6.5.1 Epidemiologic Studies of Other Birth Outcomes

Some recent epidemiologic studies have evaluated associations between perinatal oxides of nitrogen exposure and markers of fetal metabolic function, admission to the neonatal intensive care unit (NICU), and blood markers that may predict childhood allergy. These studies are summarized below. Study specific details including study population characteristics, exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 7-14.

- Lavigne et al. (2016a) reported positive associations between prenatal exposure to NO_2 and umbilical cord blood leptin and adiponectin levels, markers of fetal metabolic function, in infants in the Canadian MIREC cohort. Elevated adipokine levels in cord blood are

considered potential predicators of the early development of obesity and have been associated with higher birth weight and large for gestational age. There was 12% (95% CI: 2, 23) higher leptin levels and 13% (95% CI: 6, 20) higher adiponectin levels in umbilical cord blood for IQR (13.6 ppb) increase in NO_2. There was no evidence of effect measure modification by infant sex for either leptin or adiponectin levels. There was also no change in the associations when adjusted for $PM_{2.5}$ in a copollutant model.

- Among a small cohort (N = 136) from the University of Southern California Maternal and Child Health Study (MACHS), Alderete et al. (2017) reported that a 1-SD (2 ppb) increase in non-freeway NO_X was associated with a 33.4% (95% CI: 8.9%, 63.5%) higher leptin level and 23% (95% CI: 0.2%, 50.3%) L/A ratio (ratio of leptin to high molecular weight adiponectin).
- Howe et al. (2018) reported a 0.7 (95% CI:0.1, 1.3) µg/dL difference in total thyroxine, a marker of thyroid function, per 2-SD difference in NO_2 among newborns in the Children's Health Study from southern California. For freeway NO_X, there was null association with difference in total thyroxine and a slight inverse, but imprecise, association with non-freeway NO_X. In two-pollutant models for NO_2, there was slight attenuation (β: 0.4 µg/dL [95% CI: -0.2, 1.1]) with $PM_{2.5}$ but an increase with O_3 (β: 0.9 µg/dL [95% CI: 0.3, 1.5]).
- Seeni et al. (2019) investigated associations between acute prenatal exposure to NO_2 and NO_X and NICU admissions in the Consortium of Safe Labor (CSL: 2002 to 2008) cohort. In this case-crossover study, there was increased odds of NICU admissions for both NO_2 and NOx the day of delivery (OR for NO_2: 1.13 [95% CI: 1.10, 1.17) and OR for NO_X: 1.08 [95% CI: 1.05, 1.10]) and day before delivery (OR for NO_2: 1.13 [95% CI: 1.10, 1.17] and OR for NO_X: 1.04 [95% CI: 1.02, 1.08]), while exposure in lag week 1 had associations with lower magnitude and the CIs included the null (OR for NO_2: 1.03 [95% CI: 0.99, 1.07] and OR for NO_X: 1.01 [95% CI: 0.98, 1.05]).
- Ashley-Martin et al. (2016) reported positive associations between average maternal exposure to NO_2 and elevated cord blood concentrations of IL-33 and thymic stromal lymphopoietin, potential predictors of childhood allergy, among female newborns in the Canadian MIREC cohort.

7.6.5.1.1 Conclusions and Remaining Uncertainties

There was a small body of recent epidemiologic studies across various apical birth outcomes other than prenatal growth, preterm birth, birth defects, and infant mortality. The small number of studies and various apical endpoints limits the ability to judge coherence and consistency across these studies.

7.6.6 Synthesis Across Lines of Evidence

Overall, the associations between exposure to oxides of nitrogen, primarily NO_2, and prenatal growth were inconsistent across the different apical endpoints in the epidemiologic studies. Among the recent evidence, birth weight was the most common metric of prenatal growth evaluated. While there was a general pattern of decreased birth weight, there were inconsistent associations across other prenatal growth indicators including SGA. While the evidence is limited, there was also a consistent pattern of

decreased head circumference and abdominal circumference, but more studies are needed to judge the consistency and coherence of this pattern of association. Additional uncertainties include the lack of information on the shapes of concentration-response relationships and confidence in those relationships over the distribution of exposures, the potential modifying impact of maternal comorbidities, and the limited information available on potential confounding by copollutants, particularly those most closely associated with traffic emissions. These uncertainties limit the ability to judge coherence and consistency across studies of prenatal growth. There is limited animal toxicological evidence examining the effects of oxides of nitrogen exposure on prenatal growth. One previous study in rats and a recent study in mice both reported reductions in birth weight, suggesting this may be a potentially sensitive outcome for NO_2 exposure, but future studies are still needed to confirm and corroborate these findings. Additional studies to investigate a potential dose-response relationship and a potential mechanism of action would also meaningfully contribute to existing gaps in knowledge.

Among the recent epidemiologic evidence, the associations between exposure to NO_2 and PTB were inconsistent overall. There were different exposure periods evaluated (trimester, entire pregnancy, hourly), different exposure assessment methods (CMAQ, LUR, central site monitors, empirical Bayesian kriging, spatiotemporal/geospatial model, hybrid model), and across different geographies (United States, Canada, Australia, Europe). Additionally, some recent epidemiologic studies utilized study designs to examine short-term exposure to NO_2 in association with PTB to identify critical windows of exposure, but there was no clear critical window of exposure. A single study in Australia evaluated the concentration response relationship of NO_2 associations with PTB, reporting a threshold at 7.6 ppb. However, a single study makes it difficult to judge consistency or coherence across different study designs and evidence lines. Additionally, several recent studies also considered the association between exposure to NO_X and PTB, but the associations were inconsistent overall. Furthermore, there was limited evidence evaluating factors (SES, race/ethnicity, pre-existing comorbidities) that could modify associations between exposure to NO_2 and PTB, but the evidence was inconsistent and limited. While there were a few recent studies that evaluated the potential of copollutant confounding, there were inconsistent associations overall. Among the studies evaluating copollutants, most of the studies only considered co-adjusted models, with moderate to high correlations among copollutants, which may bias the estimates. Overall, there were inconsistent associations with PTB and exposure to NO_2 and NO_X in the recent epidemiologic evidence. There is limited animal toxicological evidence examining the effects of oxides of nitrogen on preterm birth. The only available studies are recently published studies in mice which both reported no effects on the duration of gestation. Although no effects were observed, there is a lack of sufficient evidence to determine whether there are no negative effects. Thus, additional studies are still needed to fully assess the potential impacts of NO_2 exposure on preterm birth and gestation duration in animal models.

The recent epidemiological evidence evaluating the association of exposure to oxides of nitrogen with birth defects was limited. Among the recent epidemiologic evidence, the associations across all birth defect subtypes were inconsistent. These recent studies of other birth defects did not evaluate the concentration response relationship between exposure to oxides of nitrogen and these birth defects,

factors (maternal age, race/ethnicity, SES) that may modify associations, or potential confounding by copollutants. There were no recent toxicological studies that examined the associations between exposure to oxides of nitrogen and birth defects. The small body of evidence limits the ability to judge coherence and consistency across the lines of evidence.

There was a small body of recent epidemiologic studies evaluating infant mortality although the studies lacked consistency across exposure windows (short-term vs. long-term) as well as outcome definitions (e.g., infant mortality versus neonatal mortality versus postneonatal mortality). There are remaining uncertainties regarding concentration response, lifestages and populations potentially at increased risk, and potential confounding by copollutants. Furthermore, there is limited and inconsistent animal toxicological evidence examining the effects of exposure to oxides of nitrogen on offspring mortality, with some studies reporting increased mortality and others reporting null effects as a result of gestational NO_2 exposure. The exposure window used in all the available studies was GD 0 to birth (PND 0, approximately 20 to 21 days), with the exception of one study that began dosing at GD 14. Thus, the primary driver for the observed contrast in results is likely more attributable to other differences in study design such as dose and species. Indeed, no effects were observed at doses of 2.5 ppm or lower, suggesting that higher doses may be required to elicit effects on offspring mortality. Further, effects were only observed in rats, and none were observed in mice, suggesting there may be species-dependent sensitivity to NO_2. Additional studies to confirm a dose-response relationship and any inter-species differences in sensitivity would meaningfully contribute to this gap in knowledge. Overall, the small number of studies limits the ability to judge coherence and consistency across the lines of evidence.

Overall, the epidemiologic and toxicological evidence assessing the impacts of exposure to oxides of nitrogen on birth outcomes is inconsistent, and for some apical outcomes, the evidence is limited. The strongest evidence comes from epidemiologic studies reporting a generally consistent pattern of positive associations between exposure to NO_2 and decreased birth weight. Among the epidemiologic studies, there remains uncertainty regarding the concentration-response relationship, potential differences across lifestages and populations, and the potential for copollutant confounding. Furthermore, there are limited animal toxicological studies available. While a single previous study in rats and a recent study in mice both reported reductions in birth weight, supporting the findings of the recent epidemiologic studies, their evidence is limited overall to judge consistency and coherence across the lines of evidence.

7.6.7 Biological Plausibility

This section describes biological pathways that potentially underlie effects on birth outcomes resulting from exposure to oxides of nitrogen. 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 female reproductive tissues involved in fetal development and birth outcomes (e.g., placenta, uterine tissue). Evidence supporting these proposed pathways was derived from Sections 7.6.1 through 7.6.5 of this ISA, evidence reviewed in the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016), and recent evidence collected from studies that may not meet the current PECOS criteria but contain mechanistic information supporting these pathways. Discussion of how exposure to oxides of nitrogen may lead to reproductive and developmental effects contributes to an understanding of the biological plausibility of epidemiologic results.

The 2016 Oxides of Nitrogen ISA reported that the strongest evidence for the effects of NO_X on birth outcomes was the observed associations with NO_X exposure and fetal growth restriction in a well-designed and well-conducted study in a Spanish cohort (Iñiguez et al., 2012; Estarlich et al., 2011; Aguilera et al., 2010; Ballester et al., 2010). This was supported by a previous toxicological study that reported reduced birth weight of rat pups from dams exposed for three months prior to mating (Shalamberidze and Tsereteli, 1971), and a recent toxicological study that reported reduced body weight of mouse pups on PND 1 following exposure to 2,500 ppm NO_2 for the entire duration of pregnancy (approximately 20 days) (Yan et al., 2020). However, mechanistic data are lacking, and a clear mechanism of action through which NO_X may act to negatively impact birth weight is still unclear. Some studies suggest the involvement of inflammatory processes (van den Hooven et al., 2012a), epigenetic changes (Aguilera et al., 2022; Ladd-Acosta et al., 2019; Gruzieva et al., 2016), and placental mitochondrial content (Clemente et al., 2015), but more direct links remain to be established.

7.6.8 Summary of Lifestages and Populations

Some populations and lifestages may be at greater risk of oxides of nitrogen-related health effects than the general population. Higher risks could be due to intrinsic[8], extrinsic[9], 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

[8]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.
[9]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. 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. Additionally, studies examining whether factors may result in differential exposure to oxides of nitrogen are included. Table 7-5 at the end of this section summarizes the evidence for each factor to increase the risk of oxides of nitrogen-related health effects.

7.6.8.1 Pre-existing Comorbidities

In the 2016 Oxides of Nitrogen ISA, there were no studies available that evaluated the potential for maternal pre-existing comorbidities to modify associations between exposure to oxides of nitrogen and birth outcomes. Among the recent epidemiologic evidence, maternal comorbidities were evaluated as potential modifiers on the associations between NO_2 and prenatal growth, specifically term low birth weight and SGA, and PTB; however, the evidence is limited overall. Among the studies of prenatal growth, either term low birth weight or SGA, there was no evidence of effect measure modification for maternal pre-existing comorbidities of asthma, hypertension, heart disease, diabetes, gestational diabetes, or pre-eclampsia (Lavigne et al., 2016b). Additionally, Williams et al. (2021) hypothesized that women with Type 1 diabetes and their infants would have a greater risk of poor outcomes when exposed to air pollution compared to women without autoimmune disorders and their infants. Overall, there were null associations with exposure to NO_X among women with Type 1 diabetes compared to women without autoimmune disorders for SGA across the preconception exposure period, first trimester, second trimester, or entire pregnancy.

Furthermore, several recent studies evaluated the potential modification of the association between NO_2 or NO_X exposure and PTB by maternal pre-existing comorbidities (Williams et al., 2021; Weber et al., 2019; Lavigne et al., 2016b; Li et al., 2016b; Mendola et al., 2016). While Li et al. (2016b) included adjustment for an aggregate for pre-pregnancy medical conditions (yes/no), other studies evaluated the impact of specific conditions, including asthma (Lavigne et al., 2016b; Mendola et al., 2016), hypertension (Weber et al., 2019; Lavigne et al., 2016b); diabetes (Lavigne et al., 2016b), and Type 1 diabetes (Williams et al., 2021). Some pre-existing maternal comorbidities modified the association between NO_2 or NO_X exposure and PTB, but not consistently across studies. Among women who reported having pre-pregnancy medical conditions as an aggregate measure, Li et al. (2016b) reported increased risk of NO_2-associated PTB compared to women who reported having no pre-pregnancy medical conditions in Brisbane, Australia. In a U.S.-based cohort, Mendola et al. (2016)

evaluated associations between women with and without asthma in the Consortium of Safe Labor. Overall, the pattern of association between exposure to NO_X and PTB suggested that NO_X-associated PTB risk was higher among women with asthma compared to women without asthma. Additionally, Lavigne et al. (2016b) reported that women with chronic diabetes had a larger risk of NO_2-associated PTB compared to women without chronic diabetes among births in Ontario. However, a few other studies did not find evidence of effect measure modification (Williams et al., 2021; Weber et al., 2019; Lavigne et al., 2016b), but these studies evaluated different maternal pre-existing comorbidities (asthma, hypertension, heart disease, gestational diabetes, preeclampsia, and Type 1 diabetes). Overall, the collective evidence is inadequate to determine whether maternal pre-existing comorbidities result in modification of the associations between exposure to oxides of nitrogen and birth outcomes.

7.6.8.2 Sociodemographic Factors

Sociodemographic factors encompass the broad categories of lifestages (e.g., children, adults), socioeconomic status (SES), race/ethnicity, sex, residence in an urban area, and/or proximity to roadways.

Race/ethnicity

In the 2016 Oxides of Nitrogen ISA, there were no studies available that evaluated the potential for race/ethnicity to modify associations between exposure to oxides of nitrogen and birth outcomes. Among the recent epidemiologic evidence, there were a limited number of studies that evaluated the potential for race/ethnicity to modify the associations between exposure to NO_2 and NO_X and PTB. Overall, the associations were inconsistent. While the association between exposure to NO_2 and PTB overall was null among a subset of participants in the GeoBirth Cohort in the Philadelphia area, when stratified by maternal race/ethnicity non-Hispanic Asians and other races had increased odds, although the CIs included the null (Escoto et al., 2022). Associations remained null for non-Hispanic Black, Hispanic, and non-Hispanic white mothers. Among births in Georgia, Hao et al. (2016) reported higher odds of PTB among Black mothers compared to other races (white, Asian, American Indian or Alaskan, Native Hawaiian or Pacific Islander, or multiracial). Among pregnant women in Oakland and San Jose, California, U.S., (2013 to 2015), Riddell et al. (2022) reported inconsistent and imprecise associations among non-Latina whites, non-Latina Blacks, non-Latina Asians, and Latinas. Overall, the collective evidence is inadequate to determine whether race/ethnicity results in modification of the associations between exposure to oxides of nitrogen and birth outcomes.

Socioeconomic Status

SES is a composite measure that usually consists of economic status measured by income, social status measured by education, and work status measured by occupation. In the 2016 Oxides of Nitrogen ISA, there were no studies available that evaluated the potential for SES to modify associations between exposure to oxides of nitrogen and birth outcomes. There were a few different SES metrics that were evaluated as factors that may modify associations between exposure to NO_2 and PTB. Overall, the impact

of modification by SES metrics was inconsistent. Among participants in France, Genin et al. (2022) included an ecological adjustment for social deprivation and reported higher magnitude of risk of overall PTB. However, in stratified analyses for SES index (low level and high level), Li et al. (2016b) reported similar positive associations with the adjustment for SES index. Among births in Georgia, U.S., in 2002 to 2006, Hao et al. (2016) used maternal education as a proxy measure for SES. Among mothers with less than a high school education there were higher odds for PTB, compared to mothers with higher than a high school education. Overall, the collective evidence is inadequate to determine whether SES results in modification of the associations between exposure to oxides of nitrogen and birth outcomes.

Table 7-5 Summary of evidence for populations at increased risk of effects on birth outcomes from oxides of nitrogen exposure

	Key Evidence	Key References
Inadequate evidence		
Maternal comorbidities	Prenatal growth: Limited and inconsistent evidence	†Lavigne et al. (2016b); †Williams et al. (2021)
	Preterm birth: Limited and inconsistent evidence	†Mendola et al. (2016); †Lavigne et al. (2016b); †Weber et al. (2019); †Li et al. (2016b); †Williams et al. (2021)
Race/ethnicity	Preterm birth: Limited and inconsistent evidence	†Escoto et al. (2022); †Hao et al. (2016); †Riddell et al. (2022)
SES	Preterm birth: Limited and inconsistent evidence	†Genin et al. (2022); †Li et al. (2016b); †Hao et al. (2016)

List of abbreviations in alphabetical order: SES = socioeconomic status.
†Studies published since the 2016 Oxides of Nitrogen ISA.

7.6.9 Summary and Causality Determination

The 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016)concluded that the evidence was suggestive of, but not sufficient to infer, a causal relationship between exposure to oxides of nitrogen and birth outcomes. Epidemiologic studies summarized in the 2016 Oxides of Nitrogen ISA investigated the associations between NO_2 exposure and fetal growth, PTB, birth defects, and early life mortality. Only a single animal toxicological study was available, and it investigated the effects of NO_2 exposure on birth weight and early life mortality.

In the 2016 Oxides of Nitrogen ISA, the epidemiologic studies tended to report negative associations with NO_2 exposure and fetal growth measurements such as head circumference, abdominal circumference, biparietal diameter, fetal length, and fetal weight, SGA, and IUGR. Associations between oxides of nitrogen exposure and PTB were inconsistent, and the quantity and quality of studies that reported positive associations between NO_2 exposure and PTB was similar to the quantity and quality of studies that reported null associations. Epidemiological evidence regarding the relationship between NO_2 exposure and birth weight and LBW was similarly inconsistent, with many studies reporting associations between NO_2 exposure and lower birth weight, while others report null associations. Epidemiologic studies that assessed the relationship between exposure to oxides of nitrogen sometimes reported increased odds of cardiac birth defects, but this was not entirely consistent. The associations between oxides of nitrogen exposure and early life mortality outcomes (e.g., stillbirth, spontaneous abortion, fetal loss, neonatal mortality, post-neonatal mortality) were also inconsistent. Only a single toxicological study that investigated birth outcomes was available. This study reported that NO_2 exposure reduced birth weight of pups that were gestationally exposed to NO_2, but no other birth outcomes were assessed.

Among the recent evidence, the associations between exposure to NO_2 and prenatal growth were inconsistent across the different apical endpoints in the epidemiologic studies. The most common metric of prenatal growth evaluated was birth weight. While there was a general pattern of decreased birth weight, there were inconsistent associations across other prenatal growth indicators including SGA. While the evidence is limited, there was also a consistent pattern of decreased head circumference and abdominal circumference, but more studies are needed to judge consistency and coherence of this pattern of association. Additional uncertainties result from the lack of information on the shapes of concentration-response relationships and confidence in those relationships over the distribution of exposures, the potential modifying impact of maternal comorbidities, and the limited information available on potential confounding by copollutants, particularly those most closely associated with traffic emissions. These uncertainties limit the ability to judge coherence and consistency across studies of prenatal growth. There is limited animal toxicological evidence examining the effects of NO_2 exposure on prenatal growth. One previous study in rats and a recent study in mice both reported reductions in birth weight, suggesting this may be a potentially sensitive outcome for NO_2 exposure, but future studies are still needed to confirm and corroborate these findings. Additional studies to investigate a potential dose-response relationship and a potential mechanism of action would also meaningfully contribute to existing gaps in knowledge.

Among the recent epidemiologic evidence, the associations between exposure to NO_2 and PTB were inconsistent overall. There were different exposure periods evaluated (trimester, entire pregnancy, hourly), different exposure assessment metrics (CMAQ, LUR, central site monitors, empirical Bayesian kriging, spatiotemporal/geospatial model, hybrid model), and across different geographies (United States, Canada, Australia, Europe). Additionally, some recent epidemiologic studies utilized study designs to examine short-term exposure of NO_2 in association with PTB to identify critical windows of exposure, there was no clear critical window of exposure. A single study in Australia evaluated concentration response relationship of NO_2 on associations with PTB, reporting a threshold at 7.6 ppb. However, a

single study makes it difficult to judge consistency or coherence. Additionally, several recent studies also considered exposure to NO_X in association with PTB, but the associations were inconsistent overall. Furthermore, there was limited evidence evaluating factors (SES, race/ethnicity, pre-existing comorbidities) that could modify associations between exposure to NO_2 and PTB, but the evidence was inconsistent and limited. While there were a few recent studies that evaluated the potential of copollutant confounding, there were inconsistent associations overall. Among the studies evaluating copollutants, most of the studies only considered co-adjusted models, with moderate to high correlations among copollutants, which may bias the estimates. Overall, there were inconsistent associations with PTB and exposure to NO_2 and NO_X in the recent epidemiologic evidence. There is limited toxicological evidence examining the effects of oxides of nitrogen on preterm birth. The only available studies are recently published studies in mice which both reported no effects on duration of gestation. Although no effects were observed, there is a lack of sufficient evidence to determine there are no negative effects. Thus, additional studies are still needed to fully assess the potential impacts of NO_2 exposure on preterm birth and gestation duration in animal models.

The recent epidemiological evidence was limited for birth defects in association with exposure to oxides of nitrogen. Among the recent epidemiologic evidence, the associations across all birth defect subtypes were inconsistent. The small body of evidence limits the ability to judge coherence and consistency across these studies. These recent studies of other birth defects did not evaluate concentration response relationship between exposure to oxides of nitrogen and these birth defects, factors (maternal age, race/ethnicity, SES) that may modify associations, or potential confounding by copollutants. There were no recent animal toxicological studies that examine the associations between exposure to oxides of nitrogen and birth defects.

There was a small body of recent epidemiologic studies for infant mortality although the studies lacked consistency across exposure windows (short-term versus long-term) as well as outcome definitions (e.g., infant mortality versus neonatal mortality versus postneonatal mortality). The small number of studies limits the ability to judge coherence and consistency across these studies. There are remaining uncertainties regarding concentration response, lifestages and populations potentially at increased risk, and potential confounding by copollutants. Furthermore, there is limited and inconsistent toxicological evidence examining the effects of exposure to oxides of nitrogen on offspring mortality, with some studies reporting increased mortality and others reporting null effects as a result of gestational NO_2 exposure. The exposure window used in all the available studies was GD 0 to birth (PND 0, approximately 20 to 21 days), with the exception of one study that began dosing at GD 14. Thus, the primary driver for the observed contrast in results is likely more attributable to other differences in study design such as dose and species. Indeed, no effects were observed at doses of 2.5 ppm or lower, suggesting that higher doses may be required to elicit effects on offspring mortality. Further, effects were only observed in rats, and none were observed in mice, suggesting there may be species-dependent sensitivity to NO_2. Additional studies to confirm a dose-response relationship and any species differences in sensitivity would meaningfully contribute to this gap in knowledge.

Overall, the evidence is *suggestive of, but not sufficient to infer, a causal relationship* between long-term oxides of nitrogen exposure and birth outcomes. The strongest evidence comes from epidemiologic studies reporting a generally consistent pattern of positive associations between exposure to NO_2 and decreased birth weight (see Table 7-6). Among the epidemiologic studies, there remains uncertainties regarding the concentration-response relationship, potential differences across lifestages and populations, and the potential for copollutant confounding. Furthermore, there is limited toxicological studies available. While a single previous study in rats and a recent study in mice both reported reductions in birth weight, supporting the findings of the recent epidemiologic studies, there evidence is limited overall to judge consistency and coherence across the lines of evidence.

Table 7-6 Summary of evidence contributing to causality determinations for oxides of nitrogen exposure and effects of birth outcomes

Rationale for Causality Determination[a]	Key Evidence[b]	Key References	Oxides of Nitrogen Concentrations Associated with Effects
Prenatal Growth			
Evidence from epidemiologic studies generally supportive but not consistent for birth weight	Decreased birth weight in multiple study populations, across different geographic areas, different exposure assessment metrics, and exposure windows	Section 7.6.1.1	Mean NO_2: 2.7-25 ppb for trimester and pregnancy averages
Limited evidence from epidemiologic studies for prenatal growth outcomes	Decreased head circumference and decreased abdominal circumference. Uncertainties regarding concentration response and exposure response, lifestages and population differences, and copollutant confounding.	†Leung et al. (2023) †Peterson et al. (2022) †Hjortebjerg et al. (2016) Section 7.6.1.1	Median NO_2: 15.3-23 ppb for weekly avg Median NO_2: 6-9 ppb for monthly avg
	Inconsistent associations for SGA. Uncertainties regarding concentration response and exposure response, lifestages and population differences, and copollutant confounding.	Section 7.6.1.1	Mean NO_2: 13.4-16.7 ppb for annual avg Median NO_2: 7.3 ppb for 24-hr avg
Limited evidence from toxicological studies for birth weight	Decreased birth weight in rats and mice.	Shalamberidze and Tsereteli (1971) Yan et al. (2020) Section 7.6.1.2	1.25–2.5 ppm NO_2

Rationale for Causality Determination[a]	Key Evidence[b]	Key References	Oxides of Nitrogen Concentrations Associated with Effects
Preterm Birth			
Inconsistent epidemiologic evidence for preterm birth	Some studies report positive associations with PTB, while other report no associations or negative associations.	Section 7.6.2.1	Mean NO_2: 6.52 ppb for 24-hr avg Mean NO_2: 15.4-19.15 ppb for monthly avg Median NO_2: 5.58-16 ppb for annual avg
Birth Defects			
Limited and inconsistent epidemiologic evidence for birth defects	Some studies report positive associations with birth defects, while others report no associations or negative associations.	Section 7.6.3.1	Mean NO_2: 11.3-18.3 ppb for trimester avg
Infant Mortality			
Limited and inconsistent epidemiologic evidence for infant mortality	Some studies reported positive associations with infant mortality, while other studies reported null associations.	Section 7.6.4.1	Mean NO_2: 5.72-17.94 ppb for annual avg
Limited and inconsistent toxicological evidence for infant mortality	Some studies reported increased offspring viability/mortality, while others reported null associations.	Di Giovanni et al. (1994) Tabacova et al. (1985) †Yan et al. (2020) †Yue et al. (2017) †Yue et al. (2018) Section 7.6.4.2	0.05–10 mg/m^3 NO_2 for 21 d 0.532–5.32 ppm NO_2

List of abbreviations in alphabetical order: d = day, GD = gestational day, hr = hour, NO_2 = nitrogen dioxide, NO_X = oxides of nitrogen, ppb = parts per billion, ppm = parts per million, PTB = preterm birth, SGA = small-for-gestational-age.
[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.

7.7 Effects on Postnatal Development

The 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016) concluded that the evidence was inadequate to infer the presence or absence of a causal relationship between oxides of nitrogen exposure and postnatal development. Overall, there was limited epidemiologic and toxicological studies, and the small number of studies limited the ability to judge coherence and consistency across these studies. The current epidemiologic and animal toxicological evidence is still limited. This section covers postnatal

development (growth indicators) and puberty. This section does not cover associations between exposure to oxides of nitrogen and neurodevelopmental outcomes, which are discussed in detail in Chapter 9.

7.7.1 Postnatal Growth

The 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016) included a small number of animal toxicological studies examining NO_2 exposure and physical postnatal development, with no epidemiologic studies on postnatal growth. The toxicological studies examined impaired physical development and reported some reductions in postnatal growth, but some studies reported that the dams experienced overt toxicity, increasing uncertainty in the reported results. The recent epidemiologic evidence is limited and inconsistent, with no recent toxicological studies. Study specific details including study population characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), potential confounders, and select results are in Appendix A – Appendix Table 7-15.

7.7.1.1 Epidemiologic Studies of Postnatal Growth

In the 2016 Oxides of Nitrogen ISA, there were no epidemiologic studies examining the associations between oxides of nitrogen exposure and postnatal growth. Evidence from recent studies is summarized below and described in more detail in Appendix A – Appendix Table 7-15. The recent evidence is limited, and there were inconsistent associations between oxides of nitrogen exposure and postnatal growth. These studies used different exposure assessment methods (LUR, AQS), different study locations, and different metrics of postnatal growth at different exposure time periods.

- Alterman et al. (2023) used a distributed-lag nonlinear model to evaluate the associations between weekly postnatal exposure to NO_2 and NO_X, estimated from a dispersion model, and rapid weight gain in infants in a population-based cohort in Israel. Cumulatively across gestation weeks, there was an increased risk of rapid weight gain for the prenatal NO_2 exposure period (RR: 1.04 [95% CI: 1.02, 1.05] per 7.3 ppb increase in NO_2). Additionally, cumulatively across the first four postnatal weeks, there was an increased risk of rapid weight gain (RR: 1.03 [95% CI: 1.02, 1.04] per 7.3 ppb increase in NO_2). The risks were similar for exposure to NO_X, with increased risk of rapid weight gain for the prenatal exposure period (RR: 1.048 [95% CI: 1.036, 1.061] per 11.4 ppb increase in NO_X) and postnatal exposure period (RR: 1.024 [95% CI: 1.016, 1.032] per 11.4 ppb increase in NO_X).
- Among preschoolers (age four) in the Spanish INMA (Infancia y Medio Ambiente) birth cohort, first trimester exposure to NO_2, estimated from a temporally adjusted LUR model, there were inverse associations with standardized height (zheight), standardized weight (zweight), and standardized BMI (zBMI), but the CIs included the null (Fossati et al., 2020). Furthermore, there was no associations with risk of overweight/obesity status or zBMI trajectory classes in preschoolers.
- Vrijheid et al. (2020) used an exposome approach (Ex-WAS) to systematically assess the associations between a wide array of ubiquitous environment exposures measured prenatally

and during childhood with obesity indicators in primary school aged children. Outdoor NO_2 was estimated through a LUR model developed in the context of the ESCAPE project and indoor NO_2 was estimated using passive samplers. For outdoor NO_2 exposure during childhood, there was an increased zBMI score (β: 0.23 [95% CI: 0.02, 0.43] per 9.94 ppb increase in NO_2 and an increased zBMI score (β:0.11 [95% CI: -0.04, 0.26] per 49.32 ppb increase in NO_2) with indoor NO_2, but the estimate for indoor NO_2 included the null.

- Amongst participants in the Maternal and Development Risks from Environmental and Social Stressor (MADRES) cohort, Ji et al. (2023) estimated exposure to NO_2 by using inverse distance squared weighted spatial interpolation based on AQS data and examined associations with infant growth. Comparing change in average prenatal exposure in NO_2 from the 10th percentile to the 90th percentile, the estimated growth in weight was 1.56% (95% CI: 1.17%, 2.07%) higher for exposure during the third trimester to three months. However, the estimated growth in weight was 0.90% (95% CI: 0.84, 0.98) lower for exposure from six months to two years. There was a 7.79% (95% CI: -12.00%, -3.38%) decrease in infant attained weight at two years old, when comparing change in pregnancy-averaged exposure to NO_2 from the 10th percentile to the 90th percentile. In the multipollutant model with $PM_{2.5}$, PM_{10}, and O_3, the magnitude was increased, with a 11.07% (95% CI: -16.39, -5.41) decrease in infant attained weight at two years old, when comparing change in pregnancy-averaged exposure to NO_2 from the 10th percentile to the 90th percentile.
- Among participants in the Children's Health Study, the associations between in utero and first year of life exposure to near-roadway NO_X, estimated from CALINE4, and BMI measurements were examined (Kim et al., 2018). A 40.1 ppb difference in near-road freeway NO_X in utero exposure was associated with 0.5 kg/m^2 (95% CI: -0.02, 0.10) faster increase in BMI per year and a 0.1 kg/m^2 (95% CI: -0.30, 0.50) higher BMI at age 10 years. In first year of life, a 39.1 ppb difference near-road freeway NO_X exposure was associated with a 0.1 kg/m^2 (95% CI: 0.03, 0.20) faster increase in BMI growth per year, resulting in a 0.5 kg/m^2 (95% CI: 0.02, 0.9) higher attained BMI at age 10 years. A 14.7 ppb difference in non-freeway NO_X in utero exposure was associated with 0.03 kg/m^2 (95% CI: -0.05, 0.10) faster increase in BMI per year and 0.1 kg/m^2 (95% CI: -0.30, 0.60) higher attained BMI at age 10. A 18.7 ppb different in non-freeway NO_X exposure in the first year of life was associated with a -0.02 kg/m^2 (95% CI: -0.10, 0.06) faster increase in BMI growth per year, with a -0.07 kg/m^2 (95% CI: -0.50, 0.40) higher attained BMI at age 10.
- Patterson et al. (2021) examined the associations between prenatal residential exposure to NO_2, estimated from AQS with inverse distance-squared weighting, and changes in infant measure of growth and adiposity from one to six months of life among Hispanic mother-infant dyads from the Southern California Mother's Milk Study. There was increased infant weight (β: 0.14 kg [95% CI: 0.02, 0.25]) and increased total subcutaneous fat (β: 1.69 mm [95% CI: 0.25, 3.12]). There were no associations with infant length, umbilical circumference, or the ratio of central to total subcutaneous fat. In addition, there was no evidence of effect measure modification by infant sex.

7.7.1.1.1 Conclusions and Remaining Uncertainties

There was a small body of recent epidemiologic studies across various postnatal growth metrics, mostly focused on BMI. However, the small number of studies limits the ability to judge coherence and consistency across these studies. There are remaining uncertainties regarding concentration-response relationship, lifestages and population differences, and potential confounding by copollutants.

7.7.1.2 Animal Toxicological Studies of Postnatal Growth

No animal toxicological studies that investigated the effects of oxides of nitrogen exposure on postnatal growth were reported in the 2016 Oxides of Nitrogen. A single study reported effects on postnatal growth in the 2008 Oxides of Nitrogen ISA (Di Giovanni et al., 1994). Di Giovanni et al. (1994) exposed female Wistar rats to 1.5 or 3 ppm NO_2 from GD 0 to GD 20, and observed null effects on male rat pup weight gain up to PND 21. The 1993 Oxides of Nitrogen AQCD summarized a few toxicological studies that investigated the effects of oxides of nitrogen exposure on postnatal growth, all of which reported reduced metrics of postnatal growth in offspring. One study reported a significant reduction in the skeletal length and body weight of male and female rats on PND 62 exposed continuously from GD 14 through PND 62 to 10 ppm of NO_2 (Freeman et al., 1974). However, of note is that some dams experienced overt toxicity, and authors report that some pregnant rats died prior to giving birth. Another study exposed female albino rats to 0.126 or 2.36 mg/m^3 NO_2 (0.067 or 1.25 ppm NO_2) for 12 hour/day for three months, after which they were mated to untreated male rats (Shalamberidze and Tsereteli, 1971). This pre-conception exposure reduced offspring body weights in the 1.25 ppm group (but not in the 0.067 ppm group) at all time points assessed (PND 4, 8, and 12) (Shalamberidze and Tsereteli, 1971). Similarly, Tabacova et al. (1985) exposed pregnant female Wistar rats to 1 or 10 mg/m^3 NO_2 (0.532 or 5.32 ppm NO_2) from GD 0 until birth (PND 0) and assessed pup body weight (of offspring in the 0.0532 or 5.32 ppm groups only) at multiple time points (PND 1, 4, 7, 21, 30, and 60). Offspring body weights were significantly reduced, but only in the 5.32 ppm group on PND 21 (Tabacova et al., 1985). Collectively, previous studies tended to report reduced offspring body weight following NO_2 exposures of 1.25 ppm or higher, with the exception of the results reported by (Di Giovanni et al., 1994), who reported null effects in male pups (female pups were not evaluated). No recent animal toxicological studies that investigated the effects of oxides of nitrogen on postnatal growth were available for comparison.

7.7.1.2.1 Conclusions and Remaining Uncertainties

There is limited animal toxicological evidence examining the effects of exposure to oxides of nitrogen on postnatal growth. The only available evidence comes from previously published studies in rats, and all but Di Giovanni et al. (1994) reported reductions in postnatal growth metrics. The information reported does not clearly indicate that NO_2 exposure affects postnatal growth, although further investigation is warranted. The available studies only reported effects at doses of 1.25 ppm or higher, although this was not consistent. Thus, studies that investigate a broad range of doses to further interrogate the potential dose-response relationship would be particularly informative. Further, there is uncertainty regarding possible sex-specific effects.

7.7.2 Puberty

In the 2016 Oxides of Nitrogen ISA (U.S. EPA, 2016), there were no epidemiologic or animal toxicological studies available that evaluated associations between oxides of nitrogen and puberty. There was a single recent epidemiologic study of puberty, but no recent toxicological studies evaluating exposure to oxides of nitrogen and puberty.

7.7.2.1 Epidemiologic Studies of Puberty

There were no epidemiologic studies of oxides of nitrogen and puberty in the 2016 Oxides of Nitrogen ISA. In the recent epidemiologic evidence, there was only a single study exploring the associations between exposure to oxides of nitrogen and puberty. Study specific details including study population characteristics exposure details (assessment metrics, temporal scale, and spatial scale), exposure assessment metrics, potential confounders, and select results are in Appendix A – Appendix Table 7-15.

- In a recent cross-sectional study, Zhao et al. (2021) examined the associations between long-term exposure to NO_2, estimated from the ESCAPE project LUR model, and pubertal development based on sex hormone concentrations of estradiol in females and testosterone in males among children at age 10 in Germany. There were inconsistent associations overall. Amongst females, there was decreased odds (OR: 0.807 [95% CI: 0.360, 1.806]) between exposure to NO_2 and puberal onset, represented by estradiol, but the CI included the null. Amongst males, there was increased odds (OR: 1.305 [95% CI: 0.609, 2.799]) overall with pubertal onset, represented by testosterone, but the CI was wide and included the null.

7.7.2.1.1 Conclusions and Remaining Uncertainties

A recent single study reported associations with markers of puberty in children at age 10, but the ability to judge coherence and consistency is limited. There still remains uncertainties regarding concentration-response relationship, lifestages and population differences, and potential copollutant confounding.

7.7.3 Other Postnatal Development Outcomes

There was a single recent study that evaluated associations between exposure to oxides of nitrogen and other postnatal developmental outcomes in the epidemiologic literature, and one previous toxicological study that investigated effects on developmental milestones, but no recent animal toxicological literature was available.

7.7.3.1 Epidemiologic Studies of Other Postnatal Development Outcomes

In a recent epidemiologic study in the Dutch PIAMA birth cohort examined the associations between green space, ambient air pollution, and traffic noise with diurnal cortisol slope, a marker of chronic stress, in children 12 years of age (Bloemsma et al., 2021). Exposure to NO_2 was estimated from LUR model developed for the ESCAPE project. There was a null association between NO_2 and diurnal slope cortisol (β: 0.059 nmol/L [95% CI: -0.047, 0.165]). Study-specific details including study population characteristics, exposure details (assessment metrics, temporal scale, and spatial scale), potential confounders, and select results are in Appendix A – Appendix Table 7-15.

7.7.3.1.1 Conclusions and Remaining Uncertainties

Because there was only a single study evaluating the associations between NO_2 and diurnal cortisol in children, the ability to judge coherence and consistency is limited. There still remains uncertainties regarding concentration response relationship, lifestages and population differences, and potential copollutant confounding.

7.7.3.2 Animal Toxicological Studies of Other Postnatal Development Outcomes

No animal toxicological studies that investigated the effects of oxides of nitrogen exposure on other postnatal developmental outcomes were reported in the 2016 Oxides of Nitrogen ISA or the 2008 Oxides of Nitrogen ISA. One study in the 1993 Oxides of Nitrogen AQCD exposed pregnant female Wistar rats to 0.05, 0.1, 1, or 10 mg/m^3 NO_2 (0.0266, 0.0532, 0.532, or 5.32 ppm NO_2) throughout gestation (GD 0 to PND 0) and assessed timing of developmental milestones, including incisor eruption and eye opening (Tabacova et al., 1985). Tabacova et al. (1985) reported that incisor eruption and eye opening were delayed in the 5.32 and 0.532 ppm NO_2 groups, but not in the 0.0532 or 0.0266 ppm NO_2 groups. No recent studies that investigated the effects of oxides of nitrogen on developmental milestones or other developmental outcomes were available for comparison.

7.7.3.2.1 Conclusions and Remaining Uncertainties

There is limited animal toxicological evidence on the effects of NO_2 exposure on other postnatal developmental outcomes such as developmental milestones. Only a single, previous study was available, and it reported delays in incisor eruption and eye opening of offspring born to pregnant rats dosed throughout gestation. However, no other studies are available to corroborate or expand upon these findings, and additional studies would add confidence to the evidence base. Additionally, future studies that assess any potentially sex-specific impacts on developmental milestones are also needed.

7.7.4 Synthesis Across Lines of Evidence

There remains limited evidence regarding effects related to exposure to oxides of nitrogen and postnatal development, including postnatal growth and puberty. There were no recent animal toxicological studies evaluating the effects of exposure to oxides of nitrogen on postnatal development or puberty. Furthermore, there are limited recent epidemiologic studies evaluating the associations between oxides of nitrogen and postnatal growth and puberty. In the recent epidemiologic studies, there was a small body of evidence exploring different metrics of BMI in children, but the associations were inconsistent. The small number of studies limits the ability to judge coherence and consistency across these studies. Overall, the recent epidemiologic and animal toxicological evidence remains limited. Among the epidemiologic studies, there still remains uncertainties regarding concentration-response, lifestages and population differences, and potential of confounding by copollutants.

7.7.5 Summary and Causality Determination

The 2016 Oxides of Nitrogen ISA concluded that there was insufficient evidence to infer a causal relationship between exposure to oxides of nitrogen, primarily NO_2 exposure and effects on postnatal developmental outcomes. Epidemiologic studies summarized in the 2016 Oxides of Nitrogen ISA only investigated the associations between NO_2 exposure and neurodevelopmental outcomes, which are discussed in Chapter 9. Toxicological studies investigated the effects of NO_2 exposure on postnatal body weight gain, eye opening, and incisor eruption.

No epidemiologic studies were available for the 2016 Oxides of Nitrogen ISA to inform on the effects of oxides of nitrogen exposure on postnatal physical development. Although some animal toxicological studies reported decrements in postnatal body weight gain and delays in eye opening and incisor eruption, this was not consistently observed across studies.

The recent evidence remains limited across the epidemiologic studies and animal toxicological studies (see Table 7-7). In the recent epidemiologic studies, there was a small body of evidence for postnatal developmental mainly focused on different metrics of BMI. However, the small number of studies limits the ability to judge coherence and consistency across these studies. Overall, the recent epidemiologic and animal toxicological evidence remains limited. Among the epidemiologic studies, there still remain uncertainties regarding concentration-response, lifestages and population differences, and potential of confounding by copollutants. **Overall, the evidence is *inadequate to infer the presence or absence of a causal relationship* between oxides of nitrogen exposure and postnatal development.**

Table 7-7 Summary of evidence contributing to causality determinations for oxides of nitrogen exposure and effects of postnatal development

Rationale for Causality Determination[a]	Key Evidence[b]	Key References	Oxides of Nitrogen Concentrations Associated with Effects
Postnatal Growth			
Limited and inconsistent epidemiologic evidence for postnatal growth	Inconsistent associations for postnatal growth. Uncertainties regarding concentration response and exposure response, lifestages and population differences, and copollutant confounding.	Section 7.7.1.1	Median NO_2: 12.5-15.12 ppb for weekly avg Median NO_2: 18.5 ppb for monthly avg Median NO_2: 16.4 ppb for pregnancy avg
Limited and inconsistent toxicological evidence for postnatal growth	Inconsistent effects on postnatal weight gain in rat pups.	Shalamberidze and Tsereteli (1971) Tabacova et al. (1985) Di Giovanni et al. (1994) Section 7.7.1.2	0.0532–5.32 ppm NO_2
Puberty			
Limited epidemiologic evidence for puberty	Decreased risk of pubertal onset in females, but increased risk of pubertal onset in males. Uncertainties regarding concentration response and exposure response, lifestages and population differences, and copollutant confounding	Section 7.7.2.1	Median NO_2: 11.64 ppb for annual avg
Other Postnatal Developmental Outcomes			
Limited toxicological evidence for postnatal development outcomes	Delayed developmental milestones, including incisor eruption and eye opening, in rats.	Tabacova et al. (1985) Section 7.7.3.2	0.532–5.32 NO_2

List of abbreviations in alphabetical order: avg = average, d = day, GD = gestational day, NO_2 = nitrogen dioxide, PND = postnatal day, ppb = parts per billion, ppm = parts per million.
[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.

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